Reproduction in the East African root rat, *Tachyoryctes splendens* (Rodentia: Spalacidae) from Tanzania: the importance of rainfall

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Abstract

The East African root rat, *Tachyoryctes splendens* (Rüppell, 1835) is a solitary subterranean rodent mole. The present study investigated breeding patterns in both sexes of *T. splendens* from data collected at monthly intervals over an entire calendar year. The study focused on the analyses from post mortem examination of males and females to assess the presence of foetuses, gonadal histology, reproductive tract morphometrics, measurement of gonadal steroids (plasma progesterone and oestradiol-17β in females and testosterone in males) and field observations (i.e., the presence of infants, juveniles, sub-adults and lactating females). The objective of this study was to assess if the reproductive biology of the root rat reflected the bimodal pattern of

rainfall that is characteristic of East Africa. Rainfall has been suggested to trigger breeding in many subterranean rodents and as a consequence, this study aimed to assess the relationship between rainfall and reproductive characteristics of *T. splendens*. Peaks in mean gonadal mass, increases in concentration of reproductive hormones and the presence of Graafian follicles and corpora lutea in the ovaries of females, and testes mass, seminiferous tubule diameter and testosterone titre mirrored the annual peaks of precipitation at the study area. Together with field observations of the temporal occurrence of pregnancies, infants, juveniles and sub-adults, the data show that *T. spendens* cues its breeding with the patterns of rainfall, such that offspring are born in the latter half of each rainy season, from April to July and November to December.

Introduction

Reproduction in subterranean mammals is constrained by both the prevailing ecological conditions and the burrow environment (Bennett & Faulkes, 2000). The subterranean niche precludes the use of many common cues that are normally used to maximise reproductive success, for example, photoperiod is unlikely to be an important proximate cue, whereas rainfall in the form of changing soil moisture content is used to trigger breeding (Bennett & Faulkes, 2000; Herbst *et al*., 2004; Hart *et al*., 2006). Temperature is a potentially important environmental cue that mole-rats may utilise for their daily and seasonal activity patterns. The temperatures within mole-rat burrow systems are much less variable than those above ground;

diurnal and seasonal fluctuations in temperature do occur but they are muted (Bennett *et al*., 1988; Roper *et al*., 2001).

Solitary subterranean rodents occurring at higher altitudes (particularly geomyids) exhibit marked breeding patterns and this may in part be due to the seasonal changes in temperature of their burrow environment (Kennerly, 1964). Rainfall is an important variable that can be detected underground since it softens the soil (if it reaches sufficient depths) and brings about subsequent plant growth and flushes of vegetation (Dennis & Marsh, 1997). Solitary subterranean bathyergid mole-rats time their breeding events with rainfall. These occur in winter rainfall regions and as a consequence mating occurs in the winter months, with young being born in the spring when food is abundant and the soil easily workable. This also facilitates dispersal and construction of independent burrow systems (Bennett & Jarvis, 1988; Herbst *et al*., 2004; Hart *et al*., 2006). Interestingly, the silvery mole-rat, *Heliophobius argenteocinereus* from the tropics differs in that it breeds seasonally, but not synchronously with rainfall patterns (Šumbera *et al*., 2003).

Many subterranean rodents are strictly solitary, highly xenophobic and vigorously defend the burrow system in which they reside (Bennett & Jarvis, 1988; Gazit & Terkel, 1998; Šumbera *et al.,* 2003). In solitary African mole-rats (Family Bathyergidae), courtship and subsequent copulation is brief and requires that the highly aggressive and xenophobic behaviours that are characteristic of these species are relaxed during these periods (Bennett & Jarvis, 1988; Narins *et al.,* 1992). Seismic signalling occurs with the different sexes announcing their presence and sexual status displayed by a particular frequency of foot drumming (Bennett & Jarvis, 1988; Narins *et al.,* 1992; Bennett *et al.,* 1999). Thus plural occupancy of the female's burrow system

only occurs when courtship and mating is taking place and also when the female has young (Bennett & Jarvis, 1988; Bennett *et al.,* 1991).

All solitary southern African species of bathyergid mole-rats are seasonal breeders with their reproduction acutely tied to rainfall (Bennett & Jarvis, 1988; Herbst *et al*., 2004; Sandwyk & Bennett, 2005; Hart *et al*., 2006). In marked contrast, the majority of social bathyergids reproduce throughout the year (Bennett *et al.*, 1988, Sichilima *et al.,* 2008; 2011). Two social species, one from a winter rainfall region, the common mole-rat, *Cryptomys hottentotus hottentotus* (Spinks *et al.,* 1997; 1999) and one from a summer rainfall region, the highveld mole-rat, *Cryptomys hottentotus pretoriae* (van Rensburg *et al.,* 2002) are unusual in exhibiting seasonal reproduction, with the former also occurring sympatrically with solitary species the Cape dune mole-rat, *Bathyergus suillus* (Bennett & Faulkes, 2000).

A number of studies of reproduction in solitary subterranean rodents have been conducted, but many of these have focussed on laboratory housed animals (Altuna *et al.*, 1991; Bennett *et al.,* 1991; Gazit *et al.,* 1996) given the difficulties of studying subterranean rodents in the field (Rado & Terkel, 1989; Rado *et al.,* 1998). The reproductive biology of a number of families of rodent moles have been studied and include *Geomys* (Wood, 1949; Vaughan, 1962), *Cannomys* (Eisenberg & Maliniak, 1973), *Thomomys* (Andersen, 1978), *Georychus* and *Bathyergus* (Bennett *et al.,* 1991), *Spalax* (Shanas *et al.,* 1995; Gazit *et al.,* 1998), *Ctenomys* (Weir, 1974; Camin, 2010) and *Heliophobius* (Šumbera *et al.,* 2003). Despite these, very little has been published on the East African root rat, *Tachyoryctes splendens* (Family Spalacidae). Jarvis (1973) investigated a Kenyan population of *Tachyoryctes* using field observations as well as the examination of gonadal histology from adult females. She reported that *T. splendens* has two breeding opportunities approximately 173 days apart; the gestation period was estimated to

be 38.5 days with the time interval between birth and weaning of around 43 days, and the annual recruitment rate to be 2.73 young with an average of 2.1 litters produced annually from a single female.

