The pattern of reproduction in the African giant pouched rat, *Cricetomys gambianus* from Tanzania: unravelling the environmental triggers for breeding

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Abstract

Our research represents the first extensive study of the breeding behaviour and related environmental triggers of reproduction in the African giant pouch rat (*Cricetomys gambianus*, Waterhouse, 1840) in and around the equator throughout a whole year. We measured the gross morphology and detailed histology of both female and male rats, along with plasma steroid hormone levels. Contrary to other tropical-dwelling small mammals, the African giant pouch rat is a seasonal breeder; however, rainfall is not to be the primary cue of reproduction. Our study suggests that ambient temperature and photoperiod are the primary environmental cues of reproduction, with breeding occurring during the cooler months of the year, namely in the dry season. During the wet and hot season, which succeeds the dry season, there is an increase in the availability of quality food which results in nursing mothers and weaned pups achieving a positive energy balance and increased body condition. This, in turn, increases pregnancy success and offspring survival. Climate change, particularly global warming, could harm the reproduction of African giant pouched rats as rising temperatures in and around the equator, including Tanzania, may impact their circannual reproductive cycle.

Keywords: Rainfall, Season, Rodents, Reproduction, Hormones, African giant pouched rat, *Cricetomys gambianus*

Introduction

Successful reproduction is a critical aspect of an animal's life-history, with various biotic and abiotic (environmental) factors influencing an individual's ability to produce offspring (Ims 1990; Fitzgerald and McManus 2000). Reproduction in mammals is energetically very demanding, requiring gamete production, courtship, and mating with subsequent successful fertilisation, pregnancy and offspring care. All of these stages require significant energetic investment in both sexes (Ims 1990; Fitzgerald and McManus 2000). As a consequence of this substantial energetic investment, mammals have evolved various strategies to ensure that reproduction and subsequent offspring rearing occurs when the parents, particularly the adult breeding females, and offspring have access to a surplus of energy, namely through the access to optimal food resources and/ or exposure to optimal environmental conditions.

Mammals have three primary reproductive strategies: aseasonal breeding, seasonal breeding, and opportunistic breeding (Ims 1990; Fitzgerald and McManus 2000). Aseasonal breeding happens throughout the year, while seasonal breeding occurs during specific periods of the year (Ims 1990; Fitzgerald and McManus 2000). While, opportunistic breeding occurs only under brief favourable conditions, with food availability being the primary cue for reproduction (Ims 1990; Fitzgerald and McManus 2000). Unsurprisingly, breeding strategy has been strongly linked to annual rainfall patterns because the abundance of food and quality of the food are tightly linked to rainfall (Clauss et al. 2021). Furthermore, mammals often use less energy foraging during increased rainfall as food is more easily found (Hart and Bennett 2023). As a consequence, in regions with rainfall occurring for much of the year, mammals show an aseasonal breeding strategy, whereas those inhabiting regions with defined periods of increased rainfall show a distinct seasonality to their reproduction (Ims 1990; Fitzgerald and McManus 2000). Many mammals that breed seasonally use environmental cues to determine when to reproduce. These cues can indicate when rainfall is expected, which in turn influences the activation or deactivation of reproductive processes. Photoperiod (the duration of time during which a mammal receives light) and ambient temperature are two of the most powerful zeitgebers that control the circannual breeding rhythm and are key determinants of seasonal breeding cues (Bronson and Heideman 1994;

