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Seasonal Patterns of Body Temperature Daily Rhythms in Group-Living Cape Ground Squirrels Xerus inauris

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Abstract

Organisms respond to cyclical environmental conditions by entraining their endogenous biological rhythms. Such physiological responses are expected to be substantial for species inhabiting arid environments which incur large variations in daily and seasonal ambient temperature (T_a). We measured core body temperature (T_b) daily rhythms of Cape ground squirrels Xerus inauris inhabiting an area of Kalahari grassland for six months from the Austral winter through to the summer. Squirrels inhabited two different areas: an exposed flood plain and a nearby wooded, shady area, and occurred in different social group sizes, defined by the number of individuals that shared a sleeping burrow. Of a suite of environmental variables measured, maximal daily T_a provided the greatest explanatory power for mean T_b whereas sunrise had greatest power for T_b acrophase. There were significant changes in mean T_b and T_b acrophase over time with mean T_b increasing and T_b acrophase becoming earlier as the season progressed. Squirrels also emerged from their burrows earlier and returned to them later over the measurement period. Greater increases in T_b sometimes in excess of 5°C, were noted during the first hour post emergence, after which T_b remained relatively constant. This is consistent with observations that squirrels entered their burrows during the day to ‘offload’ heat. In addition, greater T_b amplitude values were noted in individuals inhabiting the flood plain compared with the woodland suggesting that squirrels dealt with increased environmental variability by attempting to reduce their T_a-T_b gradient. Finally, there were significant effects of age and group size on T_b with a lower and less variable T_b in younger individuals and those from larger group sizes. These data indicate that Cape ground squirrels have a labile T_b which is sensitive to a number of abiotic and biotic factors and which enables them to be active in a harsh and variable environment.

Introduction

Organisms respond to cyclical variation in environmental conditions by entraining their endogenous biological rhythms [1,2]. One such rhythm in endothermic species is that of body temperature (T_b), which is considered to be a consequence of the balance between heat production and heat dissipation [3]. In many taxa, T_b daily rhythms are influenced by diel and seasonal changes in photoperiod and ambient temperature (T_a) [4–9]. Indeed, the primary cues for seasonal acclimatization of the thermoregulatory system, which include changes in T_b daily rhythms, are photoperiod and temperature [10,11]. Interestingly, little is known about which selective pressures may affect the evolution of heterothermy in endotherms. Indeed, it is unclear whether one should examine the effects of environmental variation on raw T_b data or use some index which can be comparable across species (e.g. ‘Heterothermy Index’, ‘HI’ [12]). Angilletta et al. (2010) [13] suggest that future empirical work should examine the potential “selective pressures imposed by regional and temporal heterothermy”. They identify several potential candidates which might cause T_b variations to evolve which include food and water availability, T_a and social huddling. For example, restricted food and water supplies and low T_a values should favor energy-saving reductions in T_b and temporal heterothermy. Implicit in their arguments is the fact that extremes of variation in T_a and in particular cyclical variations in T_b may result in adaptive variation in T_b daily rhythms [13–16]. For group-living animals, behaviors such as social huddling may be one mechanism to conserve water and energy [17,18]. Minimization of thermoregulatory costs and water loss are thus seen as a possible selective pressure for aggregation [19–21]. For instance, huddling in newborn rabbit (Oryctolagus cuniculus) pups not only saves energy but also affects T_b daily rhythms [22]. Hence, T_b daily rhythms are likely to be affected by group size in social animals.

The open thorn scrub savannah ecosystem of southern Africa is subject to wide diel and annual variations in temperature across seasons, often reaching above 40°C during the summer and below freezing during the winter [23]. In this habitat, large open areas...
are interspersed with occasional stands of trees and bushes that generally concentrate in depressions around pans and dry river beds [24]. These areas are likely to present different microclimatic conditions due in part to differences in exposure to solar radiation [25]. Small mammals that inhabit this region, such as the Cape ground squirrel (Xerus inaurus), exhibit typical arid adaptations including a low resting metabolic rate, a high thermal conductance and a concentrated urine [26,27]. They are active year-round and forage during the heat of the day. It has been suggested that they use both behavioral and physiological means to deal with the extremes of $T_a$ they encounter [28–30]. For example, they may be active during hot summer days because they periodically dissipate body heat by retreating to cooler burrows [31]. Therefore, it is likely that their $T_b$ will vary considerably, both on a daily and a yearly basis, as a physiological adaptation to reduce the $T_a$-$T_b$ gradient [5,32,33]. However, it is unknown how this is related to microhabitat and behavior, such as the time animals emerge in the morning and how they may interact socially with one another.

