

The pattern of reproduction in the mole-rat *Heliophobius* from Tanzania: do not refrain during the long rains!

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ABSTRACT

The genus *Heliophobius* comprises of at least six cryptic, topotypical species in the *Heliophobius argentocinereus* species complex. The current study investigated the breeding patterns of a wild-caught population from Tanzania where the putative species *H. emini* resides. Individuals were collected on a monthly basis for an entire calendar year. Assessment of foetus presence, gonadal histology, reproductive tract morphometrics in combination with gonadal steroid (plasma progesterone and oestradiol-17 β in females and testosterone in males) measurements and field observations revealed that rainfall is important for the onset of breeding. The results further confirmed that breeding is limited to a single, yearly reproductive event synchronised to the long rainfall pattern. The distinct breeding peak in July is associated with an elevation in gonadal mass, increase in concentrations of reproductive hormones and presence of Graafian follicles and corpora lutea in the ovaries of females. These reproductive parameters coincided with the end of the long rainfall period, whereas presence of young in the maternal burrow system corresponded with the start of the short rainfall of East Africa. These findings confirm *Heliophobius* has a single breeding opportunity each year, and this species is therefore vulnerable to any changes that may impact their climatically attuned breeding patterns.

Keywords: *Heliophobius*, solitary, hormones, histology, precipitation, radioimmunoassay, reproduction, cyt *b*.

Introduction

In subterranean mole-rats, breeding is constrained by the ecological conditions and the restrictive nature of the burrow system (Bennett et al. 2000). The subterranean niche prevents the use of many environmental cues used by surface occurring species, for example change in day length (photoperiod) is unlikely to act as a proximate cue because of the slight annual difference in day length. Rainfall on the otherhand is increasingly implicated as a cue for triggering breeding in several solitary subterranean mammals (Dennis and Marsh 1977; Bennett and Faulkes 2000; Šumbera *et al.* 2003; Herbst *et al.* 2004; Hart *et al.* 2006; Katandukila *et al.* 2013), but a consistent trend is yet to emerge as the importance of this cue varies geographically and across different levels of sociality.

Temperature is another important cue which subterranean mammals may utilise for their activity patterns (Lovegrove, 1989; Šklíba et al. 2007; Lovey et al. 2013; Šklíba et al. 2014; Oosthuizen and Bennett, 2015). The temperatures within the burrow systems of African mole-rats are generally more muted compared to those experienced above ground, but diurnal and seasonal fluctuations in temperature do occur *albeit* at lower amplitudes compared to those of above ground conditions (Bennett *et al.* 1988; Roper *et al.* 2001; Burda *et al.* 2007).

In South Africa the mole-rats occurring in winter rainfall regions mate and conceive only during the winter rainfall months, with the young born in the spring when food is widely available and the soil is workable enabling animals to burrow to search for food in the form of underground storage organs of geophytes. Furthermore the moist soil allows dispersal of the young and the establishment of independent burrow systems in three solitary species; the Cape mole-rat, *Georychus capensis* (Pallas, 1778), the Namaqua mole-rat, *Bathyergus janetta* (Gmelin, 1788) and the Cape dune mole-rat, *Bathyergus suillus* (Schreber, 1782) (Bennett and Jarvis 1988; Herbst *et al.* 2004; Hart *et al.* 2006). In eastern Africa these reproductive events have been found to coincide with the long and short rainfall periods in the solitary East

African root rat, *Tachyoryctes splendens* (Ruppell, 1835), an allopatric and phylogenetically diverse subterranean rodent mole (Katandukila *et al.* 2013). The solitary silvery mole-rat *Heliophobius argentocinereus* (Peters 1846) is reported to breed seasonally in Zambia, Kenya and Malawi (Copley 1950, Scharff, 2001, Šumbera *et al.* 2003). In Malawi breeding has been reported at during the end/or the second half of a rainy season (Šumbera *et al.* 2003), rather than at the beginning of the rainy season.

All solitary South African species of bathyergid mole-rats studied to date have been reported to be seasonal breeders with their reproduction linked to rainfall (Bennett & Jarvis 1988; Herbst *et al.* 2004; Hart *et al.* 2006). In contrast, the majority of social mole-rats breed throughout the year (Bennett *et al.* 1988, Sichilima *et al.* 2008; 2011; Oosthuizen and Bennett 2008). There are, however, two social species which breed seasonally with rainfall; the common mole-rat, *Cryptomys hottentotus hottentotus* (Lesson, 1812) which occurs sympatrically with the solitary mole-rats *Georchus* and *Bathyergus* mole-rat genera (Spinks *et al.*, 1997; 1999), and the highveld mole-rat, *Cryptomys hottentotus pretoriae* (Roberts, 1913)(van Rensburg *et al.* 2002, van der Walt *et al.* 2001).