97 Wube et al. 2009; Sarli et al. 2015, 2016; Hart et al. 2018, 2020a, 2021a; Kamgang et al. 2020;
98 Alagaili et al. 2017, 2020).
99 According to current climate change predictions, many environments may experience
100 changes in environmental parameters that mammals use as reproductive cues (Bronson
101 2004). This might result in a mismatch between the circannual breeding rhythm and the
102 environment, which could reduce reproductive fitness (Bronson 2004). One such region is
103 along the equator. Equatorial regions are often defined as exhibiting a muted seasonal
104 variation in terms of photoperiod, ambient temperature and rainfall; however, this is
105 predicted to change in the future (Sheldon 2019). As such, it is crucial to increase fundamental
106 research on mammal reproductive strategies and their environmental cues, especially in
107 equatorial regions (Sheldon 2019).
108 Our study is the first comprehensive study of the breeding pattern and associated
109 environmental cues of reproduction in the African giant pouch rat (*Cricetomys gambianus*,
110 Waterhouse, 1840) from in and around the Morogoro region of Tanzania (06° latitude) over
111 an entire calendar year. Detailed knowledge of their reproductive biology is still scant and
112 inconclusive (Malekani et al., 2002). The pattern of reproduction in *C. gambianus* is currently
113 reported as being aseasonal, but this is from opportunistic breeding events (Ajayi, 1975) and
114 differs from that of Rosevear (1969), who suggested that African giant pouched rats in the
115 wild may possess a distinct breeding season. Although various species of the genus *Cricetomys*
116 have been reared with some success in captivity, their overall pattern of reproductive biology
117 is not well documented (Ajayi, 1975; Malekani, 1987). To address this dearth of knowledge,
118 we examined the gross morphology and detailed histology of female and male African giant
119 pouch rat gonads (including the quantification of follicular maturation and seminiferous
120 tubules growth), along with plasma steroid hormone levels (including progesterone,
121 oestrogen and testosterone), over an entire calendar year. Circulating levels of steroid
122 hormones and the downstream gonadal changes related to hormonal changes are essential
123 for determining breeding activation or recession. For example, in males, the increase in
124 plasma testosterone levels leads to an increase in testes size, growth of seminiferous tubules,
125 and enhanced sperm production, all of which indicate reproductive activation. In females, an
126 increase in plasma levels of oestrogen and progesterone, as well as ovarian size due to
127 follicular development, ovulation, and pregnancy, indicate reproductive activation.
128

Materials and Methods

Ethics approval

We received permission to capture African giant pouched rats from all landowners. Furthermore, permission was received to conduct this research and export samples from the Ministry of Natural Resources and Tourism in Tanzania through the Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Commission for Science and Technology (COSTECH), permit number 2019-46-NA-2019-41. Our project was approved by the Animal Ethics Committee of the University of Pretoria (NAS291/2021), and we obtained Section 20 import permits from the Department of Forestry and Fisheries (12/11/1/1/8; 1816JD) and veterinary import permits (2020/7/001725 and 202102004998 South Africa). Animal experimentation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (1996; published by National Academy Press, 2101 Constitution Avenue Northwest, Washington, DC 20055, USA).

Species characterisation

We assigned clade and species to our study animals using *cytochrome oxidase subunit I (COI) gene sequencing* amplification and nucleotide sequencing of 120 individuals using previously described primers and thermal cycling conditions (Ivanova et al. (2012)). We submitted full-length gene sequences to Genbank under accession number C(OQ259530) which were complemented with homologous data from prior studies (Ngalameno and Luziga 2022) and the best-fit model of sequence evolution identified under the AICC in Mega5 (Tamura et al. 2011) was subsequently used for maximum likelihood inferences.) Two haplotypes were recovered from the individuals with good PCR nucleotides amplification selected at random for genetic characterisation. One of these had a 100% nucleotide sequence identity to Genbank sequences (MH989088.1 and MH988909.1), which correspond to *Cricetomys gambianus*. The two haplotype sequences generated in this study cluster within the *C. gambianus* clade defined by Ngalameno and Luziga (2022) with 100% bootstrap support (not shown).

Animal capture

We captured African giant pouched rats in and around the Morogoro municipality in Tanzania, namely Modeko (S06.48044°E037.38035°), Mafiga (S06.822412E037.651146°), Vibandani (S06.836657°E037.660823°), Falkland (S06.5109°E037.3951) and Mzingwi (S06.85667°E037.664752). The animals were captured from abandoned animal houses, marketplaces, nearby human settlements, and maize farms, where they are considered domestic and agricultural pests. We captured animals every month over 12 consecutive months, from July 2019 to June 2020, with five sexually mature wild male and female African giant pouched rats trapped using Havahart traps (Havahart, Woodstram Corp, Lititz, PA, USA) (85cm x 25cm x 30cm) (Igbokwe and Mbajirogu 2019), resulting in a total of 120 animals (60 adult males and 60 adult females). We conducted trapping during the night using ripe bananas as bait (Igbokwe and Mbajirogu 2019). Trapped animals were transported to the College of Veterinary and Biomedical Sciences at the Sokoine University of Agriculture for surgical procedures and sample collection.