Here we investigated the role of $T_b$ daily rhythms as a response to seasonal and diel changes in $T_a$ in Cape ground squirrels that inhabit a habitat mosaic exposed to large daily and annual temperature fluctuations. Our hypotheses were related to the middle (mesor); the amplitude and the acrophase (time of the peak) of $T_b$ daily rhythms [34]. We predicted that: (a) seasonal differences in $T_b$ daily rhythms would be apparent with higher mesor values and later acrophase times during the spring and summer; (b) rapid changes in $T_b$ would be apparent in the early mornings (after emergence) and a $T_b$ would be maintained at a constant level throughout the daylight hours because animals will move into and out of cooler locations such as their burrows as part of their thermoregulatory behavior; (c) lower mesor and amplitude values of $T_b$ would be observed in a shaded compared with an open habitat; and (d) winter mesor values would be higher in animals from larger group sizes because of the thermoregulatory benefits gained from huddling at night. In addition, we examined the potential seasonal variation in $H$ values from individuals inhabiting different locations and from different group sizes to gauge whether or not relationships that emerge when analyzing $T_b$ data are also manifest when using this index.

**Materials and Methods**

**Ethics statement**

Permission was granted from South Africa Northwest Parks and Tourism to conduct the field research. The protocol was approved by committee on the ethics of animal experiments of the Universities of Central Florida and Pretoria (permit number UCF IACUC #07-43W). The study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

**Animals and study site**

Cape ground squirrels are small (~600 g), non-hibernating, diurnal, social rodents that inhabit arid regions of sub-Saharan Africa [35–37]. They are cooperative breeders with low reproductive skew and a high operational sex ratio. Groups typically consist of 1–6 related females and their sub adult and juvenile offspring, which share a burrow cluster [35,38]. The study took place at S. A. Lombard Nature Reserve (3,660 ha, 18 km north west of Bloemhof, South Africa, 27°35’S, 25°23’E) as part of an on-going study where squirrels have been studied since 2002. The site comprises *Cymbopogon-Themeda* veld and Kalahari grasslands, and is situated on a flood plain [24]. Mean annual precipitation is 500 mm [39]. Animals were trapped from groups at two locations: an open unshaded area – “the flood plain” – and a habitat containing *Araucaria karoo* and *A. erioloba* stands – “the woodland”, which was approximately 2 km away [40]. Tomahawk wire-mesh traps (15 x 15 x 50 cm) baited with peanut butter were used to catch animals, after which they were freeze-marked for unique identification (Quick Freeze, Miller-Stephenson Chemical Co., Danbury, CT [41]) and implanted with transponders (PIT tags, AVID Inc., Norco, CA). The sides of animals were also painted with various shapes using black hair dye (Rodol D, Lowerstein & Sons Inc., New York, NY) so their identities could be seen at a distance. Body mass was recorded along with the size of the social groups to which animals belonged. Trapping took place for two one-week periods during May and October. Age was assessed by knowing dates of first emergence from the natal burrow [35,42]. Behavioral observations, including times of emergence and immersence from burrows were obtained as outlined in Waterman [37]. Briefly, this involved recording time budgets of individual animals by focal sampling in which all-occurrence data were recorded for periods of up to 20 minutes whereas the activities of all the individuals within a group were recorded every five minutes by scan sampling [43]. We were interested in many different aspects, but in particular movement and foraging activities as well as aggressive, reproductive and social/dominance interactions between individuals.

**Acquisition of body temperature ($T_b$) data**

Ten squirrels (five sub adults and five adults) were obtained from the flood plain and 10 (also five adults and five sub adults) from the woodland. Sub adults are defined as animals between six months after first emergence from the natal burrow and sexual maturity (around eight months for males and nine months for females); adults are individuals which have reached sexual maturity [38]. Miniature temperature recording iButton® data-loggers (DS1922L±0.0625°C; Thermochron, Dallas Semiconductors, Maxim Integrated Products, Inc., Sunnyvale, CA) were surgically implanted into the peritoneal cavity of each individual under anaesthesia (see below). Prior to surgery, devices were calibrated using an APPA 51 digital thermometer in a water bath. They were set to record every 60 min providing 23 weeks of continuous recordings. Data-loggers were then coated with medical grade surgical wax (ELVAX) [44] and sterilized with formaldehyde vapor. Measurements of $T_b$ were recorded between May 17th and October 28th 2006.