The *Heliophobius argenteocinereus* cryptic species complex represents an ideal study group for teasing apart variation in environmental drivers of reproduction in subterranean mammals. Members of this taxon are widely distributed throughout eastern, central and southern Africa, and were until recently believed to be monotypic. A phylogeographical study confirming the presence of multiple topotypical species within the complex, allows for a more thorough investigation of reproductive cues. As all species are solitary and share common ancestry, differences in the timing of reproduction will be strongly linked to locality-specific environmental factors. *Heliophobius emini* (Noack 1894) was selected for this study as it occurs in an area that is geographically and climatically distinct from the single *Heliophobius* species for which data are available, viz. *H. argenteocinereus* from Malawi (Šumbera *et al.*

2003) but allopatric with another solitary subterranean species *T. spendens* for which there is extensive reproductive data (Katandukila et al. 2013).

In the absence of nuclear data corroborating the topotypically discrete mitochondrial lineages (Faulkes et al. 2011) we take a conservative taxonomic approach and refer to the taxon under study as *Heliophobius*. However, it is pertinent to mention that available chromosome data, although limited, are suggestive that the species identified by Noack (1894) as *Heliophobius emini* has a $2n = 52$ whereas specimens from eastern Zambia have a $2n = 62$ whilst those from Kenya have a $2n = 60$ (George 1979, Scharff et al. 2001, Deuve et al. 2007; Faulkes et al. 2011)

By investigating the reproductive biology of *Heliophobius* from post-mortem examination of gonads and plasma steroids of animals sampled on a monthly basis for a year in Tanzania, as has been done for other subterranean mammals (e.g. van Rensburg et al. 2002; Herbst et al. 2004; Schoeman et al. 2004; Hart et al. 2006; de Bruin et al. 2012, Katandukila et al. 2013), and by recording pregnancies we can determine whether reproductive activity coincides with the rainfall patterns that are characteristic in eastern Africa; namely the long wet season from February to early June and the shorter dry season in September, October and November. The results have important implications for the species in light of changes in climate of equatorial Africa, where rainfall periods shift dramatically inter-annually in concert with El Niño Southern Oscillations, and where temperature and rainfall, have increased and decreased by an average of 0.23°C and 3.3%, respectively, per decade since 1960 (McSweeney et al. 2010).

Materials and Method

Study site and sampling

Heliophobius were captured from four main sites in Mvomero District:

S06.82036°E037.67056°, S06.97858°E037.55001°, S06.98003°E037.54636°,

S06.98005°E037.54635 in Tanzania. The study was conducted over 12 consecutive months; from January to December 2013. All sites are classified as agri-ecological zones with highly fertile soils supporting a variety of food and cash crops (Morogoro Regional Socio Economic Profile (MRSP)). The study area has two rainy seasons with a mean annual rainfall of 600 mm; the longer period of rainfall (i.e. heavy rainfall) is from March to May with a mean of 470 mm while a shorter period of rainfall of 130mm is from October to December. Dry months are, June, July, August and September (MRSP). June and July months are the transition months between heavy rains and the dry season. Annual average temperature ranges from 18 to 30°C with high humidity during March, April and May; as well as October and November (MRSP) (Figure 1).

