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ORIGINAL PAPER

Seasonal variation in energy expenditure in a rodent inhabiting a winter-rainfall desert

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Abstract

Animals that spend more energy than they obtain risk entering allostatic overload, reducing survival and fitness. They are predicted to adjust their daily energy expenditure (DEE) during periods of food scarcity. Adjustments of DEE to changes in food availability have been well-studied in species in temperate zones during winter, but less so in species enduring seasonal droughts. Likely mechanisms regulating DEE involve adjustments of activity and maintenance metabolism. Species that experience seasonal droughts and changes in food availability, like the African striped mouse (*Rhabdomys pumilio*), are appropriate model organisms to study the regulation of seasonal changes of DEE. We quantified DEE using the 'doubly labelled water' method, measured resting metabolic rate (RMR), and concomitantly determined activity levels using all-day focal observations of 69 free-living striped mice in the cold moist season with high food availability and the hot dry season with low food availability. Striped mice decreased their DEE in the food scarce dry season using multiple mechanisms, especially reductions in RMR, and reduced overall physical activity. This was further facilitated passively by reduced thermoregulatory costs. Our study demonstrates that animals reduce DEE via active and passive mechanisms in food-restricted environments, and highlights that several environmental factors should be considered simultaneously when aiming to understand how animals cope with harsh environments.

Keywords Drought · Eco-physiology · Energetics · Field metabolic rate · Physical activity level · Phenotypic flexibility

Introduction

An individual's fitness is affected by the balance between acquisition and expenditure of energy, which is essential for maintenance, growth, survival and reproduction (Krackow 1989; Boutin 1990). In nature, availability of food, the primary source of energy for animals, varies both on a spatial and a temporal scale. For many species, seasonal changes in rainfall and temperature cause significant variation in food availability, often characterized by one season with super-abundant food (often the breeding season), and one season with low food availability, which has to be survived to reach the following breeding season. While climate change might increase the likelihood of periods of food scarcity, such as droughts and extreme weather events, so far we do not know in how far animals are able to reduce their daily energy expenditure (DEE) as an adaptive response to such events. When energy expenditure exceeds energy acquisition, allostatic overload occurs, which leads to a decrease in body condition, reduced fitness, pathologies and finally, if it persists, death due to starvation occurs (McEwen and Wingfield 2003; Romero et al. 2009). When energy acquisition is reduced and migration to other areas is not possible, decreasing energy expenditure is the only adaptive solution to avoid or reduce allostatic overload.

During food shortages, energy investment into reproduction is typically the first expenditure that is ceased

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(Wingfield et al. 1983). Other energy expenditures include maintenance metabolism, behavioural activity, thermoregulation (in endothermic species) and other metabolic activities (e.g. immune, digestive and homeostatic). Endotherms are restricted in the extent to which they can reduce their metabolism (Bennett and Ruben 1979; Fuglei and Oritsland 1999) [an exception being heterotherms which can employ hibernation or daily torpor (Ruf and Geiser 2015)]. The costs of thermoregulation decrease with increasing ambient temperature (T_a), and when the period of food scarcity co-occurs with high T_a, as for example in arid habitats, decreases in DEE can coincide with reduced costs of thermoregulation (Scantlebury et al. 2006; Warnecke et al. 2010).

During periods of food scarcity, animals use mainly two, not mutually exclusive, mechanisms to reduce their DEE. Animals can reduce their maintenance metabolism (Korhonen and Harri 1984; Merkt and Taylor 1994; Corp et al. 1997; Fuglei and Oritsland 1999; Lovegrove 2005), and are able to reduce their activity level during energy shortages or adverse environmental conditions (Green and Bear 1990; Zub et al. 2009). For example, animals might focus their activity on foraging while reducing other activities such as social contacts, patrolling territory boundaries, and thus overall activity (Gutman et al. 2007). Consequently, activity modifications might reduce DEE to a larger extent than reductions in maintenance metabolism, measured as resting metabolic rate (RMR), but few studies tested this assumption. To address the question of how animals cope with food shortages and to understand the mechanisms underlying reductions of DEE, it is crucial to concomitantly measure changes in activity and RMR.

To date, few studies on free-living animals have quantified DEE, activity and RMR concomitantly. Examples include breeding blue tits *Parus caeruleus*, which spend more energy and work harder (i.e. increase their physical activity level measured as DEE/RMR) when they mismatch the timing of breeding with food supply (Thomas et al. 2001), and male weasels *Mustela nivalis*, which are able to maintain a constant winter energy expenditure across varying temperatures by adjusting their activity levels (Zub et al. 2009). But it is not well-known how small non-migratory animals from seasonally arid environments adjust their energy expenditure seasonally. Degus *Octodon degus*, which inhabit semi-arid habitats, adjust their activity patterns (Bacigalupe et al. 2003) and decrease DEE, but not basal metabolic rate (BMR), in the hot and dry summer (Bozinovic et al. 2004). However, activity patterns and the two physiological measurements were not obtained on the same individuals within the same study. Therefore, a more comprehensive approach, with concomitant measures of DEE, RMR and activity, is needed to understand which mechanism/s are employed by free-living animals to adjust their DEE to food limitation during a dry season.