Animal processing

During surgical procedures, we placed the animals under general anaesthesia using xylazine and ketamine at 5mg/kg and 50mg/kg, respectively. We then drew whole blood from the tail vein/caudal vein using a needle and heparinised vacutainer tubes, followed by centrifuging at 3000 rpm for ten minutes to obtain plasma for hormonal analysis. Plasma was separated using a pipette, transferred to a new tube and stored at -20 °C until use. We castrated the males (open castration) to remove the testes, while the females were ovariectomised and underwent hysterectomy to remove both ovaries and the uterus. After opening the abdominal cavity, we assessed the reproductive status of the females by recording the presence or absence of embryos or foetuses. We decided to perform gonadectomy to prevent unnecessary euthanasia of animals. Our post-operative care was performed on all operated animals using oxytetracycline wound spray and nursing prior to releasing them to the wild when they fully recovered at the original capture sites.

Sample processing

The testes and ovaries removed were weighed using a Sartorius scale (Zeiss, Germany) to obtain their mass (g) before fixation in Bouin's solution for 18 h, then stored in 70% ethanol (Hart et al. 2021b). The gonads were subjected to a sequential dehydration process by placing

193 them in containers of increasing alcohol concentration and subsequently embedded them in
194 a block of paraffin wax before sectioning at a thickness of 7 µm using a rotary microtome
195 (368065/2 Baird and Tatlock, London LTD, Chadwell Health, Essex. England) (Hart eat al.
196 2021b). The mounted sections were placed on microscopic slides after being dipped in water
197 at 45^{o}C mixed with gelatine as an adhesive (Hart et al. 2020b). We then stained with
198 Haematoxylin, and counter-stained with Eosin the mounted sections after dried in an oven at
199 36 °C for 72 hrs, (Drury et al. 1967). The stained sections were covered with coverslips,
200 secured with resin solution (Microscopy Entellan glue, Germany), and dried in an oven at 36°C
201 for 48 hrs.
202
203 *Testicular histology*
204 We randomly selected thirty sections from the mid-region of the testes which were used to
205 estimate the mean diameter (µm) of seminiferous tubules with a light microscope (Diaplan,
206 Ernst LeitzWetzlar GmbH, Germany) (Hart et al. 2021b). Seminiferous tubules were
207 photographed at 10× magnification using a digital camera attached to a microscope (Moticam
208 1000 1.3 M Pixel USB 2.0, Motic China Group, LTD., Xiamen, China). The diameters (µm) of
209 ~1800 seminiferous tubules per male were measured using Motic Images Plus 2.0ML software
210 (Motic China Group, Ltd., Xiamen, China) (Hart et al. 2021b).
211
212 *Ovarian histology*
213 We examined the ovary tissue sections under a light microscope for the numbers of
214 primordial, primary, secondary, tertiary and Graafian follicles as well as corpora lutea
215 following Bloom and Fawcett (1964) and Hart et al. (2020b, 2021b). The tissue sections were
216 observed consecutively at 100×, 200× and 400× magnifications. The follicles of each category
217 for each section were counted.
218
219 *Plasma progesterone*
220 We analysed the plasma samples for progesterone using a coat-a-count kit for progesterone
221 MG12171 IBL International GmbH kit. The antiserum progesterone has a cross-reactivity to
222 all naturally occurring steroids of <0.01 except for 20-α-Dihydroprogesterone 0.03, 20-β-
223 Dihydroprogesterone 3.27, 17-α-pregnan-3,20dione 3.46, 17-α-Hydroxyprogesterone1.5,
224 Pregnonele 0.03 and 11-Deoxycorcosterone 0.83. Standard concentrations ranged from 0.25

to 34.8 ng/ml. A serial dilution of a high progesterone sample paralleled the standard curve (ANCOVA: $F_{1, 10} = 0.47$, $p = 0.51$). The intra-assay coefficient of variation for the assay was 7.8%. The sensitivity of the assay was 0.05 ng/ml.

Plasma Oestradiol -17β (oestrogen)

We analysed the plasma samples for oestradiol-17β (oestrogen) using a coat-a-count kit for oestradiol-17β MG12101 IBL International GmbH kit. The antiserum oestradiol β has a cross-reactivity to all naturally occurring steroids of <0.01 except for oestrone 1.0, oestriol 0.6, ethynylestradiol 0.2 and ostradiol -17-glucoronide <0.2. Standard concentrations ranged from 0.9 to 3900 ng/ml. Steroids in the plasma were neither purified nor separated by chromatography. A serial dilution of a plasma sample with a high oestrogen concentration paralleled the standard curve (ANCOVA: $F_{1, 10} = 3.71$, $p = 0.095$). The intra-assay coefficient of variation for the assay was 6.1%. The sensitivity of the assay was 2.7 pg/ml.

