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RESEARCH ARTICLE

Diurnality as an energy-saving strategy: energetic consequences of temporal niche switching in small mammals

Vincent van der Vinne^{*,‡}, Jenke A. Gorter, Sjaak J. Riede and Roelof A. Hut

ABSTRACT

Endogenous daily (circadian) rhythms allow organisms to anticipate daily changes in the environment. Most mammals are specialized to be active during the night (nocturnal) or day (diurnal). However, typically nocturnal mammals become diurnal when energetically challenged by cold or hunger. The circadian thermo-energetics (CTE) hypothesis predicts that diurnal activity patterns reduce daily energy expenditure (DEE) compared with nocturnal activity patterns. Here, we tested the CTE hypothesis by quantifying the energetic consequences of relevant environmental factors in mice. Under natural conditions, diurnality reduces DEE by 6–10% in energetically challenged mice. Combined with night-time torpor, as observed in mice under prolonged food scarcity, DEE can be reduced by ~20%. The dominant factor determining the energetic benefit of diurnality is thermal buffering provided by a sheltered resting location. Compared with nocturnal animals, diurnal animals encounter higher ambient temperatures during both day and night, leading to reduced thermogenesis costs in temperate climates. Analysis of weather station data shows that diurnality is energetically beneficial on almost all days of the year in a temperate climate region. Furthermore, diurnality provides energetic benefits at all investigated geographical locations on European longitudinal and latitudinal transects. The reduction of DEE by diurnality provides an ultimate explanation for temporal niche switching observed in typically nocturnal small mammals under energetically challenging conditions. Diurnality allows mammals to compensate for reductions in food availability and temperature as it reduces energetic needs. The optimal circadian organization of an animal ultimately depends on the balance between energetic consequences and other fitness consequences of the selected temporal niche.

KEY WORDS: Circadian, Circadian thermo-energetics hypothesis, Daily energy expenditure, Diurnal, Energetics, Temperature cycle, Temporal niche switching

INTRODUCTION

The rotation of the Earth around its axis causes predictable daily changes in light and temperature. This results in large daily environmental changes to which organisms have adapted during evolution. Anticipation of these daily environmental changes is enabled by the presence of a circadian system, involving circadian molecular oscillators (circadian clocks). Circadian rhythms in behavior and physiology are ubiquitous in a wide range of species

and molecular circadian clocks have been described in virtually all taxa (Edgar et al., 2012; Panda et al., 2002). These circadian clocks allow animals to optimize physiology and behavior to specific times of day, resulting in species being mainly active during the day (diurnal), night (nocturnal) or around dawn and dusk (crepuscular). Circadian rhythms are believed to provide adaptive benefits by distributing conflicting metabolic and behavioral processes to different times of day (intrinsic benefits; Pittendrigh, 1993), and allow organisms to predict daily environmental fluctuations in thermal conditions, predation risk and food availability (extrinsic benefits; Daan, 1981).

For all endotherms living in temperate climates, maintaining body temperature consumes energy. Reducing thermoregulatory costs allows animals to divert energy to different tasks and so optimize growth and reproduction. Examples of behavioral energy-saving strategies typically occur during the rest phase and include daily torpor (Heldmaier et al., 2004), insulating body posture, huddling and resting in insulated nests (Gilbert et al., 2010). The energetic savings benefit of these strategies increases when ambient temperature (T_a) is lower. Thus, endothermic energy expenditure varies with time of day as a result of daily changes in T_a and solar radiation. Extrinsic benefits of circadian organization can therefore derive from optimizing the timing of activity and rest with daily environmental T_a rhythms. The circadian thermo-energetics (CTE) hypothesis predicts that diurnal activity patterns allow endothermic animals to reduce energy expenditure (Hut et al., 2012; van der Vinne et al., 2014a). As night temperatures are usually lower than day temperatures and the rest phase is associated with behavioral strategies to reduce heat loss, resting during the coldest part of the day allows animals to optimize the energetic benefits of insulation and minimize daily energy expenditure (DEE).

The energetic benefits of diurnality proposed by the CTE hypothesis would be most relevant for animals challenged to maintain energy balance. Indeed, a literature review on temporal niche switching found that most temporal niche switches occurred in typically nocturnal mammals under conditions of increased energetic requirements (e.g. winter cold or food scarcity) (Hut et al., 2012). To investigate how metabolic demand shapes circadian organization, we developed the ‘working for food’ protocol (WFF) in which mice are subjected to conditions of simulated food shortage (Hut et al., 2011). Mice subjected to WFF have to obtain their food by working in a running wheel and become increasingly day active when the workload increases (Hut et al., 2011). This allows daily torpor bouts to occur during the second half of the night in mice challenged energetically for prolonged periods (Hut et al., 2011). The increased daytime activity is more pronounced when combined with other energetic challenges such as lowered T_a (van der Vinne et al., 2014a) or lactation (Perrigo, 1987). All these findings are in line with the CTE hypothesis proposition that diurnality in endothermic animals provides energetic benefits (Hut et al., 2012).

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List of symbols and abbreviations

CTE	circadian thermo-energetics hypothesis
DEE	daily energy expenditure
k_{mouse}	thermal conductance constant of a mouse body
k_{nest}	nest temperature buffering constant
SMR	standard metabolic rate
Ext	external time
T_a	ambient temperature
T_b	body temperature
T_{HC}	higher critical temperature
T_{LC}	lower critical temperature
T_{nest}	nest temperature
T_{surface}	surface temperature
WFF	working for food protocol

An important determinant of energy expenditure for small endothermic animals is the encountered T_a (Speakman, 1997). The relationship between T_a and energy expenditure is described by Scholander curves (Scholander et al., 1950). Scholander curves have been measured in different species and environmental conditions, revealing a consistent relationship between T_a and energy expenditure (Fig. 1) (Gordon, 2012; Scholander et al., 1950). Energy expenditure is lowest in the thermoneutral zone with T_a between the lower (T_{LC}) and higher critical temperature (T_{HC}). Energy expenditure increases linearly for T_a outside the thermoneutral zone, reflecting increased thermoregulatory costs.

