Daily rhythms of body temperature and heat production of sibling *mastomys* species from different ecosystems — The response to photoperiod manipulations

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ABSTRACT

We compared body temperature (T_b) and metabolic rates, measured as oxygen consumption (VO_2), daily rhythms of two sibling species of the genus *Mastomys*. We also studied their responses to long day (16L: 8D, LD) and short day (8L: 16D, SD) photoperiod manipulations at a constant ambient temperature of 26+1 °C. We noted significant differences in T_b and VO_2 daily rhythm patterns, under SD and LD-acclimation between the sibling species. These differences explain adaptation to the climatic conditions that prevail in the different ecosystems where these species live. To the best of our knowledge, this is the first time that physiological differences between the two siblings are measured by using chronobiological methods.

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1. Introduction

Body temperature (T_b) daily rhythms have been studied in several rodent species and are the outcome of heat production and heat dissipation rhythms. In nocturnal species heat production (VO_2) and T_b increase during the period of activity, while values of both variables remain relatively low during the resting period (Refinetti and Menaker, 1992). Therefore, measuring T_b and VO_2 daily rhythms can reflect on rhythms of activity and of heat dissipation. The increase in T_b does not only follow changes in the activity pattern, but also follows changes in metabolic processes (Haim et al., 1988; Rubal et al., 1992). Both T_b and VO_2 daily rhythms also change with the seasons and such changes can be initiated by manipulating the photoperiod (Haim and Zisapel, 1995).

Multimammate mice (genus *Mastomys,* previously known as *Praomys*) are widely distributed throughout southern Africa (Skinner and Smithers, 1990). *Mastomys natalensis* (*sensue lato*) occurs commonly throughout most of South Africa. It was incorrectly considered as a single species but cytogenetically and electrophoretical studies during the late 1970s and early 1980s revealed the presence of two electrophoretically distinct types (2*n*=32/"slow" hemoglobin electromorph and 2*n*=36/ "fast" hemoglobin electeromorph), (Green

et al., 1980; Gordon and Watson, 1986). These two cytotypes have also been reported to differ in sperm and bacular morphology, reproductive behaviour, pheromones and ultrasonic vocalization (Skinner and Smithers, 1990; Musser and Carleton, 1993; Smit et al., 2001). All these differences, together with the absence of hybrids in areas of sympatry, strongly suggested reproductive isolation between the two types (Gordon and Watson, 1986). Despite the reported differences, the two cryptic species referred to the nominated species *M. natalensis* for the *n*=32 cytotype and *M. coucha* for *n*=36 cytotype (Musser and Carleton, 1993) cannot easily be distinguished by qualitative external and/or cranial morphology (Gordon and Watson, 1986). However, a subsequent study by Dippenaar et al. (1993) revealed subtle cranial morphometric differences between the two sibling species, but cautioned that such data need to be carefully evaluated before being applied, due to potential influence of geographic variation over the species' distribution ranges. Recently, a comparative study on the renal physiology of these two species did not reveal any differences in the ability of their kidneys to concentrate urine (Ntshotsho et al., 2004).

The geographic distribution of the two sibling species seems to differ. *M. natalensis* are primarily distributed in the mesic eastern parts of southern Africa, while *M. coucha* are abundant in the more arid central parts (Smit et al., 2001; Venturi et al., 2004). Therefore, Haim and Fourie (1980, 1981, and 1982) and Fourie and Haim (1981) incorrectly refer to *M. coucha* collected outside Pretoria, as *Praomys natalensis.* The mice they studied did respond to changes in photoperiod

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through changes in heat production (VO$_2$) at different ambient temperatures and in their nonshivering thermogenesis (NST) capacity (Haim and Fourie, 1980, 1981). The activity of key enzymes from different metabolic pathways, responding to photoperiod manipulations (Fourie and Haim, 1980) and to melatonin treatment, confirmed the involvement of the pineal gland (Haim and Fourie, 1982). Apparent differences in reproductive output in response to protein deprivation, as well as in growth rates, (Jackson and van Aarde, 2003) suggest that these species may differ in aspects of their physiology, which may explain the difference in their distribution patterns. However, no comparative thermoregulatory information is available for the sibling species, now known as *M. natalensis* and *M. coucha.*