Plasma testosterone

We used a coat-a-count Testosterone kit MG12191 (IBL International GmBH, Hamburg, Germany) to determine the plasma testosterone concentrations of male African giant pouched rats. The assay was performed according to the manufacturer's protocol. There was no significant difference between the serial dilution curve of a male with high plasma testosterone and the calibration curve (ANCOVA: $F_{1, 10} = 2.97$, $p = 0.16$). The specificity for the assay was <0.001 for all naturally occurring steroids except for dihydrotestosterone 0.31%, andostenedione 0.28%, progesterone 0.01% and 19- Nortestosterone 1.8%. The sensitivity of the assay was 0.18 nmols/l. The intra-assay coefficient of variation was 6.8%.

Environmental variables

We averaged (±SE) all environmental variables, namely rainfall (mm), temperature (°C) and photoperiod (number of hours of daylight), over the collection sites. We collected monthly rainfall data from the Sokoine University of Agriculture Meteorological Centre. While monthly ambient temperature and photoperiod data were collected using the methods outlined by Wallace et al. (2021) and Hart et al. (2022a) and validated by Finn et al. (2022), namely through the use of the ERA5-Land of the European Centre for Medium-Range Weather

Forecasts-the latest generation created by the Copernicus Climate Change Service (Muñoz-Sabater et al., 2021). As a result, all environmental conditions are presented in Fig. 1.

We tested the normality of the dependent variables, including rainfall, temperature and photoperiod, using Shapiro-Wilk tests. Subsequently homogeneity of normally distributed dependent variables was tested using Levene's test. Furthermore, we attempt to normalise all non-normally distributed dependent variables through log transformation. However, it was discovered that all environmental conditions were not normally distributed. We preformed spearman rank correlation tests between all environmental variables. In previous studies (Sarli et al. 2015, 2016; Alagaili et al. 2017; Hart et al. 2018, 2020a, 2021b), all environmental variables, namely monthly rainfall, temperature and photoperiod, have been found to affect reproduction in small mammals independently; consequently, we included all environmental variables in further analysis.

Data analysis

We performed all statistical analyses in R 3.5.2, and statistical significance was assumed at $p \leq 0.05$. The data in the tables and figures are presented as mean ± standard error (SE). Due to the small number of individuals captured during each month, it was decided to divide the 12 months of the year into the four well-known seasons of Tanzania, namely, the hot and dry season (January to February), wet season (March to June), dry season (July to September) and hot and wet season (October to December) (Fig. 1).

We determined the normality of the dependent variables (body mass (g), testicular and ovarian mass (g), testicular and ovarian volume (mm3), seminiferous tubules diameter (µm), plasma progesterone (ng/ml), oestrogen (pg/ml) and testosterone (ng/dl) concentration and the number of primordial, primary, secondary, Graafian follicles and corpora lutea of pregnancy and ovulation) using Shapiro-Wilk tests and the homogeneity of normally distributed dependent variables was confirmed with a Levene's test. In addition, we attempted a log transformation to normalise all non-normally distributed dependent variables.

We analysed normally distributed dependent variables (Log transformed testicular volume (mm3)) using linear models (LMs). While all non-normal dependent variables (body mass (g), testicular and ovarian mass (g), ovarian volume (mm3), seminiferous tubules diameter (µm) plasma progesterone (ng/ml), oestrogen (pg/ml) and testosterone (ng/dl)

concentration as well as the number of primordial, primary, secondary, Graafian follicles and corpora lutea of pregnancy and ovulation) were analysed using Generalised linear models (GLMs) that were fitted with gamma distributions and log-link functions, or negative binomial distributions with log-link functions using the *lme4* package. We conducted post-hoc comparisons using Tukey's honestly significant difference (HSD) tests. Furthermore, in order to investigate the variation in body mass (g) of male and female African giant pouched rats we used GLM containing season (hot and dry; wet; dry; hot and wet) and sex (male; female) and their two-way interaction. All models that investigated the various male parameters (testicular mass (g), testicular volume (mm3), seminiferous tubules diameter (μm) and plasma testosterone (ng/dl) concentration) or female reproductive parameters (ovarian mass (g), ovarian volume (mm3), plasma progesterone (ng/ml) and oestrogen (pg/ml) concentration and the number of primordial, primary, secondary, Graafian follicles and corpora lutea of pregnancy and ovulation) contained the dependent variable with season (hot and dry; wet; dry; hot and wet) and body mass as the predictor and covariant respectively.