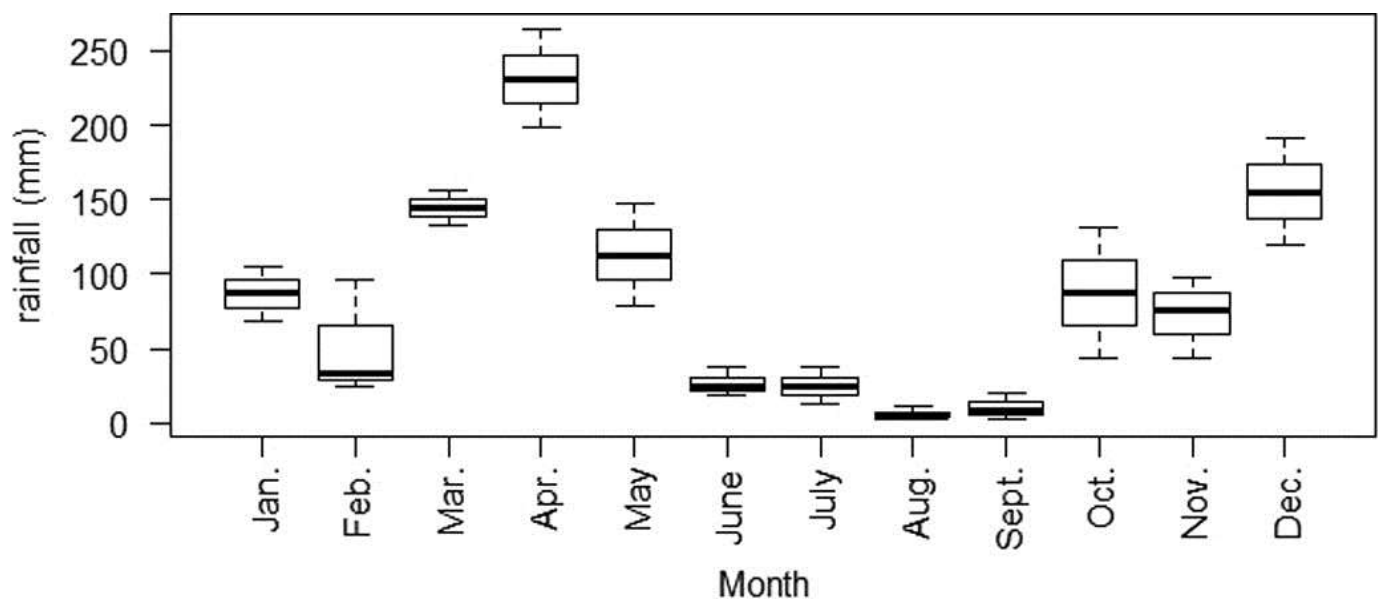


Fig. 1. Mean (\pm SD) monthly rainfall (mm) of Mvomero District, Morogoro, Tanzania, for January until December 2014. Data from Tanzania Meterology Agency.

Animals were collected from areas subject to widespread crop destruction, where the mole-rat is considered an agricultural pest. Animals were collected using Hickman live traps or by excavation of burrow systems (Hart *et al.* 2006; Sichilima *et al.* 2008). Five adult female and five adult male mole-rats were captured monthly for one calendar year resulting in a total of 120 animals (i.e. 60 adult males and 60 adult females). Burrows for capturing animals were selected randomly by opening a distinct burrow system at least 100m from one another. The animals were euthanized with an overdose of chloroform and the body mass subsequently recorded. Burrow entrances were left open to check for subsequent blocking and if arising traps were inserted (multiple occupancy usually resulted when there was a mother and offspring).

The presence or absence of elongated teats and/ or a perforate/imperforate vagina were recorded in females. Blood was drawn from the heart of the sacrificed animal using heparin coated syringes and centrifuged at 500g (3000 rpm) for 10 minutes. Following centrifugation plasma was removed using a pipette, transferred to a new tube and stored at -20 °C. Gonads (testes/ovaries) were removed and fixed in Bouin's fixative for a period of 18 h prior to being stored in 70% ethanol. The reproductive status of females was further assessed by recording the presence or absence of embryos/foetuses following the opening of the abdominal cavity. Lactating females could be identified by the presence of prominent teats which released milk on squeezing and / or the occupancy of young, either juvenile(s) or infant(s) in their burrows. Juveniles and sub-adults of both sexes co-habiting their mother's burrow were recorded on capture to assess recruitment to the population of *Heliophobius* from reproductive animals collected.

The mass (g) of the gonads was measured using a Sartorius scale (Zeiss, Germany).

Following weighing the gonads were stored in picric acid for 24h and subsequently stored in 70% ethanol until being used for histological preparation. The gonads were then sequentially

dehydrated by placing them in containers of increasing alcohol concentration and subsequently embedded in a block of paraffin wax before sectioning at a thickness of 7 μm using a rotary microtome (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, New York, U.S.A.). Sections were mounted on microscopic slides after being dipped in water at 45°C mixed with gelatine as an adhesive. The mounted sections were subsequently dried in an oven at 36 °C for a period of 72 hrs and then stained with Ehrlich's haematoxylin and counter-stained with eosin (Drury & Wallington 1967). The stained sections were covered by slide cover slips secured with resin solution (Microscopy Entellan glue, Germany) and dried in an oven at 36 °C for 48 hrs.

Testicular histology

Thirty randomly selected sections taken from the mid region of the testes were used to estimate the mean diameter of seminiferous tubules with a light microscope (Diaplan, Ernst LeitzWetzlar GmbH, Germany). Seminiferous tubules were photographed at 10 \times magnification using a digital camera attached to a microscope (Moticam 1000 1.3 M Pixel USB 2.0, Motic China Group, LTD., Xiamen, China). The diameters (μm) of 1800 seminiferous tubules were measured using Motic Images Plus 2.0ML (Motic China Group, Ltd., Xiamen, China). The larger diameter of the seminiferous tubules implies an active testes with greater sperm production.

Ovarian histology

The sectioned ovaries were examined under a light microscope for the numbers 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). 100 \times , 200 \times and 400 \times magnifications were used consecutively (van Rensburg *et al.* 2002; Hart *et al.* 2006). The follicles of each category for each section were counted. Counting error was taken into

account using the method of Borgeest et al. (2004). The sections were photographed using a digital camera attached to a light microscope (Moticam 1000 1.3 M Pixel USB 2.0, Motic China Group, LTD., Xiamen, China).

Radioimmunoassay

Progesterone

Plasma progesterone concentrations were measured using a commercially available kit (Coat-a-Count MG12171, IBL International GmbH, Hamburg, Germany). The assay enabled the determination of testosterone concentrations over the range 6 to 1200 ng/dl. The cross-reactivity of the antibody with other naturally occurring steroids was < 0.1% except for 5 α -Pregnan-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.

