Seasonal patterns of body temperature daily rhythms in group-living cape ground squirrels Xerus inauris

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Organisms respond to cyclical environmental conditions by entraining their endogenous biological rhythms [1,2]. One such rhythm in endothermic species is that of body temperature (Tb), which is considered to be a consequence of the balance between heat production and heat dissipation [3]. In many taxa, Tb daily rhythms are influenced by diel and seasonal changes in photoperiod and ambient temperature (Ta) [4–9]. Indeed, the primary cues for seasonal acclimatization of the thermoregulatory system, which include changes in Tb daily rhythms over time with mean Tb increasing and Tb 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 Tb, sometimes in excess of 5°C, were noted during the first hour post emergence, after which Tb remained relatively constant. This is consistent with observations that squirrels entered their burrows during the day to ‘offload’ heat. In addition, greater Tb 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 Ta-Tb gradient. Finally, there were significant effects of age and group size on Tb with a lower and less variable Tb in younger individuals and those from larger group sizes. These data indicate that Cape ground squirrels have a labile Tb which is sensitive to a number of abiotic and biotic factors and which enables them to be active in a harsh and variable environment.
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 to different distances from the main center of solar radiation [25]. Animals that inhabit this region, such as the Cape ground squirrel (*Xerus inauris*), exhibit typical arid adaptations including a lower resting metabolic rate, a high thermal conductance, and a concentrated urine [26,27]. They are active year-round and move into and out of cooler locations such as their burrows as part of their thermoregulatory behavior; (c) lower mesor values and later acrophase times during the spring and summer; 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 HI values from individuals inhabiting different locations and from different group sizes to gauge whether or not relationships that emerge when analyzing T<sub>b</sub> 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. Only eight of the total 20 animals implanted were recaptured. After removal of dataloggers, T<sub>b</sub> data were down-sampled. Dataloggers were then coated with medical grade surgical wax (ELVAX) and sterilized with formaldehyde vapor. Measurements of T<sub>b</sub> 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), cetamine (Anatel V, Centaur Laboratories (PTY) Ltd, Isando) (13.6 ± 1.9 mg/kg) and buprenorphine (Temensic, Ricketts Laboratories, Isando) (0.5 ± 0.06 µg/kg) [45]. Anesthesia was induced by Antisedan (Pfizer Laboratories (PTY) Ltd, Sandton) (232 ± 92 µg/kg) and for lasting 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 dataloggers. The linea alba was closed with 4/0 polydioxanone (PDS, Ethicon, Midrand) and the skin was closed with an intercuticular suture. Surgical procedures were performed by committee on the ethics of animal experiments of the Universities of Central Florida and Pretoria with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Acquisition of body temperature (T<sub>b</sub>) 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]. Microtelemetry recording iButton<sup>®</sup> data-logger (DS1922L±0.0625°C; Thermochron, Dallas Semiconductors, Maxim Integrated Products, Inc., Sunnyvale, CA) were surgically implanted into the peritoneal cavity of each individual under anesthesia (see below). Prior to surgery, devices were calibrated using an AAPP 51 digital thermometer in a water bath. They were set to record every 60 min providing 23 weeks of continuous recordings. Dataloggers were then coated with medical grade surgical wax (ELVAX) and sterilized with formaldehyde vapor. Measurements of T<sub>b</sub> were recorded between May 17th and October 28th 2006.

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Body Temperature Daily Rhythms in Squirrels

The study investigated the daily rhythms of body temperature (Tb) in squirrels, with a focus on ambient temperature and daylight measurements. Ambient temperature (Ta) was determined using dataloggers placed in the study sites, and the measurements were analyzed using cosinor analysis. The Tb daily rhythms were calculated for each individual, and the cosinor parameters, including mesor, amplitude, and acrophase, were determined for each week.

**Methods**

- **Ambient temperature and daylight measurements**
  - Ambient air temperature (Ta) was determined using dataloggers placed in Stevenson screens located 90 cm above the ground. Ta was measured every hour for the first 84 days (12 weeks) of the sampling period. Dataloggers were placed inside the study sites.
  - To obtain data over a longer time period, a second set of dataloggers was used, and the Ta measurements were 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 the burrows of the squirrels. However, these devices did not provide useful information because the burrows were not vacant; squirrels removed them from the burrows and found in spoil heaps on the surface.