We further evaluated the effects of the environmental variables on reproduction and body mass of male and female African giant pouched rats using GLMs fitted with gamma distributions and log-link function or negative binomial distributions with log-link functions, with each environmental variable (rainfall, ambient temperature and photoperiod) and body mass (only for reproductive parameters dependent variables models) run for each dependent variable (Bates et al. 2015). Model selection was conducted for each model using the dredge function of the *Mumin* package (Barton and Barton, 2015). Model suitability was assessed using Akaike information criterion values corrected for a small sample size (AICc). Models with ΔAICc <2 were considered equally parsimonious, the coefficients of which were subsequently averaged to construct a final model. Conditional average values were reported for final models with more than one competing model. The final models selected are presented in Table 2.

Results

Environmental variables

The number of hours of daylight experienced by the African giant pouched rats varied slightly between seasons; namely, the hot and wet (12.4±0.02 hours) and hot and dry (12.4±0.03 hours) seasons possessed the longest days, while the dry (11.9±0.03 hours) and wet

(11.9±0.03 hours) seasons possessed the shortest days (Fig. 1A). The average ambient temperature across all capture sites was greatest during the hot and dry season (25.0±0.02°C), while the coldest temperatures were experienced in the dry season (22.8±0.2°C) (Fig. 1B). Furthermore, both the wet season (23.±0.20°C) and the hot and wet season (23.7±0.20°C) experienced similar average temperatures (Fig. 1B). The average rainfall across all capture sites was the highest during the wet season (828±119mm), followed by the hot and wet season (364±79.4mm), then the hot and dry season (14.4±3.71mm) with the dry season (5.17±1.95mm) receiving the least rainfall (Fig. 1C).

Monthly rainfall did not correlate with either monthly temperature or photoperiod ($r \leq -0.17$, $p \geq 0.2$). In contrast, monthly ambient temperature and photoperiod were directly proportional to one another ($r = 0.84$, $p < 0.0001$, Fig. 1).

Body mass

We found that body mass was unaffected by sex ($t_{1, 119} = -1.51$, $p = 0.13$), season ($t_{3,119} = -0.60$, $p = 0.55$) or their two-way interaction (Sex*Season: $t_{7, 119} = 1.84$, $p = 0.07$, Table 1). Likewise, no environmental variable significantly affected body mass (Table 2).

Pregnancy occurrence

A total of 14 pregnant females were captured in two (number of pregnant females: dry season - 6; hot and wet season – 8) out of the four seasons (Fig. 2).

Ovarian morphology and histology

We found no relationship between body mass and ovarian mass or volume of female African giant pouched rats ($t_{1,59} \leq -0.08$, $p \geq 0.76$). Similarly, we found that season did not affect ovarian volume ($t_{3, 59} = 1.40$, $p = 0.17$; hot and dry: $2.38 \pm 0.42 mm^3$; wet: $1.87 \pm 0.19 mm^3$; dry: $1.47 \pm 0.23 mm^3$; hot and wet: $1.92 \pm 0.20 mm^3$), but did significantly affect ovarian mass ($t_{3, 59} = -3.24$, $p = 0.002$ Fig. 3 A & B). The ovarian mass of females captured in the hot and dry season (0.03 ± 0.005g) was significantly less than those captured in the other three seasons (wet: 0.06 ± 0.01g; dry: 0.11 ± 0.01g; hot and wet: 0.04 ± 0.005g) (HSD: $p \leq 0.01$, Fig 3A). While all females caught in the remaining seasons possessed similar ovarian mass (HSD: $p \geq 0.05$. Fig 3A). Interestingly, ovarian mass was significantly affected by rainfall and photoperiod, while ovarian volume was only affected by temperature (Table 2).