To date, there has been no in-depth study on the reproductive biology of both male and female *Tachyoryctes* and this study addresses this deficiency on a population from Tanzania, East Africa where the species that is considered to be an agricultural pest. In this study we aimed to assess the reproductive biology of *T. splendens* using post-mortem data and investigation of plasma steroids from animals sampled on a monthly basis for an entire calendar year, as has been done for other subterranean mammals (e.g., Wood, 1949; Herbst *et al.,* 2004; Schoeman *et al.,* 2004; Hart *et al.,* 2006; de Bruin *et al.,* 2012). We predicted that peaks in reproductive activity and pregnancy would coincide with the bimodal rainfall patterns characteristic in East Africa, namely the long wet season from February to early June and the shorter wet season in September, October and November.

Materials and Methods

Tachyoryctes splendens were sampled from the Mamba Komakundi village which is located on the foot slopes of Mount Kilimanjaro in Moshi Rural District, north-east Tanzania, East Africa (03˚16.54' S, 037˚32.49' E; 1495 m above sea level). The study was conducted over 12 consecutive months, from January to December 2011. The study site is classified as agri-ecological zone with highly fertile volcanic soils supporting a variety of food and cash crops (Kilimanjaro Regional Profile [KRP], 1998). The study area has two rainy seasons (Figure 1) with a mean annual rainfall of 1250 mm; a longer period of rainfall (i.e., heavy rainfall) occurs in the latter half of February, March, April, and May while a shorter period of rainfall occurs in late

September, October and November. The dry months are December, January, June, July, and August (KRP, 1998). June and July are the transition months between the heavy rains and dry season. Annual average temperature ranges from 15°- 40° C with high humidity during March, April, May and October (KRP, 1998).
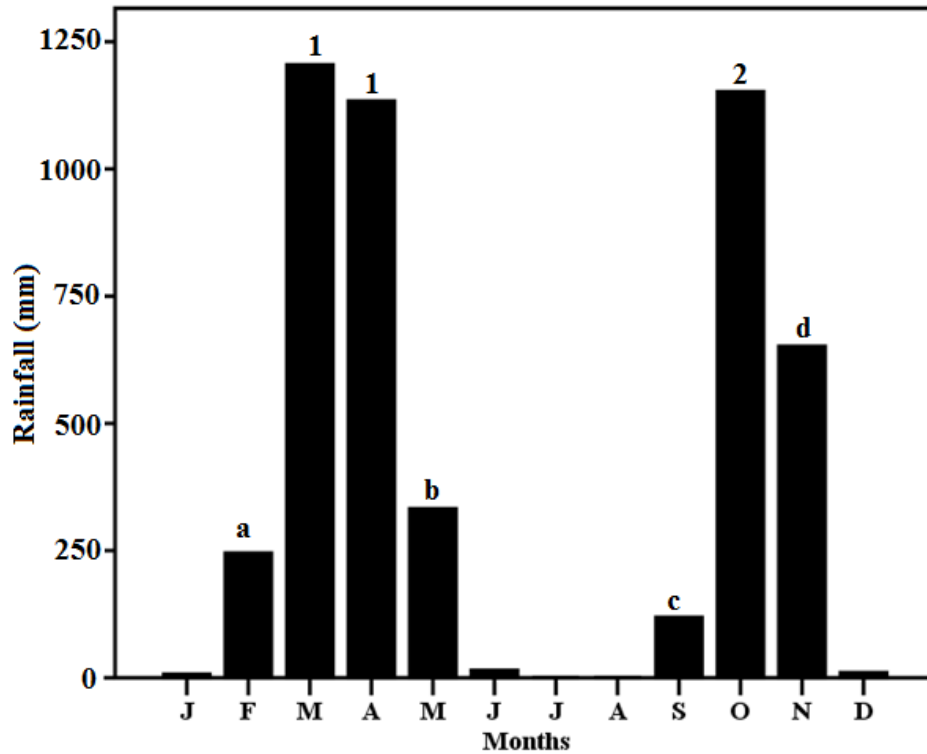

Figure 1: Mean monthly rainfall (mm) of Marangu ward, Kilimanjaro region Tanzania from January to December 2011. Data were obtained from the Tanzania Meteorology Agency (TMA): a = onset of heavy rainfall (wet season 1), b = offset of heavy rainfall, c = onset of short rains (wet season 2), d = offset of short rains, 1 = heavy rains, 2 = short rains. July, August and January are driest months.

Animals were sampled from areas subject to widespread crop destruction by *T. splendens*, and were sampled from their burrows by either using Hickman live traps or by manual excavation of burrow systems (Hart *et al*., 2006; Sichilima *et al.,* 2008). Ten adult female and ten adult male *T. splendens* were sampled each month for one calendar year resulting in a total of 240 animals (i.e., 120 adult males and 120 adult females). Burrows for sampling animals

were selected randomly (Sichilima *et al.,* 2008; 2011). Animals were euthanized with an overdose of chloroform (Merck, Johannesburg, South Africa). Following expiration, standard body measurements were recorded including body mass, head-body length, tail length and the length of hind foot (Hart *et al*., 2006).

The external reproductive characteristics such as presence of teats and a perforate vagina were recorded in females. Blood was drawn from the heart of the sacrificed animal using heparinized syringes and centrifuged at 500 g for 10 minutes. Plasma was separated using a pipette and subsequently stored at -20° C. Gonads (testes/ovaries) were removed and fixed in Bouin's fixative for 18 hrs before storing in 70% ethanol. The reproductive status of females was further assessed by recording either the presence or absence of embryos/foetuses after post-mortem dissection. Lactating females were identified by assessing the presence of prominent teats and occupancy of either juvenile(s) or infant(s) in their burrows.

Juveniles and sub-adults of both sexes were recorded on sampling to assess recruitment to the population of *T. splendens* from reproductive animals sampled, but their blood and gonads were not included in the hormonal and histological analyses of this study because their reproductive systems were not fully developed. Categorisation of individuals into either juveniles or sub-adults was based on tooth eruption, based on the right maxillary molar tooth-row using the method described by Taylor *et al.*, (1985), with modification appropriate to the specific dental formula of *T. splendens* (1/1, 0/0, 0/0, 3/3; Katandukila *et al.,* submitted). Infants were classed by having either two cheek teeth or less, with no eruption of the third tooth, and had less fur present on their body than juveniles. Juveniles were classed as having two fully-developed cheek teeth and an erupting third molar, and still inhabited the maternal burrow. Sub-adults had

three fully-developed teeth and occupied an independent burrow adjacent to maternal burrow systems with immature reproductive organs (i.e., small testes and ovaries).

