

## The Interplay of Energy Balance and Daily Timing of Activity in a Subterranean Rodent: A Laboratory and Field Approach

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### ABSTRACT

The tuco-tuco (*Ctenomys* aff. *knighti*) is among the rodent species known to be nocturnal under standard laboratory conditions and diurnal under natural conditions. The circadian thermoenergetics (CTE) hypothesis postulates that switches in activity timing are a response to energetic challenges; daytime activity reduces thermoregulatory costs by consolidating activity to the warmest part of the day. Studying wild animals under both captive and natural conditions can increase understanding of how temporal activity patterns are shaped by the environment and could serve as a test of the CTE hypothesis. We estimated the effects of activity timing on energy expenditure for the tuco-tuco by combining laboratory measurements of metabolic rate with environmental temperature records in both winter and summer. We showed that, in winter, there would be considerable energy savings if activity is allocated at least partially during daylight, lending support to the CTE hypothesis. In summer, the impact of activity timing on energy expenditure is small, suggesting that during this season other factors, such as predation risk, water balance, and social interaction, may have more important roles than energetics in the determination of activity time.

**Keywords:** activity patterns, metabolic rate, respirometry, *Ctenomys*, tuco-tuco, diurnality, nocturnality, circadian thermoenergetics (CTE) hypothesis.

### Introduction

Daily rhythms of physiology and behavior are driven by outputs of endogenous circadian clocks, which are synchronized to environmental cycles. Recent studies, combining investigations of animals under laboratory and natural conditions, are providing new insights into the adaptive significance of daily rhythmicity and its plasticity (Kronfeld-Schor et al. 2013). The immediate and dramatic switch from diurnality when in the field to nocturnality when in the laboratory displayed by some rodent species is a striking example of this plasticity (Levy et al. 2007; Blanchong et al. 1999; Daan et al. 2011; Hut et al. 2012; Tomotani et al. 2012). Survival and fitness of free-living animals requires integration of a far more complex suite of biotic and abiotic factors than is found in the laboratory, and that can serve to shape the expression of daily rhythms (Hut et al. 2012). These factors include environmental conditions known to impact energy balance, particularly food availability and ambient temperature ( $T_a$ ). A recently formulated circadian thermoenergetics (CTE) hypothesis (van der Vinne et al. 2014, 2015) posits that switching from nocturnal to diurnal activity is a response to energetic challenges; diurnality reduces thermoregulatory costs by consolidating activity and higher body temperature ( $T_b$ ) to the warmest part of the day and rest and lower  $T_b$  to the coldest hours of the night.

Most evidence supporting the CTE hypothesis comes from studies of laboratory mice, which clearly respond to energetic challenges of low  $T_a$  and working for food by increasing daytime activity (Hut et al. 2011; van der Vinne et al. 2014). Utilizing wild animals under both captive and natural conditions can increase understanding of how temporal activity patterns are influenced by the environment and could serve as a test of the CTE hypothesis. The tuco-tuco (*Ctenomys* aff. *knighti*) is a subterranean rodent that is known to peak in activity and  $T_b$  during the day under natural conditions, whereas under standard laboratory conditions it is strongly nocturnal (Tomotani et al. 2012; Tachinardi et al. 2014).

Energetic challenges are much greater in the field than in the laboratory. In field conditions, this herbivorous rodent relies on intense digging through hardened soils in a semiarid habitat where vegetation is sparse (Luna et al. 2002, 2009). Moreover, daily

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changes in  $T_a$  can exceed 15°C and might present thermoregulatory challenges at certain times of the day and year. To investigate whether consolidation of activity during the day could provide energy savings in this species, we measured metabolic rate (MR) across a range of  $T_a$ 's relevant to natural conditions (i.e., the Scholander curve; Scholander et al. 1950). Additionally, we recorded air ( $T_{air}$ ), operative ( $T_e$ ), and soil ( $T_{soil}$ ) temperatures across one year. Using these field data in conjunction with estimates of MR from laboratory-held animals, we estimated the impact of aboveground activity timing on minimum daily energy expenditure (MDEE), taking into consideration  $T_e$ ,  $T_{soil}$ , and daily durations animals were active above- or belowground. This study adds a subterranean rodent perspective to the recent body of studies modeling energetics in light of the plasticity of activity timing (Levy et al. 2012, 2016; Van der Vinne et al. 2014, 2015).