Squirrels were anaesthetized with medetomidine (Domitor, Pfizer Laboratories (PTY) Ltd, Sandton) (67.6±9.2 μg/kg), ketamine (Anaket V, Centaur Laboratories (PTY) Ltd, Isando) (13.6±1.9 mg/kg) and buprenorphine (Temgesic, Ricketts Laboratories, Isando) (0.5±0.06 μg/kg) [45]. Anaesthesia was induced after 3.1±1.4 minutes. The abdomen was surgically prepared with a chlorhexidine scrub (Hibiscrub, ICL Laboratories), then with chlorhexidine and alcohol (Hibitane, ICI Laboratories). A midline celiotomy was performed for insertion of the data-loggers. The linea alba was closed with 4/0 polydioxanone (PDS, Ethicon, Midrand) and the skin was closed with an intercuticular suture. The procedure for each individual lasted approximately 20 minutes. At the end of the surgical procedure, anaesthesia was reversed with atipamezole (Antisedan, Pfizer Laboratories) (232±92 μg/kg). Recovery occurred within 3.5±2.2 minutes. This procedure was followed for removal of data-loggers for the case of five animals that were recaptured. Three other recaptured animals were euthanized with an overdose of halothane upon recapture as part of a different study [46]. Only eight of the total 20 animals implanted were recaptured. After removal of data-loggers, $T_b$ data were down-
Ambient temperature and daylight measurements

Ambient air temperature ($T_a$) was determined using two methods. We set dataloggers to record every hour for the first 84 days (12 weeks) of the sampling period. One datalogger was used per study site. Dataloggers were placed inside Stevenson screens located 90 cm above the ground. To obtain data over a longer time period, we used daily minimum, maximum and mean ambient temperatures recorded at Bloemhof 27.65 S, 25.60 E, GMT +2 (South African Weather Bureau, Pretoria) for the entire 23 weeks of the sampling period; mean hours of sunlight as well as the times of sunrise (civil dawn) and sunset (civil dusk) were also noted. In an attempt to measure underground temperatures, we also placed two dataloggers inside what we thought were disused squirrel burrows. However, these devices did not provide useful information because the burrows were not vacant; squirrels removed them from the burrows and they were found in spoil heaps on the surface.

Data analyses

Cosinor analysis was used to determine the $T_b$ daily rhythms of the individuals measured [34,47]. The mean mesor, amplitude and acrophase values of the $T_b$ daily rhythms were calculated for every individual for each of the 23 weeks of the study period (‘$T_b$mesor’, ‘$T_b$amplitude’ and ‘$T_b$acrophase’, respectively). The significances of the fitted curves were tested against the null hypothesis that the amplitude was zero [48]. The variability in the data that could be accounted for by the fitted curve (percentage rhythm) was calculated. In addition, we calculated the HI values for each animal for each week of the study and assessed whether there were any relationships between HI and season, age or group size. Statistical analyses were performed using SPSS 17 (SPSS Inc., Chicago, Ill., U.S.A.). Mean values are reported ± standard deviations.

(1) Seasonal variation in $T_b$ daily rhythms. Linear mixed models were used to examine the variation in $T_b$ cosinor parameters (mesor, amplitude, acrophase) as a function of time (over the 23 week period). Each dependent variable was analyzed separately. ‘Individual ID’ was included as a random factor to avoid pseudoreplication and to correct for repeated measurements. ‘Week’ was included as fixed covariate. As several explanatory terms and their interactions were investigated, models were selected in a stepwise backward fashion, removing the least significant explanatory terms sequentially until the most parsimonious model was obtained, determined by Akaike’s information criterion (AIC). Interaction terms were only included when they were significant.