The rate of energy expenditure increase (Scholander slope) with lowering T_a depends on the animal's thermal conductance. Rest-

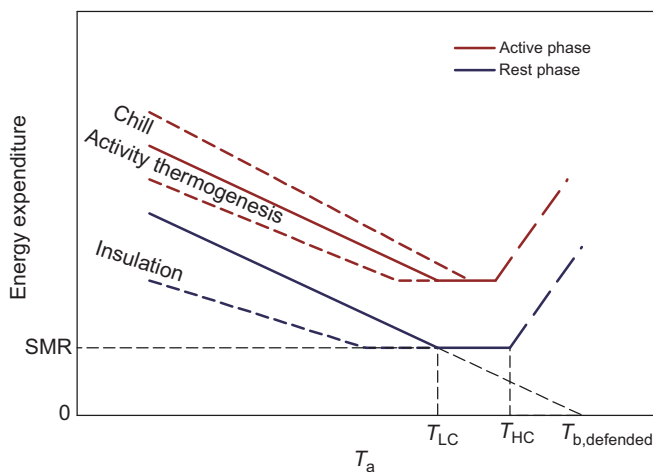


Fig. 1. Scholander curves describe the relationship between ambient temperature and energy expenditure in mammals and birds. Energy expenditure increases linearly with decreasing ambient temperature (T_a) when T_a is below the lower critical temperature (T_{LC}). The slope of this part of the Scholander curve extrapolates to the defended body temperature ($T_{\text{b,defended}}$). Energy expenditure in the thermoneutral zone is relatively constant (standard metabolic rate, SMR). Above the higher critical temperature (T_{HC}), energy expenditure increases rapidly as a result of the energetic requirements of active cooling. Energy expenditure during the active phase is higher than that during the rest phase. Energy-saving strategies associated with the rest phase (nest insulation, huddling, torpor) reduce the energy expenditure increase when T_a decreases below T_{LC} and thereby also lower the T_{LC} . Elevated heat loss due to exposure to the outside elements during the active phase (chill) augments the energy expenditure increase for $T_a < T_{\text{LC}}$ and raises T_{LC} . The use of heat generated by activity for thermogenesis (activity thermogenesis) reduces the energy expenditure increase when T_a decreases below T_{LC} and reduces T_{LC} .

associated energy-saving strategies, such as huddling and nest insulation, reduce thermal conductance, thereby decreasing energy expenditure at T_a below T_{LC} (Fig. 1) (Gilbert et al., 2012). During the active phase, energy expenditure is elevated. An interspecies comparison estimates that thermal conductance is approximately 50% higher during the active phase compared with the rest phase (Aschoff, 1981). However, this does not account for changes in the shape of the Scholander curve during the active phase. A quantitative assessment of the energetic consequences of changes in circadian organization requires an understanding of the modulation of Scholander curves during the active phase in combination with the effects of energy-saving strategies during the rest phase.

This study presents a fully quantified model that estimates how the temporal organization of behavior influences DEE of mice under natural conditions in a temperate climate. The model takes into account the different relationship between T_a and energy expenditure during the rest and active phase, the energetic consequences of rest-associated energy-saving strategies (nest insulation, huddling, rest posture, daily torpor) and the differences in daily surface (T_{surface}) and nest (T_{nest}) temperature rhythms. Combining these factors in a computational model (Fig. 2) allows for the quantification of the energetic benefit of diurnality under different environmental conditions. Finally, the expected energetic consequences of diurnality are assessed for different European geographical locations and for different days separately to assess the impact of daily fluctuations in the T_{surface} rhythm on the energetic benefit of diurnality.

RESULTS**Energy expenditure during active and rest phase**

Energy expenditure of mice was measured by respirometry throughout the day and night to ensure that every phase of the daily cycle was measured (Fig. 3A,B). During both the active and rest phase, a linear increase of energy expenditure was found with decreasing T_a at temperatures below thermoneutrality. As expected, energy expenditure was higher during the active phase. The relative difference in slope of the Scholander curve below T_{LC} between the rest and active phase depended on running wheel availability, which resulted in a greater difference in energy expenditure between the two phases. While energy expenditure decreased with increasing T_a at the same rate during the active and rest phase for mice housed without a running wheel (Fig. 3A), mice with access to a running wheel showed a reduced Scholander slope during the active phase (Fig. 3B) compared with the resting phase. This is in line with the hypothesis that heat generated by muscle activation during activity can be used to maintain body temperature (T_b) and thereby reduce thermoregulatory costs during the active phase (Humphries and Careau, 2011).

The measurement of energy expenditure in the laboratory should be adjusted for the altered heat loss under natural conditions by adjusting the slope of the measured Scholander curve. Heat loss under natural conditions will typically be increased by the different environmental conditions (e.g. humidity, wind). The thermal conductance constant of a mouse body (k_{mouse}) was therefore measured under natural and laboratory conditions, and division of k_{mouse} obtained under natural conditions by that under laboratory conditions provided a chill factor to adjust the slope of the measured active phase Scholander curve. The chill factor was determined for three mouse models to be 1.423 ± 0.184 (mean \pm s.d.).

Energy saving during rest phase

Energy expenditure can be reduced by minimizing heat loss to the environment. Strategies to do so during the rest phase include

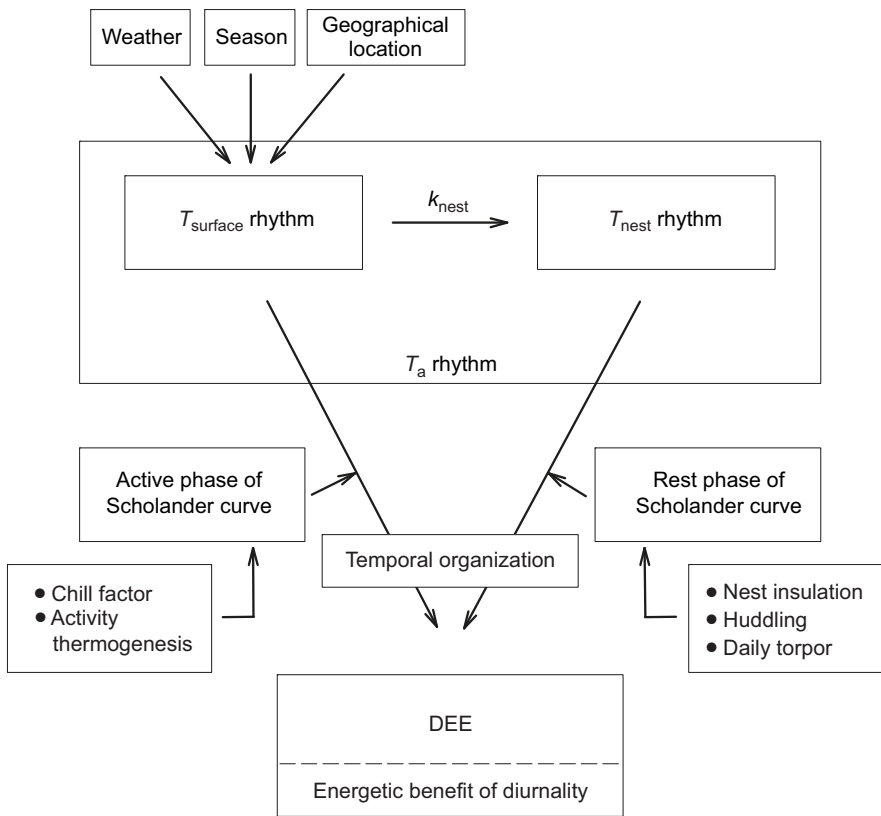


Fig. 2. Model for calculation of the energetic benefit of diurnality under natural conditions. The daily surface temperature (T_{surface}) rhythm and thermal buffering of the nesting location (k_{nest}) determine the daily nest temperature (T_{nest}) rhythm. The encountered ambient temperature (T_{a}) rhythm depends on the animal's temporal organization. Environmental conditions shape the Scholander curves during both the active and rest phases. The daily energy expenditure (DEE) and energetic benefit of diurnality depend on the encountered T_{a} rhythm and the shape of the Scholander curves during the rest and active phase.

huddling with nest mates and nest insulation. The effectiveness of such strategies depends on the absolute difference between T_{b} and T_{a} and is thus greatest when T_{a} is low. This is reflected in a decreased slope of the resting phase Scholander curves measured for mice living in larger litters and/or provided with more nest insulation material (Fig. 3C,D; supplementary material Fig. S1). The slope of the resting phase Scholander curve decreased in steps of $8.21 \times 10^{-3} \text{ kJ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$ when the amount of nest insulation material was increased from 0 to 0.5 to 2.5 g cotton wool. Increasing the group size from 1 to 3 mice per nest reduced the Scholander slope by

$4.34 \times 10^{-3} \text{ kJ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$ for each additional mouse (Fig. 3C). With a constant defended T_{b} and standard metabolic rate (SMR), this resulted in a lowered T_{LC} with increasing insulation (Fig. 3D).