The African striped mouse *Rhabdomys pumilio* (Sparrman, 1784) inhabits an environment with significant seasonal changes in the availability of food, such that energy saving strategies would be adaptive. This species occurs in the Succulent Karoo, where periods of high T_a coincide with periods of low food availability, and RMR is predicted to decrease during both conditions. We reported in a recent study that food availability, not T_a, is the main driver of seasonal changes in RMR of striped mice (Rimbach et al. 2018). These characteristics make the species a suitable model organism to examine the influence of RMR and T_a on DEE independent of each other and to assess whether changes in physical activity enable reductions in DEE in periods of low food availability. We asked two questions. (1) Do striped mice decrease DEE in the dry season when T_a is high and food availability is low? (2) If so, is the reduction in DEE due to a reduced RMR, reduced costs of thermoregulation and/or a reduction in activity?

Materials and methods

Study site and animals

Data were collected from October 2014 until August 2015, covering two moist seasons (October to December 2014; June to August 2015) and the intervening dry season (January to May 2015) in Goegap Nature Reserve, Northern Cape Province, South Africa (29°41′56″S, 18°1′60″E). The study site lies within the semi-desert biome of the Succulent Karoo, which is characterized by cold and moist winters in which most of the annual rainfall (mean 160 mm) falls. Striped mice breed in spring when food is abundant, and summers are hot and dry with low food availability (Schradin and Pillay 2005, 2006).

At the study site, striped mice construct nests above ground under shrubs and live in social groups, which typically consist of one breeding male, two to four breeding females and their philopatric offspring of both sexes (Schradin and Pillay 2004). During the dry season, individuals can lose 10% or more of their body mass and subsequently gain body mass during the moist season, when food becomes abundant again (Schradin and Pillay 2005). To avoid the confounding effect of reproduction on DEE, data were collected outside the breeding season.

Food availability

We recorded the number of palatable food plants biweekly in eight monitoring plots of 4 m^2 each (Schradin et al. 2010). Palatability of plants was known from behavioural observations (Schradin and Pillay 2006).

Resting metabolic rate (RMR)

Using an open circuit respirometry system (Foxbox, Sable Systems, New Jersey, USA), we measured oxygen consumption (ml $O_2 h^{-1}$) in a respirometry laboratory at the research station. This system has been validated for the study species using comparisons between post-absorptive and fed individuals, lactating and non-lactating females and via repeated measurements and measurements at 20 and 30 °C, which found highly significant correlations between repeated measures of individuals. Metabolic chambers were immersed in a propylene container and the temperature was controlled using a temperature controller (Pelt5, Sable Systems). Outside atmospheric air was pumped (PP-2 v2, Sable Systems International) through a mass flow metre to four different air-tight metabolic chambers at a rate of 700 ml min^{-1}. Air flow was controlled by a flow regulator (FB8 Multichannel Mass Flow Meter, Sable Systems) placed upstream. From the chambers, air was sub-sampled at a rate of 300 ml min^{-1} and drawn into a multiplexer (RM8 Intelligent Multiplexer, Sable Systems International), and was used to direct air from the chambers to a portable O_2 and CO_2 analyser (FoxBox, Sable Systems International) and a relative humidity analyser (RH-300, Sable Systems International), which measured water vapour pressure (WVP). Fractional O_2 and CO_2 concentrations, WVP, barometric pressure (BP), ambient temperature (T_a) and flow rate (FR) were recorded every 3 s. To minimise drift, we calibrated the FoxBox weekly and the RH-300 every 3 months. We calibrated O_2 at 20.91% by scrubbing outside air with drierite and we zeroed CO_2 and WVP with N_2 (AFROX, South Africa). We spanned CO_2 with 0.92% CO_2 (AFROX, South Africa). We spanned WVP by subtracting O_2 moist from O_2 dry (with drierite), then multiplying it with BP and subsequently dividing it by O_2 dry. We checked the spanned values using a gas cylinder containing 21% O_2 and 0.5% CO_2 (AFROX, South Africa). To account for any drift in the analyser, we measured oxygen concentration in an empty baseline chamber for 5 min before and for 10 min after measurements were taken in the mouse chamber. We analysed metabolic records using the data acquisition software ExpeData (v 1.8.5; Sable Systems). In a first step, we corrected O_2, CO_2, and flow rate for water vapour dilution using the equations (Lighton 2008):

$$\text{Dry-corrected gas concentration} = \frac{\text{Gas concentration wet} \times \text{BP}}{(\text{BP} - \text{WVP})},$$

$$\text{Dry-corrected FR} = \frac{\text{FR wet} \times (\text{BP} - \text{WVP})}{\text{BP}}.$$