From the studies of Haim (1987) and Haim and Izhaki (1993), we expected xeric species to have lower resting VO$_2$ and T_b values than those of mesic species with a same body mass. Therefore, we expect that *M. natalensis* from the mesic environments will have higher metabolic rates and higher T_b values than *M. coucha* from the more xeric environments. We used the chronobiological method, which is useful when comparing related species (Rubal et al., 1992), or populations of the same species from different habitats (Shanas et al., 2002), to compare T_b and VO$_2$ daily rhythms of the two sibling species (Gordon, 1978) under long and short photoperiod regimes simulating summer and winter acclimatization.

2. Materials and methods

2.1. Mice

Individuals of *Mastomys natalensis* were trapped alive in Sherman traps set in the coastal dune forests north of Richards Bay, KwaZulu-Natal while *Mastomys coucha* were trapped at the University of Pretoria's Experimental Farm, Gauteng, South-Africa. These sites represent the core distributional range of each of the species see Venturi et al. (2004). Electrophoresis and chromosomal analyses of individuals from these two sites confirmed their taxonomic status.

In the laboratory we kept the mice separately in cages placed in a climate room with a constant ambient temperature (T_a) of 25+1 °C and under long photoperiod (16L: 8D; LD-acclimation; with lights on between 06:00 and 22:00 h) for 3 weeks prior to the onset of our experimental procedures. During the photophase white light (450lux) and red dim lights (25lux) were on and during scotophase only the dim lights were on. Sawdust served as bedding, food (Epol mouse pellets) and water were offered *ad lib*. Fresh carrots were provided daily as an additional water source. Mice were weighed before and at the end of acclimation to each of the photoperiod regime. Following the measurements of T_b and VO$_2$ daily rhythms of mice acclimated to a long photoperiod, a three week period of acclimation to a short photoperiod (8L:16D) commenced. Lights were on between 08:00 and 16:00 h.

2.2. Body (rectal) and ambient temperature

Both body and ambient temperature (T_a) were measured with a copper-constantan thermocouple connected to an APPA 51 digital thermometer (Appa Technology Comp., Talpel, Taiwan.). For assessing T_b-daily rhythms, the thermocouple coated with medical Vaseline, was inserted to the rectum of the tested individual to a depth of 3 cm for a period of 30 s. The tested mouse was held in a dark cotton bag after having been removed form its holding caged. Twelve individuals of each species were randomly placed into one of two groups and within each group mice were measured at 6 h intervals, with the starting point for each of the groups differing by 3 h (Haim et al., 1995, Shanas et al., 2002).

2.3. Oxygen consumption

We measured oxygen consumption with an open circuit system (Depocas and Hart, 1957) in which the airflow rate (450–460 mL/min)

to the system was determined by using a bubble flow meter mounted on a modified burette containing water and soap (Bennett et al., 1993). The cylindrical metabolic (respiratory) chamber (volume of 1000 mL) was made out of transparent Perspex. A negative pressure flow-through the system was used. Outside, air was pulled through the respirometer after removing water, by using a color-indicator silica gel. The percent of oxygen in exhalent samples was recorded every two minutes from an Applied Electrochemistry S-3A oxygen analyzer. All values were corrected to STPD and calculated as mass-specific.

To assess VO$_2$ daily rhythms, mice were placed into the metabolic chamber, which contained mice pellets and fresh carrots as well as tissue paper used for bedding. The metabolic chamber was connected to a high-pressure air pump (RENA, Annecy, France) and dried air (silica gel) was pumped into the metabolic chamber as described earlier. Mice were habituated to these conditions for at least 12 h before VO$_2$ measurements started. Each mouse was measured at intervals of 4 h over a period of 30 h. In order to avoid disturbance, the metabolic chamber and silica gel column were removed together from the pump and within few seconds connected to the oxygen consumption monitoring system. Mice were weighed before entering and after being removed from the metabolic chamber to the accuracy of 0.l g, using a digital scale (OHAUS Precision Advanced, Model GT 410D, N.J, USA). T_a and photoperiod regimes in the laboratory were the same as those that mice were acclimated to.