We did not observe a relationship between body mass and the number of any ovarian follicle types produced by the ovary of female African giant pouched rats ($t_{1,59} \leq -0.44$, $p \geq 0.36$). Furthermore, we found that the season did not affect the number of primordial ($t_{3, 59} = -1.81$, $p = 0.114$), tertiary ($t_{3, 59} = -1.59$, $p = 0.16$) or Graafian ($t_{3, 59} = -2.11$, $p = 0.07$) follicles in ovaries of female African giant pouched rats (Table 3). In contrast, season had an effect on the number of primary ($t_{3, 59} = -2.66$, $p = 0.03$) and secondary ($t = -4.80$, $p = 0.002$) follicles produced as well as the number of corpora lutea of ovulation and pregnancy ($t_{3, 59} = -3.11$, $p = 0.02$) (Table 3). The females in the dry season possessed a significantly higher number of primary follicles than those in the hot and wet season (HSD: $p = 0.04$, Table 3). All females captured in the remaining seasons possessed a similar number of primary follicles (HSD: $p \geq 0.15$; Table 3). Similarly, the females captured in the dry season possessed a significantly higher number of secondary follicles compared to those captured in the other three seasons (HSD: $p \leq 0.01$, Table 3). Furthermore, females captured in the hot and wet season possessed a significantly higher number of secondary follicles compared to those captured in the wet season (HSD: $p = 0.004$, Table 3). The females captured in the hot and dry season possessed a similar number of secondary follicles to those captured in the hot and wet season (HSD: $p = 0.18$) and wet season (HSD: $p = 0.80$) (Table 3). Corpora lutea of ovulation were observed throughout the year, yet an increase in the total number of corpora lutea was observed in the dry as well as the hot and wet season, likely due to the formation of corpora lutea of pregnancy (Fig. 3C, Table 3). The females captured in the dry, and hot and wet seasons similarly exhibited higher numbers of corpora lutea (HSD: $p = 0.53$, Table 3) compared to the hot and dry season and the wet season (HSD: $p \leq 0.02$, Table 3). The hot and dry season and the wet season possessed equally low levels of corpora lutea (HSD: $p = 0.90$, Table 3).

The number of primordial, primary, secondary, tertiary and Graafian follicles was greatest during the periods of the lowest rainfall (Table 2). During periods of the shortest day lengths, the number of primary, tertiary and Graafian follicles was at its highest (Table 2). Similarly, during the coolest periods of the year, the numbers of primordial follicles were at their highest (Table 2). We found that during the coolest and shortest day lengths, the number of corpora lutea of ovulation and pregnancy observed in female African giant pouched rats were at their highest (Table 2).

Female hormonal profiles

We again found no relationship between body mass and plasma progesterone concentrations of female African giant pouched rats ($t_{1, 59}$ = 0.43, p = 0.67), however, season significantly affected plasma progesterone concentrations of African giant pouched rats ($t_{3, 59}$ = -2.03, p = 0.04, Fig. 3A). The females captured in the hot and wet season (3.85 ± 1.55ng/ml) possessed significantly higher plasma progesterone than those captured in the wet season (1.26 ± 0.22ng/ml) (HSD: p = 0.04, Fig. 3D). In contrast the hot and dry (3.83 ± 1.11ng/ml) and dry (3.10 ± 1.20ng/ml) seasons and all remaining comparisons were not significantly different from one another (HSD: p ≥ 0.18, Fig. 3D). We also found that plasma progesterone was significantly affected by rainfall, but not temperature or photoperiod (Table 2).

While we found that both body mass (t1$_{, 59}$ = -2.79, p = 0.01) and season ($_{3, 59}$ t = 2.75, p = 0.01) significantly affected plasma oestrogen concentrations of female African giant pouched rats (Fig. 3D). Interestingly, heavier females had lower concentrations of oestrogen (Table 2). Females captured in the wet season (329 ± 168pg/ml) possessed higher plasma oestrogen concentrations compared to the hot and dry (29.9 ± 7.40pg/ml) (HSD: p = 0.001) and dry season (84.4 ± 43.3pg/ml) (HSD: p = 0.03) (Fig. 2D). The hot and wet season (247 ± 111pg/ml) and all remaining comparisons were not significantly different from one another (HSD: p ≥ 0.07, Fig. 4D). Only ambient temperature significantly affected the plasma oestrogen concentrations of female African giant pouched rats (Table 2).

Testicular morphology and histology

No relationship between body mass and testicular mass, volume or seminiferous tubule diameter of male African giant pouched rats was found ($t_{1, 59}$ ≤ -0.37, p ≥ 0.08). Similarly, season had no effect on the testicular mass of male African giant pouched rats of this study ($t_{3, 59}$ = -0.03, p = 0.98, Fig. 4A). However, season did affect testicular volume ($t_{3, 59}$ = 6.19, p < 0.0001, Fig. 4B) and seminiferous tubule diameter ($t_{3, 59}$ = -19.3, p < 0.0001, Fig. 4D). The males captured in the dry season (4.21 ± 0.21mm^3) possessed the lowest testicular volume compared to the other three seasons (Hot and dry: 7.68 ± 0.4mm^3; hot and wet: 6.60 ± 0.54mm^3; wet: 5.55 ± 0.27mm^3)(HSD: p ≤ 0.01, Fig. 4B). While we found the males captured in the hot and dry season possessed larger testicular volumes than those captured in the wet season (HSD: p = 0.004, Fig. 4B). The males captured in the hot and wet season possessed similar testicular volumes to those captured in the hot and dry season (HSD: p = 0.26) and wet season (HSD: p = 0.27) (Fig. 4B). In contrast, males captured in the dry season (263 ± 48.5µm)

possessed the largest seminiferous tubule diameters, followed by the males captured in the hot and dry season (209 ± 17.9µm), then by the males captured in the wet season (163.3 ± 27.0µm), with the males captured in the hot and wet season (152.7) ± 28.3µm) possessing the smallest seminiferous tubule diameters (HSD: $p < 0.0001$, for all, Fig. 4D).