- **Data analyses**
  - Cosinor analysis was used to determine the Tb daily rhythms of the individuals measured. The mesor, amplitude, and acrophase values of the Tb daily rhythms were calculated for each individual, and the cosinor parameters were determined for each week.
  - To examine the variation in Tb daily rhythms, linear mixed models were used to examine the variation in Tb, cosinor parameters (mesor, amplitude, acrophase) as a function of time (over the 23 week period). Each dependent variable was analyzed separately. The ‘individual’ ID was included as a random factor to avoid pseudoreplication and to correct for repeated measurements. ‘Week’ was included as fixed covariate. As a random factor and week as a factor. Models were selected in a stepwise manner using AIC as the dependent variable.

**Results**

Of the 20 individuals originally implanted with dataloggers, eight were recaptured; six from the flood plain (two adults, four 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 sunset, the weekly average time of sunrise 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 Tb, models and the individual light models. Again, for each analysis the best model was obtained using AIC.

(3) Relationship between emergence and immergence times and Tb daily rhythms.

Emergence and immergence times for the two habitats were calculated as the mean observed emergence and immergence time 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 immerged. 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 immergence times. Temporal variation in mean emergence and immergence times was investigated using linear regressions. In order to determine how daily variations in Tb were related to the times of emergence and whether this differed throughout the year, we computed, for each day, the mean Tb of each individual one hour before the time of emergence and the mean Tb one hour after the time of emergence. The difference in Tb between these two values was then calculated as a percent of the maximum amplitude difference in Tb for that individual for that day. The mean percent Tb 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 Tb and Ta daily rhythms.

To examine whether mean Tb differed between the flood plain and the woodland, we conducted linear mixed models with habitat as a fixed factor, week as a time and Tb measured at both study sites as the dependent variable. To determine whether high values of Tb 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 Tb obtained at both study sites was considered as being ‘daytime’ Tb if the measurement was taken between the sunrise and sunset of a given day, and ‘night-time’ Tb if the measurement was taken between sunset and sunrise time between two consecutive days. An average Tb 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 Tb, Tb 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 Tb daily rhythms.

Effects of age and group size on Tb, mesor, Tb amplitude, Tbamplitude 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.

**Discussion**

The study findings suggest that ambient temperature and daylight measurements play a crucial role in shaping the Tb daily rhythms in squirrels. The cosinor analysis allowed for the determination of mesor, amplitude, and acrophase values, which are essential for understanding the circadian rhythms. The inclusion of ‘light’ variables such as sunset, sunrise, and photophase helped in elucidating the effect of light on Tb. The results also highlight the importance of habitat on Tb and Ta daily rhythms, with significant differences observed between the flood plain and woodland.

**Conclusion**

The study provides valuable insights into the Tb daily rhythms in squirrels, which can be further utilized in understanding the physiological responses of these animals to environmental factors.
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 \( T_b \)mesor and \( T_b \)acrophase (\( F_{1,175} = 35.86, p < 0.001 \) and \( F_{7,175} = 8.51, p < 0.001 \) 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 \( T_b \)amplitude (\( F_{7,160} = 2.60, p < 0.05 \); 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 ± 6.4°C during the first week to 21.2 ± 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 ± 0.11°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 \( T_b \)min, \( T_b \)mean and \( T_b \)max) that significantly influenced \( T_b \)mesor was \( T_b \)max (\( F_{1,60} = 23.87, p < 0.001 \)). Similarly, the only ‘light’ variable that significantly affected \( T_b \)mesor was the time of sunset (\( F_{1,99} = 23.72, p < 0.001 \)). When both explanatory terms were included into the same model, neither had a significant effect (\( p > 0.1 \) in both cases). In contrast, although \( T_b \)max had a significant effect on \( T_b \)amplitude (\( F_{1,53} = 12.43, p < 0.01 \)), \( T_b \)mean and sunrise were the factors that significantly affected \( T_b \)acrophase (\( F_{1,64} = 29.80, p < 0.001 \) and \( F_{1,78} = 42.05, p < 0.001 \) respectively), with sunrise being the most important factor in the combined model (\( F_{1,45} = 10.90, p < 0.01 \)).