The average resting, VO$_2$ values of each 30 min, measured for each mouse were used for further calculations. In case of mice moving during measurements the period of 30 min. was extended. For establishing the daily rhythm figures mice were measured between 75 min before and after the indicated hour in each figure. Total daily energy expenditure (DEE) was estimated by adding all values of oxygen consumption from each species maintained under either photoperiod during 24-h period. Afterwards, these values were converted to caloric values using the factor 4.99 kcal per liter of O$_2$ utilized (20.92 KJ per liter of O$_2$) presuming that the respiratory quotient is 1, as describe by Speakman (2000). Experiments were carried out in the period of 5 months, between October 1999 and January 2000.

2.4. Statistics

All values are given as mean+one standard error of the mean (SE). All statistical analyses were performed using the SPSS for windows (Release 11.0.0.; SPSS Inc., 1989-2001). The T_b and VO$_2$ rhythms data were evaluated by ANOVA repeated measures (ANOVARAM) and differences between sample times for each group were analyzed with Bonferoni pairwise comparison test. Unpaired *t*-test was used to test the significance of means between the two species at each sample time of the LD or SD regimes and for assessing differences in body mass before and after acclimation.

Data for each variable were also evaluated for rhythm detection by the single cosinor method (Minors and Waterhouse, 1989; Nelson et al., 1979). The method is based on nonlinear regression analysis, which involves fitting time series data to a cosine function using the least square procedure. A computer program written in Excel spreadsheet, made available to us, was used in computing this estimate. Rhythm parameters estimated from the cosinor procedure included mesor (24 h rhythm-adjusted mean of the fitted cosine curve fitted to the data), amplitude (difference between the maximum and the cosine calculated mesor), acrophase (peak of the fitted cosine curve with reference to 00:00 h), and percent rhythm (PR; percentage of overall variance attributed to the best fitted cosine curve). An *F*-test was used to determine the significance of a 24 h rhythm by rejection of the zero amplitude hypothesis ($P<0.05$). Species differences in the 24 h rhythm estimates for each variable under a given photoperiod were ascertained by Bingham's test (Bingham et al., 1982). Statistical differences were established at a significance level of $P<0.05$ (two-tailed), except for unpaired *t*-test comparisons. In this case, the Bonferroni

correction was used to adjust the P-value threshold by the number of comparisons between two selected curves ($P \leq 0.05/6$; one-tailed).

Experiments were carried out between November 1999 and January 2000. All experiments, holding and handling procedures were approved by ethical committee of the Faculty of Natural and Agricultural Sciences of the University of Pretoria.

3. Results

3.1. T_b-daily rhythms

Under the two different photoperiod regimes, both sibling *Mastomys* species showed a T_b-daily rhythm of a nocturnal species, as their T_b patterns were characterized by higher values during the scotophase of the light/dark cycles (Fig. 1). Moreover, under a LD-acclimation both species showed significant time-related variations in T_b values (ANOVARM; $F_{9,45} = 25.49$ (*nalalensis*) and 41.38 (*coucha*), $P < 0.0001$). Bonferoni *post hoc* analysis suggested that the lowest values for both species at 12:00 h were significantly different ($P < 0.03$) from the maximal values obtained at 24:00 h (38.53±0.13 °C) for *M. coucha* and at 03:00 h (38.72±0.15 °C) for *M. nalalensis*. The differences between mean minimum and maximum T_b values were similar (≈3 °C) in both

Table 1
Mean 24 h rhythm parameters estimated by cosinor analysis for T_b of each *Mastomys* species during LD or SD regimes