In a second step, we calculated RMR from the lowest level of O_2 consumption recorded for 89 consecutive readings, equalling 4.45 min., using the equation (Withers 2001; Lighton 2008):

$$VO_2 = \frac{FR \times \left(\left(FiO_2 - FeO_2\right) - FeO_2 \times \left(FeCO_2 - FiCO_2\right)\right)}{(1 - FeO_2)},$$

where FiO_2 and $FiCO_2$ are input fractional concentrations of O_2 and CO_2 to the chamber, respectively; FeO_2 and $FeCO_2$ are excurrent fractional concentration of O_2 and CO_2 from the chamber, respectively.

We captured individuals in the morning using Sherman-type traps (26 × 9 × 9 cm), took them to the laboratory, where we weighed (± 0.1 g) them. Between 10:00 and 11:00 we placed them in one of four metabolic chambers (1000 cm^2 each). We initiated oxygen measurements and measured metabolic rates at five different temperatures (5–25 °C; results not reported here) for 20 min each. This procedure took 6–7 h due to the time needed to change the temperature in the chambers. Subsequently, we measured oxygen consumption for 20 min at 30 °C ± 1 °C, which lies within the species' thermoneutral zone (Scantlebury et al. 2006). We confirmed the resting state of individuals by visual inspection of oxygen consumption rates and of video footage of the animals in the chambers recorded during metabolic measurements. We considered measurements only when individuals were inactive and did not show any signs of piloerection. After the measurements (between 17:00 and 18:00), we weighed mice again (± 0.1 g) and used the average body mass.

Our measures represent RMR and not BMR, because all individuals used in our study were young adults that were still growing, and thus our measures are violating the strict criteria used to determine BMR. Measures of BMR are obtained from post-absorptive, inactive, normothermic, non-reproductive, adult individuals during their inactive period and in their thermoneutral zone (McNab 1997). RMR is a slightly less rigorous measure than BMR because it allows violation of some of the 'basal' conditions, but it still requires that animals are resting within their thermoneutral zone (Speakman et al. 2004). One alternative could have been to measure sleeping metabolic rate. However, thermoregulatory benefits gained from sleeping in huddling groups are the main reason for group-living in striped mice (Schradin et al. 2012). Thus, separating individuals during the night might cause stress and consequently influence metabolic rate. RMR values reported in this study are lower (Schradin et al. 2009) or comparable (Haim and Fourie 1980) to values reported in previous studies on striped mice. Striped mouse's sleeping metabolic rate (SMR) measured in our field respiratory laboratory is not significantly different from our measures of RMR (paired data from $N = 25$; individuals RMR: mean ± SD = 0.76 ± 0.25 ml O_2 g^{-1} h^{-1}; SMR: 0.70 ± 0.17 ml O_2 g^{-1} h^{-1}; paired t test $t = -0.91$, $df = 24$, $P = 0.36$).

In total, we measured RMR of 69 individuals from 14 different groups (mean 5.1 ± SD 3.2 individuals per group), 39 in the dry season (20 females and 19 males) and 30 during the moist season (14 females and 16 males; Table 1). Of these 69 individuals, 40 remained restless in the metabolic chambers. Because activity during metabolic measurements leads to elevated metabolic rates (Jäger et al. 2017), RMR could not be measured reliably for these individuals. Thus, we modelled RMR data of these individuals using a LMM and a long-term dataset of 659 RMR measurements collected from 2014 to 2017, which included data from breeders and philopatrics (young adults remaining in their natal group) measured in dry and moist seasons. Although data for this study were collected exclusively on philopatric individuals, we included RMR data of breeders in the RMR dataset because a larger dataset should increase the predictive power for the modelled data. We used measured RMR as the response variable in the LMM. As predictor variables we used season (dry/moist), social category (breeder/philopatric), sex (male/female), the interaction between sex and body mass, body mass, square of the body mass and the interaction between body mass and square of the body mass. As random factors (intercept only) we used individual ID and year. To validate the accuracy of RMR values predicted by the LMM, we excluded 104 measured RMR data points, 52 per season (13 female breeders, 13 female philopatrics, 13 male breeders, 13 male philopatrics). Subsequently, we used the 'predict' function of the package 'stats' to predict the RMR values we excluded in the previous step based on the LMM. Measured and predicted RMR values correlated positively ($t_{102} = 6.57$, $P < 0.0001$, $r = 0.54$, Online Resource Fig. S1) and were not statistically different from each other (*t* test $t_{177.7} = -0.70$, $P = 0.48$). The root mean square error, the standard deviation of the residuals, determined using the package 'Metrics' in R3.3.3 (https://www.r-project.org/), was 7.06 and the mean difference between predicted and measured values was −0.69 kJ day^{-1} (± SD 7.06 kJ day^{-1}). Finally, we used the validated LMM to predict the missing 40 RMR measurements (i.e. individuals that we restless) for the DEE study.