(2) Effect of light and ambient temperature ($T_a$) on body temperature ($T_b$) daily rhythms. Linear Mixed Models were used to examine the effects of light and $T_a$ on the mean weekly cosinor parameters. First, we obtained several measures of $T_a$: the daily minimum ($T_a$min), the daily maximum ($T_a$max) and the daily mean value ($T_a$mean) (South African Weather Bureau). We then calculated weekly averages of $T_a$min, $T_a$max and $T_a$mean and included each of these in a model with individual identity as a random factor and week as a fixed effect. This corrected for repeated measurements and differences in mean values between individuals. All potential interactions between temperature variables were included. Models were selected by removing the least significant explanatory terms sequentially until the most parsimonious model was obtained using AIC. Each dependent cosinor variable was analyzed separately. Second, we assessed the effects of various ‘light’ variables on the cosinor variable. The light variables we used were: the weekly average time of sunrise, the weekly average time of sunset and the weekly average length of the photophase. As before, models were selected using AIC by removing least significant explanatory terms sequentially. Finally, for each of the dependent cosinor variables, combined models were undertaken which included the factors with most explanatory power from both the individual $T_a$ models and the individual light models. Again, for each analysis the best model was obtained using AIC.

(3) Relationship between emergence and immurgence times and $T_b$ daily rhythms. Emergence and immurrence times for the two habitats were calculated as the mean observed emergence and immurrence time of groups of squirrels inhabiting both areas [35]. Data were collected over seven months of detailed observation time recording when individual squirrel groups from the two habitats emerged or immurred. An average of 8.1±0.65 squirrels from different groups were observed every week to calculate emergence times and 5.7±0.81 squirrels from different groups were observed every week to calculate immurrence times. Temporal variation in mean emergence and immurrence times was investigated using linear regressions. In order to determine how daily variations in $T_b$ were related to the times of emergence and whether this differed throughout the year, we computed, for each day, the mean $T_b$ of each individual one hour before the time of emergence and the mean $T_b$ one hour after the time of emergence. The difference in $T_b$ between these two values was then calculated as a percent of the maximum amplitude difference in $T_b$ for that individual for that day. The mean percent $T_b$ change for each individual was then calculated for each week, after which the mean change for all individuals was calculated for the 23 weeks.

(4) Effect of habitat on $T_a$ and $T_b$ daily rhythms. To examine whether mean daily $T_a$ differed between the flood plain and the woodland we conducted linear mixed models with habitat as a fixed factor, week as a time and $T_a$ measured at both study sites as the dependent variable. To determine whether high values of $T_a$ obtained during the day or low values obtained during the night differed between the two habitats we included day/night as an additional fixed factor. The hourly $T_a$ obtained at both study sites were considered as being ‘daytime’ $T_a$ if the measurement was taken between the sunrise and sunset of a given day, and ‘night-time’ $T_a$ if the measurement was taken between sunset and sunrise time between two consecutive days. An average $T_a$ was then determined for each daytime and each night-time period for the 84 days (12 weeks) of the sampling period. To examine the effect of habitat on mean weekly $T_b$ values and cosinor parameters, we included ‘habitat’ and ‘day/night’ as a fixed factors, ‘individual’ as random variable and ‘week’ as factor.

(5) Effect of age and group size on $T_b$ daily rhythms. Effects of age and group size on $T_b$mean, $T_b$mesor, $T_b$amplitude, $T_b$acrophase and HI were conducted using linear mixed models with ‘individual’ as a random variable and ‘week’ as factor. Models were selected in a stepwise manner using AIC as described previously. Age (adult/sub adult) was included as a categorical factor and group size as a continuous variable.

Results

Of the 20 individuals originally implanted with dataloggers, eight were recaptured; six from the flood plain (two adults, four...
sub adults) and two from the woodland (two adults). Group sizes (i.e. the sizes of groups in which the eight animals lived) ranged from one to nine individuals. The implanted animals were regularly observed during the two weeks following implantation and no mortality or immigration was observed. We observed no signs of different behavior of the implanted squirrels compared to the others. There were significant daily rhythms of T_b in all of the eight individuals measured (Table 1, Fig. 1) with mean ± SD values of the mesor, amplitude and acrophase for the 23 week measurement period of 37.51±0.13°C, 1.13±0.08°C and 12:33±2 min, respectively.

(1) Seasonal variation in T_b daily rhythms
There were significant effects of both ‘week’ and ‘individual’ on T_bmesor and T_bacrophase (F1,175 = 35.86, p<0.001 and F1,175 = 8.51, p<0.001 respectively; Fig. 2A, 2C) indicating that mesor values increased significantly and acrophase values became earlier over the time period, and that these values differed between individuals. There was also a significant interaction between individual and week on T_bamplitude (F7,168 = 2.60, p<0.05; Fig. 2B), indicating that changes in amplitude differed between individuals over time.