Computational modeling of DEE

The expected DEE of mice under natural conditions can be calculated by combining energetic measurements with daily rhythms in T_{surface} and T_{nest} . Shifting activity to the day (diurnality) increases the encountered T_{a} during both the light and dark phase of the day (van der Vinne et al., 2014a). In addition, rest-

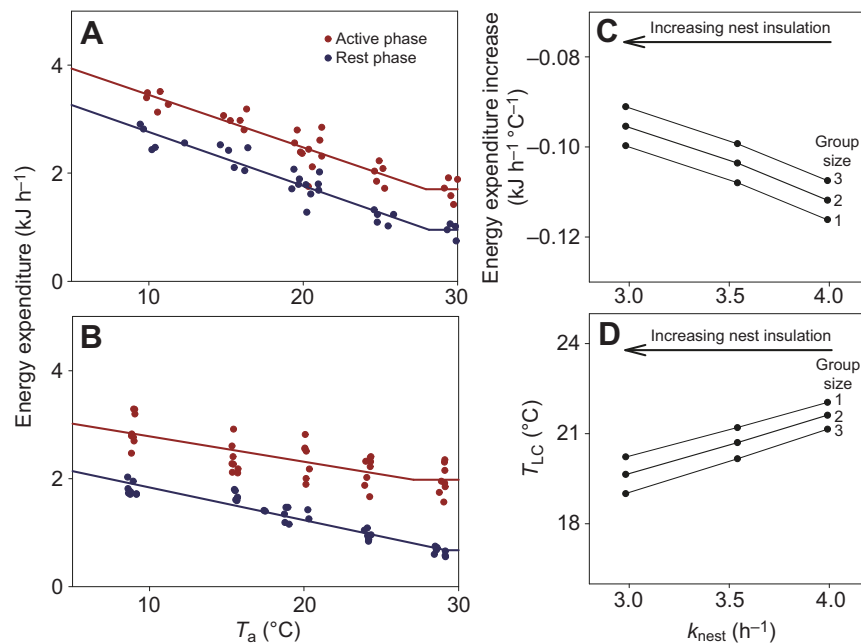


Fig. 3. Energy expenditure of mice during rest and activity. Scholander curves show the relationship between energy expenditure and T_{a} during the rest and active phase for mice housed without (A) or with (B) access to a running wheel. Energy expenditure was increased during the active phase, especially in the presence of a running wheel. (C) During the rest phase, energy-saving strategies (nest insulation and huddling) reduced the energy expenditure increase with decreasing T_{a} . (D) Because of the decreased slope, increased nest insulation and huddling also lowered T_{LC} .

associated energy-saving strategies provide a larger relative reduction in energy expenditure at lower T_a . Therefore, the energetic benefit will be maximal when rest is synchronized with the colder night while the disadvantages of energetically detrimental conditions (humidity, wind) can be reduced by being active during the warmer light phase. Therefore, diurnality is associated with a lowered DEE (Fig. 4).

The quantitative model used here to calculate the expected DEE assumes a 12 h continuous active phase. Although the daily activity pattern of small mammals does not consist of a continuous active phase (Long et al., 2005), using the average activity pattern of mice fed *ad libitum* or exposed to simulated food shortage in our modeling did not result in a change in the optimal activity midpoint, expected DEE and energetic benefit of diurnality (supplementary material Fig. S2). Therefore, further calculations were done using the simplified 12 h active–12 h rest activity profile.

Environmental factors influencing the energetic benefit of diurnality

The energetic consequences of changes in circadian organization depend on several environmental factors. The contribution of these factors is assessed systematically by calculating the expected energetic benefit of diurnality (Fig. 5; supplementary material Fig. S3) after their incorporation into the model. For mice housed at a constant T_a , changes in temporal organization did not alter the expected DEE. When subjected to a daily temperature cycle, nocturnality was energetically beneficial (energetic benefit of diurnality: -0.2% ; Fig. 5C) as it utilizes activity thermogenesis to reduce thermoregulatory costs during the cold night. The energetic benefit of nocturnality was increased (-1.4%) by the presence of a running wheel. Access to a running wheel allowed an increased intensity of activity, resulting in a greater difference in heat production between the rest and active phase. Diurnality became energetically beneficial when the T_{nest} rhythm was dampened by the thermal capacity of the nest (4.7%). Increased thermal capacity of the nest results in a reduced amplitude and delayed peak phase of the T_{nest} rhythm (Fig. 4). Diurnality allows an animal to leave the nest during the day when it is warmer outside ($T_{\text{surface}} > T_{\text{nest}}$) and to be in



Fig. 4. DEE depends on daily timing of activity. The difference between surface (T_{surface}) and nest (T_{nest}) temperature rhythms (inset) allows diurnal mice to reduce DEE by encountering the relatively warm T_{surface} during the active phase and the relatively warm T_{nest} during the rest phase. The light–dark cycle is represented by the yellow–gray bar. The hatched bar represents the timing of the active phase for a mouse with an activity midpoint at an external time (ExT) of 16 h and the associated expected DEE.