Oestradiol -17 β

Oestradiol -17 β assays were performed as described by Herbst et al. (2004) using the Coat-a-Count MG12101 oestradiol kit (IBL International GmbH, Hamburg, Germany). The cross-reactivity of the antibody to all naturally occurring steroids was 10% with oestrone, <5% with oestriol, oestrone- β -D-glucuronide, oestone-3-sulphate, d-equilenin, 17 β -oestradiol-3-monosulphate, testosterone and androsterone. The assay was validated for plasma of *H. emini* 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, $F = 4.4$, $P = 0.09$, $n = 4$). The intra-assay coefficient of variation for repeated determinations of a quality control was 8.3% and sensitivity of the assay was 10pg/ml.

Testosterone assays

The plasma testosterone concentrations were measured using the commercially available Coat-a-Count MG12191 testosterone kit (IBL International GmbH, Hamburg, Germany). The assay was able to determine testosterone concentrations of 6 to 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 silvery mole-rat 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 5ng/dl or 0.175nmols/l.

Descriptive and statistical analyses were performed using R i386 3.2.2 and Excel 2007. The relationship between variables such as gonadal mass, volumes and rainfall were evaluated using correlation statistical analyses. The normality of the data within each month was tested using the Shapiro-Wilk normality test (Lilliefors 1967) as a consequence parametric tests were used in subsequent analyses. Annual variation in hormone concentrations and gonadal metrics was investigated using ANOVA with Tukey's honest significance difference (HSD) to evaluate significant differences. All descriptive results in the figures are presented as mean \pm SD (standard deviation of mean).

Species designation

Clade and species assignment was determined by *cyt b* gene amplification and nucleotide sequencing of 10 randomly selected individuals (5 males and 5 females), using previously described primers and thermal cycling conditions (Bastos *et al.* 2011). Full-length gene sequences submitted to Genbank under accession numbers KX060579-KX060580, were complemented with homologous data from two prior studies (Faulkes *et al.* 2004 and 2011) and the best-fit model of sequence evolution identified under the AIC_C in Mega5 (Tamura *et al.* 2011) was subsequently used for maximum likelihood inferences.

Two haplotypes were recovered from the 10 individuals selected at random for genetic characterisation. One of these had 100% nucleotide sequence identity to AY425935 and AY425938, which correspond to clade 3 *Heliophobius* museum voucher QM1026 and QM1023, respectively and the other represents a new haplotype that differs from the aforementioned reference strains at nucleotide position 604 (G/A). This first base position mutation results in a non-synonymous amino acid substitution at position 202 (Ala/Thr). The two haplotype sequences generated in this study cluster within the *H. emini* clade defined by Faulkes *et al.* (2011), with 100% bootstrap support (not shown).

Results

Testicular metrics and histology

The testicular mass increased over the period February to March and decreased from July to August and remained reduced until January (Figure 2A). The mass of the testes was significantly different between months; $F_{(11,48)} = 4.811$, $n=60$, $P < 0.0001$, with the months of the long wet season having males with heavier testes per gram mass of animal than those of the dry months and the short rains of October and November ($F_{(1,58)} = 15.58$, $n= 60$,

$P < 0.0002$) (Figure 2B). Significant differences were clearly observed between June and August ($p = 0.012$), June and December ($p = 0.005$), March and December ($p = 0.02$), May and December ($p = 0.033$), October and June ($p = 0.008$) and October and May ($p = 0.04$). Testicular mass of the breeding and non breeding animals was not influenced by body mass; $F(56,3) = 2.033$, $n = 60$, $p = 0.16$).