(3) Relationships between emergence and immersgence 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, \( F_{1,46} = 63.25, r^2 = 0.579, p < 0.001 \)). In contrast, immersgence times occurred earlier in the day at the beginning of the measurement period (17:24) than at the end (18:17) \( F_{1,45} = 103.02, r^2 = 0.696, p < 0.001 \) (Fig. 3). There were no differences in emergence and immersgence times between animals that inhabited the flood plain and the woodland (emergence: \( F_{1,46} = 0.19, p = 0.662 \); immersgence: \( F_{1,45} = 0.17, p = 0.685 \)). 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 \( T_b \) (Fig. 4).

Changes in \( T_b \) over 24 h periods were greatest at around the times of emergence and immersgence, 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 +0.72 ± 0.12°C compared to +0.48 ± 0.10°C during the hour prior to emergence, respectively. There was a significant difference in the \( T_b \) increase between the beginning and the end of the measurement period, with a 52% 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 (\( F_{1,28} = 4.99, r^2 = 0.20, p < 0.05 \)). \( T_b \) 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 immersgence and −0.16 ± 0.06°C during the hour prior to immersgence for week 1; this compared to changes of −0.08 ± 0.04°C and −0.20 ± 0.04°C, for post-and pre-immersgence times during week 22, respectively.

The mean time at which \( T_b \) began to decrease in the mornings across all seasons was 10:12 ± 0.19 minutes and 38.70 ± 0.06°C (Fig. 6). This time became earlier as the measurement period progressed from week 1 to week 22. For the weeks 1, 8, 15 and 22, the mean times when \( T_b \) first decreased were 10:59 ± 0.23, 10:14 ± 0.27, 10:22 ± 0.33 and 9:14 ± 0.28 minutes which corresponded to mean \( T_b \) values of 38.49 ± 0.07, 38.82 ± 0.11, 38.75 ± 0.14 and 38.72 ± 0.18°C, respectively.

(4) Effect of habitat on \( T_b \) and \( T_b \) daily rhythms

Mean daily \( T_a \) values were not significantly different between the two habitats (\( F_{1,167} = 0.188, P = 0.665 \)). However, there were significant differences between habitats when day and night temperatures were specified in the model (Habitat: \( F_{1,335} = 0.939, p = 0.333 \); Day/night: \( F_{1,335} = 1131.06, p < 0.001 \)). Similarly, the only day/night variable that significantly affected \( T_b \)mesor was \( T_b \)max (\( F_{1,335} = 33.310, p < 0.001 \)) indicating that the flood plain was significantly hotter during the day and colder during the night than the woodland. Mean \( T_b \) 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).

There was a significant effect of habitat on \( T_b \)mesor and \( T_b \)amplitude values. Values recorded for individuals from the flood plain were higher than those from the woodland (\( F_{1,150} = 10.23, p < 0.01 \) and \( F_{1,150} = 81.58, p < 0.001 \) respectively; Fig. 2A, 2B). However, there was no significant difference between \( T_b \)acrophase values of individuals from the two habitats (\( F_{1,127} = 1.59, p = 0.210 \); Fig. 2C).