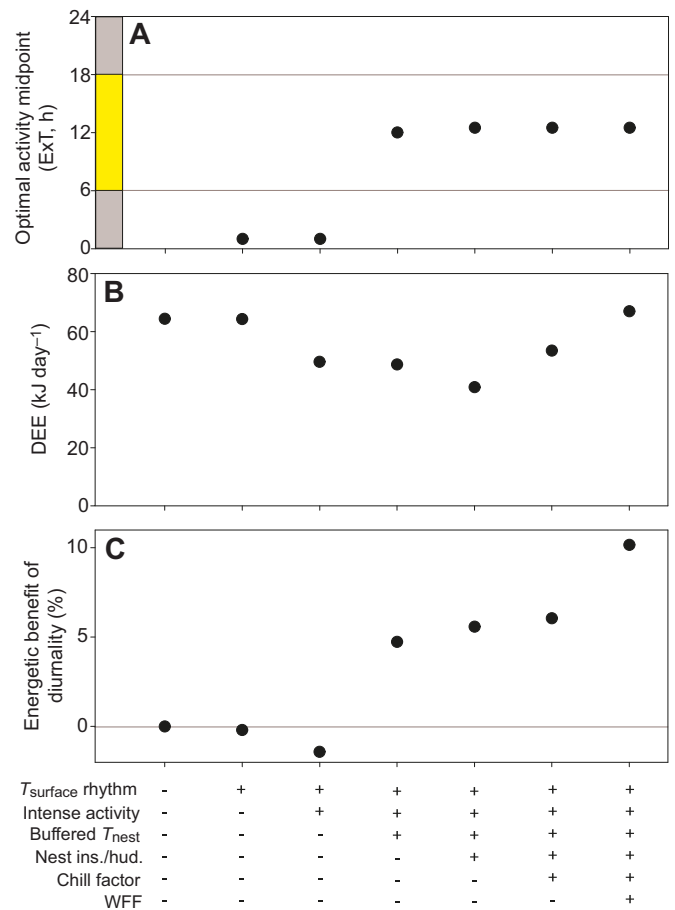


Fig. 5. Impact of environmental factors on the energetically optimal daily timing of activity. Addition of environmental factors to our energetic model allows the assessment of the importance of each factor (see supplementary material Fig. S3, for effects of individual factors). (A) The optimal activity midpoint indicates the time of day (ExT) associated with the lowest DEE for a mouse with an active phase starting 6 h before and ending 6 h after the indicated time. (B) DEE associated with the optimal activity midpoint. (C) Percentage energetic savings associated with diurnality compared with a nocturnal mouse. (nest ins., nest insulation; hud., huddling; WFF, working for food protocol).

the nest during the night when it is warmer inside ($T_{\text{nest}} > T_{\text{surface}}$). A buffered T_{nest} rhythm thus makes diurnal animals encounter higher T_a throughout the 24 h cycle compared with nocturnal animals. Incorporating nest insulation and huddling during the rest phase (5.6%), and the added energetic costs of outside chill (humidity, wind) during the active phase (6.0%) increased the expected energetic benefit of diurnality.

Mice placed under conditions of simulated food shortage (WFF protocol) reduce T_b primarily during the rest phase, to approximately 32°C (van der Vinne et al., 2014a). These changes further increased the energetic benefit of diurnality (10.2%; Fig. 5C). Prolonged energetic challenges can even induce daily torpor bouts in mice (Hut et al., 2011), but the energetic measurements of WFF mice used here did not involve torpid mice (van der Vinne et al., 2014a). Assuming negligible energy expenditure during daily torpor, a 6 h torpor bout during the late rest phase would provide an additional 9.6% saving for a nocturnal and a 10.3% energetic saving for a diurnal mouse. The shift to diurnality and daily torpor bouts observed during prolonged simulated food shortage (Hut et al., 2011) would thus reduce

DEE by $\sim 20\%$ under natural conditions. As is the case with other energy-saving strategies associated with the rest phase (huddling, nest insulation), the energetic benefits of daily torpor are maximized by diurnality.

The length of the active phase also influences the energetic benefit of diurnality (supplementary material Fig. S4). In nature, foraging yield is a major determinant of the length of the active phase. Here, the energetic benefit of diurnality was assessed for different active phase lengths with an activity midpoint at noon or midnight. The calculated maximal energetic benefit of diurnality was reached at an active phase length of 9.3 h (6.3%) with lower energetic benefits for both shorter and longer active phases. Being completely diurnal with an active phase length of 12 h provided an energetic benefit of 5.9% compared with a completely nocturnal mouse.

Energetic consequences of thermal buffering of T_{nest}

Because the thermal buffering of T_{nest} was the most important environmental factor in making diurnality energetically beneficial (supplementary material Fig. S3), the energetic consequences of changes in the T_{nest} buffering constant (k_{nest}) were explored using computational modeling (Fig. 6). k_{nest} is the Newtonian cooling law constant for the complete nest compared with T_{surface} . k_{nest} depends on the thermal conductance and heat capacity of the nesting location. Lower values of k_{nest} indicate better buffering of T_{nest} . While diurnality was energetically beneficial at all k_{nest} values (Fig. 6A) and changing k_{nest} decreased DEE by only 1.7% for a diurnal mouse (Fig. 6B), the energetic benefit of diurnality over nocturnality increased from 3.0% to 6.2% when the nest became better buffered from T_{surface} (Fig. 6C). The critical k_{nest} range determining the diurnal benefit of diurnality was between $\sim 2 \text{ h}^{-1}$ and $\sim 0.1 \text{ h}^{-1}$, representing nesting situations with a small nest on the ground surface and a nest 20 cm inside a hay-filled box, respectively. Highly buffered nests such as underground burrows do not encounter a daily T_{nest} rhythm and the constant T_{nest} depends on the surrounding ground temperature (supplementary material Fig. S5). The absolute energetic benefit of diurnality is constant in these conditions as diurnality still allows an animal to encounter the higher T_{surface} during the active phase (supplementary material Figs S6, S7).

Energetic benefits of diurnality in different geographical locations

The generality of the expected energetic benefit of diurnality was assessed for different geographical locations. T_{surface} data along a European east–west longitudinal and a north–south latitudinal transect were collected for 36 weather stations around the March and September equinox (Fig. 7C; supplementary material Fig. S8, Table S1, S2; Mundomanz.com, 2014). Variation in average T_{surface} was limited along the east–west transect ranging from European Russia to The Netherlands. Average T_{surface} decreased along the north–south transect from southern Spain to Svalbard. On both clines, average T_{surface} was higher in September than in March and the daily T_{surface} range was correlated with the average T_{surface} (supplementary material Fig. S8).

The expected energetic benefit of diurnality for the observed daily T_{surface} rhythms on the east–west longitudinal transect would be around 5% in March and 8% in September (Fig. 7A). On the north–south latitudinal transect, the expected energetic benefit of diurnality decreased from around 8% to 2% with latitude in March and from around 11% to 2% in September. These diurnal benefits were calculated based on the Scholander curves measured for *ad libitum* fed mice. When challenged energetically by situations of