Photoperiod	Mesor (°C)	Amplitude (°C)	Acrophase (hh:min)	PR* (%)	P-value#
16L:8D					
M. natalensis	37.3±0.16	1.5±0.15	03:47±0:15	81	0.003
M. coucha	36.69±0.11	1.5±0.09	01:50±0:22	85	0.001
$P^{\#\#}$	0.01	0.87	0.0001		
8L:16D					
M. natalensis	38.02±0.14	0.51±0.14	23:35±10:18	50	0.09
M. coucha	37.04±0.08	0.95±0.09	01:54±1:19	53	0.07
$P^{\#\#}$	0.003	0.03	0.09		

*Percent rhythm (percentage of overall variance attributed to the fitted cosine curve). #P value from zero amplitude. ## Bingham's test.

species. However, T_b values for *M. nalalensis* at 09:00 h and 03:00 h were significantly higher ($P < 0.001$ and 0.005, respectively) than those for *M. coucha* at the same sampling times. This was not the case for the remaining sample times (Fig. 1 A).

Consistently with the LD-acclimation, the T_b daily rhythms in both species under SD-acclimation were also significantly associated with the light/dark regime (ANOVARM; $F_{9,45} = 3.68$, $P < 0.002$ (*nalalensis*) and 18.63, $P < 0.0001$ (*coucha*)). While no significant differences were crevealed between T_b values at the different sample times for *M. natalensis* pattern, Bonferoni pairwise comparison indicated that mean T_b values for *M. coucha* at 03:00 h were significantly higher ($P < 0.03$) than those at 12:00 h and 15:00 h. Generally, mean T_b levels were higher for *M. natalensis* than for *M. coucha* at all time intervals, though significant differences were only noted at 12:00 h, 18:00 h and 06:00 h (Fig. 1 B).

Cosinor analysis demonstrated significant 24 h rhythms of T_b in the two species only under the LD regime (Table 1). Under LD-acclimation, T_b mesor of *M. natalensis* was 37.3±0.16 °C and of *M. coucha* was 36.7± 0.11 °C and these differences were statistically significant ($P < 0.01$). The acrophase for the 24 h rhythm of *M. coucha* occurred at 01:50±0:22 (h:min) while that of *M. natalensis* occurred at 03:47±0:15 (h:min) and these differences were also highly significant ($P < 0.0001$). In contrast, T_b amplitudes of both species were similar. Under the SD regime, differences were revealed between the 24 h rhythm parameter values obtained by the cosinor analysis for both species, but only the mesors and amplitudes differed significantly ($P < 0.003$ and $P < 0.03$, respectively; Table 1).

3.2. VO_2-daily rhythms

The VO_2 values of LD-acclimated mice of both species were at minimal during the light-phase while, maximal values were recorded during the dark phase. For *M. natalensis* the minimal VO_2 values (1.3± 0.05 mLO$_2$/g h) were recorded at 08:00 h, and they increased between 20.00 to a peak (3.25±0.06 mLO$_2$/g h) at 24:00 h while, for *M. coucha* the minimal values (1.21±0.05 mLO$_2$/g h) were recorded at 12:00 h and values thereafter increased gradually to a peak (2.90±0.08 mLO$_2$/g h) at 04:00 h, (Fig. 2A). The patterns of both species varied with time of day during the 24 h light/dark period (ANOVARM; $F_{9,45} = 121.18$, $P < 0.0001$ (*nalalensis*) and 61.21, $P < 0.001$ (*coucha*)). Post hoc analysis suggest that the 24:00 h levels of *M. natalensis* were significantly higher ($P < 0.0001$) than both 08:00 h and 12:00 h. In *M. coucha*, the minimal and maximal VO_2 values at 12:00 h and 04:00, respectively, also differed significantly ($P < 0.001$). VO_2 values of both species differed significantly ($P < 0.0001$) from each other only at 24:00 h, 08:00 h, and 12:00 h. Furthermore, at these sample times except 08:00 h, *M. natalensis* levels were higher (Fig. 2 A).

