

# **Locomotor activity and Tb rhythms in the Mahali mole-rat (*C. h. mahali*): the effect of light and ambient temperature variations**

Barry van Jaarsveld, Nigel C. Bennett, Daniel W. Hart, Maria K. Oosthuizen\*

**Affiliation:** Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa.

**Corresponding Author email:** moosthuizen@zoology.up.ac.za

**Running title:** (Circadian rhythms in the Mahali mole-rat)

## **Highlights**

- Mahali mole-rats display endogenous rhythms of locomotor activity and body temperature.
- Mahali mole-rats can use both light and ambient temperature to entrain their locomotor activity rhythms.
- This is the first study to use ambient temperature ramps simulate natural temperature cycles.
- There are indications that ambient temperature may be a prominent zeitgeber for circadian rhythms in this mole-rat.

## **List of Abbreviations**

T<sub>b</sub> – core body temperature

T<sub>c</sub> – constant ambient temperature

T<sub>n</sub> – natural ambient temperature

LD<sub>s</sub> – square wave 12L:12D

LD<sub>n</sub> – natural wave 12L:12D

DD – constant darkness

## Abstract

African mole-rats (family: Bathyergidae) are strictly subterranean mammals that reside in extensive networks of underground tunnels. They are rarely, if ever, exposed to light and experience muted temperature ranges. Despite these constant conditions, the presence of a functional circadian clock capable of entraining to external light cues has been reported for a number of species. In this study, we examine a social mole-rat species, *Cryptomys hottentotus mahali*, to determine if it possesses a functional circadian clock that is capable of perceiving light and ambient temperature cycles, and can integrate these cues into circadian rhythms of locomotor activity and core body temperature. Eight male and eight female, non-reproductive individuals were subjected to six cycles of varying light and temperature regimes. The majority of the individuals displayed daily rhythms of locomotor activity and body temperature that are synchronised to the external light and temperature cycles. Furthermore, endogenous rhythms of both locomotor activity and core body temperature were displayed under constant conditions. Thus, we can conclude that *C. h. mahali* possesses a functional circadian clock that can integrate external light and temperature cues into circadian rhythms of locomotor activity and core-body temperature.

## Keywords

body temperature, *C. h. mahali*, circadian rhythms, locomotor activity, Mahali mole-rat

## Introduction

Almost all organisms, from bacteria to mammals, have evolved the ability to synchronise internal biological processes to predictable changes in environmental conditions such as changes in light and temperature associated with the day-night cycle (Patel et al., 2016). Metabolism, endocrine secretion, gastrointestinal tract motility, reproduction, feeding, and of particular relevance to this study – locomotor activity and body temperatures ( $T_b$ ), are all examples of biological processes that are expressed in a rhythmic fashion (Froy, 2011).

Biological rhythms that coincide with the day-night cycle and can ultimately entrain to these cues are called circadian rhythms (*circa*: approximately; *diēm*: day) (Patel et al., 2016). The ubiquity of circadian rhythmicity suggests that it provides organisms with an adaptive advantage. The adaptive value of circadian rhythms arises from fitness benefits related to the ability to anticipate environmental changes associated with the day-night cycle and

optimising both behavioural and physiological processes accordingly (Schibler et al., 2015). DeCoursey et al. (2000) reported decreased fitness in chipmunks (*Tamias striatus*) following a disruption of the circadian system through lesioning of the superchiasmatic nucleus (SCN). Lesioned diurnal chipmunks displayed uncharacteristic night-time restlessness which resulted in increased predation rates by weasels.

The mammalian circadian system comprises a number of endogenous timing mechanisms, termed “oscillators”, and signalling networks that dictate the temporal expression of behaviour and physiological processes (Foulkes et al., 2016). Under constant environmental conditions, such as complete darkness and uniform temperature, circadian oscillators express circadian rhythms at periods ( $\tau$ ) that typically range from 20 to 28 hours (Benstaali et al., 2001). Two features of the circadian system ensure synchrony between circadian rhythms and the environment – hierarchical organisation of circadian oscillators and its ability to entrain to external stimuli.

The master circadian oscillator entrains to external stimuli (termed: *zeitgebers*) (Aschoff, 1960). Entrainment is defined as a phase-shift in circadian rhythms in response to external stimuli so that endogenous rhythms and environmental cycles are in synchronisation (Golombek and Rosenstein, 2010). Feeding times, social cues, and of particular importance to this study – light and temperature, are amongst the cues that result in entrainment of the circadian system (Golombek and Rosenstein, 2010).

Light is arguably the most important environmental cue in the entrainment of circadian rhythms to the day-night cycle, presumably due to light being the most accurate indicator of the Earth’s position relative to that of the Sun (Kregel and Sieck, 2002). The master circadian oscillator is entrained on a daily basis by light entering the retina and reaching the SCN via the retinohypothalamic tract; the master clock subsequently synchronises peripheral oscillators in other regions of an organism (Schibler et al., 2015).

Daily variation in ambient temperature is an important non-photocue that circadian rhythms have been shown to entrain to, albeit to a lesser degree; it has been identified in a wide range of taxa from cyanobacteria to mammals (López-Olmeda, 2017). The strength of ambient temperature as a *zeitgeber* is species specific and depends largely on their thermoregulatory capabilities. Rhythms of melatonin secretion in *Anolis carolinensis*, an ectothermic lizard, and *Molossus ater*, a heterothermic bat, readily entrain to ambient

temperature; while in contrast, in homeotherms ambient temperature appears to be a weaker *zeitgeber* and consequently entrainment occurs to a lesser degree (Rajaratnam and Redman, 1998). It is important to note that light and ambient temperature may have a compounding effect on the entrainment of circadian rhythms and together may result in a more sensitive entrainment scenario (Francis and Coleman, 1990). Photic cues result in much more rapid entrainment compared to non-photic cues, further illustrating the dominant role of light as a *zeitgeber* (Golombek and Rosenstein, 2010).