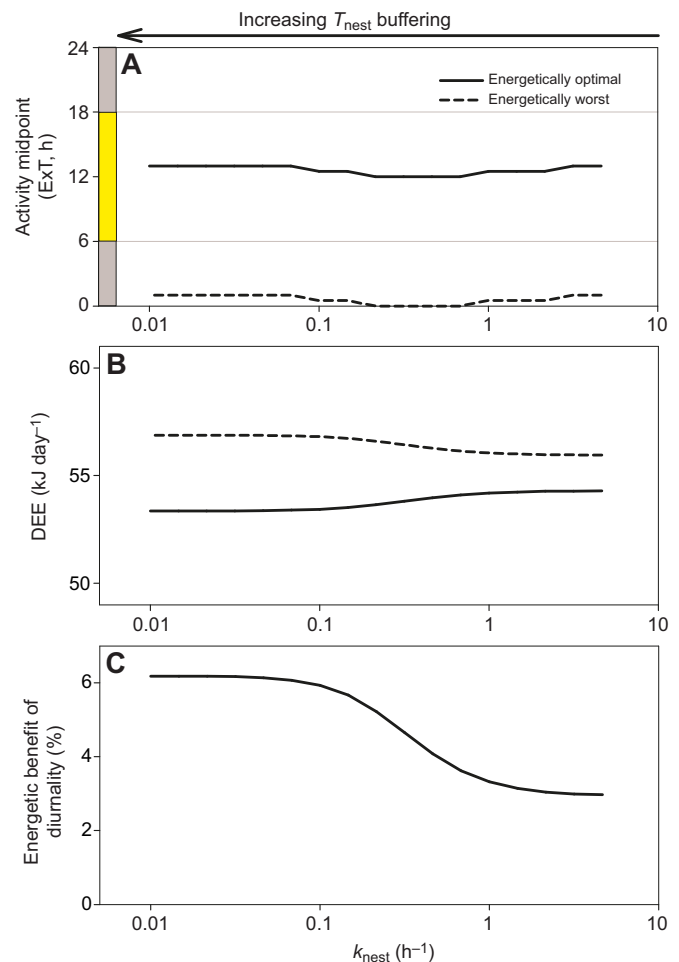


Fig. 6. Energetic consequences of thermal buffering of nest temperature. (A) Increasing thermal buffering of nest temperature (T_{nest}) by reducing the nest temperature buffering constant k_{nest} results in limited changes in the timing of the energetically optimal and worst activity midpoint, which remain at around noon and midnight, respectively. (B) DEE decreases with increasing thermal buffering of T_{nest} (decreasing k_{nest}) for mice active at the energetically optimal time (diurnal) and increases for mice active at the energetically worst time (nocturnal). (C) The energetic benefit of diurnality increases from 3.0% to 6.2% when going from a small nest on the ground surface ($k_{\text{nest}} \sim 3 \text{ h}^{-1}$) to a nest 20 cm inside a hay-filled nest box ($k_{\text{nest}} \sim 0.07 \text{ h}^{-1}$). Lower values of k_{nest} indicate better buffering of T_{nest} .

simulated food shortage (WFF), the difference in energy expenditure during the rest and active phase increased (van der Vinne et al., 2014a) (Fig. 7B). The observed T_{surface} rhythms on the east–west longitudinal transect would provide an expected energetic benefit of around 7% and 13% for diurnal mice in March and September, respectively. The expected energetic benefits of diurnality on the north–south latitudinal transect would range from 14% to 3% in March and from above 14% to 3% in September when moving from southern Spain to Svalbard.

Day-to-day differences in temperature cycle

Day-to-day variation in weather conditions can cause substantial variation in the encountered T_{surface} rhythm. To investigate the day-to-day variability in the energetic benefit of diurnality, the diurnal benefit and optimal activity midpoint were calculated for all days in September and March in the years 1991–2010. Diurnality was energetically beneficial on 98.5% (591/600) of all September days with an average energetic benefit of $5.69 \pm 3.06\%$ compared with a

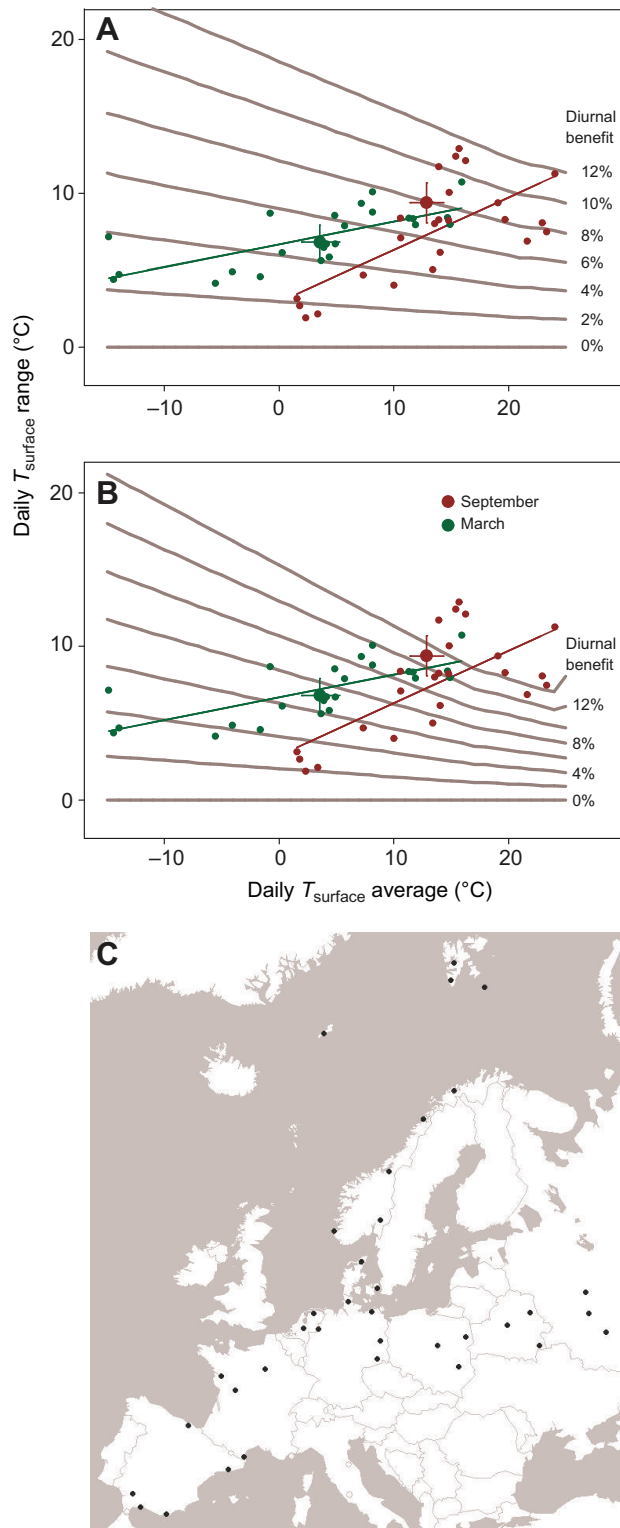


Fig. 7. Diurnal benefit depends on average and range of the daily surface temperature rhythm. Gray lines indicate the expected energetic benefit of diurnality for specified daily surface temperature (T_{surface}) average and range for mice under *ad libitum* (A) or simulated food shortage (B) feeding conditions. The bold green and red dots indicate the mean (with s.d.) T_{surface} on a longitudinal transect in March and September, respectively (see supplementary material Fig. S8). Green and red lines indicate expected T_{surface} on a latitudinal transect in March and September, respectively, with small dots indicating the T_{surface} values measured for individual weather stations on the latitudinal transect. (C) The geographical locations of the weather stations used for the longitudinal and latitudinal transects.

completely nocturnal mouse (Fig. 8A). An activity midpoint during the light phase was energetically optimal in 98.5% (591/600) of September days with an activity midpoint at or shortly after midday as the optimum (Fig. 8B). In March, complete diurnality was energetically beneficial on 94.4% (585/620) of all days with an average benefit of $3.27 \pm 2.47\%$ (supplementary material Fig. S9A). In 95.6% (593/620) of all March days, an activity midpoint during the light phase was energetically optimal (supplementary material Fig. S9B).