In both species under SD-acclimation, clear time-related variations in VO_2 levels were established by the ANOVARM analysis ($F_{9,45} = 9.53$, (*nalalensis*) and 17.03 (*coucha*), $P < 0.0001$). Maximal and minimal values of the two species were recorded at 24:00 h and 12:00 h,
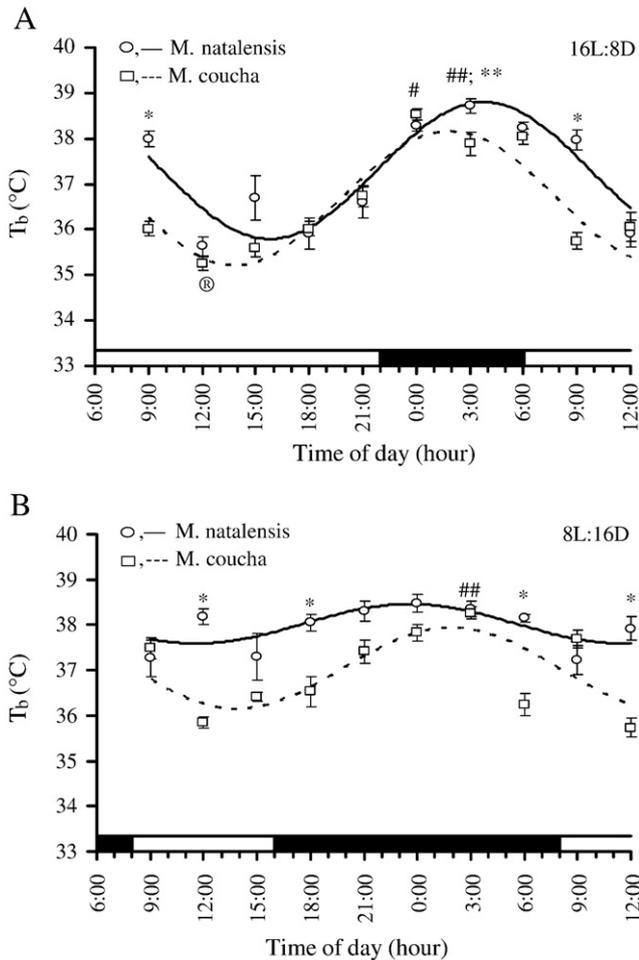

Fig. 1. Body temperature (T_b) daily rhythms of Sibling *Mastomys* species under two photoperiods, long day (A, 16L:8D light–dark cycle with lights on at 06:00 h) and short day (B, 8L:16D light–dark cycle with lights on at 0800 h). T_b values were recorded with rectal prop at 3 h intervals for 30 h. Values are means±SE of $n=6$ per group. Solid and hatched lines denote the best cosine equation that models the data. Scotophase is represented by dark horizontal bars. [(A): # $P < 0.02$ (*coucha*) and ## $P < 0.03$ (*natalensis*) vs. 12:00; * and ** $P < 0.001$ and 0.005 vs. *coucha*, respectively; (B): ## $P < 0.03$ vs. 12:00 (*coucha*); * $P < 0.001$ vs. *coucha*].

respectively, and these differences were statistically significant ($P<0.001$ for *natalensis*) and $P<0.03$ for *coucha*). There were no significant differences in mean VO_2 values between the two species at all times except at 08:00 h and 12:00 h, which were highest ($P<0.005$) for *M. natalensis* (Fig. 2 B). SD-acclimated mice of both species showed higher VO_2 values respectively to those of LD-acclimation. The mean minimal VO_2 values under LD-acclimation were at a rate of 1.2 and 1.3 mLO_2/g h for *M. natalensis* and *M. coucha*, respectively, and under SD-acclimation they were 1.75 and 1.50 mLO_2/g h, respectively.

Total DEE values of *M. natalensis* maintained under either LD (261.63 ± 2.78 kcal kg^{-1} day^{-1}) or SD (255.53 ± 5.73 kcal kg^{-1} day^{-1}) photoperiod regimes were significantly higher than those of *M. coucha,* under LD-acclimation (229.38 ± 4.49 kcal kg^{-1} day^{-1}, $P<0.0001$) while for SD-acclimation (235.23 ± 6.23 kcal kg^{-1} day^{-1}, $P<0.02$). In contrast, no significant intraspecific differences were observed for the two species between LD and SD photoperiod regimes.