African mole-rats of the family Bathyergidae are a group of subterranean rodents endemic to the African continent (Bennett and Faulkes, 2000). The family comprises of six genera and approximately 30 species (Davies et al., 2015). Social organisation varies remarkably within the family, ranging from strictly solitary to eusocial (Bennett, 2009).

The subterranean niche provides two main advantages to mole-rats, shelter from above-ground predators and muted temperature ranges (Bennett et al., 1988; Burda et al., 2007). However, lack of sensory cues and the disadvantages associated with high relative humidity, poor ventilation, low O<sub>2</sub> and high CO<sub>2</sub> partial pressures make it simultaneously a challenging environment (Burda et al., 2007). African mole-rats are morphologically, physiologically and behaviourally well adapted to cope with these challenges given their reduced resting metabolic rates, low core body temperatures, high thermal conductance and array of behavioural thermoregulatory mechanisms (Bennett, 2009).

Studies investigating the effect of light on locomotor activity patterns showed evidence of circadian rhythmicity in African mole-rats. They have revealed that African mole-rats possess endogenous rhythms of locomotor activity which are capable of entraining to light and allude to the presence of a functional circadian system (Oosthuizen et al., 2003; Hart et al., 2004; Vasicek et al., 2005; Schöttner et al., 2006; de Vries, et al. 2008). Congruently, Němec et al. (2008) showed that although the visual system of many members of the Bathyergidae is severely regressed, the subsystem involved in photoreception is well-developed and comparable to those exhibited by surface dwelling rodents. However, these studies have also revealed that the degree of locomotor activity rhythmicity and its ability to entrain to light, varies between species and within species (Oosthuizen et al., 2003) and sex (Haupt et al., 2017). Solitary species, such as the Cape mole-rat (*Georychus capensis*), generally have more robust rhythms of locomotor activity that entrain to light more readily compared to those in the social highveld mole-rat (*Cryptomys hottentotus pretoriae*)

(Oosthuizen et al., 2003) and eusocial naked mole-rat (*Heterocephalus glaber*) (Ricchio and Goldman, 2000a). Furthermore, there is intra-individual variation within species; Oosthuizen et al. (2003) reported that although the majority of *C. h. pretoriae* individuals were nocturnal, some showed a preference for diurnal activity. Šklíba et al. (2014) suggest that desynchronised activity patterns within a colony may result in more effective foraging, territory maintenance and protection against predators.

All previous studies examining the effect of light on locomotor activity in African mole-rats have utilised square wave (LD<sub>s</sub>) light regimes and did not consider the effect of the twilight phases in natural day-night cycles (LD<sub>n</sub>) on locomotor activity patterns. The gradual change in spectral composition and intensity of light during dawn and dusk periods can provide animals with important temporal information required for accurate entrainment of the circadian system (Tang et al., 1999; Sosniyenko et al., 2009).

Kavanau (1967) reported that white-footed mice (*Peromyscus leucopus*) were unable to entrain their activity patterns to different light regimes under LD<sub>s</sub>, but when subjected to LD<sub>n</sub> entrainment of activity patterns took place. Similarly, Syrian hamsters (*Mesocricetus auratus*) were shown to entrain more rapidly and showed more robust activity patterns under LD<sub>n</sub> compared to LD<sub>s</sub> (Sosniyenko et al., 2009). Temperature changes have been shown to influence total locomotor activity levels in African mole-rats (Šklíba et al., 2014; Oosthuizen and Bennett, 2015; Haupt et al., 2017). Locomotor activity in the highveld and female Damaraland mole-rats were inversely related to ambient temperature (Oosthuizen and Bennett, 2015; Haupt et al., 2017). Reproductive status also played a role in the temporal distribution of locomotor activity in the Damaraland mole-rat (*Fukomys damarensis*), with non-reproductive females showing more day time activity (Oosthuizen and Bennett, 2015).

In addition to rhythms of locomotor activity, body temperature in some African mole rats has been shown to display circadian rhythmicity. As with circadian rhythms of locomotor activity, circadian T<sub>b</sub> rhythms in African mole-rats show a degree of species specificity. No evidence of circadian rhythms of T<sub>b</sub> were reported for the Cape mole-rat, whereas the naked and highveld mole-rats showed clear circadian rhythms of T<sub>b</sub> that entrain to external lighting regimes. (Lovegrove and Muir, 1996; Ricchio and Goldman, 2000b; Haupt et al., 2017). Haupt et al. (2017) showed that T<sub>b</sub> rhythms entrained to different temperature

regimes. A wide range of thermoregulatory abilities are present in Bathyergidae, ranging from heterothermy in the naked mole-rat (*Fukomys darlingi*) and homeothermy in the common, highveld, as well as the Damaraland mole-rat (Buffenstein and Yahav, 1991; Faulkes et al., 2004; Bennett et al., 1993; Boyles et al., 2012; Haupt et al., 2017).