Table 1 Overview of sample sizes per season and sex for measurements of DEE, RMR and all-day observation in 69 striped mice

	DEE, RMR, observation	DEE, RMR (without observation)
Moist		
Females	8	6
Males	8	8
Dry		
Females	11	9
Males	8	11
Total	35	34

Daily energy expenditure (DEE)

Using the two-points doubly labelled water (DLW) method (Lifson and McClintock 1966; Speakman 1997b), we determined DEE in a total of 69 philopatrics. Originally, we injected a total of 72 philopatrics, but three individuals had to be excluded (two males did not re-enter traps and one female was lost to predation by a raptor). We determined DEE for 30 individuals in the moist season and for 39 individuals during the dry season (Table 1). We weighed (± 0.01 g) individuals, anesthetized them with diethyl ether and subsequently took a first blood sample (100 µl) from the sub-lingual vein (Heimann et al. 2009) into non-heparined glass capillaries, which we then flame-sealed. To determine individual background isotope enrichment of ^{2}H and ^{18}O, we used the first blood sample which we took directly after RMR measurements. Subsequently, after disinfecting the individual´s abdomen, we injected a known dose [1.0 g of 97% ^{18}O and 0.35 g of 99.9% ^{2}H per kg of total body water (TBW)] of DLW intraperitoneally. To calculate the exact mass of DLW injected, we weighed syringes immediately before and after administration (± 0.0001 g, Mettler-Toledo balance). We kept individuals in Sherman-type traps without food or water, and after 1 h equilibration, we took a second blood sample (100 µl) from the sub-lingual vein to determine the maximum isotope enrichment. Subsequently, as a compensation for lost foraging opportunities, each individual received a food reward which comprised 7% of its body mass and consisted of 40% sunflower seeds and 60% apple. After another 30–45 min, we released individuals at their nests. We recaptured 53 individuals 24 h after the DLW injection, one individual after 36 h, 12 individuals after 48 h, and 3 individuals 60 h after injection, and collected a third blood sample (100 µl) to estimate the isotope elimination rate. In all animals recaptured, the enrichment in ^{18}O remained > 10 delta per mils above background. We kept blood samples in sealed glass capillaries in a fridge at 4 °C until transport to the IPHC-DEPE laboratory at CNRS, Strasbourg, France for isotope analysis.

Isotope analysis

We vacuum distilled blood samples for 5 min and injected 0.1 µL distillate into an elemental analyser with thermal conversion (TC/EA, Thermo, Bremen, Germany) which was connected to a continuous flow isotope ratio mass spectrometer (IRMS-DELTA V PLUS, Thermo, Bremen, Germany), as previously described (Chery et al. 2015). In the TC/EA, distillates were pyrolyzed at 1400 °C into H_2 and CO gas in a glassy carbon tube under pure He flow at 90 ml min^{-1}.

We further separated H_2 and CO at 110 °C on a molecular sieve GC column before sequential analysis in the isotope ratio mass spectrometer. We first drift-corrected results and optionally applied a memory effect correction. We normalized results versus the VSMOW2/SLAP2 international scale. We performed all analyses in quadruplet and re-analysed samples if SD exceeded 2‰ for ^{2}H and 0.2‰ for ^{18}O in more than three out of the four analyses per sample. We calculated TBW from the ^{18}O dilution space divided by 1.007 to correct for in vivo isotopic exchange (Racette et al. 1994). The average isotope dilution space ratio was 1.040 ± 0.009 (mean ± SD). We calculated the production rate of CO_2 from the single pool model as recommended for the body size of striped mice (Speakman 1997b; Speakman and Hambly 2016). Following Speakman (1997a) we calculated CO_2 production and converted it to DEE using Wier's equation assuming a food quotient (FQ) of 0.925 (July–December) or 0.945 (January–May). We calculated FQ (Black et al. 1986) based on proportions of the macronutrients protein, fat and carbohydrates in seasonal diets.

Physical activity level (PAL)

We determined the physical activity level (PAL) of individuals by dividing DEE by RMR (Speakman 1997a). To assess whether an individual's PAL was lower or higher than the expected activity level, we compared our results with the results obtained using a standard reference, the allometric equation ($DEE/BMR = 4.79 M_b^{-0.133}$) for PAL in relation to body mass (M_b) for eutherian mammals (Degen and Kam 1995) (see "Results").

Ambient temperature

We recorded average T_a experienced by individuals during the DLW experiment (T_{DEE}: average temperature between release of the individual after DLW injection and the collection of the third blood sample) using a radio-controlled weather station (Orion Weather Science, South Africa) which recorded T_a every 10 min. The weather station was located in the open ca. 20 cm above ground.