(5) Effect of age and group size on \( T_b \) daily rhythms

There were significant interactions between age and body mass on \( T_b \)mesor (\( F_{1,111} = 75.8, p < 0.001 \)) and a significant increase in \( T_b \)mesor with increasing mass whereas \( T_b \) was independent of body mass in younger animals. There was also a significant effect of group size on \( T_b \)mesor with individuals from larger groups having lower \( T_b \)mesor values than those from smaller groups (\( F_{1,150} = 18.70, p < 0.001 \)) and \( F_{1,150} = 81.58, p < 0.001 \) respectively; Fig. 7A). There was a significant effect of group size (\( F_{1,150} = 22.29, p < 0.001 \)) and a significant interaction between age and body mass on \( T_b \)amplitude (\( F_{1,153} = 9.22, p = 0.003 \)). Individuals from larger group sizes had lower \( T_b \)amplitude values and older animals decreased in \( T_b \)amplitude with increasing mass whereas \( T_b \)amplitude 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 \( T_b \)acrophase (\( F_{1,127} = 44.26, p < 0.001 \) and \( F_{1,120} = 36.25, p < 0.001 \)). 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 $1.23 \pm 0.29 \degree C$ and ranged from 0.68 to 2.32 $\degree C$. 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 $F_{1,175} = 1.15$, $p=0.286$). However, individuals from larger group sizes had lower HI values (least squares regression $F_{1,182} = 20.33$, $p<0.001$) and there was a significant interaction between age and group size on HI ($F_{1,180} = 15.03$, $p<0.001$); 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 $T_b$ values, including both maximal and minimal $T_b$’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 [20,56]. Ground squirrels in general have labile $T_b$’s [2,5,57–61], $T_b$-amplitudes of different species may vary by 4–5 $\degree C$ and be accompanied by bouts of torpor or hibernation. This compares with $T_a$ amplitude values of up to 4.1 $\degree C$ in Arabian oryx (Oryx leucoryx) [51] and 2.6 $\degree C$ in Arabian sand gazelles (Gazella subgutturosa marica) [52]. We found no evidence of torpor and recorded daily variation in $T_b$, of 5–6 $\degree C$, which is greater than that noted in most other species and greater than noted by Wilson et al. (2010) [33] for Cape ground squirrels which is greater than that noted in most other species and greater than noted by Wilson et al. (2010) [33] for Cape ground squirrels in a more mesic area (3.8 $\degree C$ amplitude); hence this probably reflects adaptation to an environment with high $T_a$ values and large daily variations in $T_a$.

(3) Relationship between $T_b$ daily rhythms, $T_a$ and daylight
Peak ambient temperature ($T_{a_{max}}$) 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

| Week | Begin date | End date | Mesor | Amplitude | Acrophase hh:mm | Percentage rhythmicity |
|------|------------|----------|-------|-----------|-----------------|------------------------|
| 1    | 21/05/2006 | 28/05/2006 | 37.37 (0.12) | 0.93 (0.08) | 12:55 (0.11) | 50.32 (4.27) |
| 2    | 28/05/2006 | 04/06/2006 | 37.47 (0.10) | 1.16 (0.10) | 12:36 (0.05) | 58.84 (4.74) |
| 3    | 04/06/2006 | 11/06/2006 | 37.36 (0.08) | 1.22 (0.09) | 12:37 (0.04) | 64.63 (2.80) |
| 4    | 11/06/2006 | 18/06/2006 | 37.35 (0.11) | 1.11 (0.10) | 12:36 (0.12) | 56.12 (4.94) |
| 5    | 18/06/2006 | 25/06/2006 | 37.38 (0.09) | 1.07 (0.11) | 13:01 (0.16) | 53.71 (4.66) |
| 6    | 25/06/2006 | 02/07/2006 | 37.28 (0.09) | 1.07 (0.13) | 12:31 (0.10) | 46.42 (4.19) |
| 7    | 02/07/2006 | 09/07/2006 | 37.42 (0.09) | 1.18 (0.12) | 12:41 (0.10) | 62.49 (3.38) |
| 8    | 09/07/2006 | 16/07/2006 | 37.48 (0.12) | 1.10 (0.10) | 12:40 (0.05) | 60.63 (3.86) |
| 9    | 16/07/2006 | 23/07/2006 | 37.46 (0.12) | 1.13 (0.10) | 12:43 (0.11) | 64.48 (2.89) |
| 10   | 23/07/2006 | 30/07/2006 | 37.51 (0.12) | 1.22 (0.07) | 12:39 (0.05) | 68.00 (1.78) |
| 11   | 30/07/2006 | 06/08/2006 | 37.27 (0.14) | 1.00 (0.07) | 12:43 (0.06) | 51.43 (3.55) |
| 12   | 06/08/2006 | 13/08/2006 | 37.46 (0.11) | 1.21 (0.09) | 12:32 (0.06) | 63.94 (3.24) |
| 13   | 13/08/2006 | 20/08/2006 | 37.55 (0.11) | 1.22 (0.07) | 12:31 (0.09) | 65.30 (1.58) |
| 14   | 20/08/2006 | 27/08/2006 | 37.44 (0.12) | 1.04 (0.10) | 12:38 (0.11) | 54.78 (4.70) |
| 15   | 27/08/2006 | 03/09/2006 | 37.55 (0.14) | 1.16 (0.09) | 12:40 (0.09) | 56.88 (3.80) |
| 16   | 03/09/2006 | 10/09/2006 | 37.79 (0.11) | 1.27 (0.08) | 12:23 (0.05) | 69.15 (1.85) |
| 17   | 10/09/2006 | 17/09/2006 | 37.76 (0.12) | 1.20 (0.08) | 12:29 (0.06) | 68.12 (1.75) |
| 18   | 17/09/2006 | 24/09/2006 | 37.72 (0.12) | 1.22 (0.09) | 12:19 (0.08) | 67.99 (2.19) |
| 19   | 24/09/2006 | 01/10/2006 | 37.68 (0.15) | 1.09 (0.08) | 12:21 (0.07) | 59.17 (3.02) |
| 20   | 01/10/2006 | 08/10/2006 | 37.61 (0.14) | 1.12 (0.09) | 12:13 (0.05) | 65.07 (2.88) |
| 21   | 08/10/2006 | 15/10/2006 | 37.61 (0.15) | 1.09 (0.06) | 12:15 (0.05) | 61.46 (2.74) |
| 22   | 15/10/2006 | 22/10/2006 | 37.59 (0.14) | 1.15 (0.06) | 12:27 (0.08) | 63.18 (2.33) |
| 23   | 22/10/2006 | 28/10/2006 | 37.65 (0.12) | 1.06 (0.07) | 12:17 (0.10) | 50.39 (4.56) |
| Mean | 21/05/2006 | 28/10/2006 | 37.51 (0.03) | 1.13 (0.02) | 12:33 (0.02) | 60.11 (1.34) |
defining Tb_acrophase which may suggest that sunrise acted to temporally entrain the thermoregulatory system [62]. Indeed Tb_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 Tb 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 Tb [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 Tb 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) Tb_Mesor (°C); (b) Tb_Amplitude (°C); (c) Tb_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 Tb values are shown in (d) as top, middle and lower lines. doi:10.1371/journal.pone.0036053.g002