(2) Effect of light and T_a on T_b daily rhythms
Mean T_a values ranged from 7.0±1.4°C during the first week to 21.1±0.43°C during the last with daily minimum and maximum values of −3°C and 22°C, and 9°C and 36°C respectively (Fig. 2D). By comparison, mean T_b ranged from 37.37±0.12°C during the first week to 37.70±0.12°C during the last. This corresponded to minimum and maximum T_b values of 34.28 and 40.11°C, and 35.64°C and 41.23°C, respectively (Fig. 2A).

When the effects of ambient conditions on T_b were examined the only ‘temperature’ variable (of T_bmin, T_bmean and T_bmax) that significantly influenced T_bmesor was T_bmax (F1,60 = 23.87, p<0.001). Similarly, the only ‘light’ variable that significantly affected T_bmesor was the time of sunset (F1,99 = 23.72, p<0.001). However, there was no significant effect of group size on T_bmesor with individuals from both habitats showing similar T_bmesor values regardless of group size.

(3) Relationships between emergence and immersion times and T_b daily rhythms
Animals emerged later in the day at the beginning of the measurement period (07:44) (May), than at the end (October) (06:40) (least-squares regression, F1,46 = 63.25, ̄r²=0.579, p<0.001). In contrast, immersion times occurred earlier in the day at the beginning of the measurement period (17:24) than at the end (18:17) (F1,46 = 103.02, ̄r²=0.696, p<0.001; Fig. 3). There were no differences in emergence and immersion times between animals that inhabited the flood plain and the woodland (emergence: F1,46 = 0.19, p = 0.662; immersion: F1,46 = 0.17, p = 0.685). However, there was an indication that variation in T_b on a day-by-day basis reflected variation in T_b with depressions in T_b occurring at similar times to depressions in T_a (Fig. 4).

Changes in T_b over 24 h periods were greatest at around the times of emergence and immersion, sometimes in excess of 5°C, highlighting the potential relationship between T_b and whether or not the animals were above or below ground (Fig. 5). During the winter (week 1), mean increases in T_b for the hour following emergence were +1.10±0.12°C, which were greater than changes in T_b which occurred in the hour preceding emergence of −0.14±0.13°C. During the end of the measurement period at week 22, increases in T_b following emergence were less at +0.67±0.12°C compared to +0.18±0.10°C during the hour prior to emergence, respectively. There was a significant difference in T_b increase between the beginning and the end of the measurement period, with a 32% increase in T_b during the first hour following emergence (relative to the total change in T_b during that day) during week one and only a corresponding 20% increase in T_b during week 22 (F1,20 = 4.99, ̄r²=0.20, p<0.05). T_b values stabilized when animals returned to their burrows in the evening; changes in T_b of −0.01±0.06°C were recorded during the hour post immersion and −0.16±0.06°C during the hour prior to immersion for week 1; this compared to changes of −0.08±0.04°C and −0.20±0.04°C for post-and pre-immersion times during week 22, respectively.

(4) Effect of habitat on T_bmesor and T_bacrophase values. Recorded for individuals from the flood plain were higher than those from the woodland (F1,127 = 1.59, p = 0.210; Fig. 2C). There was a significant effect of habitat on T_bmesor and T_bacrophase values. There was a significant difference between habitats when day and night temperatures were specified in the model (Habitat: F1,335 = 0.939, p = 0.333; Day/night: F1,335 = 113.1, p<0.001; Habitat * Day/night: F1,335 = 33.310, p<0.001) indicating that the flood plain was significantly hotter during the day and colder during the night than the woodland. T_b values in the flood plain were 18.00±0.41°C during the day and 2.46±0.42°C during the night which compared with values of 15.34±0.40°C during the day and 4.35±0.37°C during the night in the woodland (Fig. 2D).