Histology: Mass (g) of fixed gonads was recorded using a Sartorius scale (Zeiss, Germany), while length (mm) and width (mm) were measured using a pair of digital calipers (Mitutoyo American Corporation Aurora, Illinois). Testicular and ovarian volumes were evaluated using the formula for the volume of an ellipsoid: $V = 4/3\pi ab^2$ where a = half the maximum length and b = half the maximum width as detailed by Woodall & Skinner (1989). All measured gonads were sequentially dehydrated in increasing concentrations of ethanol baths and embedded in a cube of paraffin wax before being sectioned at a thickness of 7 μm using a rotary microtome (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, New York, U.S.A.). Gonad sections were mounted on microscopic slides after being dipped in warm water mixed with gelatine as an adhesive. Mounted sections were dried in an oven at 36° C for about 72 hrs and subsequently stained using Ehrlich's haematoxylin and counter-stained with eosin (Drury & Wallington, 1967). Stained sections were covered by a slide cover-slip and glued with resin solution (Microscopy Entellan glue, Germany) as adhesive and then dried in an oven at 36° C for about 48 hrs.

Testicular histology: 30 randomly selected sections from the mid region of the testes were chosen in order to measure the diameter of seminiferous tubules with a light microscope (Diaplan, Ernst Leitz Wetzlar GmbH, Germany). Seminiferous tubules were then photographed at 10× magnification with a digital camera (Moticam 1000 1.3 M Pixel USB 2.0, Motic China Group, LTD., Xiamen, China) attached to a microscope. The diameters (μm) of 3600 seminiferous tubules were measured using Motic Images Plus 2.0ML (Motic China Group, Ltd.,

Xiamen, China). It is assumed that greater diameter of the seminiferous tubule indicate active testes with higher production of spermatozoa.

Ovarian histology: Ovarian sections were examined under a light microscope at 100×, 200× and 400×magnification (van Rensburg *et al.,* 2002; Hart *et al.,* 2006). Each ovary was sectioned in its entirety and sections were examined in consecutive order using a light microscope and checked for the presence of primordial, primary, secondary, tertiary and Graafian follicles as well as corpora lutea following Bloom & Fawcett (1962), van Rensburg *et al.* (2002) and Hart *et al.* (2006). Follicles of each developmental stage per section were counted, avoiding double counts by matching follicles split between consecutive sections using the method of Borgeest *et al.* (2004). Sections were photographed using a digital camera (Moticam 1000 1.3 M Pixel USB 2.0, Motic China Group, LTD., Xiamen, China) attached to a light microscope.

Radioimmunoassay: In the laboratory, plasma from female animals was assayed for progesterone and oestradiol-17β while plasma from males was assayed for testosterone using Coat-A-Count kits following the manufacturer's specifications. All hormone assays were validated for use in mole-rats as described in Bennett *et al.* (1994) and the total hormonal concentrations were determined using a gamma counter.

Progesterone: A volume of 100μL of plasma was dispensed in duplicate into polypropylene tubes following the Coat-A-Count progesterone kit (Progesterone Diagnostics Products Corporation, U.S.A.) procedures. The Coat-A-Count progesterone antiserum is highly specific for progesterone with a particularly low cross-reactivity (< 1%) to other naturally occurring steroids except in 5α-Pregnan-3, 20-dione (9%), 17α-Hydroxyprogesterone (3.4%), β-pregnan-3, 20-dione (3.2%) and 11-Deoxycorticosterone (2.2%). A blood plasma sample with a high concentration of progesterone was double-diluted using the assay buffer as a matrix 1:1 to 1:8

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then assayed. The slopes of serial double dilution and standard curve were compared to check for parallelism (Analysis of covariance (ANCOVA): $F = 2.54$; $n = 4$; $P > 0.05$) following a log-logit transformation of the data (Chard, 1987). The intra-assay coefficient of variation for the plasma pool was 2% whereas the inter-assay coefficient was 9.9% and sensitivity of the assay was 1.35 nmol/L.

Oestradiol -17β: Oestradiol -17β assays were performed as described by Herbst *et al*. (2004) using a Coat-a-Count oestradiol kit (Diagnostic Products Corporation). Cross-reactivity of the antibody to all naturally occurring steroids was 10% with oestrone, <5% with oestriol, oestrone-β-D-glucoronide, oestone-3-sulphate, d-equilenin, 17β-oestradiol-3-monosulphate, testosterone and androsterone. The assay was validated for plasma of *T. splendens* by testing the slope of the curve produced using serial dilutions of un-extracted mole-rat plasma obtained from a female with high oestradiol concentrations (over the range 1:1 to 1:32) against the standard curve. After logit-log transformation of the data (Chard, 1987), slopes of the lines were compared and found not to differ significantly from the reference preparation (ANCOVA: $F = 0.72$; $n = 6$; $P > 0.05$). The intra-assay coefficient of variation (CV) for repeated determinations of a quality control was 8.3% and sensitivity of the assay was 10pg/ml.

Testosterone: A volume of 50 μl of plasma was dispensed in duplicate into polypropylene tubes following Coat-A-Count Total Testosterone kit (Testosterone Diagnostics Products Corporation, U.S.A.) procedures. The Coat-A-Count Total Testosterone antiserum is highly specific for testosterone with cross-reactivity for 19-Nortestosterone (22%), 4-Estren-17-ol-3-one (20%) and 11-Ketotestosterone (16%) whereas 5α-Dihydrotestosterone, 19-Hydroxyandrostenedione and other steroids has low cross-reactivity of < 3.5%. A blood plasma sample with highest testosterone concentration was double diluted from 1:1 to 1:32 then assayed. The slopes of serial

double-dilution and that of standard curve was compared to the double-dilutions to check for parallelism (ANCOVA: $F = 2.87$; n = 6; $P > 0.05$) following a log-logit transformation of the data (Chard, 1987). The intra-assay coefficient of variation for the plasma pool was 3.2% whereas the inter-assay coefficient was 4.7% and sensitivity of the assay was 1.39 nmol/L.