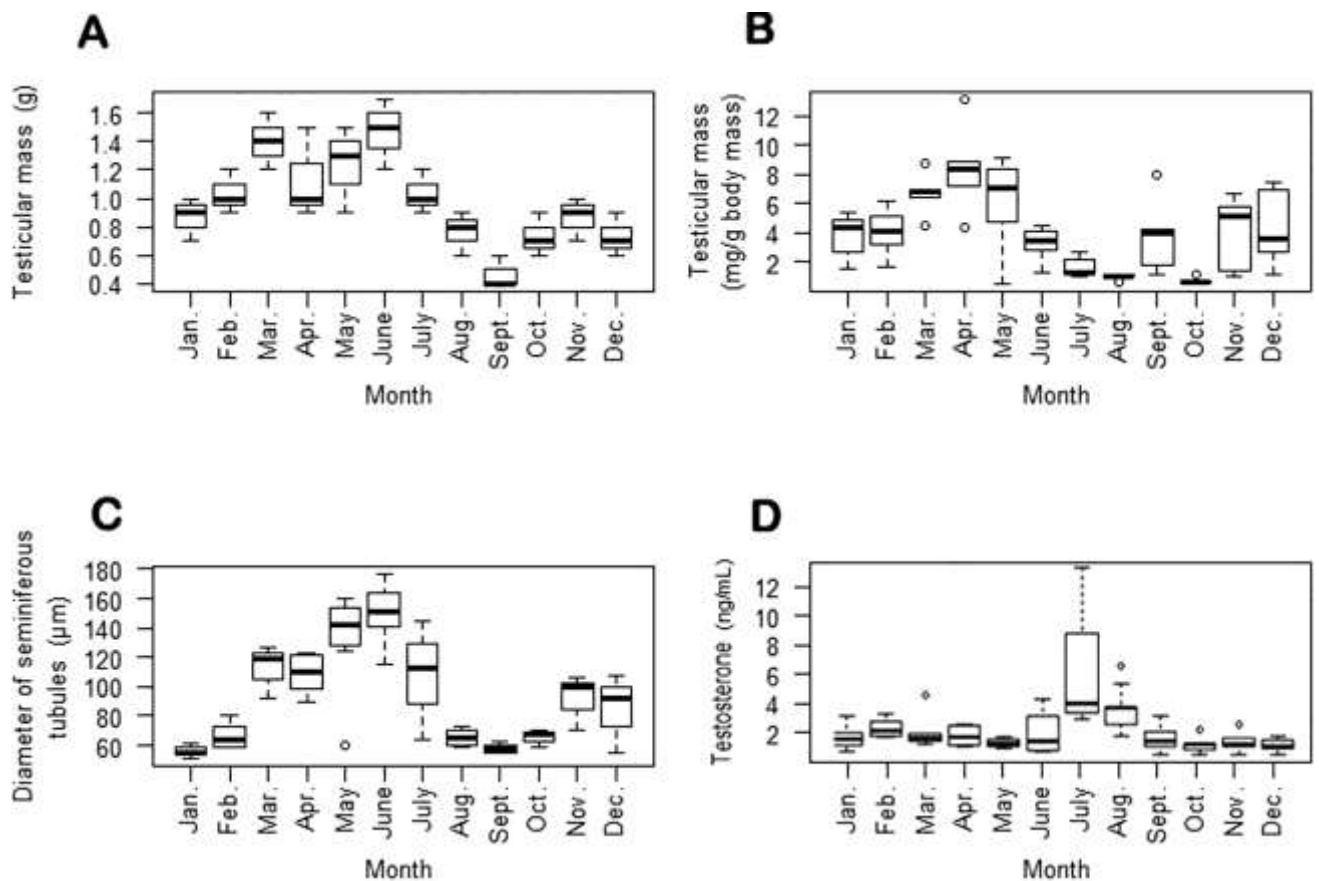


Fig. 2. Monthly fluctuations (mean \pm SD) for raw testicular mass (A), testicular mass milligrams per gram body mass (B), diameter of seminiferous tubule (C), and plasma testosterone concentration (D) in Emin's mole-rat (*Heliophobius argenteocinereus emini*) from January until December 2014.

The mean diameter of the seminiferous tubules was greatest in May, June and July, whereas the lowest mean diameter was in August, September and October and again in January (Figure 2C). Variation in the diameter of seminiferous tubules between months was significant; ($F_{(11,1788)} = 115.9$, $n = 1800$, $P < 0.0001$) with breeding months (the long rains) showing males with increased diameter of seminiferous tubules than non-breeding months

(dry months and short rains) ($F_{(1,1328)} = 390.9$, $n = 1330$, $P < 0.0001$). Significant differences were observed between; September and April, October and April, June and August, March and August, May and August, June and December, March and December, May and December as well as May and February ($P < 0.0001$). The width of the seminiferous tubules was positively correlated with the peak of rainfall (Pearson's correlation; $r = 0.123$, $n = 1799$, $P < 0.0001$).

Ovarian metrics and histology

The mass of the ovaries of females were greatest in March, April and May, but remained relatively small for the rest of the year (Figure 3A). Ovarian mass varied between breeding and non-breeding months ($F_{(1,58)} = 82.91$, $n = 60$, $P < 0.0001$) (Figure 3B). Significant differences were observed between; August and April, December and April, November and April, March and December, May and January and May and July ($P < 0.0001$).

The follicular development varied over the year, and as expected, primordial follicles were uniform and showed no clear pattern, but the highest numbers were recorded in March through to July (Table 1). Primary follicles increased from June to March increasing dramatically in April and then decreased, reaching the lowest number in October (Table 1). The numbers increased almost three-fold in November and then decreased to almost by half the amount in December (Table 1). The number of secondary follicles increased in January, peaking in June, then decreased gradually through to September. The numbers increased again in October and November and finally dropped off again in December (Table 1). Tertiary follicles increased sharply from January to April, slightly decreased in May and gradually decreased in November whereafter they increased sharply (Table 1). Graafian follicles increased in number from January reaching a peak in April. The number decreased gradually from May to October, increased in November and decreased again in December (Table 1). The number of corpora lutea increased gradually from February to May, followed by a sharp

increase in June and July. The numbers decreased to zero in September then increased marginally from October to December (Figure 3C).