Focal observations during the entire activity period

At first capture, we marked all mice permanently with ear tags (National Band and Tag Co., Newport, KY, USA) and fitted individuals with radio-collars (Holohil, Canada) at least 3 days before focal observations. Radio-collars weighed 2.3–2.6 g (6.6–7.5% body mass); a previous study at the same field site indicated that carrying of radio-collars did not affect the behaviour of striped mice (Schradin 2008).

At the field site, striped mice are well-habituated to the presence of observers, due to the continuous monitoring of the population. To determine activity levels, we conducted a total of 35 all-day focal observations (a total of 395.7 h) (Schradin 2006), 16 in the moist season and 19 during the dry season. We observed individuals from the time they emerged from their nest in the morning until they returned in the evening and used radio-tracking to follow focal individuals and to determine their location when they were not visible (e.g. in shrubs). Every minute, we recorded all behaviours shown by the individual during the previous minute (Rimbach et al. 2016). We determined the activity level as the percentage of minutes each individual was physically active (i.e. traveling, foraging, feeding, climbing, jumping, digging, chasing another mouse, was chased by or fighting with another mouse) during the all-day focal observation. Using the track function on a GPS (eTrex Venture, GARMIN International, Olathe, Kansas), we determined the distance travelled by the observer as a proxy of the distance travelled by the observed individual (Rimbach et al. 2016). To establish whether the presence of the observer affected an individuals' DEE, we compared DEE of individuals on which focal observations ($N = 35$) were conducted with the DEE of individuals on which no focal observations were conducted, but that otherwise underwent the same procedures ($N = 34$).

Statistical analyses

We conducted all analyses with R3.3.3 (https://www.r-project.org/). All statistical tests were two-tailed, and the statistical threshold was set at $P < 0.05$. We used Shapiro–Wilk tests to test for normality and log-transformed DEE to meet the assumption of normality. In a first step, we used *t* tests to assess whether environmental and physiological factors differed between the moist and the dry season, the basic assumptions of this study. T_{DEE}, food availability, daily water turnover rate and TBW could not be transformed to reach normality. Thus, we used Mann–Whitney *U* tests to analyse seasonal differences in these factors and to determine whether focal observations influenced DEE. Due to the strong influence of body mass on DEE and RMR (Meerlo et al. 1997), we used residual DEE and residual RMR, calculated as the residuals of the regression of log-DEE (or log-RMR) on log-body mass, in all analyses and all models.

1. Does DEE change seasonally?

To assess whether DEE changed seasonally and whether focal observations influenced DEE we used a LMM (Model 1). We used season, sex and whether a focal observation was conducted (yes/no) as fixed factors, and individual ID as a random factor.

2. Influence of RMR, activity and T_{DEE} on seasonal changes in DEE

(a) Which proxy of activity should be used?

To determine whether PAL (Model 2), activity (Model 3) or distance travelled (Model 4) explained more variation in DEE, we compared three LMMs. In addition to the estimate of activity (PAL, activity or distance travelled), we included RMR and T_{DEE} as explanatory variables and individual ID and group ID as random factors. We compared the Akaike Information Criterion (AIC) of the three models and Model 2 (PAL) had the lowest AIC and also the highest R^2 (see "Results"), which is why in subsequent models we used PAL as a proxy for activity. Using a Mann–Whitney *U* test, we examined seasonal differences in PAL.

For all LMMs, we standardized (z-transformed) all numeric predictors for more accurate model fitting and to facilitate comparisons of model estimates (Schielzeth 2010). We tested two-way interactions between all factors and dropped non-significant interaction terms in all LMMs. We verified models by inspecting *Q*–*Q* plots and by plotting model residuals against fitted values. We checked variance inflation factors (Zuur et al. 2010) using the 'vif' function in the car package (Fox and Weisberg 2011), which did not indicate collinearity (all vifs < 2). To assess model stability, we run diagnostics (dfbetas), which did not suggest the existence of influential cases. We determined $R^2_{(c)}$ with the function 'r.squaredGLMM' from the MuMIn package (Bartoń 2013). $R^2_{(c)}$ ('*c*' stands for conditional) indicates the variance explained by both fixed and random factors.

3. Relationship between DEE and RMR

We used Pearson's product-moment correlations to assess the relationship between (1) RMR and DEE, (2) PAL and RMR, (3) PAL and body mass and (4) PAL and T_{DEE}.

Results

Seasonal changes in environmental and physiological factors

T_{DEE} was higher and food availability was lower in the dry season than in the moist season (Table 2; Fig. 1a, b). DEE, RMR, body mass and activity level of striped mice were higher in the moist season than in the dry season (Table 2; Fig. 1c–e, h). Distance travelled and PAL did not vary significantly between seasons (Fig. 1f–h).