Data analyses

The number of young produced from adult females per annum was determined using the following population equation from Jarvis (1973) with modification:

$$\text{L} = Y/T \quad \text{where} \quad T = \frac{G+W}{1-(\frac{n}{N})}$$

Where L= litter(s) born to one adult female per annum
  Y= days in the year ($\approx$ 365 days)
  T= duration of one breeding cycle
  G= Gestation period ($\approx$ 38.5 days)
  W= time between birth and weaning ($\approx$ 43 day)
  $\frac{n}{N}$= Proportional of number of adult females in the sample neither pregnant nor lactating
    to the total number of females in the sample

Descriptive and statistical analyses were performed using Statistical Package for the Social Sciences (*SPSS*; Schneider, 1988) version 20 (*IBM® SPSS® Statistics 20)* statistical packages and Excel 2007. The relationship between variables such as gonadal mass, volumes and rainfall were evaluated using Pearson's correlation analysis. Normality of data within each month was initially tested using Kolmogorov-Smirnov (KS) test (Lilliefors, 1967) and parametric tests were used in subsequent analyses after the normality of the data were confirmed. Monthly variation in hormone concentrations and gonadal metrics was investigated using analysis of variance (ANOVA) with Student Newman Keuls (S-N-K: testosterone, progesterone and gonadal metrics) and Tukey's honest significant difference (HSD: oestradiol-17β) to evaluate

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significant differences *post hoc*. All descriptive statistics in the illustrations are presented as mean ± one standard deviation (SD) except for rainfall.

Results

The plasma and gonads of 120 adult males and 120 adult females were sampled, while 5 pups and 121 juveniles were sampled in maternal burrows. Among juveniles, 60 were females and 61 were males. Juvenile and infant samples increased from April to May, thereafter decreasing from June to July, none in February, August, and September, but increasing again from October to December. Samples of juveniles and infants were thus restricted to the mid and latter end of peaks of rains (Figure 1). 83 sub-adults were sampled of which 43 were females and 40 were males, they were sampled in their burrow systems which were adjacent to their maternal burrows. Of the 120 adult females, 32.97% ($n = 40$) were lactating whereas 16.20% ($n = 19$) were pregnant. Pregnancies increased from February to March and decreased from April, June and July then increased again from September to early November (Figure 2). Pregnancies in June and July were considered as late pregnancies as they occurred when many other females were lactating rather than pregnant. Post-mortem dissection of pregnant females revealed that three females were carrying 2 foetuses, while the remaining 16 were pregnant with a singleton (giving a mean litter size of 1.1 pups). With two conception periods separated by 166 days, fitting the parameters into the equation of Jarvis (1973), the mean reproductive output was calculated as 2.2 pups per female per year.
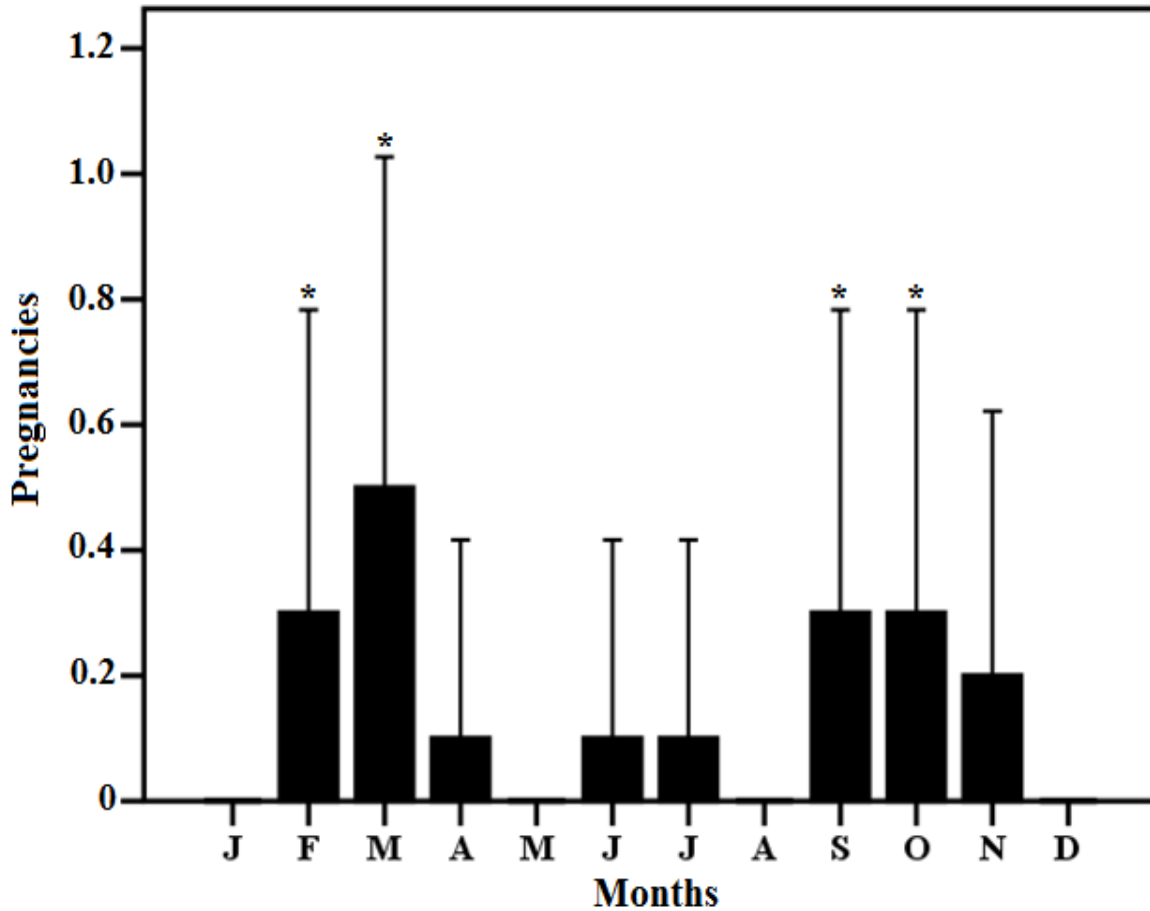
Figure 2: The mean ± SD number of pregnancies observed from post mortem examination in female *Tachyoryctes splendens* (*n* = 120) sampled from Tanzania from January to December 2011: * = Months having the highest number of pregnancies.