## Methods

### *Animals, Measurement of Core $T_b$ , and General Considerations*

Four male and four female *Ctenomys* aff. *knighti* (150–212 g) were trapped within a 3-km radius of the town of Anillaco (28°48'S, 66°56'W; 1,350 m) in the Monte Desert, Argentina. Animals were initially housed individually in plastic cages under 12L:12D conditions at 23° ± 2°C and provided ad lib. sweet potato, carrot, and commercial pellets for 8–12 mo. Animals were implanted with temperature-sensitive transponders (G2 E-Mitters, accuracy of ± 0.1°C; Mini-Mitter, Bend, OR) for continuous measurement of  $T_b$  (details in Tachinardi et al. 2014). Data were recorded every 5 min and analyzed using VitalView software (Mini-Mitter, Bend, OR).

Throughout the article time is expressed as UTC–3 (the time zone of the study area). When averages are mentioned, mean values are reported with standard deviations.

### *Metabolic Measurements*

We measured rates of  $O_2$  consumption ( $\dot{V}O_2$ ) and  $CO_2$  production ( $\dot{V}CO_2$ ) at rest using open flow respirometry (Sable Systems, Las Vegas, NV; detailed in Tachinardi et al. 2015) to estimate MR across a range of  $T_a$ 's, thus obtaining a Scholander curve (Scholander et al. 1950). In brief, individual animals were placed in a 7.4-L plastic respirometry cage within an environmental chamber at a fixed  $T_a$ . Animals were allowed to acclimate for 2 h, after which  $\dot{V}O_2$  and  $\dot{V}CO_2$  were recorded each minute simultaneously with telemetric measures of  $T_b$ . Measurements were made at  $T_a$ 's approximating 8°, 16°, 20°, 24°, 28°, and 32°C, as recorded within the respirometry chamber with a temperature logger (HOBO U10/003, accuracy of ± 0.53°C; Onset Computer Corporation, Bourne, MA).  $\dot{V}O_2$  was calculated using LabGraph (Toien 2013) and was described by Withers' (1977) equation (3b) with the approximation of  $\dot{V}CO_2 = \dot{V}_E \times (FICO_2 - FECO_2)$ , where  $\dot{V}_E$  is the airflow exiting the chamber (mL/min),  $FICO_2$  is the fraction of  $CO_2$  entering the chamber, and  $FECO_2$  is the fraction of  $CO_2$  exiting the chamber.  $\dot{V}O_2$  and the respiratory quotient

( $RQ = \dot{V}CO_2/\dot{V}O_2$ ) were used to calculate energy expenditure in watts (Withers 1977). For each individual, measurements of MR at each  $T_a$  were conducted at the same hour of the day during light hours, corresponding to the resting phase of tuco-tucos under standard laboratory conditions.

The average MR of the last hour of measurement at each  $T_a$  was used for data analysis. We performed linear regression analysis by means of least squares for several  $T_a$  ranges, all comprising measurements between the lowest  $T_a$  measured and a given tested breakpoint  $T_a$  ( $T_a$ 's between 20° and 30°C were tested, at 1°C steps). The best-fit model was used for the slope of the Scholander curve below thermoneutrality, and the breakpoint  $T_a$  for that model was considered the lower critical temperature (LCT). We tested the correlation between  $T_b$  and  $T_a$  using Pearson's correlation test to assess whether animals exhibited hypothermia, torpor, or hyperthermia at the various  $T_a$ 's. All analyses were performed with R (ver. 3.3.2; R Development Core Team 2013).

### *Daily Variation of Environmental Temperature in the Tuco-Tuco Habitat*

All environmental temperatures were measured in a location where tuco-tucos naturally occur and previous field experiments took place (Tomotani et al. 2012) using data loggers (HOBO UA-002-08).  $T_{air}$  was measured 1 m aboveground, inside a radiation shield (RS3; Onset Computer Corporation). For  $T_{soil}$ , the temperature loggers were buried and placed in the soil at depths tuco-tucos of this region are known to occupy (20, 40, and 60 cm; P. Tachinardi, V. S. Valentinuzzi, G. A. Oda, and C. L. Buck, personal observations). Loggers were not placed inside the burrow system itself by design to avoid damage from the animals and the potential acquisition of spurious results should an animal rest on or near the logger. Although the loggers were not placed directly in the burrow chamber, temperature data obtained should correspond to burrow temperature, since the airspaces belowground are sealed and quite small, in thermal equilibrium with the soil.