Table 2 Data for striped mice in the dry season and moist season

	Dry season ($N=39$)	Moist season ($N=30$)	*P* value
Body mass (g)	32.9 ± 4.2	35.3 ± 4.8	**0.041**
N_O ^{18}O (mol)	1.23 ± 0.2	1.3 ± 0.2	**0.045**
N_H ^{2}H (mol)	1.28 ± 0.2	1.4 ± 0.2	0.073
TBW (%)	66.7 ± 2.8	67.0 ± 6.5	0.116
rH_2O (mL day^{-1})	4.7 ± 3.1	12.4 ± 5.6	**< 0.0001**
DEE (kJ day^{-1})	25.2 ± 8.5	36.1 ± 9.1	**< 0.0001**
RMR (kJ day^{-1})	20.3 ± 4.6	28.6 ± 6.3	**< 0.0001**
Food availability	1.3 ± 0.4	4.6 ± 1.9	**< 0.0001**
T_{DEE}	24.1 ± 2.9	17.9 ± 5.6	**< 0.0001**

Significant seasonal differences are in bold

N_O dilution space for ^{18}O, N_H dilution space for ^{2}H, *TBW* total body water, rH_2O daily water turnover rate, *DEE* daily energy expenditure, *RMR* resting metabolic rate, T_{DEE} average ambient temperature (T_{DEE}) experienced by individuals during the DLW experiment. Data are means ± SD

Does DEE change seasonally?

DEE differed seasonally (LMM, estimate ± SD 0.283 ± 0.049, $t = 5.75$, $P < 0.0001$) and was higher in the moist season (Table 2). DEE was not influenced by sex (LMM, estimate ± SD −0.063 ± 0.050, $t = -1.25$, $P = 0.21$) and whether or not a focal observation was conducted (LMM, estimate ± SD 0.046 ± 0.050, $t = 0.91$, $P = 0.35$; Online Resource Fig. S2).

Which environmental and physiological factors explain seasonal changes in DEE?

(a) What is the best proxy of physical activity?

Of the three proxies of physical activity, only PAL significantly influenced DEE, and the model with PAL had the lowest AIC and highest R^2 (Table 3). Using PAL as a proxy of activity indicated that the effect of PAL on DEE was dependent on T_{DEE} (Table 3a; Fig. 2). RMR significantly influenced DEE for all three proxies (Table 3). T_{DEE} alone did not influence DEE, but RMR influenced DEE to a greater extent at lower temperatures than at higher temperatures in the model using activity (Table 3b).

Relationship between DEE and RMR

RMR positively correlated with DEE when data from both seasons were combined (Pearson's product-moment correlation $t_{67} = 2.05$, $P = 0.04$, $r = 0.24$), but not when seasons were examined separately (dry $t_{37} = 0.95$, $P = 0.34$, $r = 0.15$; moist