(5) Effect of age and group size on T_b daily rhythms
There were significant interactions between age and body mass on T_bmesor (F1,111 = 75.8, p<0.001 respectively). Older individuals decreased T_b with increasing mass whereas T_b was independent of body mass in younger animals. There was also a significant effect of group size on T_bmesor with individuals from larger groups having lower T_bmesor values than those from smaller groups (F1,156 = 18.70, p<0.001 respectively; Fig. 7A). There was a significant effect of group size (F1,154 = 22.29, p<0.001) and a significant interaction between age and body mass on T_bamplitude (F1,153 = 9.22, p = 0.003). Individuals from larger group sizes had lower T_bamplitude values and older animals decreased in T_bamplitude with increasing mass whereas T_bamplitude was independent of body mass in younger animals (Fig. 7B). There were significant interactions between age and body mass and between group size and body mass on T_bacrophase (F1,74 = 44.26, p<0.001 and F1,120 = 36.25, p<0.001 respectively; Fig. 7C). Young animals which were large for their age tended to
Figure 1. Body temperature ($T_b$) daily rhythm of an adult Cape ground squirrel (605 g) for the first (21 to 28 May), eighth (09 to 16 July), fifteenth (27 August to 03 September) and twenty-second week (15 to 22 October) of a 23-week measurement period. ‘M’ indicates the mesor (37.41°C), ‘A’ the amplitude (0.92°C) and ‘Ø’ the acrophase (189.11° or 12:36 h) of the fitted cosine curve. SR and SS show times of sunrise and sunset.

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have $T_a$ acrophase values which occurred earlier in the day whereas larger adults had $T_a$ acrophase values which occurred later. Finally, $T_a$ acrophase values tended to occur later in the day as group size increased but was earliest for a group size of nine.

(6) Effect of season, age and group size on the heterothermy index (HI)

Mean HI value across all individuals was 1.23 ± 0.29°C and ranged from 0.68 to 2.32°C. While there were significant differences in HI values between individuals, there was no significant effect of ‘week’ ($F_{7,175} = 22.91$, $p<0.001$) and there was no significant interaction between age and group size on HI ($F_{1,175} = 15.03$, $p<0.001$); older animals decreased in HI with increasing group size whereas for young animals HI was independent of group size.

Discussion

Living in hot arid environments can be stressful for small diurnal mammals since the availability of free water necessary to reduce body heat by evaporation is limited [49]. Consequently, evaporative cooling is often accompanied by behavioral and physiological mechanisms to dissipate heat such as the use of a thermal refuge or substrate [50] or heterothermy [13,51–53]. In the current study, Cape ground squirrels were exposed to a wide seasonal and daily range of $T_a$ and the $T_b$ mesor of all individuals increased significantly as the season progressed. This indicates that $T_a$ values, including both maximal and minimal $T_a$’s were higher on average when $T_a$ values were higher. This will presumably serve to conserve their water and energy as a reduced $T_a$-$T_b$ temperature gradient minimizes the need to keep cool by evaporation [15,54,55]. In addition, acrophase values became earlier over the measurement period, indicating that activity periods also became earlier [20,36]. Ground squirrels in general have labile $T_b$’s [2,5,57–61], $T_b$ amplitudes of different species may vary by 4–5°C and be accompanied by bouts of torpor or hibernation. This compares with $T_a$ amplitude values of up to 4.1°C in Arabian oryx (Oryx leucoryx) [51] and 2.6°C in Arabian sand gazelles (Gazella subgutturosa marica) [52]. We found no evidence of torpor and recorded daily variation in $T_b$, of 5–6°C, which is greater than that noted in most other species and greater than noted by Wilson et al. (2010) [53] for Cape ground squirrels in a more mesic area (3.8°C amplitude); hence this probably reflects adaptation to an environment with high $T_a$ values and large daily variations in $T_{b}$.

(3) Relationship between $T_b$ daily rhythms, $T_a$ and daylight

Peak ambient temperature ($T_{a,\text{max}}$) was the primary factor that explained both $T_b$ mean and $T_b$ amplitude, which suggests that this is the most thermally challenging period of the day. By comparison, sunrise provided the greatest explanatory power