Histology

Testicular metrics: Testicular mass increased from February to March and decreased from April to August then increased again from September to October with the lightest testes mass observed in August (Figure 3). The mass of testes was significantly different between months ($F_{(11,108)}$ = 7.38; *n* = 120; *P* = 0.0001) with months of wet seasons having heavier testes than dry months ($F_{(1,118)}$ = 47.08; *n* = 120; *P* = 0.0001 = 120). The volumes of testes (*n* = 120) were highest in

January but decreased from February to April and increased again in August and then decreased from September to November; however, volumes increased again in December (Figure 3). Testicular volumes were significantly higher during non-breeding than breeding months ($F_{(1,118)}$ = 12.34; n = 120; P = 0.010) inversely to testicular mass. Testicular mass increased in parallel with rainfall (Pearson's correlation: r = 0.78; n = 12; P = 0.001) in contrast to testicular volume (Pearson's correlation: r = -0.69; n = 12; P = 0.004).

The mean diameter of seminiferous tubules (n = 3600) was greatest in September, October and November; whereas the lowest mean diameter was in January and August (Figure 3). Variation in the diameter of seminiferous tubules between months was significant ($F_{(11,3588)}$ = 118.72; n = 3600; P = 0.0001) with breeding months (i.e., wet seasons) showing widened diameter of seminiferous tubules than non-breeding months (i.e., dry season: $F_{(1,3598)}$ = 185.77; n = 3600; P = 0.0001). The width of seminiferous tubules was positively correlated with peaks of rainfall (Pearson's correlation: r = 0.68; n = 12; P = 0.005).
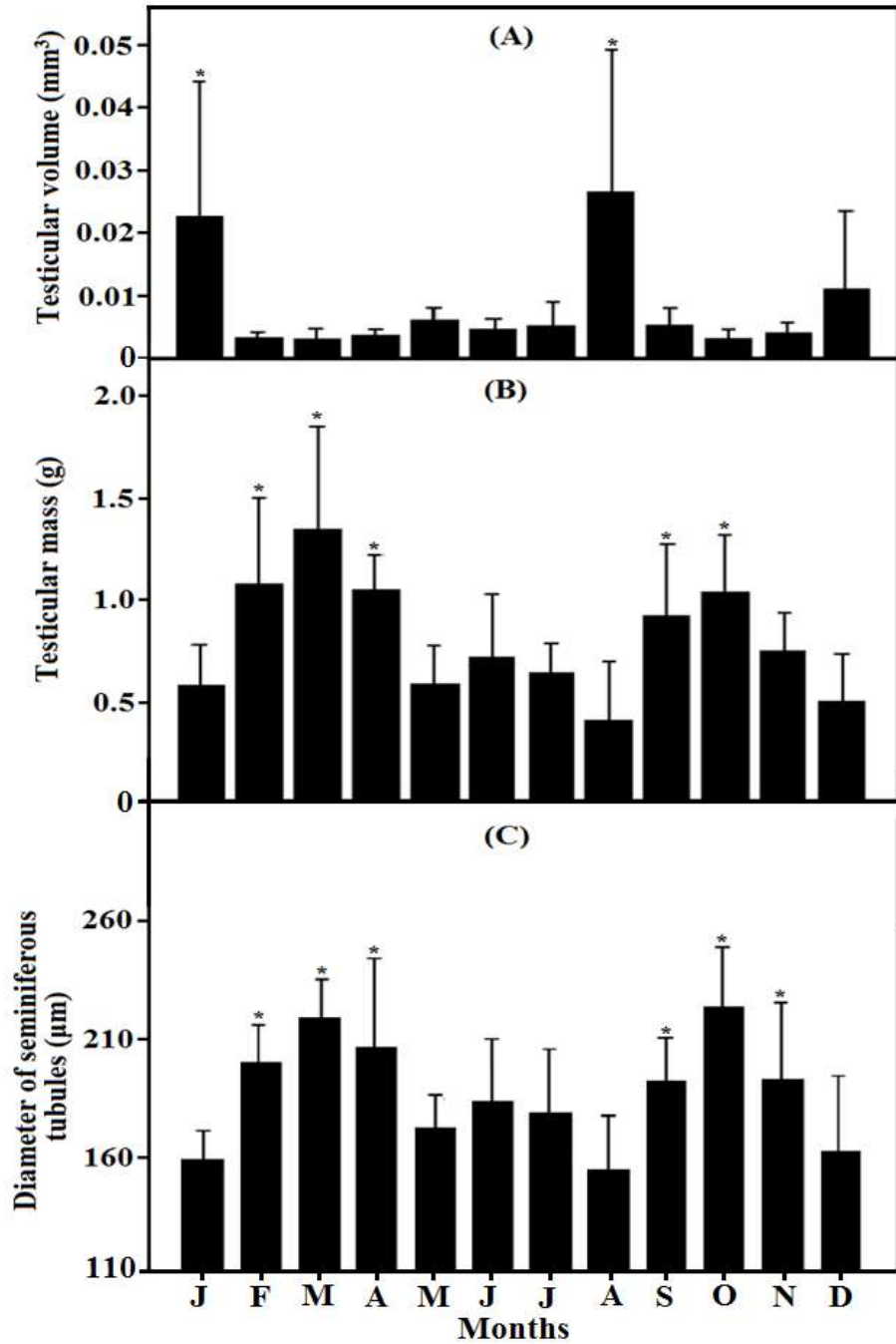
Figure 3: Monthly measurements (mean ± SD) of testicular volume (A), mass (B) and diameter of seminiferous tubules (C) in male *Tachyoryctes splendens* (*n* = 120) sampled in Tanzania from January to December 2011). * = Months of the greatest measurements

Ovarian metrics: The mass of the ovaries was greatest in February, March and April; and again in September, October and November with the lowest mean ovarian mass occurring in August (Figure 4). Ovarian mass varied between breeding and non-breeding months ($F_{(1,118)}$ = 9.88; n = 120; P = 0.002). Ovarian volumes were higher in January, March, August, November and December (Figure 4). Ovarian volumes varied significantly between months ($F_{(11,108)}$ = 3.76; n = 120; P = 0.0001) whereas non-breeding months (dry season) had highest volumes than breeding months. The mass of ovaries increased with increasing rainfall (Pearson's Correlation: r = 0.61; n = 12; P = 0.021) and was inversely proportional to ovarian volume (Pearson's correlation: r = -0.68; n = 12; P = 0.005).