DISCUSSION

The CTE hypothesis aims to explain observed temporal niche switches from nocturnal to diurnal activity patterns in terms of energetic benefits (Hut et al., 2012; van der Vinne et al., 2014a). The current study shows that diurnal activity rhythms in mice are indeed associated with reduced DEE. Systematic evaluation of the different factors that modulate DEE identified thermal buffering of T_{nest} as being the most important factor in making diurnality energetically beneficial (Fig. 5; supplementary material Fig. S3). Thermal buffering of T_{nest} dampens the amplitude and delays the peak phase of the T_{nest} rhythm relative to the T_{surface} rhythm

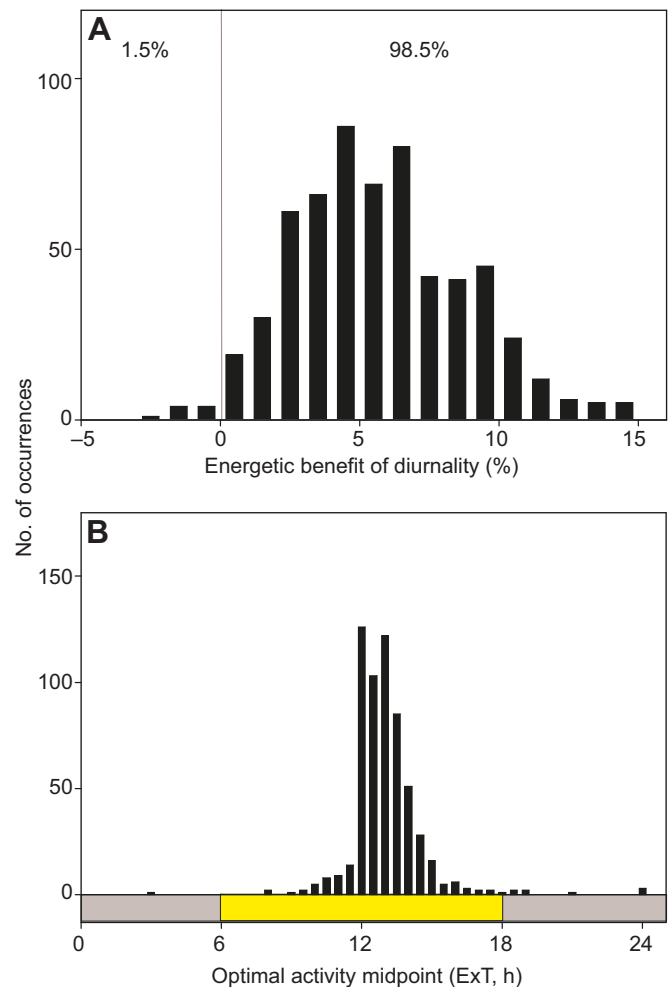


Fig. 8. Diurnality is associated with energetic benefits on most individual September days. (A) The expected energetic benefit of a completely diurnal compared with a completely nocturnal mouse based on hourly T_{surface} data. Diurnality is associated with energetic savings on 98.5% of all days. (B) The energetically optimal activity midpoint of a 12 h active phase is during the light phase on 98.5% of all days.

(supplementary material Fig. S5). Diurnality allows an animal to use these relative differences in T_{nest} and T_{surface} rhythms to encounter higher T_a during both day and night. Although the energetic quantification performed in this study was based on mice data, in temperate climates, diurnality will be energetically beneficial to all endothermic animals resting in sheltered locations.

Based on the quantifications performed in the present study, diurnality is expected to reduce DEE by approximately 6–10% under natural conditions (Fig. 5). Lowering DEE reduces foraging requirements and allows a shorter active phase, thereby further reducing DEE. Diurnality can thus reduce foraging time by more than 6–10%, with the exact reduction depending on the encountered foraging yield. Foraging time will be reduced most under conditions that require a long active phase (low foraging yield). Diurnality provides energetic benefits for endothermic animals resting in sheltered locations, but the optimal distribution and time spent on activity and rest will depend on foraging opportunities and predation risk.

The present study quantified the consequences of different energy-saving strategies to calculate the energetic benefit of diurnality. In addition to diurnality, energetically challenged mammals use other strategies to reduce DEE. Mice exposed to prolonged periods of simulated food shortage show daily torpor bouts during which T_b drops to T_a (Hut et al., 2011). Combining daily torpor with becoming diurnal reduces DEE by approximately 20%. Further energetic benefits could be obtained by mammals using sun basking to warm up (Warnecke et al., 2008). As solar radiation can only be utilized during the light phase, including sun basking in our computations could only have increased the energetic benefit of diurnality.

The assessment of energy expenditure in the natural environment based on laboratory measurements requires a correction for altered thermal conductance. Comparing passive cooling of a warmed body in the laboratory and in the natural environment provides a scaling factor to adjust energy measurements from laboratory to natural conditions. In our opinion, this method provides a simpler and more accurate scaling factor than using a copper animal model with complex elements determining the thermodynamic properties of the whole model (Bakken, 1976; Long et al., 2005). By simultaneously assessing all components contributing to heat loss in a dead mouse, the total measurement error is minimized. Similarly, differences in energy expenditure measured during the active and rest phase might result from changes in different aspects of an animal's physiology and behavior (e.g. T_b and sleep–wake state). As temporal niche switching induced by energetic challenges results in simultaneous phase shifts of the physiology and behavior of mice (Hut et al., 2011; van der Vinne et al., 2014a), energy expenditure was measured during the active or rest phase to combine the influence of all of these factors simultaneously and reduce the total measurement error. In contrast, measuring changes of separate factors (e.g. T_b and sleep–wake state) during the day would allow the quantification of the influence of these separate factors on the altered energy expenditure between rest and activity.

An extensive assessment of the consequences of environmental conditions (humidity, precipitation, wind, sun, cover) on the thermal conductance of an animal was beyond the scope of the current study but would provide a more accurate description of DEE in nature. All of these environmental factors either do not show predictable daily fluctuations or peak at a phase that would increase the energetic benefit of diurnality. Therefore, taking these environmental factors into account in the assessment of the consequences of different temporal strategies would, if anything, increase the benefit of diurnality. The insulation from daily T_{surface} cycles provided by

snow cover in winter would result in a reduced energetic benefit of diurnality, but as daily T_{surface} amplitudes are low in winter and at high latitudes (supplementary material Fig. S8), our model already predicted low benefits of diurnality in these environments.

The current study shows that becoming diurnal allows typically nocturnal endothermic animals to conserve energy. Indeed, in the wild, diurnality is observed in typically nocturnal mammals in response to demanding energetic conditions (reduced food availability, lowered temperatures) (Hut et al., 2012). Under controlled laboratory conditions, diurnality can be induced by challenging mice energetically (van der Vinne et al., 2014a). This suggests that shifting to diurnality is a strategy employed by typically nocturnal mammals to compensate for environmental fluctuations in energy availability.