In this study, we assessed whether *C. h. mahali* exhibits endogenous circadian rhythms of locomotor activity and core body temperature, and whether these rhythms can synchronise to artificial light and temperature cycles. In addition, we investigated the effect of square-wave and natural-wave light cycles and constant and varying ambient temperature cycles on rhythms of locomotor activity and  $T_b$ . The effects of natural-wave light and temperature cycles on locomotor activity and body temperatures of African mole-rats have not previously been investigated in the laboratory. We anticipated that *C. h. mahali* would express endogenous circadian rhythms of locomotor activity that would synchronise external light and temperature cycles. In response to different regimes of light and temperature, we predicted quantitative differences in rhythms locomotor activity and core body temperature.

## Material and Methods

### *Ethical Statement*

All experimental procedures were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (National research Council, 2011). The protocol was approved by the Animal Ethics Committee of the University of Pretoria (EC067-16).

### *Capture and Housing*

Eight male (mean body mass =  $130.95 \pm 27.69$ g) and eight female (mean body mass =  $92.07 \pm 8.33$ g), non-reproductive, *C. h. mahali* mole-rats were captured using modified Hickman traps (Hickman 1979), baited with sweet potatoes. All animals were captured from the north western most region of Gauteng, close to Brits (S  $25^{\circ} 09.827'$ , E  $27^{\circ} 57.356'$ ). Trapping was conducted during the summer (January to March) of 2017.

Captured animals were transported back to the Department of Zoology and Entomology at

the University of Pretoria where they were kept in a temperature constant room (26°C) on a 12L (330 lux):12D (0 lux) lighting regime. Animals were individually housed in plastic containers (50 x 28 x 24cm) lined with wood shavings and supplied with paper towels for nesting material. Animals were fed *ad libitum* on sweet potatoes that were replaced daily; and in addition, received apples once every two weeks. A study conducted by Bennett and Jarvis (1995) showed that sweet potatoes provide mole-rats with more than 90% of their nutritional needs. Since mole-rats obtain all their necessary moisture from food sources, no free water was provided (Bennett and Faulkes, 2000). A 10-day acclimation period was allowed before the commencement of the experimental procedures.

During the experimentation period animals were housed individually in plastic containers (83 x 44 x 37cm) and maintained on an *ad libitum* diet of sweet potatoes which were replaced on a daily basis, to avoid any increases in activity levels associated with foraging (Vasicek et al., 2005). To prevent entrainment to a fixed feeding time, the animals were fed at irregular times of the day and night. (Golombek and Rosenstein, 2010). At the end of each experimental cycle, plastic containers were cleaned, and wood shavings and paper towels replaced. Rooms were fitted with double doors to ensure the integrity of the light cycles inside the room. A dim red light was used if feeding times occurred during the dark phase as well as during the constant dark conditions (Light intensity < 1 lux).

#### *Recording of locomotor activity patterns*

Locomotor activity of each mole-rat was recorded with an infrared motion detector (Quest PIR internal passive infrared detector; Elite security products (ESP), Electronic lines, UK). The motion detectors were situated over the containers so that any movement along the container's floor space was detected. Activity readings detected by the motion detectors were summed per minute and relayed to a computer using the programme Vitalview (Vital View™, Minimitter Co., Inc., Sunriver, OR, USA; [www.minimitter.com](http://www.minimitter.com)). The computer was situated in a separate room.

#### *Body temperature recordings*

iButtons (DS1922L iButtons, MaximIntegrated Products, Dallas, TX, USA) were used to track core body temperature. iButtons were coated with surgical wax and implanted into the abdominal cavities of the animals by a registered veterinarian. All surgeries were performed under Isoflurane and all efforts were made to minimize suffering of the

animals. Animals were monitored post-surgery; all animals were upright and grooming 10 minutes after the surgery. iButtons recorded core body temperatures at hourly intervals with an accuracy of 0.0625°C. The temporal resolution was a little low, but given the constraints of the study, was our best option at the time. The iButton combined with the surgical wax weighed around 4.5% of the animals' body mass thus adhering to the general consensus that implanted devices should not exceed 5% of an animal's body mass (Lovegrove, 2009). Upon completion of the experiment, animals were euthanized via overdose of isoflourane and iButtons were extracted.

### *Experimental setup*

For the duration of the experiment, animals were maintained in a climate-controlled room. Light was provided by 5 light bulbs (3x Eurolux 320 lumens, 1 x Eurolux 350 lumens, 1 x Verbatim 350 lumens) mounted over the midline between each two cages. The light bulbs provided different intensities of light such that when all 5 were switched on, 500 lux were measured at the bottom of each cage. During the square wave light cycles, all 5 lights were switched on and off simultaneously, whereas for the simulated dawn and dusk, lights were switched on and off consecutively with timers (4x TopTronic TDT7 Digital timer, 1x MajorTech MTD7 Digital timer) over the course of an hour. The lowest increment was 50 lux, followed by 100, 250, 400 and a maximum of 500 lux measured at the bottom of the animal cages. Fifty lux as a starting increment for a simulated 'dawn-dusk' cycle may appear rather high, however, although the mole-rats may be able to detect light levels at 50 lux, they show an increased in response in the SCN to higher light irradiances (Oosthuizen et al., 2010), thus we believe that the incremental increase in light levels simulates a natural light cycle. The ambient temperature could be controlled externally and may be set either to a constant 25°C, or programmed to follow a natural cycle varying between 18 and 24°C. The temperatures were verified with a HOBO pendant temperature/light data logger (Onset computer corporation, MA, USA).