<table>
<thead>
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<th>Week</th>
<th>Begin date</th>
<th>End date</th>
<th>Mesor (°C)</th>
<th>Amplitude (°C)</th>
<th>Acrophase hh:mm</th>
<th>Percentage rhythmicity</th>
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<td>12:55 (0.11)</td>
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<td>04/06/2006</td>
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<td>12:36 (0.05)</td>
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<td>18/06/2006</td>
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<td>12:36 (0.12)</td>
<td>56.12 (4.94)</td>
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<td>25/06/2006</td>
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<td>1.07 (0.13)</td>
<td>12:31 (0.10)</td>
<td>46.42 (4.19)</td>
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Table 1. Mean (±SE) of the mesor (°C), amplitude (°C), acrophase (time hh:mm) and percentage rhythmicity obtained from 24 h cosine functions of hourly $T_b$ recordings of eight Cape ground squirrels during a 23-week sampling period.
defining T_a acrophase which may suggest that sunrise acted to temporally entrain the thermoregulatory system [62]. Indeed T_b mean increased rapidly (4–5 °C) post-emergence. The sensitivity of organisms to the timing of first light is exemplified by the fact that light ‘pollution’ during the dark phase can alter the seasonal acclimation of thermoregulatory, reproductive and immune systems of small mammals [63,64]. Interestingly, increases in T_b during the first hour post-emergence were faster and greater earlier in the measurement period, indicating that animals gained thermal energy more rapidly during the winter. This indicates that as well as endogenous rhythms, mechanisms such as sun-basking might also be important in raising T_b [28,31,65,66]. Whether or not squirrels preferentially orientate themselves to maximize heat uptake whilst basking, for example as in Raccoon dogs (Nyctereutes procyonoides) [67], remains unclear. By comparison, after initial increases, the time at which T_b stabilized in the mid-morning is likely to be indicative of another regulatory behavior: seeking shelter in burrows or in shade [31,68]. This effect also became

![Figure 2. Mean ±SE daily rhythm parameters of eight Cape ground squirrels during the 23 week measurement period for: (a) T_b Mesor (°C); (b) T_b Amplitude (°C); (c) T_b Acrophase (time of day and degrees). Individuals inhabiting the flood plain and the woodland are denoted by solid and open circles. Maximum, minimum and mean T_b values are shown in (d) as top, middle and lower lines. doi:10.1371/journal.pone.0036053.g002](image)

![Figure 3. Mean ±SE immergence and emergence times in the flood plain (solid circles and bold line) and woodland (open circles and light line). Mean number of animals observed at any one time was 8.1 ± 4.5 at emergence and 5.6 ± 2.6 at immergence. doi:10.1371/journal.pone.0036053.g003](image)

![Figure 4. T_b (open circles) and T_a (solid circles) and fitted cosine curves for a Cape ground squirrel during the 9th week of the sampling period illustrating the variation in T_b and T_a. The difference between the lowest T_b value recorded (33.39 °C at 19:08) and the highest T_b during the previous day (39.32 °C at 16:08) was 5.93 °C. Over the 23 week period, extreme changes in T_b included one individual that decreased in T_b by 5.56 °C and another that increased in T_b by 5.98 °C in one hour. doi:10.1371/journal.pone.0036053.g004](image)
earlier as the season progressed (Fig. 7) suggesting that animals were using thermal refuges to offload heat earlier, allowing periodic bouts of foraging. There was also an indication that $T_b$ tracked $T_a$ (Fig. 4) highlighting the thermal lability of these animals. It is likely that Cape ground squirrels were allowing their $T_b$ to vary to defend both water loss and energy expenditure as the greatest amplitudes of variation were noted during the winter.

Alpine ibex ($\text{Capra ibex ibex}$) also show the greatest amplitude of variation of $T_b$ during the winter which the authors suggested promoted a ‘thrifty’ use of body reserves [9]. By comparison, desert ungulates showed the greatest daily variation in $T_b$ during the summer (2.6±0.8°C in Arabian sand gazelles and 4.1±1.7°C in Arabian oryx); this is the season that is most stressful for them when they benefit most by minimizing evaporative water loss [51,52]. It is noteworthy that $T_{b\text{mean}}$ decreased just before evening immergence and remained steady once the squirrels were within their burrows. It seems that the major stimulus to enter burrows could be the prevention of a further decrease in $T_b$ or an increase in energy expenditure due to increased thermoregulation, rather than other possible cues, such as light intensity.

(4) Influence of habitat on $T_b$ daily rhythms

As expected, $T_a$ was more variable in the flood plain than in the woodland, with the former habitat exhibiting both colder nights and hotter days. Although the sample size was reduced because we were not able to capture many of the individuals that were
implanted, the results obtained suggest that \( T_a \) values were also greater in animals inhabiting the flood plain than the woodland. This may reflect a physiological strategy to minimize the \( T_a - T_b \) temperature gradient and save on thermoregulatory costs [55]. There were also significant differences between \( T_b \) mesor values of animals inhabiting the two habitats, with higher values recorded in those from the flood plain. This is interesting because \( T_b \) mesor values did not differ between the two habitats. Therefore, the high \( T_a \) experienced during the day must have had a greater effect on the squirrels’ physiology than the \( T_a \) experienced during the night in their burrows; moreover the flood plain was more thermally challenging than the woodland. Presumably squirrels are not exposed to the lowest \( T_a \) values during the night because they shelter in burrows, whereas they are exposed to high \( T_a \) values during the day even though they may use of temporary thermal refuges [68]. This corroborates our previous finding that \( T_{b\text{max}} \) held the greatest explanatory power for and \( T_b \) mesor.