Follicle development varied over the year, and as expected, primordial follicles were recorded in all ovaries (n = 120), with no significant variation in number over the year ($F_{(11,108)}$ = 0.94; n = 120; P = 0.36). The number of primary follicles was highest for *T. splendens* sampled in August and lowest in December. Secondary follicles showed the highest numbers in the animals sampled in January, March and April and lowest in May and August (Table 1); however, the number of tertiary follicles was highest in January, February and June with none recorded in August and in December. February and September had the greatest numbers of Graafian follicles while January, May and August had fewer Graafian follicles with none in November and in December (Figure 4). The number of corpora lutea were highest in March than in February, April, June and July with none observed in January, May, August and December. The maturation of Graafian follicles to corpora lutea matched the peaks of rainfall (Figures 1 and 4).
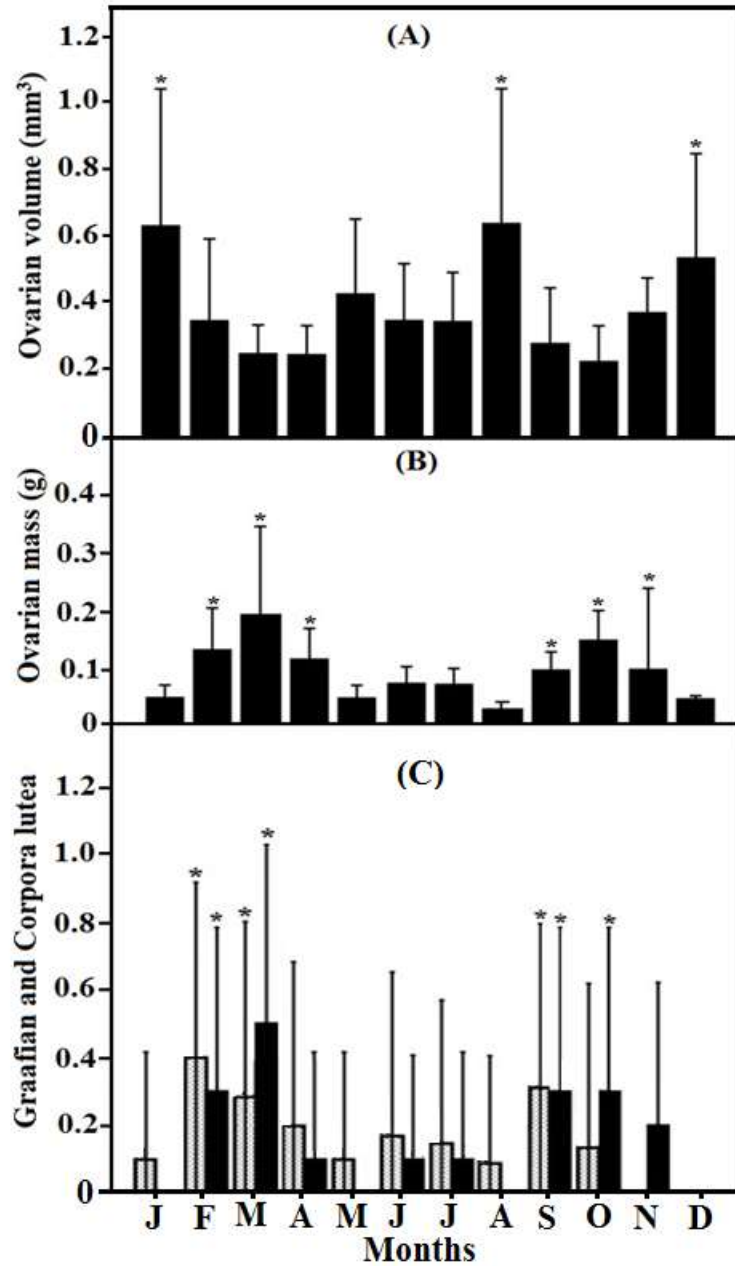
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Figure 4: Monthly fluctuations (Mean ± SD) in ovarian volume (A), mass (B) and number of Graafian follicles and Corpora lutea (C) in female *Tachyoryctes splendens* (*n* = 120) sampled in Tanzania from January to December 2011; (C), hatched bars = Graafian follicles and solid black bars = Corpora lutea: * = Months of the greatest measurements.

Radioimmunoassay

Testosterone: The mean concentration of testosterone was highest in November and lowest in January and August (Figure 5). Concentration of testosterone varied significantly between months ($F_{(11,108)}$ = 8.15; n = 120; P = 0.001) whereas higher concentrations were observed in breeding than in non-breeding months. The concentration of testosterone in male *T. splendens* increased during the wet season and was lowest during the dry season with maxima coinciding with peaks of precipitation (see Figures 1 & 5). There was a positive correlation between rainfall and testosterone concentration (r= 0.58; n = 12; P = 0.03).
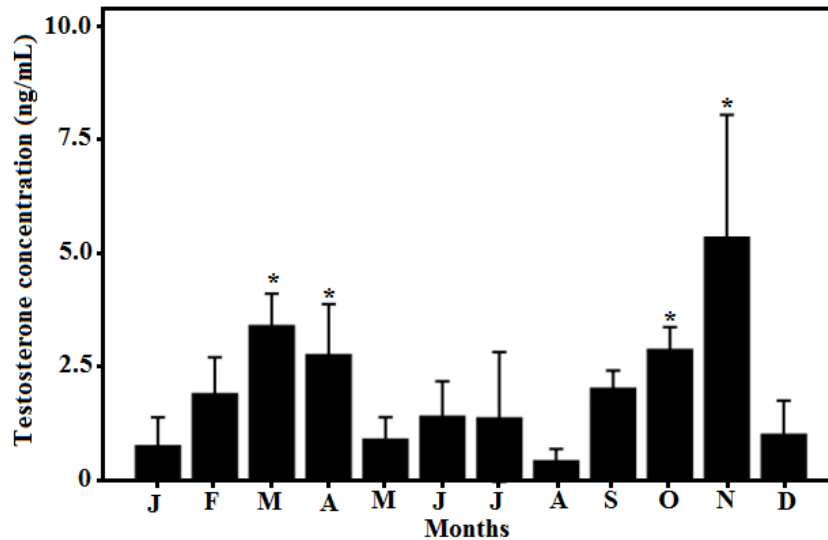

Figure 5: The concentration (mean ± SD) of testosterone (ng/mL) in male *Tachyoryctes splendens* (n = 120) sampled in Tanzania from January to December 2011: * = Months having the highest concentrations of testosterone.