The cosinor analysis detected significant 24 h rhythms for both species under the two light/dark regimes, except for LD-acclimated *M. coucha* (Table 2). The 24 h mesors and acrophases for both species differed significantly when compared either with LD or SD photoperiod regimes, while no significant differences were observed be-
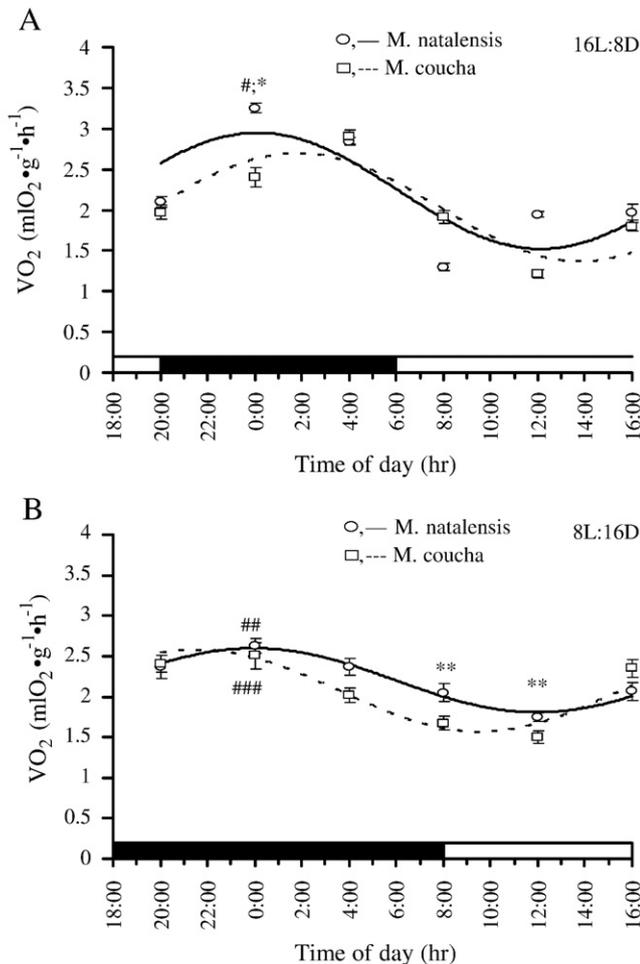
Table 2
Mean 24 h rhythm parameters estimated by cosinor analysis for VO_2 of each *Mastomys* species during LD or SD regimes

Photoperiod	Mesor (mLO_2 g^{-1} h^{-1})	Amplitude (mLO_2 g^{-1} h^{-1})	Acrophase (hh:min)	PR* (%)	P-value#
16L:8D					
M. natalensis	2.24 ± 0.02	0.72 ± 0.03	$00:07\pm0:26$	62	0.23
M. coucha	2.04 ± 0.05	0.67 ± 0.05	$01:48\pm0:25$	81	0.04
P##	0.001	0.41	0.001		
8L:16D					
M. natalensis	2.21 ± 0.05	0.45 ± 0.03	$23:58\pm1:53$	98	0.004
M. coucha	2.08 ± 0.05	0.55 ± 0.08	$21:31\pm1:47$	89	0.03
P##	0.048	0.13	0.012		

*Percent rhythm (percentage of overall variance attributed to the fitted cosine curve). #P value from zero amplitude. ## Bingham's test.

tween amplitudes of the two species at each of the photoperiods (Table 2).

3.3. Body mass

LD-Acclimation did not have any effect on body mass of *M. natalensis* ($43.9+11.7$ g before acclimation and $44.6+12.0$ g after) or for *M. coucha*, ($47.3+9.7$ g before acclimation and $46.4+11.2$ g after). However, under SD-acclamation body mass of both species increased significantly ($P<0.001$; $t=6.51$ for *M. natalensis* and $t=7.20$ for *M. coucha*) compared with LD-acclimated mice. *M. coucha* ($51.4+12.8$ g) was not significantly heavier than *M. natalensis* (49.3 ± 10.5 g) under SD-acclimation.