### *Experimental protocol*

Animals were subjected to six different light and temperature cycles, each 25 days long. Ambient temperature was maintained at a constant 25°C ( $T_c$ ) for the first three cycles. During the last three cycles, a natural temperature cycle ( $T_n$ ) was simulated to mimic typical daily temperatures within a burrow system (Bennett et al., 1988; Roper et al., 2001). For



cycle 1 and 4, animals were exposed to a square wave 12L:12D light cycle ( $LD_s$ ), where lights were abruptly switched on at 06:00 and abruptly turned off at 18:00. For cycles 2 and 5, animals received a natural light cycle ( $LD_n$ ) where dawn and dusk were simulated over the course of an hour. The light increased progressively in intensity from first light at 06:00 reaching a maximum light intensity at 07:00; and then decreased progressively from maximum intensity at 17:00 to darkness at 18:00. During cycles 3 and 6, animals were subjected to constant darkness (DD). Between cycles 3 and 4, animals were subjected to an extra week of  $LD_s$  to allow re-entrainment of rhythms after constant darkness.

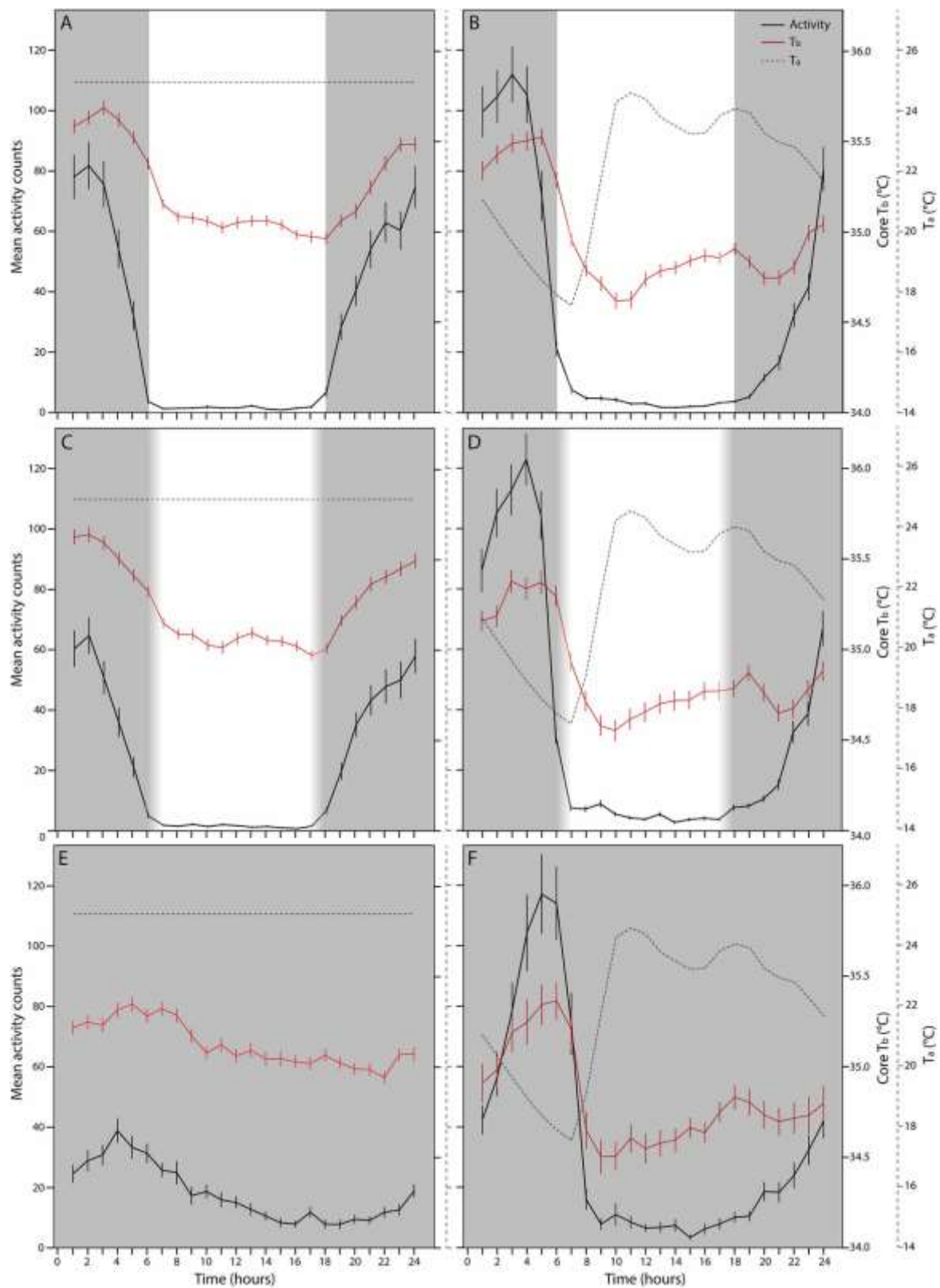
### *Statistical Analysis*

Due to animal deaths from natural causes and iButton failures, sample sizes were not equal for all regimes (Table 1). The recording resolution of the iButtons was one hour, thus activity counts were also converted to hourly values. Hourly activity counts and core body temperatures were averaged over experimental days in order to obtain a representative value, all analysis was conducted on these. The first five days of each cycle, for each individual, was omitted from the statistical analysis to avoid potential after effects from previous cycles. The lengths of the free-running rhythms ( $T$ ) of locomotor activity and core body temperature were calculated in BioDare2 (FFT-NLLS algorithm); the rhythms of some individuals broke down therefore we only used the first 10 days (biodare2.ed.ac.uk). These days were constant for all individuals. The correlation between core body temperature and locomotor activity was determined by Spearman-rank correlation across all six regimes. To test for differences in activity and core  $T_b$  between the different light regimes and potential effect of temperature; generalised linear mixed models were conducted in SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Activity count data were heavily over dispersed thus a negative binomial distribution, with log link function, was used. Core body temperature values were modelled using a gamma distribution with a log link function. The predictor variables used were cycle, sex, phase, all two-way and three-way interactions were included, animal I.D was used as a random effect. Body mass was included in all models, however, in all cases it was shown to have no effect on the results. In the event of a predictor variable having a significant effect on the response variable, a Tukey-Kramer test was conducted for a post-hoc multi-comparison. Regime DD  $T_c$  was excluded from the statistical analysis since the period lengths ( $T$ ) of individual animals with free-