The analysis of individual daily T_{surface} rhythms revealed that diurnality decreased DEE on 94–99% of all days in September and March (Fig. 8; supplementary material Fig. S9). This near-certain diurnal benefit could provide an evolutionary selection pressure for a mechanism whereby animals use the light–dark cycle as a proxy for the ultimately energetically optimal time of day to be active (as suggested by Baker, 1938, for seasonal rhythms). Animals thus respond to a negative energy balance by becoming diurnal without continuously assessing energy expenditure throughout the day. Indeed, mice subject to conditions of simulated food shortage use the light–dark cycle and not the T_a cycle to select their temporal organization (van der Vinne et al., 2014a).

The energetic benefit of diurnality increases with average T_{surface} and daily T_{surface} range, both of which are affected by geographical location and season (Fig. 7; supplementary material Fig. S8). As both average T_{surface} and daily T_{surface} range decrease with latitude but longitude only has a small effect on the average T_{surface} in our European transects, the energetic benefit of diurnality is expected to increase from north to south but remain relatively constant from east to west. A clear season effect is discernible on the longitudinal transect. The energetic benefit of diurnality substantially increases during spring and summer compared with autumn and winter. These observations lead to the prediction that diurnality in response to energetically challenging conditions is more likely to occur in southern (warmer) locations and during the warmer seasons. The lowered DEE associated with higher average T_{surface} , however, makes it less likely that animals require the energetic benefits of diurnality.

As all measurements in this study were performed at T_a below 30°C, the present model cannot quantify the optimal circadian organization for animals facing heat dissipation difficulties (Speakman and Król, 2010). However, the CTE hypothesis can predict the energetic consequences of diurnality at T_a above T_{HC} in qualitative terms. Because energy expenditure increases dramatically when T_a increases above T_{HC} (Gordon, 2012; Scholander et al., 1950), avoiding activity during the warmest part of the day would be beneficial. Studies in multiple species suggest that such behavioral adaptations indeed occur in response to high T_a . The diurnal European ground squirrel (*Spermophilus citellus*) reduces activity during the warmest part of the day on sunny days (Váczai et al., 2006), while lactating common voles (*Microtus arvalis*) shift lactation to the night when challenged by a high T_a , presumably to reduce the risk of overheating (van der Vinne et al., 2014b). These examples illustrate that different species of small mammals adjust the distribution of activity and rest to reduce the risk of hyperthermia.

This study describes the optimal distribution of activity and rest from an energetic point of view. Under natural conditions, the optimal circadian organization will depend on the consequences it has on all fitness components (e.g. energy expenditure, foraging efficiency,

sleep length, reproductive success and predation risk). The relative importance of each of these fitness components depends on the environmental conditions an animal encounters. The ultimately optimal circadian organization of activity and rest will be such that the sum of costs and benefits for all these factors is optimized. Although diurnality would nearly always be beneficial from an energetic standpoint, other fitness components make nocturnality beneficial under most environmental conditions. The shift to diurnality observed in response to energetic challenges suggests that the increased importance of energetic savings under these conditions adjusts the overall balance and ultimately makes diurnality beneficial.

MATERIALS AND METHODS

Animals

CBA/CaJ male mice (5–9 months old) housed in standard macrolon mouse cages (15×32×13 cm) equipped with or without a running wheel were placed on a 12 h light:12 h dark cycle at least 10 days before energetic measurements. Food (AM II diet rodent chow 10 mm, 17.3 kJ g⁻¹, Arie Blok, Woerden, The Netherlands) and water were provided *ad libitum*. Animals were housed on sawdust bedding (Lignocel hygienic animal bedding, Rettenmaier, Rosenberg, Germany). Procedures involving animals were approved by the Animal Experimentation Committee of the University of Groningen (DEC 5454).

Scholander curves during activity and rest

T_a was set to the temperature of interest (Fig. 3) 24 h before respirometry measurements (O₂, CO₂ analyses) started. Solitary housed mice ($N=8$ for each of the five T_a treatments) were transferred in their home cage to the respirometry setup at external time (ExT) of 17 h. The mice stayed in their home cage during the respirometry measurements, eliminating the need for a long acclimatization period. Energy expenditure was determined every 10 min for at least 23 h by measuring O₂ use (for formulas, see Hill, 1972) and CO₂ production using a dried air open-circuit respirometry system at a constant T_a throughout the day (O₂: Servomex Xentra 4100, CO₂: Servomex 1440, Crowborough, UK; cage volume: 20 l; flow: 30 l h⁻¹, type 5850 Brooks mass flow controller, Rijswijk, The Netherlands). O₂ use and CO₂ production concentrations were converted to energy expenditure using the formula: $16.18 \times \dot{V}_{O_2} + 5.02 \times \dot{V}_{CO_2}$ (Romijn and Lokhorst, 1961). Average energy expenditure during the active and rest phase was determined as the maximum and minimum value, respectively, of the 2 h running average of measured energy expenditure. To obtain average estimates for the energy expenditure during the rest and active phase, energy expenditure rate was averaged over this 2 h time interval, thereby obtaining reliable estimates for energy expenditure during the full rest and active phase. SMR (i.e. energy expenditure in the thermoneutral zone), T_{LC} and the energy expenditure increase per °C at $T_a < T_{LC}$ (Scholander slope) were determined simultaneously by fitting a Scholander curve (Scholander et al., 1950) to the pooled energetic measurements of all individuals using a least squares parameter estimation procedure for these three parameters (hockey stick regression procedure).

Energy expenditure effects of energy-saving strategies

The energetic consequences of nest insulation and group size were quantified in a crossover design. Three different group sizes were used (group size: 1, 2 or 3 mice; $N=7$, 8 and 6 groups, respectively). These three group sizes were provided in random order with three different nest insulation conditions (0, 0.5 or 2.5 g of standard medical cotton wool). Nest material was provided at least 2 days before the energetic measurements started, which gave enough time for nest building. All nests incorporated all the wool provided. Cooling curves of these nests showed that nest insulation depended significantly on the amount of cotton wool ($F_{2,40}=12.34$, $P<0.0001$; cooling constants: 0 g: 3.99 h⁻¹, 0.5 g: 3.54 h⁻¹, 2.5 g: 2.98 h⁻¹). All groups of mice were measured in the three nest material conditions at 1 week intervals during the light (rest) phase. Energy expenditure of groups of mice was measured between ExT of 7 and 17 h, and T_a was lowered from 30°C to 10°C in steps of 5°C every 2 h. The T_a was applied in the same time order to disentangle the effects of huddling and insulation, excluding the effect of time of day. Scholander

curves were calculated for all nest insulation and group size combinations based on the average energy expenditure per animal in the second hour of measurements in each T_a . In this analysis, the effects of nest insulation and huddling on the T_{LC} and energy expenditure increase at $T_a < T_{LC}$ were estimated as linear effects using a least squares fitting procedure while SMR was kept constant (Fig. 1).