Progesterone: The concentration of progesterone increased from February, March and April while the lowest occurred during the dry season in January, May and August (Figure 6). Progesterone concentration differed significantly between months ($F_{(11,108)}$ = 23.15; n = 120; P = 0.0001). There was a significant positive correlation between progesterone concentration and

rainfall ($r = 0.69$; $n = 12$; $P = 0.004$) mirroring the periods with the highest number of pregnancies (Figure 2).

Oestradiol-17β: Oestradiol-17β concentration was significantly higher during the rains in February and March as well as in September and October with the lowest concentration observed in the dry month of May (Kruskal Wallis test: $H_{(11)} = 23.51$; $n = 10$; $K = 12$; $P = 0.02$). Although these elevations in oestradiol-17β mirrored rainfall peaks, the correlation was not significantly different ($r = 0.06$; $n = 12$; $P = 0.67$; Fig. 6).
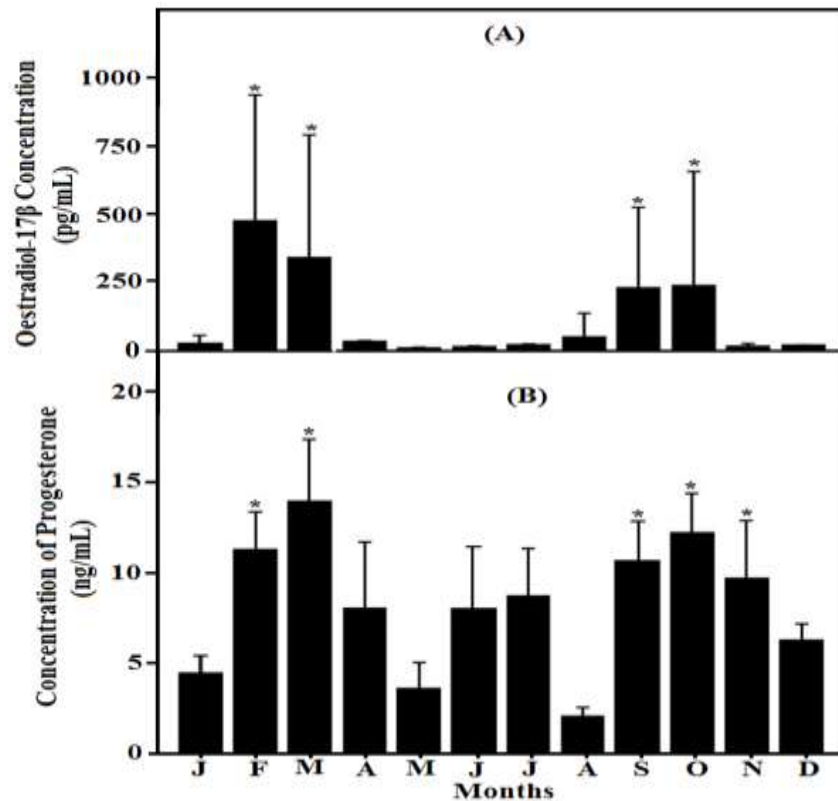

Figure 6: Monthly concentrations (mean ± SD) of Oestradiol-17β (A) and progesterone (B) in female *Tachyoryctes splendens* ($n = 120$) sampled in Tanzania from January to December 2011: * = Months having significant the highest concentrations.

Discussion

Post-mortem examination of a variety of reproductive parameters in *T. splendens* from Tanzania shows that it clearly synchronises its breeding with periods of precipitation. The reproductive tract morphometrics, gonadal histology and measurement of gonadal steroids revealed two distinct peaks of breeding that mirrored rainfall peaks at the study area. Plural occupancy of burrows generally only occurs either when adult male and female come together to mate or when females are caring for their young (Jarvis, 1973; Flynn 1990; Nowak, 1999). Coincident peaks of rainfall, oestradiol-17β and the presence of Graafian follicles in February, March, September and October indicate that female *T. splendens* ovulate during the onset of the wet months. Likewise, male *T. splendens* also show peaks in concentration of testosterone, have larger seminiferous tubule diameters and heavier testicular mass during the wet months. This suggests that it is during wet months that adult males and females prepare for mating, the raised testosterone levels coincident with the onset of the rains.

The mating period may therefore reflect increased territoriality and active mate searching as has been reported in other solitary subterranean mammals during breeding (see Bennett & Jarvis, 1988; Bennett *et al.*, 1991; Narins *et al.*, 1992). Furthermore, intra-male competition may be enhanced during this period as males actively seek female mates, as has been reported for the African mole-rat *Bathyergus suillus*, which may fight to death to acquire mates (Bennett & Faulkes, 2000; Hart *et al*., 2006). As with other subterranean rodents, adult *T. splendens* of both sexes synchronise the maturation of their reproductive cells to increase the chances of conception before the end of wet months so that when young are born food is abundant. This has also been reported for gophers, *Geomys* sp. (Wood, 1949; Villa-Cornejo & Engeman, 1995), the Middle Eastern mole-rat, *Spalax ehrenbergi* (Heth *et al.*, 1987; Rado *et al.*, 1987), African mole-rats,

Bathyergus suillus and *Georychus capensis* (Bennett & Jarvis, 1988; Sandwyk & Bennett, 2005; Hart *et al.,* 2006), *Bathyergus janetta* (Herbst *et al.,* 2004) and the tuco-tuco, *Ctenomys talarum* (Fanjul *et al.,* 2006). In such instances, rainfall results in soils becoming softer so that digging can occur and the caching and harvesting of both subterranean (geophytes) and sprouting above-ground vegetation can be utilised by the animals during these periods of resource abundance.