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

Figure 4. Tb (open circles) and Ta (solid circles) and fitted cosine curves for a Cape ground squirrel during the 9th week of the sampling period illustrating the variation in Tb and Ta. The difference between the lowest Tb value recorded (33.39°C at 19:08) and the highest Tb during the previous day (39.32°C at 16:08) was 5.93°C. Over the 23 week period, extreme changes in Tb included one individual that decreased in Tb by 5.56°C and another that increased in Tb by 5.98°C in one hour. doi:10.1371/journal.pone.0036053.g004
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 Tb tracked Ta (Fig. 4) highlighting the thermal lability of these animals. It is likely that Cape ground squirrels were allowing their Tb to vary to defend both water loss and energy expenditure as the greatest amplitudes of variation were noted during the winter. Alpine ibex (Capra ibex ibex) also show the greatest amplitude of variation of Tb 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 Tb 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 Tb 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 Tb or an increase in energy expenditure due to increased thermoregulation, rather than other possible cues, such as light intensity.

(4) Influence of habitat on Tb daily rhythms

As expected, Ta 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

Figure 5. Mean ±SE Tb changes between successive hours across all eight individuals during the first, eighth, fifteenth and twenty-second weeks of the measurement period. Grey bars represent the mean ±SE times of emergence (left-hand bar) and immergence (right-hand bar).
doi:10.1371/journal.pone.0036053.g005

Figure 6. Mean ±SE Tb of the eight individuals for the first, eighth, fifteenth and twenty-second weeks of the sampling period. Tb values rose rapidly in the morning before reaching a plateau during the day.
doi:10.1371/journal.pone.0036053.g006
Implanted, the results obtained suggest that $T_{amax}$ amplitude 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 $T_{b}$ 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 $T_{bmax}$ held the greatest explanatory power for $T_{b}$ 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 (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 (Apodemus mystacinus) from different sides of the African Great Rift valley [70,71]. $A. cahirinus$ inhabiting a xeric environment had later $T_{b}$ 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 $T_{b}$ mean and body mass in older but not in younger animals. In our case, heavy young animals also tended to have earlier $T_{b}$ 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 $T_{b}$ mesor 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 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 (Cryptomys hottentotus natalensis and 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.

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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.

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