We used a taxidermic mount to obtain  $T_e$ , which is a result of the combined effects of conduction, convection, and radiation on an inert body of the same size, shape, and color as our study animal (Bakken 1980; Chappell and Bartholomew 1981; Long et al. 2005). The taxidermic mount consisted of a temperature logger surrounded by copper that was fitted inside a tuco-tuco pelt and placed adjacent to the experimental outdoor enclosures.

$T_{air}$  and  $T_{soil}$  were measured at hourly intervals for 12 consecutive months (from January 1 through December 31, 2016).  $T_e$  was measured at 20-min intervals from February 16 through June 6, 2016, and at 30-min intervals from July 3 through November 9, 2016.

### *Estimation of MDEE for Nocturnal and Diurnal Strategies*

We modeled MDEE in both summer and winter for aboveground activity allocated at different times of the day. To estimate the temperature to which our model animals would be exposed ( $T$ ), we calculated hourly averages of  $T_e$  and  $T_{soil}$  60 cm belowground

from February 17 through March 17 (summer) and from August 15 through September 13 (winter), as 60 cm belowground is the presumed depth at which animals rest (fig. S1; figs. S1–S4 are available online). We assumed the duration aboveground was the average of the duration observed for individuals released in semi-natural enclosures during each season (winter:  $186 \pm 71$  min/day,  $n = 8$ , data from Flôres et al. 2016; summer:  $82 \pm 36$  min/day,  $n = 8$ , data from Jannetti et al. 2015). For simplicity, we assumed that activity would occur in a single continuous block of time. To estimate the impact of aboveground activity time on energy expenditure, we estimated MDEE 24 times, each having the aboveground activity centered at one hour of the day. Using the equation obtained for the slope of our tuco-tuco Scholander curve ( $DEE = 9.09955 - 0.25063 \times T$ ), the average  $T_e$  or  $T_{soil}$  and the hypothetical information on whether the animal was below- or aboveground at that given time, we calculated the amount of energy the animal would spend each hour of the day and then summed the 24-h values to obtain MDEE (minimum daily energy expenditure excluding the energetic costs of activity). To estimate the impact of timing of aboveground activity, we estimated MDEE 24 times, each having the aboveground activity centered at one hour of the day except for the hours of the day in which  $T_e$  exceeded the  $34^\circ\text{C}$ , above which tuco-tucos cannot maintain constant euthermic  $T_b$  and become hyperthermic (Tachinardi 2012).

## Results and Discussion

We estimated the potential effects of activity time on energy expenditure in a subterranean rodent by combining laboratory measurements of MR and field environmental temperatures in both winter and summer. The LCT of the tuco-tuco was  $23^\circ\text{C}$ , below which MR increased linearly with decreasing  $T_a$  ( $r^2 = 0.78$ ,  $P < 0.05$ ; fig. 1; table S1, available online), suggesting that the thermoneutral zone (TNZ) for these animals ranges from approximately  $23^\circ$  to  $33^\circ\text{C}$ . MR did not increase with decreasing  $T_a$  across this range, and the  $T_b$  of tuco-tucos increases at  $T_a$ 's above  $34^\circ\text{C}$  (Tachinardi 2012).  $T_b$  did not correlate with  $T_a$  across the measured range of  $4^\circ$ – $32^\circ\text{C}$  (Pearson's  $r = 0.36$ ,  $P > 0.01$ ) and averaged  $35.87^\circ \pm 0.36^\circ\text{C}$ . The TNZ and LCT for *Ctenomys* aff. *knighti* are similar to those described for another *Ctenomys* species (TNZ for *C. talarum* =  $25^\circ$ – $30^\circ\text{C}$ ; Busch 1989; Baldo et al. 2015).

$T_{air}$  and  $T_e$  exhibited wider daily and seasonal variation than  $T_{soil}$  at any depth (fig. 2). Across the year, minimum daily  $T_e$  and  $T_{air}$  were lower than the LCT of tuco-tucos. Tuco-tucos spend more time outside the burrows during the coldest months, when  $T_a$  is always below their LCT. This might be due to increased foraging needs; winter is the dry season in the Monte Desert and food availability is lower, with higher energy requirements due to low temperature. Alternatively, tuco-tucos may spend less time aboveground in summer to avoid daytime high temperatures and incident solar radiation, since in summer  $T_e$  is often above the TNZ.