The presence of corpora lutea, higher circulating levels of progesterone and greater ovarian mass through the period when rainfall is high reflects the observations of pregnancies during this time (February-March and September-October). Although the majority of conceptions occurred at the onset and subsequent pregnancies were through the period of heavy rains, interestingly, some pregnancies were also recorded at the end of heavy rains (June and July months). These pregnancies possibly represent a period when an opportunistic chance to mate occurs as a consequence of lower constraints in mate acquisition, as suggested in other studies of solitary mammals (Emlen & Oring, 1977; Schulte-Hostedde *et al*., 2001; Isaac & Johnson, 2003). The late season pregnancies were validated by observations of a few new born pups at the end of June, early July and January. Although food resources might be more limiting during these months of the dry season (June, July and January) the adult females may cache food within their burrow and female *T. splendens* extend their burrows to increase foraging efficiency particularly when they have pups.

The estimation of 2 litters born per annum per adult female *T. splendens* corresponds well with the findings of Jarvis (1973). Jarvis (1973) documented two conception cycles of 173 days apart and the successful births of 2.1 litters per adult female, whereas in this study there is a similar cycle of 166 days with the birth of an average 2.2 litters per adult female a year. However, post-mortem observations revealed that three pregnant *T. splendens* had twin foetuses,

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although pregnancy with a single foetus was by far the most common condition in pregnant females. Birth of pups during periods of good rainfall ensures the acquisition of quality food resources for lactating females and their young as the soil is softened and burrowing is made more efficient. Since precipitation is a major influence for the re-sprouting, regeneration and an overall increase in plants succession and subsequently increased vegetation cover (Bennie, 1991; Barber, 1995; Pregitzer & King, 2005), the numbers of pup births in *T. Splendens* over the periods of rainfall might be an ultimate factor for timing of their births. This observation is parallel to reports documented for bathyergids, geomyids, ctenomyids, Middle East spalacids and other arboreal small mammals (Vaughan, 1962; Andersen, 1978; Bronson, 1985; Lovegrove & Jarvis, 1986; Bennett & Jarvis, 1988; Ims, 1990; Bennett *et al.,* 1991; Malizia *&* Busch, 1997; Bennett & Faulkes, 2000; Herbst *et al.* 2004; Camin, 2010; Tassino & Passos, 2010).

Juvenile and/or sub-adult *T. splendens* were recorded throughout the year in this study demonstrating that although litters are most often a single pup, *T. splendens* has a steady recruitment rate, and that predation pressure may be low and competition for key resources such as food and space also low (Jarvis & Sale, 1971; Hickman, 1983; Kokiso & Bekele, 2008). To ensure young are adequately provisioned, female *T. splendens* construct a more complex burrow system (length range = 5.85–13.51 m; fractal dimension range = 1.16–1.29) compared to male burrows (length range = 4.51–8.50 m long; fractal dimension range = 1.14–1.24) (Katandukila *et al.*, submitted). The increased burrow length and fractal dimension ensures efficient foraging, and is similar to observations in females of other solitary subterranean rodents (Bennett & Faulkes, 2000; Herbst *et al.,* 2004; Sandwyk & Bennett, 2005; Hart *et al.*, 2006).

In this study, the dispersal of sub-adult *T. spendens* peaked during rainy season months. This observation implies that the timing of dispersal of sub-adult *T. splendens* from their natal

burrow systems occurs at the onset of next rains to ensure that the offspring have maximal vegetation and food resources available for independence when setting up their own tunnel system. This breeding strategy and the associated dispersal from natal burrows have been well-documented for other solitary subterranean rodents (e.g., *Georychus capensis*: Jarvis & Bennett, 1991; *Bathyergus* species: Bennett & Faulkes, 2000; Herbst *et al.,* 2004). The dispersal of sub-adults during periods of rain when food is plentiful (Rado *et al.,* 1992; Le Galliard *et al.,* 2012) and the soil is workable (Williams & Cameron, 1984) enables sub-adult *T. splendens* to successfully establish their own independent burrow system (Bennett *et al.,* 1991; Rado *et al*., 1992; Selås 1997; Herbst *et al.,* 2004; Maher & Burger, 2011).

Breeding seasonally enables animals to time their reproductive events such that young are born when environmental conditions are favourable and they can maximise their reproductive success (Ims, 1990). Photoperiod plays a very important role as a proximate factor in triggering reproductive events in above-ground organisms with developed visual systems, living at higher latitudes (Lofts, 1970, Karsch *et al*., 1984; Nelson *et al*., 1998), but its role in the seasonality of breeding in visually regressed subterranean rodents is probably either limited or not important at all. Spalacid mole-rats are known to respond to photoperiod, such as effecting thermoregulation in *S. ehrenbergi* (Haim *et al*. 1983). African mole-rats also entrain their locomotory activity rhythms to different lighting schedules (Oosthuizen *et al.,* 2003; Hart *et al*., 2004; de Vries *et al*., 2008), but it is unlikely that it is important in reproduction. Rainfall is probably the most important environmental factor acting on subterranean mammals in that it can be detected underground since it softens the soil (if a sufficient amount falls), and brings about subsequent plant growth and flushes of vegetation (Dennis & Marsh, 1997). All solitary subterranean southern African bathyergid mole-rats studied to date time their breeding events with rainfall.

These occur in winter rainfall regions and as a consequence mating occurs in the winter months, with young being born in the spring when food is abundant and the soil easily workable. This also facilitates dispersal and construction of independent burrow systems (Bennett & Jarvis, 1988; Herbst *et al*., 2004; Hart *et al*., 2006). Interestingly, the silvery mole-rat, *Heliophobius argenteocinereus* from the tropics differs in that it breeds seasonally, but not synchronously with rainfall patterns (Šumbera *et al*., 2003). Likewise, *S. ehrenbergi* exhibits seasonal reproduction with breeding occurring in the winter months where they likely cue into rainfall (Heth *et al*., 1995).

Our findings from the reproductive hormone profiles, histological assessment of gonadal characteristics and field observations have all clearly revealed that *T. splendens* is a seasonal breeder having two periods of heightened reproductive activity within a year, which is consistent with the findings of studies undertaken in Kenyan by Jarvis (1973). *Tachyoryctes splendens* from Tanzania shows peaks of reproductive hormone concentrations, pregnancies, births and sub-adult dispersals concomitant with peaks of precipitation. This suggests that precipitation is the key factor which triggers their seasonal reproduction. The overall results in the present study confirm our earlier prediction that peaks in reproductive activity and pregnancy would coincide with the bimodal rainfall patterns characteristic in East Africa, namely the long wet season from February to early June and the shorter dry season in September, October and November.

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