running rhythms would deviate from 24h, and only referred to for evidence of endogenous circadian rhythms of locomotor activity and core body temperature. Means are presented as mean  $\pm$  standard error.

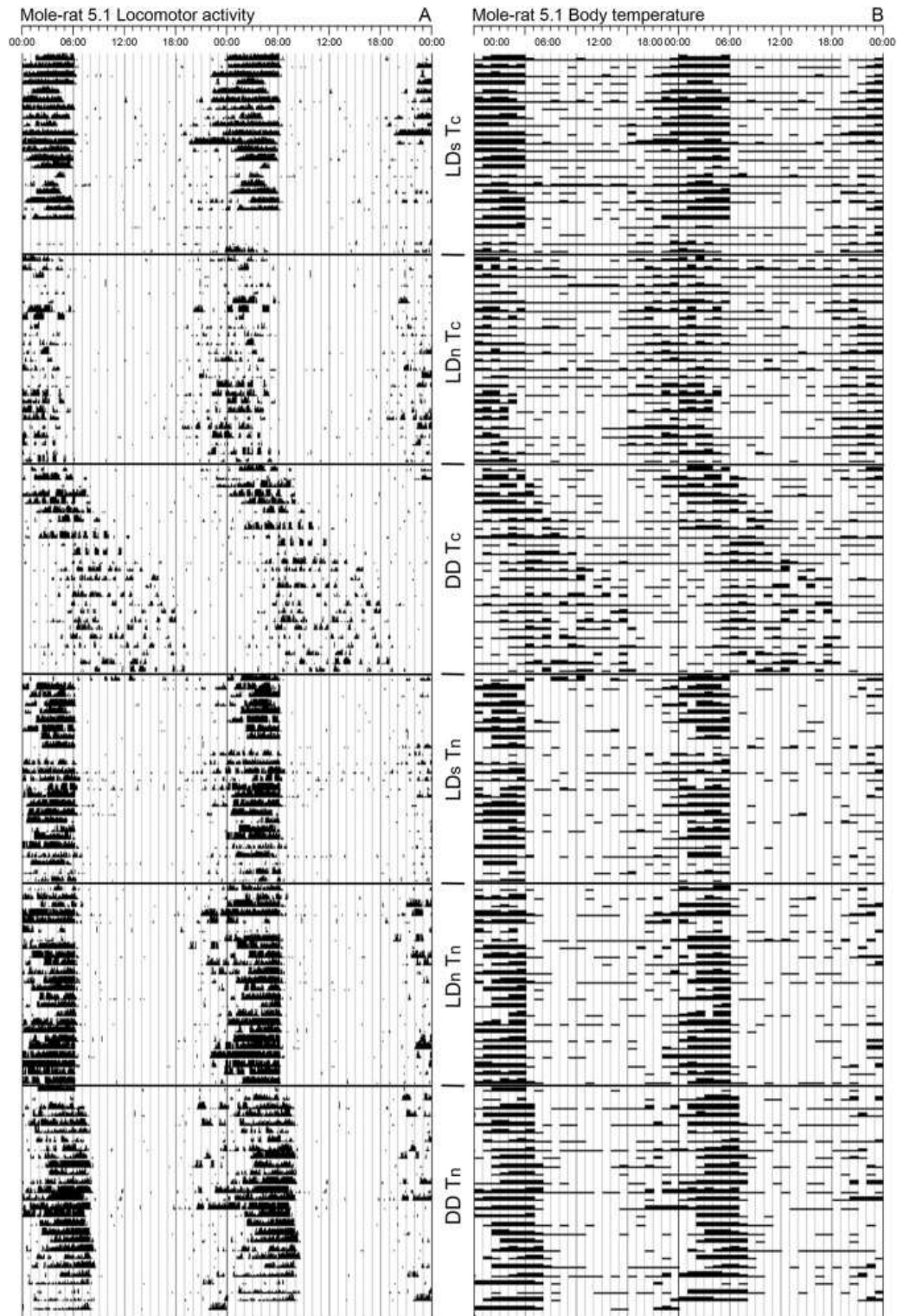
**Table 1:** Sample sizes for *C. h. mahali* during the different light and temperature regimes in the experiment. LD<sub>s</sub> and LD<sub>n</sub> represents square-wave and natural-wave light, respectively. T<sub>c</sub> and T<sub>n</sub> represent constant temperature and a natural temperature cycle, respectively.