The results of our model indicate that the effect of activity timing varied between seasons (fig. 3). In summer, there is almost no difference in MDEE related to the timing of activity; the time

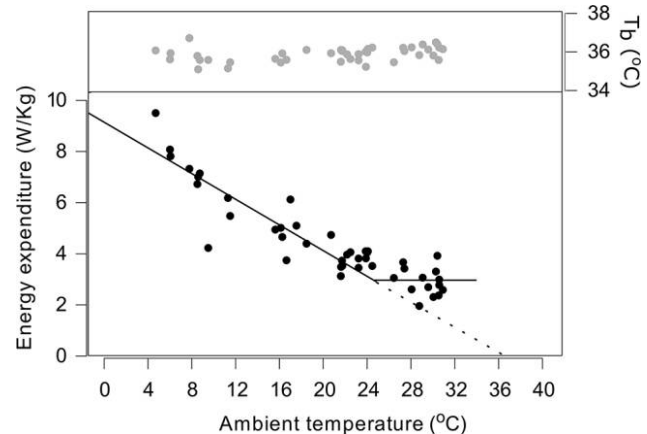


Figure 1. Metabolic rate (MR) and body temperature ( $T_b$ ) as a function of ambient temperature ( $T_a$ ). Each point represents the MR (black) or  $T_b$  (gray) measured for one individual at a given  $T_a$ . The horizontal line is traced along the thermoneutral zone ( $MR = 3.3$  W/kg). The inclined line represents the linear regression model calculated for  $T_a$  below  $23^\circ\text{C}$ . The dashed line extrapolates the linear model to  $MR = 0$  and  $T_a = T_b$  (average  $T_b$  of individuals over the 5 d before the metabolic measurements =  $36.3^\circ \pm 0.4^\circ\text{C}$ ). Tuco-tucos maintained a stable  $T_b$  throughout all of the  $T_a$ 's used during the MR measurements.

of activity with the highest MDEE (295.6 kJ/kg; 10:00) was only 2.68% higher than the time with the lowest MDEE (287.7 kJ/kg; 21:00). In winter, the differences in MDEE are much larger, with a difference of 11.48% between the highest (463.2 kJ/kg; activity at 09:00) and the lowest (410 kJ/kg; activity at 20:00) values. In summer,  $T_e$  exceed  $34^\circ\text{C}$  from 13:00 until 20:00, which we considered nonpermissive for aboveground activity. In both summer and winter, the time of aboveground activity with the highest MDEE is early morning. In winter, MDEE reached a low constant in the afternoon and early evening (14:00–20:00). Because activity phase in winter averages 186 min, the onset of activity when the center is 20:00 is at least 1 h before sunset. This indicates that aboveground activity during daylight hours can be energetically beneficial for tuco-tucos. Besides the seasonal differences of temperature, the duration of the activity phase has quite an important impact on the magnitude of the differences in MDEE between seasons (fig. S2).

We acknowledge that our model has limitations. MR was measured during the rest phase of the animals but was used for both activity and rest phases in our MDEE estimations. MR is higher during the active phase than during the rest phase (up to 50% in nonprimate mammals; Aschoff 1982), and taking this into account would further increase the impact of activity timing on MDEE (fig. S3). Although  $T_e$  integrates the effects of conduction, convection, and radiation, it assumes that the animal is metabolically inert and does not take into account wind-induced changes in resistance to heat flow (Bakken 1980; Chappell and Bartholomew 1981). Adding those variables to the model would also increase the differences in MDEE according to timing of activity (fig. S4). Finally, we estimate MDEE in field settings while using MR measured in laboratory conditions, which is known to change