Table 1: Mean \pm SD of follicular types found in ovary over a single calendar year

Month	Follicular cells				
	Primordial	Primary	Secondary	Tertiary	Graffian
January	19 \pm 2.14	0.38 \pm 0.0	0.23 \pm 0.43	0.15 \pm 0.37	0.08 \pm 2.7
February	18.75 \pm 2.06	0.25 \pm 0.5	0.25 \pm 0.5	0.5 \pm 0.57	0.25 \pm 0.5
March	20 \pm 3.29	0.67 \pm 0.54	0.33 \pm 0.51	0.5 \pm 0.54	0.5 \pm 0.54
April	18.4 \pm 2.7	0.8 \pm 0.48	0.4 \pm 0.54	0.6 \pm 0.54	0.8 \pm 0.44
May	19.4 \pm 2.44	0.57 \pm 0.53	0.43 \pm 0.53	0.57 \pm 0.53	0.57 \pm 0.53
June	19.4 \pm 2.38	0.25 \pm 0.5	0.5 \pm 0.5	0.25 \pm 0.5	0.5 \pm 0.57
July	19 \pm 1.63	0.25 \pm 0.5	0.25 \pm 0.5	0.25 \pm 0.5	0.25 \pm 0.5
August	15 \pm 4.47	0.33 \pm 0.5	0.22 \pm 0.44	0.11 \pm 0.33	0.11 \pm 0.33
September	18.5 \pm 2.73	0.25 \pm 0.46	0.25 \pm 0.46	0.13 \pm 0.35	0.13 \pm 0.35
October	14.6 \pm 3.78	0.2 \pm 0.44	0.4 \pm 0.54	0.2 \pm 0.44	0.2 \pm 0.44
November	18.2 \pm 2.59	0.6 \pm 0.54	0.4 \pm 0.54	0.6 \pm 0.54	0.4 \pm 0.54
December	12.67 \pm 2.5	0.33 \pm 0.49	0.17 \pm 0.4	0.17 \pm 0.4	0.33 \pm 0.51