Chill factor

The laboratory-based Scholander curve for the active phase was adjusted to account for the different rate of heat loss under outside surface conditions in nature. The total effect of different environmental influences (e.g. humidity, wind) on heat loss was determined by comparing the k_{mouse} of pre-heated dead mice ($N=3$) in the respirometry setup versus our outdoor enclosures (supplementary material Fig. S10). The k_{mouse} was determined under each condition by direct comparison between T_b during passive cooling and T_a using the Newtonian cooling model: $T_{mouse}(t) = T_{a,initial} + (T_{mouse,initial} - T_{a,initial}) \times \exp(-k_{mouse} \times t)$. T_b and T_a were measured every 2 min using wax-sealed Thermochron iButton abdominal data loggers (Lovegrove, 2009). The cooling constant of mice outside was divided by the cooling constant of mice inside to obtain the chill factor.

Surface and nest temperature

The hourly $T_{surface}$ values for the months September and March were obtained from the Koninklijk Nederlands Meteorologisch Instituut (KNMI) weather station (Eelde, The Netherlands; WMO: 06280; 53°07'N, 06°35'E) for the years 2001–2010. An average daily $T_{surface}$ profile was determined by averaging all data per time point per month for all years and linearly interpolating the average hourly values to obtain a $T_{surface}$ profile in 10 min intervals. The daily T_{nest} rhythm depends on thermal buffering of the nest (k_{nest}) against $T_{surface}$. k_{nest} depends on the thermal conductance and heat capacity of the nesting location. k_{nest} was determined for four nest locations in our outdoor mouse enclosures at the laboratory in Groningen, The Netherlands (53°14'N, 6°32'E). The night-time cooling rate of these nests compared with $T_{surface}$ was modeled using the Newtonian cooling law equation: $T_{nest} = T_{nest,previous} + k_{nest} \times (T_{surface,previous} - T_{nest,previous})$, in 1 min steps ($k_{nest} = 0.0722 \pm 0.0178$ h⁻¹, mean ± s.d.). The expected T_{nest} was calculated in 10 min iterative steps: $T_{nest} = T_{nest,previous} + k_{nest} \times (T_{surface,previous} - T_{nest,previous})$ using k_{nest} for 30 days; the T_{nest} profile on day 31 was used as the daily T_{nest} rhythm (Fig. 4). The reduction in amplitude and phase delay of T_{nest} (supplementary material Fig. S5) with increasing thermal buffering was similar to measurements of underground T_{nest} (Škliba et al., 2014).

DEE model

DEE was calculated (Fig. 2) for a mouse with a 12 h active and 12 h rest phase in 144 bins of 10 min. For each bin, energy expenditure was calculated based on whether the mouse was active or resting, using the corresponding T_a ($T_{surface}$ when active, T_{nest} during rest). During the rest phase, the energetic benefits of nest insulation and huddling were presumed to equal those of mice huddling in groups of 3 with 2.5 g of cotton wool. During the active phase, the negative slope of the Scholander curve increased (more negative) because of multiplication of the slope by the chill factor. Energy expenditure was calculated for all possible temporal niches in 30 min steps. The optimal activity midpoint was defined as the time of the lowest DEE; the least optimal activity midpoint was defined as the time of the highest DEE. The energetic benefit of diurnality was calculated as a percentage of the nocturnal DEE, by comparing the daily minimal and maximal DEE, which typically occurred around noon or around midnight. All energetic calculations described in this paper were performed using custom-written scripts in SciLab 5.5.0.

The energetic consequences of changes in T_{nest} buffering were assessed by varying k_{nest} . The assessed k_{nest} constants ranged from nests built by mice in standard laboratory cages ($k_{nest} = 3–4$ h⁻¹) to underground burrows ($k_{nest} = 0.018–0.0004$ h⁻¹; Strijkstra, 1999). The nests built by mice in our outdoor enclosures were found to have intermediate T_{nest} buffering constants ($k_{nest} = 0.0722$ h⁻¹). Stepwise addition of the parameters [the energetic effects of a $T_{surface}$ rhythm, running wheel access, T_{nest} rhythm, nest insulation, huddling, chill and simulated food shortage (WFF)] was used to quantify the contribution of these factors to the energetic benefit of diurnality.

Activity profile validation

To assess the validity of using a 12 h active, 12 h rest (block) activity profile in our energetic model, we compared this profile with the average activity profile of nocturnal and diurnal mice (supplementary material Fig. S2). The average activity profiles of 12 mice during the 5 days preceding start of the WFF protocol (all mice nocturnal) and the last 5 days of the WFF protocol (all mice diurnal) (van der Vinne et al., 2014a) were used to calculate a diurnal and nocturnal activity profile normalized to the total activity levels.

The validity of using a 12 h active phase was assessed by comparing the energetic benefit of diurnality for different activity phase lengths (4–20 h). The activity midpoints were fixed at noon (ExT of 12 h) for the diurnal DEE and at midnight (ExT of 24 h) for the nocturnal DEE (supplementary material Fig. S4).

Predicted energetic benefits of diurnality along longitudinal and latitudinal transects

Daily maximal and minimal temperatures in different European geographical locations on an east–west longitudinal transect (supplementary material Table S1) and north–south latitudinal transect (supplementary material Table S2) were obtained from Mundomanz.com (2014). For each location, daily maximal and minimal temperatures were collected over 6 days around the September and March equinox for the years 2006–2010. From the daily extremes, the average daily T_{surface} and daily T_{surface} range were calculated.

The expected energetic benefits of diurnality for combinations of average T_{surface} and daily T_{surface} range were calculated by optimizing the daily T_{surface} range for each average T_{surface} to obtain the specified diurnal benefit of 0–14%. This analysis assumed an energetic model where the shape of the daily T_{surface} rhythm (Eelde weather station, The Netherlands) was scaled by the T_{surface} average and daily T_{surface} range. The T_{nest} rhythm was derived from the T_{surface} rhythm in 10 min iterative steps using the measured nest cooling constant ($k_{\text{nest}}=0.0722$), while the effects of nest insulation, huddling and chill factor were incorporated in the energetic model. The expected diurnal benefits were calculated for mice under energetically relaxed (*ad libitum* fed, with running wheel Scholander curves; Fig. 3B) and demanding (WFF Scholander curves; van der Vinne et al., 2014a) conditions.

Variation in daily temperature rhythm

The energetic consequences of T_{surface} variation between days was assessed by calculating the energetic benefit of a completely diurnal mouse over a completely nocturnal mouse and the optimal activity midpoint for all days in September and March in the years 1991–2010. Hourly T_{surface} data were obtained from the Eelde weather station (The Netherlands) for all days in September and March 1991–2010.

Competing interests

The authors declare no competing or financial interests.

Author contributions

V.v.d.V. and R.A.H. designed the research. V.v.d.V. and J.A.G. performed the measurements. V.v.d.V. and S.J.R. performed the modeling analyses. V.v.d.V. and R.A.H. wrote the manuscript.

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Supplementary material

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