4. Discussion

The timing of light within the 24 h light/dark cycle is a signal that entrains the endogenous circadian clock, whereas changes in photoperiod are used as a predictor of approaching seasons. In most ecosystems outside of the tropics, changes in photoperiod initiate the seasonal acclimatization of heat production and thermoregulatory mechanisms as well as changes in reproductive activity (Reiter 1991, 1993). Therefore, the response of different species, or different populations of the same species, inhabiting different ecosystems, seems an efficient tool to assess adaptations to environmental conditions (Shanas et al., 2002).

Multimammate mice of the genus *Mastomys* in southern Africa (*M. natalensis* and *M. coucha*) are morphologically similar and field identification is impossible (Smit et al., 2001). From earlier studies (Gordon, 1984; Dippenaar et al., 1993; Smit et al., 2001; Jackson and van Aarde, 2003; Venturi et al., 2004) it seems that *M. natalensis* is confined to the mesic warm regions of the savana biome, while *M. coucha* is confined to the drier grasslands (Rutherford and Westfall, 1993).

Winter is challenging, especially in the grasslands where night T_as may drop to zero or below. Therefore, it is expected that species occurring here, should rapidly increase their heat production from the lower RMR values, as expected for arid adapted species (Haim and Levi, 1990; Haim and Izhaki, 1993). The results of previous studies indeed showed that SD-acclimated *M. coucha* increase their VO_2 values in a short time, presumably by the significant increase of NST as a response to SD-acclimation (Haim and Fourie, 1980). *M. natalensis* that occur in the mesic savanas of South Africa is not exposed to such extremes and therefore the VO_2 daily changes of LD-acclimated mice can be gradual as supported by our findings.

The validity of using thermocouples for measuring rectal T_b in rodents has been discussed (Adams et al., 2001; Shanas et al., 2002). We chose this method because we could study a larger sample of mice, divided into two groups, so that each group was measured every 6 h. As measurements in the second group started with a delay of 3 h, we could collect T_b data every 3 h as was done in Haim et al. (1995). Furthermore, we have shown in the past that implantation of transmitters into the

Fig. 2. Oxygen consumption (VO_2) daily rhythms of Sibling *Mastomys* species under either long day (A, 16L:8D light–dark cycle with lights on at 06:00 h) or short day (B, 8L:16D light–dark cycle with lights on at 0800 h). Oxygen consumption was measured between 75 min before and after the indicated hour in the figure. Values are means±SE of $n=6$ per group. Solid and hatched lines denote the best cosine equation that models the data. Scotophase is represented by dark horizontal bars. [(A): # $P<0.0001$ vs. 8:00 (*natalensis*); ## $P<0.001$ vs. 12:00 (*coucha*); * $P<0.001$ vs. (*coucha*); (B): ## $P<0.001$ (*natalensis*) and ### $P<0.03$ (*coucha*) vs. 12:00; ** $P<0.005$ vs. *coucha*].

abdomen of golden spin mice *Acomys russatus* resulted in a shift of VO_2 daily rhythms (Haim and Zisapel, 1995). Recently Adams et al. (2001) showed that implanted transmitters have a long term effect on the mice physiology.

The results of our study show that *M. coucha* individuals, under SD-acclimation, manifested a robust T_b daily rhythm, relatively to *M. natalensis*. Maintaining lower T_b values when inactive, under dry and cold conditions, can be of great advantage for energy and water conserving, as these low values can emerge, from lower heat production (Aschoff, 1982). Similar robust T_b daily rhythms were reported for desert adapted species1ike in *A. russatus* (Haim and Zisapel, 1995).