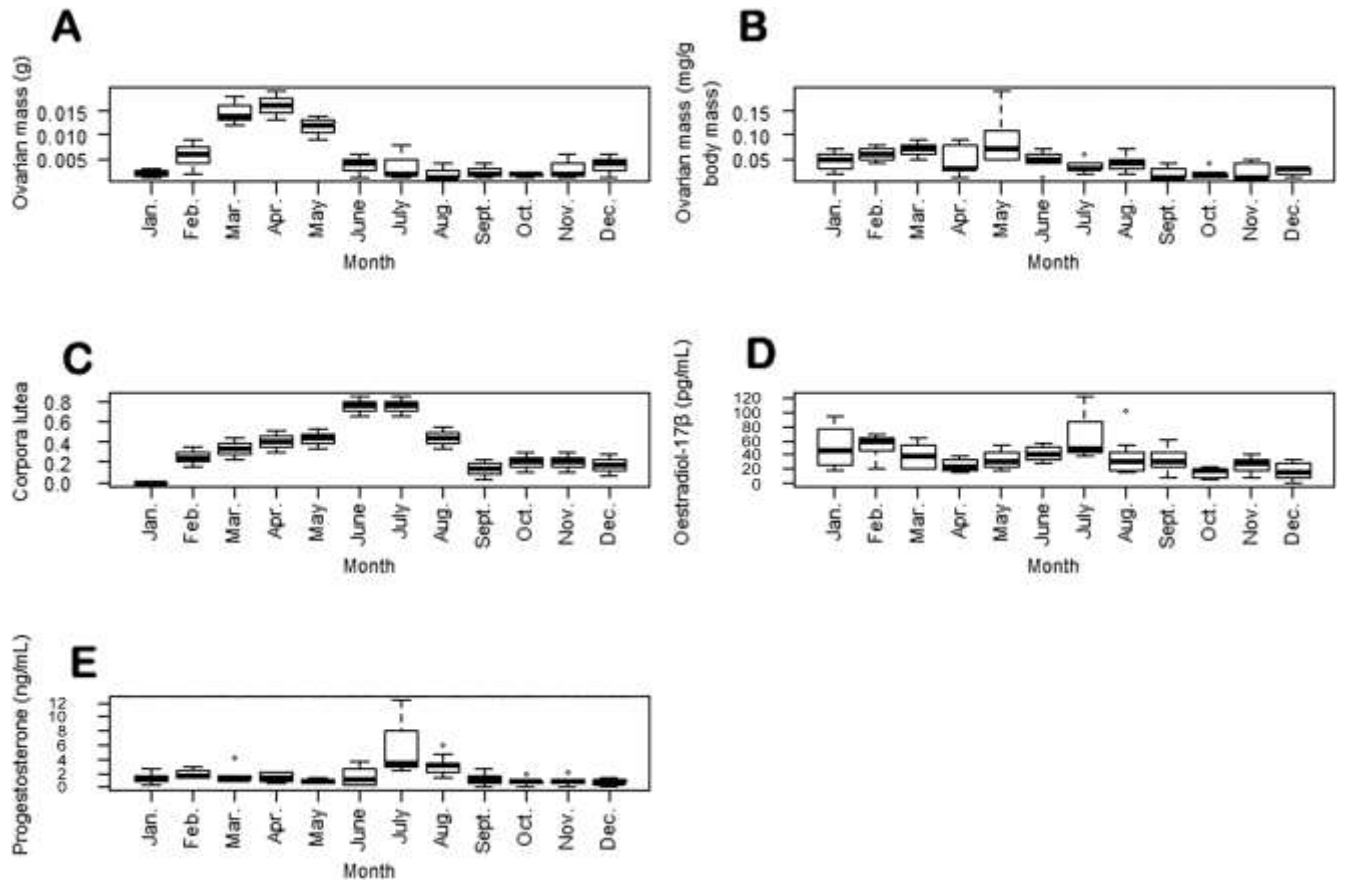


Fig. 3. Monthly fluctuations (mean \pm SD) for raw ovarian mass (A), ovarian mass milligrams per gram body mass (B), number of corpora lutea (C), plasma oestradiol-17 β concentration (D), and plasma progesterone concentration (E) in Emin's mole-rat (*Heliophobius argenteocinereus emini*) from January until December 2014.

Hormonal profiles

The concentration of testosterone in the males was generally low throughout the year, but increased in April and peaked in June thereafter the concentrations dropped through to March (Figure 2D) . The concentration of testosterone varied between months ($F_{(11,46)}= 3.212, n=58, P=0.00261$).

The concentration of testosterone in male mole-rats increased during the long rains and was lowest during the dry season with maxima coinciding with peaks in precipitation (see figures 1 and 2D).

Levels of oestradiol increased from April, peaking in July whereafter there was a gradual decline through to October, with a slight increase observed in November and December (Figure 3D). Progesterone was high in June, July and August then declined from September, remaining very low for all other months (Figure 3E). There was a significant positive correlation between progesterone concentration and number of corpora lutea ($r = 0.42$, $n = 75$, $P < 0.0002$).

Pregnancies and lactation

A total of 120 mole rats were sampled (60 males and 60 females). Out of 60 sampled female animals, 12 (20%) were pregnant and 13 (21.7%) were lactating. Pregnancies were observed initially in June when two of five females (40%) were pregnant. The percentage increased in July when all five female animals sampled (100%) were pregnant. Pregnancies decreased in the following months, whereby three females (60%) and two females (40%) were found to be pregnant in August and September respectively. No pregnancies were observed after September or before June. This is reflected in the number of corpora lutea, which increased from March to May (these are probably corpora lutea of ovulation). In June and July there was peak in numbers of corpora lutea, the corpora lutea of pregnancy, which dropped gradually in August and reached the lowest number from September through to February (Figure 3C).

A total of 50% of pregnant females were found to have five embryos, three on the left uterine horn and two on the right, 35% had six, three on either uterine horn and 15% were found to have five embryos four on the left horn and one on the right. Based on the number of embryos the mean litter size of *Heliophobius* from Tanzania was 5.3 ± 0.5 . However the number of pups found in maternal burrows was lower with a the mean litter size of 4.4 ± 0.5 . Lactating animals were first observed in September in which four of the five females were lactating (80%). All female animals sampled in October and November were lactating and this

decreased dramatically in December, whereby only one out of five sampled females were found to be lactating (20%).

Presence of juveniles.

A total of 47 juveniles (20 male and 27 female) were sampled in maternal burrow systems. Juveniles were captured in the months of September, October and November. The presence of juveniles coincided with the short rainfall. We predict that in the months of December to February that these individuals would disperse to establish their own independent burrow systems dispersing off from the maternal burrow system.

Discussion

A field-based study based on post-mortem examination of animals using a number of reproductive indices in *Heliophobius* from Tanzania showed a single clear breeding period. The reproductive tract morphometrics, gonadal histology and measurement of circulating gonadal steroids revealed one single distinct breeding period that coincided with the precipitation pattern of the long rains in Tanzania. Oestradiol 17β and the presence of Graafian follicles in April and May imply that the females ovulate during the height of the long wet season. Likewise, male *Heliophobius* also showed peaks in testosterone around April and June, had larger seminiferous tubules and heavier testicular mass around April to June. These findings suggest that the adult males and females prepare for mating during the period of April to June. The mating period may reflect a time when the sexes are more tolerant of one another to promote copulation and ensure maximal reproductive success, but is also a period when increased territoriality between same-sex individuals may arise, as has been found in other subterranean mammals during the breeding season (Bennett and Jarvis, 1988; Bennett *et al.* 1991). It is unknown if the sexes use seismic communication as has been

reported for other solitary bathyergids (Bennett and Jarvis 1988; Narins *et al.* 1992; Herbst *et al.* 2004; Hart *et al.* 2006) and spalacids (Heth *et al.*, 2007; Rado *et al.* 1987; Hrouzkova *et al.* 2013; Katandukila *et al.* 2013).