<b>Regime</b>	<b>Circadian Measure</b>	<b>Overall</b>	<b>Male</b>	<b>Female</b>
LD <sub>s</sub> T <sub>c</sub>	Locomotor activity	15	8	7
	T <sub>b</sub>	14	8	6
LD <sub>n</sub> T <sub>c</sub>	Locomotor activity	16	8	8
	T <sub>b</sub>	14	8	6
DD T <sub>c</sub>	Locomotor activity	16	8	8
	T <sub>b</sub>	14	8	6
LD <sub>s</sub> T <sub>n</sub>	Locomotor activity	15	7	8
	T <sub>b</sub>	13	7	6
LD <sub>n</sub> T <sub>n</sub>	Locomotor activity	15	7	8
	T <sub>b</sub>	13	7	6
DD T <sub>n</sub>	Locomotor activity	8	5	3
	T <sub>b</sub>	6	4	2



**Fig. 1.** Mean activity and core body temperature over the 24-h day under different light and temperature regimes for *C.h. mahali*. Light and temperature conditions for each respective panel were as follows: A – LD<sub>s</sub> T<sub>c</sub>; B – LD<sub>s</sub> T<sub>n</sub>; C – LD<sub>n</sub> T<sub>c</sub>; + T<sub>n</sub>; E -DD T<sub>c</sub>; F – DD T<sub>n</sub>. Dotted lines indicate the T<sub>a</sub> profile whereas grey bars show the lighting cycle. Solid red lines represent core T<sub>b</sub> profile whereas grey bars show the lighting cycle. Solid red lines represent core T<sub>b</sub> and solid black lines indicate locomotor activity. LD<sub>s</sub>

and LD<sub>n</sub> represent square-wave and natural-wave light, respectively. T<sub>c</sub> and T<sub>n</sub> represent constant temperature and a natural temperature cycle, respectively. Error bars represent standard error .



**Fig. 2.** An example of an animal that shows good entrainment of its (A) locomotor activity and (B) body temperature. Actograms are double plotted with the time on the x-axis. Lights were on at 06h00 and went off at 18h00 for both square wave and the natural light cycles. For natural light cycles lights became progressively brighter from 06h00 to 07h00 and faded from 17h00 to 18h00. The number of days is on the Y-axis and the experimental conditions are illustrated between the two actograms. LD<sub>s</sub> = square wave, LD<sub>n</sub> = natural wave, T<sub>c</sub> = constant ambient temperature, T<sub>n</sub> = natural ambient temperature cycle.

**Table 2.** Mean activity counts (per hour) ( $\pm$ SE) and mean core body temperature ( $^{\circ}$ C) $\pm$  SE) for *C. h. mahali* under different light and temperature regimes. LDs and LDn represent square-wave and natural-wave light, Tc and Tn represent constant and natural ambient temperature cycles respectively.

Regime	Circadian Measure	Overall	Male	Female
LDs Tc	Mean Activity Counts	26.15 $\pm$ 0.98	35.15 $\pm$ 1.54	16.43 $\pm$ 1.14
	Tb	35.24 $\pm$ 0.01	35.21 $\pm$ 0.01	35.27 $\pm$ 0.01
LDn Tc	Activity Counts	20.02 $\pm$ 0.78	30.96 $\pm$ 1.45	9.08 $\pm$ 0.50
	Tb	35.22 $\pm$ 0.01	35.18 $\pm$ 0.01	35.29 $\pm$ 0.01
DD Tc	Activity Counts	15.21 $\pm$ 0.489	16.74 $\pm$ 0.810	13.77 $\pm$ 0.564
	Tb	35.11 $\pm$ 0.01	35.04 $\pm$ 0.10	35.21 $\pm$ 0.01
LDs Tn	Activity Counts	10.92 $\pm$ 0.63	36.88 $\pm$ 1.75	13.64 $\pm$ 0.81
	Tb	34.95 $\pm$ 0.01	34.93 $\pm$ 0.01	34.98 $\pm$ 0.01
LDn Tn	Activity Counts	28.6 $\pm$ 0.91	45.28 $\pm$ 1.77	14.00 $\pm$ 0.61
	Tb	34.85 $\pm$ 0.01	34.98 $\pm$ 0.01	34.7 $\pm$ 0.02
DD Tn	Activity Counts	32.16 $\pm$ 1.31	41.42 $\pm$ 1.89	15.08 $\pm$ 0.99
	Tb	34.77 $\pm$ 0.02	34.60 $\pm$ 0.03	35.13 $\pm$ 0.02

## Results

### *Phase preference, sexual dimorphism and endogenous rhythmicity*

Locomotor activity and body temperature showed daily rhythmicity under all five lighting and temperature regimes (DD excluded), with activity counts ( $F = 1\ 404.08$ ,  $df_1 = 1$ ,  $df_2 = 1\ 622$ ,  $p < 0.001$ ) and core body temperature ( $F = 109.39$ ,  $df_1 = 1$ ,  $df_2 = 1\ 408$ ,  $p < 0.001$ ) being higher during the dark phase compared to the light phase (Fig. 1,2). Sex had a significant effect on locomotor activity ( $F = 48.38$ ,  $df_1 = 1$ ,  $df_2 = 28\ 938$ ,  $p < 0.001$ ), males were more active than females across all regimes (Table 2). In addition, the sex and phase interaction was found to be significant; ( $F = 45.96$ ,  $df_1 = 4$ ,  $df_2 = 1\ 622$ ,  $p < 0.001$ ); post-hoc analysis revealed there was no significant difference between male and female locomotor activity during the light phase ( $p = 0.278$ ); whereas males were more active

than females during the dark phase ( $p < 0.001$ ). Core body temperature, however, did not significantly differ between the two sexes ( $F = 0.48$ ,  $df_1 = 1$ ,  $df_2 = 1408$ ,  $p = 0.488$ ) (Table 2).