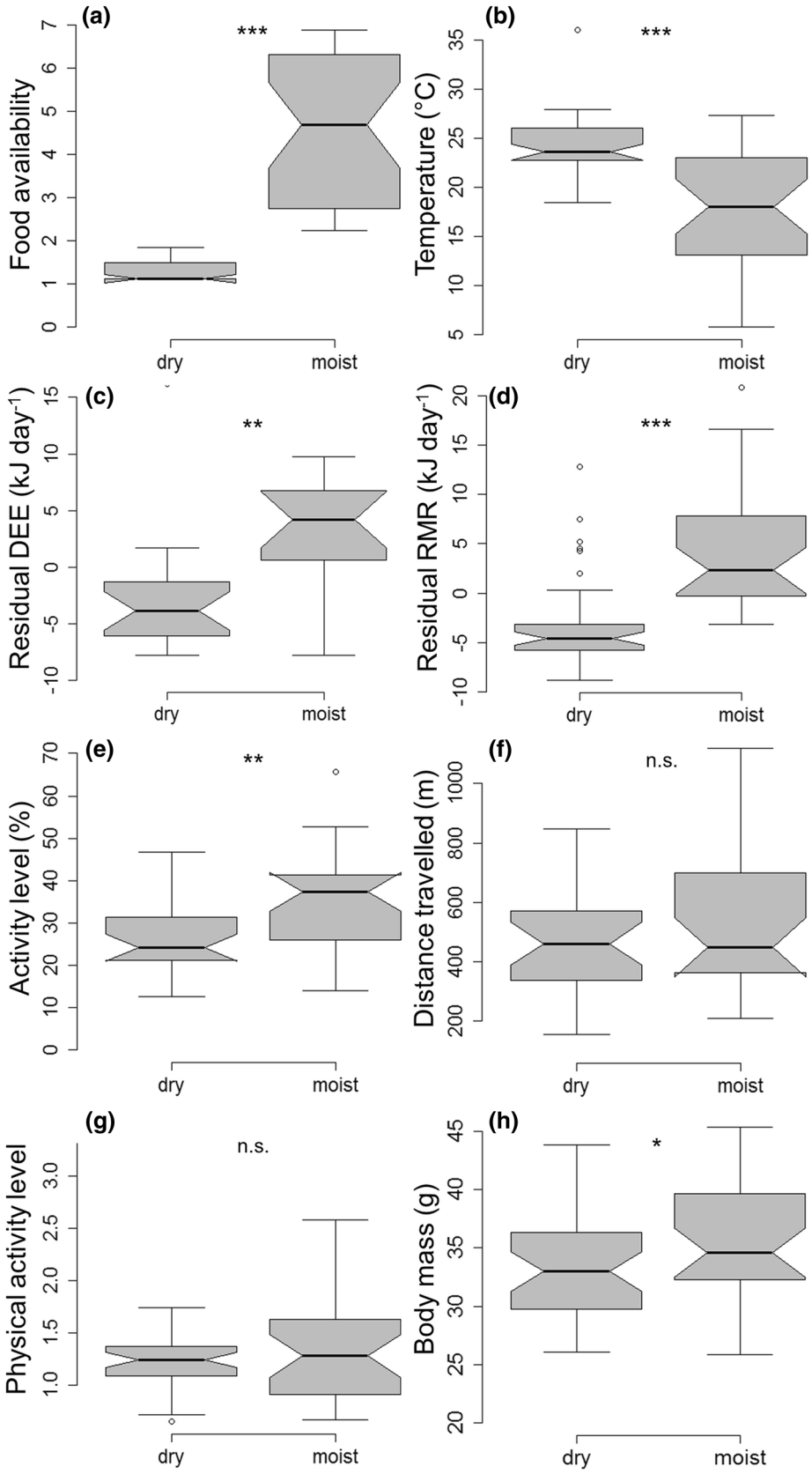

Fig. 1 Changes in physiological, behavioural and environmental factors. Panels show **a** food availability ($N=69$), **b** T_{DEE} ($N=69$), **c** residual DEE ($N=69$), **d** residual RMR ($N=69$), **e** % of time spent active ($N=35$), **f** distance travelled ($N=35$), **g** physical activity level ($N=69$) and **h** body mass ($N=69$) of striped mice in the dry season and moist season (black lines indicate the median, notches show the 95% confidence intervals, whiskers show minimum and maximum values of non-outlier data and open circles show outliers). Significant differences are illustrated (*$P<0.05$; **$P<0.01$, ***$P<0.001$)

Table 3 Relationship between DEE and different proxies of activity

Model 2: $R^2_{(c)}=0.63$; AIC = 134.0	Estimate	SE	*t* value	*P*
(a) PAL				
Intercept	6.339	0.581	10.90	**< 0.0001**
Residual RMR (kJ day^{-1})	3.363	0.630	5.33	**0.0006**
T_{DEE}	−0.885	0.630	−1.40	0.1966
PAL	2.851	0.647	4.40	**0.0020**
PAL*T_{DEE}	−1.025	0.475	−2.15	**0.0309**
Model 3: $R^2_{(c)}=0.32$, AIC = 218.8	Estimate	SE	*t* value	*P*
(b) Activity				
Intercept	6.959	0.803	8.66	**< 0.0001**
Residual RMR (kJ day^{-1})	2.398	0.797	3.00	**0.0121**
T_{DEE}	−0.379	0.873	−0.43	0.6730
Activity	0.649	0.862	0.75	0.4680
Residual RMR*T_{DEE}	2.538	1.154	2.19	**0.0278**
Model 4: $R^2_{(c)}=0.33$; AIC = 218.4	Estimate	SE	*t* value	*P*
(c) Distance travelled				
Intercept	6.874	0.860	7.99	**< 0.0001**
Residual RMR (kJ day^{-1})	2.173	0.874	2.48	**0.0460**
T_{DEE}	−0.803	0.854	−0.93	0.3826
Distance travelled	0.759	0.840	0.90	0.4003
Residual RMR*T_{DEE}	2.222	1.121	1.83	0.1148

Results of LMMs examining the influence of residual RMR, T_{DEE} and (a) PAL, (b) activity and (c) distance travelled on residual DEE (kJ day^{-1}) in 35 striped mice. Significant contrasts are given in bold