The VO_2 maximal values obtained for *M. coucha* under LD-acclimation are significantly ($P<0.05$) lower than those obtained under SD-acclimation. The increase in heat production during the dark phase of SD-acclimated mice by 20%, at the maximal value compared with LD-mice, at the same T_a, points out to the importance of the response to photoperiod changes which are earlier than those of environmental temperature. For *M. coucha* winter is the challenging season as it is dry and cold while the summer is warm and wet (in years with no drought) and is relatively a comfortable season, during the period of activity. The fact that under SD-conditions in T_b daily rhythms, *M. coucha* has another peak at 09:00 h, may suggest that it can be active also in the early hours of the day during the winter time. This point should further be studied. For *M. natalensis* winter is a comfortable season as it is not dry and not very cold during nighttime, when the mice are active. This is well manifested in its T_b-daily rhythm curve, which is almost of an arhythmic (Table 1), while in summer, the hot season, a clear T_b daily rhythm is noted with a T_b peak at the middle of the dark period. The increase in T_b follows that of VO_2 supports the idea that VO_2 is the driving force for the T_b daily rhythms in rodents with low metabolic rates, as the Pouch mouse *Saccostomus campstris* (Haim et al., 1988) and the golden spiny mouse *A. russatus* (Rubal et al., 1992).

Physiological regulation in energy expenditure to specific climatic regions is a well known phenomenon among small rodents, and thus a negative correlation between energy expenditure and ambient temperature is expected. Generally, energy expenditure is expected to decrease in hot and arid environments, whereas increased energy expenditure is a common adaptive strategy in mesic counterparts (Lovegrove, 2003; Scantlebury et al., 2004; Vaanholt et al., 2007). Accordingly, our results demonstrate that total DEE values were 8% and 12% higher in *M. natalensis* than those in *M. coucha* under either LD or SD photoperiods, respectively. Additionally, higher levels were also estimated for both T_b and VO_2 mesors of *M. natalensis* compared with *M. coucha* (Tables 1 and 2).

The ecological significance of adjusting DEE to specific environments suggests that in arid environments the reduced DEE enables rodents, such as *M. coucha*, to contend with high ambient temperatures and aridity by decreasing heat production, which may reduce evaporative heat dissipation in hot environments, and evaporative water loss. While the enhanced DEE of mesic rodents, such as *M. natalensis*, facilitates low ambient temperatures survival by allocating more energy for thermo-regulation (Bennett, 1988; Tracy and Walsberg, 2000).

Although we found no significant differences in total DEE of each species between the two photoperiods, each species showed significant photoperiod-related differences in rhythm's estimates (Tables 1 and 2). In T_b experiment, mean SD-mesors levels of the two species were significantly elevated compared with LD-levels. However, VO_2 mesors did not differ between the two photoperiods. Since the elevated mean T_b levels were reached with out a comparable enlargement in mean VO_2 levels, it would seem that heat dissipation has been attenuated allowing the animals to regulate relatively higher T_b values with out significant increase in heat production. The SD-elevated T_b mesor levels are most likely reflecting improved thermo-regulatory responses during the cold winter season.

The acrophase occurrences of T_b and VO_2 of each species under SD conditions were considerably delayed when compared with those of LD groups. The shift in acrophase occurrence suggests that both variables are under the control of the circadian clock and both can be entrained by the light dark cycle.

Although the mice were not seasonally acclimatized but rather challenged by photoperiod manipulations, our results show a significant difference between the two sibling species in their VO_2 and T_b daily rhythm responses to SD and LD-acclimations, which presumably, at least partly, mimics winter and summer conditions. To the best of our knowledge, this is the first study manifesting chronobiological differences of physiological variables between the two sibling species and therefore the results of this study are a significant contribution to our understanding the separation between them. Furthermore, the chronobiological differences do indeed reflect physiological adaptations to environmental challenges.

4.1. Epilogue

In the year 2004 we had the opportunity of measuring T_b of SD-acclimated mice of progenies, fourth generation born in captivity, of the two sibling species. As under SD-acclimation, a significant difference in T_b values was revealed at 12:00 h. The results we obtained were similar to those of trapped wild SD-acclimated mice studied in 1999/2000. The values were 38.3 ± 0.3 °C and 36.2 ± 0.4 °C for *M. natalensis* and *M. coucha* respectively. These results support our suggestion that the differences revealed in our study are adaptations.

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