During constant conditions (DD  $T_c$ ), all but two of the 16 individuals displayed some degree of circadian drift in locomotor activity; the average  $\tau$  for the individuals that showed drifting is  $24\text{h } 50\text{m} \pm 0\text{h } 11\text{m}$ . The period for core body temperature rhythms could be calculated for 10 of the 14 individuals for which there was core body temperature data, the average  $\tau$  for the individuals that did show drifting was  $24\text{h } 50\text{m} \pm 0\text{h } 15\text{m}$ . Not all individuals that exhibited endogenous rhythms of locomotor activity expressed endogenous rhythms of core body temperature. Core body temperature was significantly positively correlated to activity counts ( $r = 0.176$ ,  $p < 0.001$ )

#### *Effect of different light and temperature regimes on locomotor activity and $T_b$ rhythms*

Different light and temperature regimes had a significant effect on the amount of locomotor activity displayed ( $F = 90.32$ ,  $df_1 = 4$ ,  $df_2 = 1622$ ,  $p < 0.001$ ). When subjected to constant temperature ( $T_c$ ) there was no significant difference in mean activity counts between square wave and natural wave light cycles,  $LD_s$  and  $LD_n$ , ( $p = 0.849$ ) (Table 2). Whereas, under natural temperature ( $T_n$ ) mean activity counts were lower under the square wave ( $LD_s$ ) than natural wave ( $LD_n$ ) light cycles ( $p < 0.001$ ) (Table 2). Mean activity counts were greater under natural temperature ( $T_n$ ) than constant temperature ( $T_c$ ) when subjected to natural wave light ( $LD_n$ ) (Table 2) ( $p < 0.001$ ). Conversely, under square wave light ( $LD_s$ ), mean activity counts were lower under natural ( $T_n$ ) than constant temperature ( $T_c$ ) ( $p < 0.001$ ) (Table 2). As with locomotor activity, core body temperature varied in response to different light and temperature regimes ( $F = 48.39$ ,  $df_1 = 4$ ,  $df_2 = 1408$ ,  $p < 0.001$ ). Under  $T_c$ , core body temperature was not different between  $LD_s$  and  $LD_n$  ( $p = 0.996$ ) (Table 2). Conversely core body temperature was lower under  $LD_n$  than  $LD_s$  when subjected to  $T_n$  ( $p = 0.012$ ) (Table 2). Core body temperature lower under  $T_n$  than  $T_c$  when subjected to  $LD_s$  ( $p < 0.001$ ) as well as  $LD_n$  ( $p < 0.001$ ) (Table 2).

## Discussion

### *Constant conditions and endogenous rhythms*

The presence of endogenous rhythms of locomotor activity and core body temperature have previously been reported in a number of other African mole-rat species (Lovegrove and Muir, 1996; Riccio and Goldman, 2000b; Haupt et al., 2017). Likewise, *C. h. mahali* showed evidence of free-running endogenous rhythms for locomotor activity and core body temperature, both expressing periods slightly longer than 24 hours. The temporal resolution of the body temperature recordings was a little low, thus it was not possible to determine whether the body temperature rhythm lagged the locomotor activity rhythm. Free-running rhythms showed variations in robustness, where some individuals maintained free-running rhythms throughout the 25-day cycle, whereas others disintegrated progressively to complete arrhythmia. Locomotor activity and Tb were expressed in a circadian fashion in subsequent cycles, thus suggesting that rhythm degradation is downstream of the pacemaker and not due to the deterioration of the pacemaker itself (Refinetti, 2006). Similar patterns have been reported in *Georychus capensis* and *Cryptomys darlingi* (Lovegrove and Muir, 1996; Vasicek et al., 2005).

The adaptive advantage of a functional circadian clock is thought to arise from synchronising behaviour and physiology to predictable daily changes in the external environment (extrinsic advantage). However, it has been suggested that the adaptive value of a functional circadian clock may arise from intrinsic advantages by coordinating internal metabolic cycles (Vaze and Sharma, 2013). The presence and adaptive value of a functional clock in organisms inhabiting arrhythmic environments such as caves, deep seas, and polar regions has been attributed to benefits derived from intrinsic advantages rather than extrinsic advantages (Abhilash et al., 2017; Beale et al., 2016). The subterranean niche of African mole rats has been included into this group of organisms that experience arrhythmic environments and would therefore mostly derive intrinsic adaptive advantages. However, although the subterranean niche is mostly constant in terms of light, a rhythmic temperature profile is present, and African mole rats are exposed to this cycle within their tunnel systems (Bennett et al., 1988; Roper et al., 2001). This leads to the possibility that, in addition to intrinsic advantages, a functional circadian clock in African

mole rats may also provide extrinsic advantages derived from synchronizing behaviour and physiology to daily changes in environmental temperatures.

#### *Temporal distribution of activity and core body temperature*

When subjected to 12L:12D lighting regimes, *C. h. mahali* displayed predominantly nocturnal, monophasic locomotor activity patterns (Fig. 2). This result is in congruence with several similar studies performed on other species of African mole-rats (Schöttner et al., 2006; de Vries et al., 2008; Oosthuizen and Bennett, 2015; Haupt et al., 2017). Core  $T_b$  closely followed the locomotor activity rhythm when animals were subjected to 12L:12D light regimes, with core  $T_b$  being higher during the dark phase than the light phase. A circadian rhythm of core  $T_b$  has previously been reported in the closely related *C. h. pretoriae* (Haupt et al., 2017) as well as in the more distantly related *H. glaber* and *Spalax ehrenbergi* (Goldman et al., 1997; Riccio and Goldman, 2000b). When subjected to constant darkness in the presence of a natural temperature cycle that is typically experienced within burrows, the locomotor activity and core  $T_b$  of *C. h. mahali* were expressed as monophasic nocturnal rhythms with a period of 24 hours. Since both of these rhythms free-run in constant conditions, these results suggest that the mole-rats may be able to use temperature to entrain locomotor activity and core body temperature to the 24-hour day. However, it is important to note that the current experimental design prevents conclusive differentiation of entrainment to ambient temperature from that of masking. Another DD Tc cycle would have to be included after the DD Tn cycle to definitively distinguish between entrainment and masking. Thus, we cannot make inference on the role of ambient temperature as a *zeitgeber*.