The fact that variation in physiological characteristics occurred within a small geographical area suggests that Cape ground squirrels are able to regulate their \( T_b \) according to local environmental conditions. Similar patterns have been recorded in other small mammals albeit over different scales. Common spiny mouse (\textit{Acomys cahirinus}) populations a mere 2–300 m apart on either side of a valley in the Mediterranean ecosystem exhibit a suite of physiological differences which include variations in their chronobiology [15,69], as do populations of the broad-toothed field mouse (\textit{Apodemus mystacinus}) from different sides of the African Great Rift valley [70,71]. \textit{A. cahirinus} inhabiting a xeric environment had later \( T_a \) acrophase and greater \( T_b \) amplitude values than those inhabiting a mesic cooler environment [15]. It was suggested that individuals from the former population allowed their \( T_b \) to vary considerably, rather than waste water by controlling \( T_b \) through evaporation or waste energy using endogenous heat sources, a strategy noted elsewhere [72–74]. Since no physical barrier exists between the two sites in the current study, one can assume that there is relatively high within-site fidelity [40].

(5) Effects of age and group size on \( T_b \) variation

Across taxa, younger animals generally have less prominent \( T_b \) daily rhythms than older animals, in part because \( T_b \) daily rhythms need time to mature [75,76]. Larger animals also tend to have smaller \( T_b \) amplitude values as a presumed consequence of their greater thermal inertia and reduced susceptibility to changes in food availability [76,77]. Although our results must be interpreted with caution because of the small sample sizes, these relationships are corroborated as a negative correlation was noted between \( T_{b\text{mean}} \) and body mass in older but not in younger animals. In our case, heavy young animals also tended to have higher \( T_a \) acrophase values, indicating earlier activity periods in these individuals. If emergence times are driven by thermoregulatory constraints, it is possible that older individuals and those large for their age may emerge earlier because of their lower surface area to volume ratios and greater thermal capacities. An alternative explanation might be that larger animals might simply have more fat reserves, allowing them to emerge earlier and expend more energy on thermoregulation.

The fact that \( T_{b\text{mean}} \) values decreased with increasing group size suggests that squirrels were expending less energy on thermoregulation in larger groups. Previous studies have suggested that aggregation/huddling behavior can significantly reduce thermoregulatory costs [17,78] and daily averaged energy expenditure [79] in some groups of small mammals. For example, \( T_b \) values were found to be lower in large groups of roosting bats \textit{Noctilio albiventris} [80]. It was suggested that individual bats in larger groups might be less prone to predation and hence could benefit by lowering their \( T_b \)’s further than those within smaller groups. In contrast, for two species of African mole-rat (\textit{Cryptomys hottentotus natalensis} and \textit{Fukomys damarensis}), individuals in experimentally increased group sizes had greater \( T_b \) values [78]. In this case a crowded burrow which is thermally buffered might make it difficult to cool down and consequently \( T_b \) values are greater. Because Cape ground squirrels forage during the day as a spaced group [35], any thermoregulatory benefits of group size would presumably occur during the night [68] and hence a larger group size could facilitate a lower and more stable \( T_b \).

Finally, both \( T_b \) amplitude and HI were negatively associated with group size and older animals had lower HI values in larger group sizes whereas younger animals did not. This is also consistent with our predictions that individuals in larger groups benefit by being thermally buffered and that older animals are better at regulating their \( T_b \). In this instance, both metrics (\( T_b \) amplitude and HI) appear to provide similar results, i.e. that there are significant effects of age and group size on \( T_b \) variation.
Overall, these data confirm that the thermal physiology of Cape ground squirrels is sensitive to changes both in the abiotic and biotic environment. Many factors are observed to affect their $T_b$, which can be modified, enabling them to survive in arid, hostile environments.

Acknowledgments

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Author Contributions

Conceived and designed the experiments: MS. Performed the experiments: MS MBM KEJ. Analyzed the data: MS MDG. Contributed reagents/materials/analysis tools: NCB JMW. Wrote the paper: MS MDG PWB NCB MBM KEJ JMW.

References