Testicular mass and seminiferous tubule diameter of male African giant pouched rats were both greatest during periods of the year with the lowest day lengths and ambient temperature (Table 2). Likewise, the diameter of the seminiferous tubules was greatest during periods of the least rainfall (Table 2). In contrast, the testicular volume of male African giant pouched rats was greatest during increased day lengths and temperature (Table 2).

Male hormonal profiles

There was no relationship between body mass and plasma testosterone concentrations of male African giant pouched rats ($t_{1, 59} = -1.27$, $p = 0.31$). Season, however, did affect plasma testosterone titre ($t_{3, 59} = -3.02$, $p = 0.004$). The males captured in the hot and wet season (1.33 ± 0.23ng/dl) possessed a lower plasma testosterone titre compared to the dry season (10.2 ± 5.47ng/dl) (HSD: $p = 0.01$), the hot and dry season (HSD: $p = 0.04$) and the wet season (HSD: $p = 0.002$, Fig. 4C). All males captured in the remaining three seasons possessed similar plasma testosterone concentrations (HSD: $p \geq 0.96$, Fig. 4C).

Plasma testosterone concentrations of male African giant pouched rats were both greatest during periods of the year with the lowest day lengths and temperature (Table 2).

Discussion

In this study, we sought to investigate the reproductive strategy and the possible environmental cues that may influence reproduction in a small mammal that resides around the equator, namely the African giant pouched rat. As with many other small mammal species with a short gestation period, our *a priori* prediction for the African giant pouched rat was for it to exhibit a seasonal breeding strategy, confining its breeding to the months that experienced rainfall. We further predicted that rainfall would bring about reproductive activation in this rodent species, while other environmental factors, namely photoperiod and ambient temperature cues, would not significantly influence reproductive activation or regression. The finding from our study supported the prediction that the African giant pouch rat is a seasonal breeder; however, our remaining predictions, mainly that rainfall is the

448 primary cue of reproduction, were not supported. Our study suggests that ambient
449 temperature and photoperiod (which were observed to be significantly linked) are the
450 primary environmental cues of reproduction, whereas rainfall, which is likely still important,
451 is less significant than the other environmental cues investigated in this study.

452 Interestingly, we found that during periods of reduced ambient environmental
453 temperature and photoperiod, namely, the last month of the wet season and most of the dry
454 season, both males and females showed increased reproductive activation, including
455 increases in ovarian and testicular mass, seminiferous tubule diameter and plasma
456 progesterone, oestrogen and testosterone concentrations. This would have increased mating
457 events during these periods, resulting in pregnancy events during the dry season, as observed
458 in our study. As the gestation period of the African giant pouched rat is between 27 to 42 days
459 (Rosevear, 1969), the offspring of the dry season mating period would be born during the hot
460 and wet seasons. Therefore, during this period of increased rainfall, temperature and
461 photoperiod, primary productivity and food availability would be significantly greater (Ims
462 1990). As a consequence, nursing mothers would have an increased quantity and quality of
463 food, increasing their energy intake and reducing energy expenditure, thus resulting in a
464 positive energy balance in these females that would allow sufficient nutrient (energy) transfer
465 to nursing pups. Furthermore, as the average weaning time of African giant pouched rats is
466 approximately 28 days (Rosevear, 1969), if pups are born at the end of the dry season and
467 early in the hot and wet season, they would be weaned within the hot and wet season and
468 still have sufficient time to achieve a positive energy balance, by accessing the increased
469 resource availability of the hot and wet season, before the hot and dry season begins. Similar
470 patterns of increased reproductive activation and even pregnancy during the cooler dry
471 seasons have been observed in many small mammals that inhabit arid regions near the
472 equator (Yamaguchi et al. 2013; Sarli et al. 2015, 2016; Alagaili et al. 2017; Hart et al. 2018,
473 2020a, 2021b). As with these arid-dwelling small mammal species, the African giant pouch rat
474 likely uses the decreasing temperature and photoperiod to time the birth of their pups at the
475 beginning of the hot and wet season when there are sufficient resources to maintain the body
476 condition of the nursing mother and weaned pup.