The sexes synchronise the maturation of their reproductive organs and the sperm and ova to maximise conception before the end of the wet months. The rainfall results in the soil becoming more workable so that excavation and digging can arise and hence the sexes pair up, however, we currently have little information of how this arises in the field.

Patzenhauerova *et al.* (2010) using microsatellites report *Heliophobius argenteocinereus* to exhibit polygyny. The population they studied was found to have a female biased sex ratio. Due to the large distances between burrow systems it is speculated that males might seek females aboveground.

The presence of numerous corpora lutea of pregnancy, higher circulating progesterone concentrations and presence of embryos suggest that maximum pregnancies occur in June and July, falling off in August, following the long rains. These pregnancies probably reflect the optimal period when mating could arise and when constraints on burrowing are minimal as has been suggested for other subterranean mammals (Emlin and Oring 1977; Isaac and Johnson 2003). Lactation was first reported in September, but was frequent in October and November, this coincides with the short rainfall period. As a consequence the soils would be soft and workable during this time and would allow lactating females with young to access the geophytes and grass rhizomes and thus enable these mothers to care for the juveniles over this period. The span of first pregnancy to first lactation and maximal pregnancy to maximal lactation is around three months, which fits with the estimated gestation of 87 days as reported by Jarvis (1969) and Šumbera *et al.* (2003) for *Heliophobius* from Kenya and Malawi, respectively. By breeding seasonally animals can time their reproduction in order

that the pups are born at a point which maximises offspring survival in the environment (Ims 1990).

Many organisms use environmental proximate cues to trigger the onset of reproductive activity. In this study it would appear that the onset of the long rains triggered reproductive activity. In contrast to the East African root rat, mating did not take place early in the rains, but moreover during the latter part of the long rains. The role of photoperiod in subterranean rodents appears to be relatively unimportant. In the spalacid, *Spalax ehrenbergi* (Nehring 1898) photoperiod has a role in thermoregulation (Haim et al. 1983), similarly several species of bathyergid are able to entrain their locomotory activity patterns to different lighting schedules (Oosthuizen *et al.* 2003; Hart *et al.* 2004; de Vries *et al.* 2008). However, rainfall is undoubtedly the most important environmental cue acting on subterranean mammals in that it can be detected underground because it softens soil and also brings about plant growth and flushes of vegetation (Dennis & Marsh 1997). Indeed, all solitary South African bathyergid mole-rats studied to date gauge their breeding events with extended periods of rainfall (Bennett and Jarvis 1988; Herbst *et al.* 2004; Hart *et al.* 2006). The results of our study are consistent with those for *Heliophobius* from Malawi (Šumbera *et al.* 2003) in that reproduction arises in the latter part of the long rains.

The finding of a single litter born per annum per adult female corresponds well with the findings of Copley (1950) and Šumbera *et al.* (2003) who found a single litter to be produced during the long rains in Kenya and Malawi respectively. The litter size of *Heliophobius* from Tanzania was higher compared to 3.2 ± 0.9 observed in *Heliophobius* from Malawi (Šumbera *et al.* 2008). Birthing 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. Precipitation is important for the re-sprouting, regeneration and an overall increase in plants succession and vegetation cover (Bennie 1991; Pregitzer and King,

2005). Juvenile and/or sub-adults were found in the maternal burrow system during the months of the short rains and dispersal of sub-adult mole-rats probably occurs from December onwards as no juveniles were caught with their mothers after this time. This observation implies that the timing of dispersal of sub-adult mole-rats from their natal burrow systems occurs prior to the onset of the next long rains to ensure that the offspring are fit enough and have access to maximal vegetation and food resources for independence when setting up their own tunnel system. This breeding strategy and the associated dispersal from natal burrows is documented for other solitary subterranean rodents from South Africa (e.g. *Georychus capensis*: Jarvis & Bennett, 1991, *Bathyergus* species: Bennett and 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 and Cameron 1984) enables sub-adult *Heliophobius* to successfully establish their own independent burrow system (Jarvis and Sale 1971; Bennett *et al.* 1991; Herbst *et al.* 2004).

Our findings from the reproductive hormone profiles, histological assessment of gonadal characteristics and field observations have clearly revealed that this population of *Heliophobius* is a strictly seasonal breeder having a single period of reproductive activity within a year. *Heliophobius* 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 long gestation period precludes the production of a second litter during the short rains. In contrast, the East African root rat is capable of such fecundity due to the much shorter gestation period of around 38 days (Katandukila *et al.* 2013). The restriction of reproduction to a single event a year makes this species susceptible to climate instability as a result of the action of El Niño and El Niña weather oscillations in the Indian ocean. These

weather phenomena may bring about either higher and lower rainfall in regions of Tanzania occupied by *Heliophobius* (McSweeney *et al.* 2010).

Additional studies on other members of the *Heliophobius* species complex are required to understand how reproduction varies between species and localities. As these species are cryptic it is important that these initiatives are conducted within a phylogeographical framework.

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