Haupt et al. (2017) reported that male highveld mole-rats were more active during the light phase, whereas females were more active during the dark phase. The authors also found that females displayed higher body temperatures when exposed to 20°C and males displayed higher body temperatures at an ambient temperature of 30°C. As with the highveld mole-rats, a clear sexual dimorphism is evident in *C. h. mahali* for locomotor activity. However, in contrast to the highveld mole-rats, *C. h. mahali* males are more active during the dark phase and there is no difference between locomotor activity intensity during the light phase. Sexual dimorphism in core body temperature is not evident for *C. h. mahali*. Sexual dimorphism in locomotor activity and core body temperature amongst rodents is uncommon and the reason behind the difference remain



unknown (Demas et al., 2001; Haupt et al., 2017).

### *Effect of different light and temperature regimes on locomotor activity and core $T_b$*

Core body temperature and locomotor activity intensity does not differ in response to the different light regimes when subjected to constant ambient temperature; whereas core body temperature is lower and locomotor activity higher under natural-wave light than square-wave light in the presence of a natural temperature cycle. This suggests that *C. h. mahali* is sensitive to the difference between square wave and natural wave light regimes only in the presence of a natural temperature cycle. For both square-wave and natural wave-light regimes there is a significant difference between core body temperature and locomotor activity intensity between natural and constant temperature; thus, irrespective of light cycle the inclusion of a natural temperature cycles influences core body temperature and locomotor activity intensity in *C. h. mahali*. Simulated natural ambient temperature and light cycles have not been previously investigated in mole-rats, therefore these results are novel and provide some insight toward the relative importance of photic and non-photoc cues for entrainment in mole-rats. Ambient temperature is generally considered to be a weak zeitgeber for circadian rhythms. Some, but not all, marmosets are able to entrain their locomotor activity to trapezoidal ambient temperature cycles (Pálková et al., 1999). This effect may be related to the amplitude of the body temperature cycle of the species. Camels can entrain their core body temperature rhythms to ambient temperature cycles, but light remains a stronger zeitgeber for circadian rhythms (El Allali et al., 2013).

Temperature variations over the 24-hour period of the day may be perceived as a form of enrichment for the animals, given the otherwise relatively impoverished environment they were maintained in. Enrichment is known to increase levels of activity in animals (Benarova-Milshtein et al., 2004).

In other rodent species, specific levels of illuminance have been shown to trigger the onset and offset of locomotor activity, independent of the temporal timing of the light (Boulos et al., 1996, Kavanau and Ramos, 1975). Our experimental setup may not have been sensitive enough to expose the animals to low enough levels of light for these responses to occur, the dimmest light that *C. h. mahali* was exposed to during the natural wave regime may have been above this threshold. In addition to light intensity and timing, spectral

intensity of light plays an important role in the integration of environmental light into the circadian system (Walmsley et al., 2015; Bonmati-Carrion et al., 2017, Ashley et al., 2014). The finding thus provides scope for the further investigation of the role of light intensity and spectral composition in entrainment.

## Conclusions

The importance of circadian rhythmicity in strictly subterranean mammals has been the subject of much discussion. All mole-rat species investigated thus far has been shown to have circadian rhythms of locomotor activity (De Vries et al., 2008; Oosthuizen et al., 2003; Riccio and Goldman, 2000a; Schöttner et al., 2006; Vasicek et al., 2005). It is possible that mole-rats were originally surface dwelling and that their circadian rhythms are an evolutionary remnant thereof. Temporal rhythms of activity may be useful to species that inhabit extreme habitats where superficial foraging tunnels get very hot during the day, and animals have to retreat to deeper, cooler nesting chambers for thermoregulatory purposes. Tunnels are not breached frequently enough to allow for light to entrain circadian rhythms *pers. obs.*), emphasizing the potential for another zeitgeber to be dominant cue in these animals.

In the present study, *C. h. mahali* displayed monophasic, nocturnal rhythms of both locomotor activity and core body temperature when presented with external light and temperature cues. This is indicative of a functional circadian clock that is capable of perceiving and integrating external light and temperature cues into daily rhythms of locomotor activity and core body temperature. This study provides the first experimentally-based indication that locomotor activity and  $T_b$  rhythms may be capable of synchronizing to ambient temperature cycles, thus supplementing anecdotal evidence suggesting temperature may be an important stimulus for circadian entrainment in these subterranean rodents (Oster 2002; Šklíba et al., 2014; Lövy et al., 2013). However, with the present experimental setup, we were unable to differentiate between masking and entrainment. Sexual dimorphism is present in locomotor activity, but not in core body temperature; however, physiological mechanisms and ultimate causes of this difference remain obscure. Future studies should focus on the role of spectral composition and light intensity as well as investigating potential differences between  $LD_s$  and  $LD_n$ . Furthermore, entrainment to ambient temperature cycles needs to be investigated and whether light or temperature acts as the dominant

zeitgeber in *C. h. mahali* could be investigated.

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## **Competing Interests**

No competing interests are declared.

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