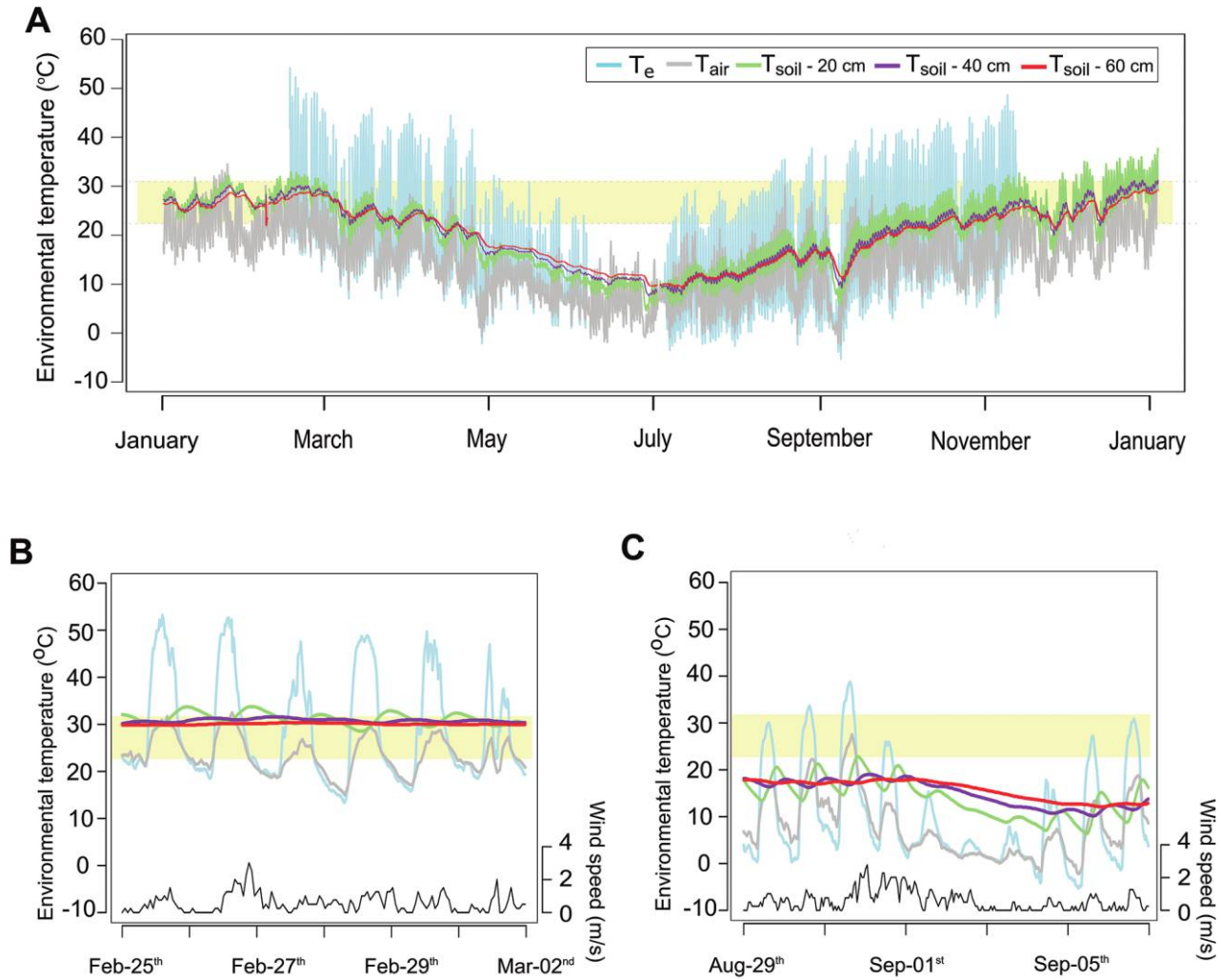


Figure 2. Environmental temperatures in the tuco-tuco's natural habitat. The blue line indicates the operative temperature ( $T_e$ ) measured using a taxidermic mount, the gray line indicates the air temperature ( $T_{air}$ ) 1 m aboveground (measured inside a radiation shield), the green line indicates the soil temperature ( $T_{soil}$ ) at a depth of 20 cm, the purple line indicates  $T_{soil}$  at a depth of 40 cm, and the red line indicates  $T_{soil}$  at a depth of 60 cm. The black line in B and C depicts wind speed. The shaded yellow area indicates the thermoneutral zone (TNZ) of the tuco-tuco.  $T_e$  was measured only from February 16 through June 6, 2016, and from July 3 through November 9, 2016. A, Temperatures measured from January 2016 through December 2016. B, Close-up of temperatures during six summer days (February 27–March 2). C, Close-up of temperatures during 10 winter days (August 29–September 7). From May through September,  $T_{air}$  and  $T_{soil}$  remained mostly below the TNZ of the tuco-tuco.  $T_e$  was above the TNZ during daylight hours on most measured days. Most of the time, underground  $T_{soil}$  was higher than  $T_{air}$ , and the daily variation in  $T_{soil}$  was minimal compared with that in  $T_{air}$  and  $T_e$ .