477 In conclusion, the African giant pouched rat is a seasonal breeder which undergoes
478 reproductive activation during the coolest months of the year, namely the dry season. Since
479 the dry season is succeeded by the wet and hot season, which are characterised by an

480 increase in the availability of quality food, nursing mothers and weaned pups would achieve
481 a positive energy balance and increased body condition. This begs one question: why were no
482 pregnant females captured in the wet season or in the beginning dry season? We speculate
483 that during the wet season, there could be a successive increase in primary productivity (plant
484 material) over several months. This would enable both male and female animals to achieve a
485 positive energy balance, which is needed for them to attain the required body condition and
486 fat storage. This, in turn, would allow for the activation of reproduction during the dry season.
487 However, as the results of our study suggest, they do not become active; other possible
488 explanations may be at play. Our study suggests that increased rainfall alone is not the sole
489 cue responsible for reproductive activation in mammals around the equator. Therefore,
490 further research is needed to determine the cause of a lack of reproductive activation
491 following a period of increased rainfall in the African giant pouched rat.
492 The findings from our study highlight the possible consequences of climate change,
493 particularly global warming, as temperatures in and around the equator, including Tanzania,
494 are expected to rise significantly under future climate change scenarios (Luhunga et al. 2018).
495 Therefore, since temperature may play a vital role in the circannual control of reproductive
496 activation in African giant pouched rats, global warming may have a detrimental effect on the
497 reproduction of this rodent species. In particular, due to the predicted rise in temperatures
498 of the future, African giant pouched rats may not achieve the necessary energy balance to
499 allow for reproductive activation.
500
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509
510 **Data availability**

The datasets presented in this article are not readily available because rights to this raw data are held by the institute and further permission is needed to release such data. Requests to access the datasets should be directed to DWH, u10022725@tuks.co.za

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

Fig. 1: The climatic conditions of the Morogoro municipality, Tanzania, from July 2019 to June 2020. A) The photoperiod (number of hours of daylight per day), B) average monthly temperatures (◦C) and C) average monthly rainfall (mm). Climatic conditions were averaged over the five capture sites in the Morogoro municipality, namely Modeko (S06.48044°E037.38035°), Mafiga (S06.822412E037.651146°), Vibandani (S06.836657°E037.660823°), Falkland (S06.5109°E037.3951) and Mzingwi (S06.85667°E037.664752). The colour red indicated the months of the hot and dry season; the colour blue indicated the months of the wet season; the colour brown

635 indicated the months of the dry season, and the months of the hot and wet season were
636 indicated by green.
637
638 **Fig. 2: Percentage of pregnant female African Pouched Rat (*Cricetomys gambianus*,**
639 **Waterhouse, 1840) captured [(Number of pregnant females captured per month/total**
640 **number of females captured per month) * 100].** The colour red indicated the months
641 of the hot and dry season; the colour blue indicated the months of the wet season; the
642 colour brown indicated the months of the dry season, and the months of the hot and
643 wet season were indicated by green.
644
645 **Fig. 3: Monthly variation of reproductive parameters (mean ± SE) of female African**
646 **Pouched Rat (*Cricetomys gambianus*, Waterhouse, 1840). A) Ovarian mass (g), B)**
647 **ovarian volume (mm3), C) plasma progesterone concentration (ng/ml) and D) plasma**
648 **oestrogen concentration (pg/ml).** The colour red indicated the months of the hot and
649 dry season; the colour blue indicated the months of the wet season; the colour brown
650 indicated the months of the dry season, and the months of the hot and wet season were
651 indicated by green. *: indicates significant ($p \leq 0.05$).
652
653 **Fig. 4: Monthly variation of reproductive parameters (mean ± SE) of male African**
654 **Pouched Rat (*Cricetomys gambianus*, Waterhouse, 1840). A) Testicular mass (g), B)**
655 **testicular volume (mm3), C) plasma testosterone concentration (ng/dl) and D)**
656 **seminiferous tubule diameter (µm).** The colour red indicated the months of the hot
657 and dry season; the colour blue indicated the months of the wet season; the colour
658 brown indicated the months of the dry season, and the months of the hot and wet
659 season were indicated by green. *: indicates significant ($p \leq 0.05$). (↔) indicate all
660 possible combinations were significant ($p \leq 0.05$).