many aspects of physiology (Calisi and Bentley 2009). However limited, this approach allows fair estimations and has been widely used as a first step toward estimating energy budgets in the field (e.g., Kenagy and Hoyt 1989; Kenagy et al. 2002). Ideally, this approach should be followed and validated by other independent estimates of energy expenditure in the field, such as doubly labeled water (e.g., Weather et al. 1984; Buttemer et al. 1986; Goldstein et al. 1988; Kronfeld-Schor et al. 2001), heart rate (e.g., Portugal et al. 2016), or accelerometry (e.g., Williams et al. 2016a, 2016b). Furthermore, while the absolute MDEE values are probably not precise, our goal was to assess the relative differences in energy expenditure for different times of activity.

While we did not take into account seasonal changes in both animal and nest insulation, we think that changes in these parameters would have a minor impact on MDEE. Seasonal variations in nest insulation was not observed in excavated burrows (V. S. Valentinuzzi and C. L. Buck, personal observations), and model calculations based on data from marmots (Webb and Schnabel 1983) and arctic ground squirrels (Buck and Barnes 1999) suggest it is unlikely that variation in conductivity of nests of subterranean rodents plays a significant role in energy expenditure. Although seasonal changes in fur density and length have been observed in other tuco-tuco species (Cutrera and Antinuchi 2004), they are quite small compared with those in species from temperate and



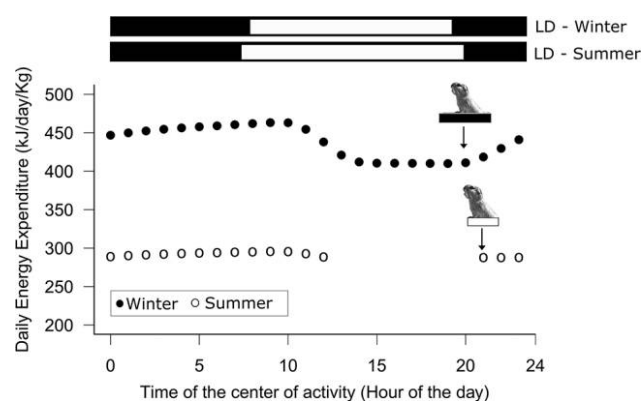


Figure 3. Estimated minimum daily energy expenditure (MDEE) for different times of activity. Points are the MDEE values estimated by our model when aboveground activity is centered around a given time, winter (filled circles) and summer (open circles). Small bars above the points indicate the duration of the aboveground activity phase centered at the time of the lowest MDEE value (winter activity phase length: 186 min, lowest MDEE at 19:00; summer activity phase length: 82 min, lowest MDEE at 21:00). Bars above the plot indicate the average light (white) and dark (black) phases for the days used in the model (summer: sunrise at 07:20, sunset at 19:58; winter: sunrise at 07:40, sunset at 19:14).

arctic climates (Scholander et al. 1950; Underwood and Reynolds 1980). In addition, it has been noted that seasonal pelage variations have only modest effects on mass-specific metabolism in small mammals (Steudel et al. 1994), whereas they can have substantial effects in large mammals.

Collectively, our results suggest that, in winter, there would be considerable energy savings if activity was allocated at least partially during daylight, lending support to the CTE hypothesis; these results are similar to those reported for house mice (van der Vinne et al. 2015). In summer, however, the impact of activity timing on energy expenditure is small, suggesting that, in this season, some combination of other factors, such as predation risk (Tyler 2016), water balance (Rezende et al. 2003; Levy et al. 2016), and social interaction (Kronfeld-Schor and Dayan 2003), may play more important roles than energetics in the timing of activity. Interestingly, our model indicates that duration of aboveground activity time can greatly increase the impact of daytime activity on MDEE, which has also been reported for mice (van der Vinne et al. 2015). Since food is scarce and ambient temperatures are low (especially at night) during winter, tuco-tucos spend more time foraging aboveground (Tomotani et al. 2012). Consolidation of aboveground activity to the daytime decreases thermoregulatory costs and increases the animal's ability to cope with these energetic challenges. This would be especially evident in years with low precipitation and suggests that projected changes in rainfall in South America for the next decades (Boulanger et al. 2007; Labraga and Villalba 2009) may ultimately impact activity patterns of tuco-tucos. Finally, energetic benefits of daytime aboveground activity might be more significant for tuco-tucos inhabiting higher latitudes and altitudes (van der Vinne et al. 2015).

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