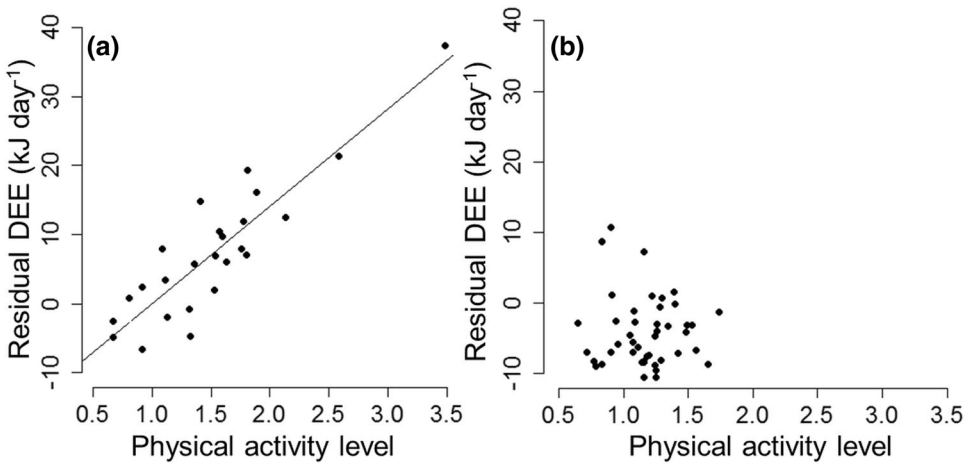

Fig. 2 Relationship between residual DEE and PAL at **a** cold temperatures (below the average of 21.4 °C) and **b** warm temperatures (above the average of 21.4 °C) in striped mice. At cold temperatures PAL and DEE positively correlated (Pearson's product-moment correlation $t=9.25$, $df=23$, $N=25$, $P<0.0001$, $r=0.88$), whereas there was no relationship at warm temperatures ($t=-0.33$, $df=42$, $N=44$, $P=0.73$, $r=-0.05$)

$t_{28}=-1.15$, $P=0.25$, $r=-0.21$, Fig. 3a). Striped mice had an average PAL of 1.29 (±SE 0.03), which resembled an averaged 43.2% (±SE 3.3%) of the values predicted by the allometric equation for eutherian mammals (Degen and Kam 1995). PAL significantly decreased with increasing RMR (Pearson's product-moment correlation $t_{67}=-3.05$, $P=0.003$, $r=-0.35$; Fig. 3b) and increasing T_{DEE} ($t_{67}=-2.12$, $P=0.03$, $r=-0.25$), and increased with increasing body mass ($t_{67}=2.88$, $P=0.005$, $r=0.33$).

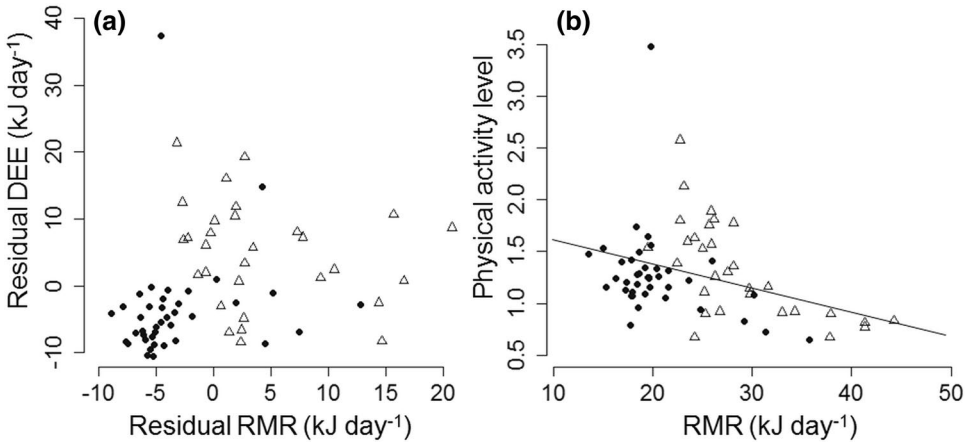

Fig. 3 **a** Relationship between residual RMR and residual DEE (Pearson's product-moment correlation dry season: $t_{37} = 0.95$, $P = 0.34$, $r = 0.15$; moist season: $t_{28} = -1.15$, $P = 0.25$, $r = -0.21$) and **b** relationship between RMR and PAL (Pearson's product-moment correlation: $t_{67} = -3.05$, $P = 0.003$, $r = -0.35$). Filled circles represent the dry season ($N = 39$) and open triangles the moist season ($N = 30$)

Discussion

To adjust to a seasonally changing environment, animals are predicted to reduce their energy expenditure during periods of low food availability. Here, we showed that DEE of African striped mice decreased in the food-restricted dry season mainly due to reduced RMR and reduced thermoregulatory costs. While they also reduced their activity in the dry season, our behavioural estimates of activity level (activity and distance travelled) were not significantly related to DEE, but our indirect estimate of behavioural activity (PAL) was. To our knowledge, this is the first study that simultaneously estimated DEE, RMR and activity in a small mammal living in a seasonally arid environment, offering comprehensive insights into the seasonal regulation of energy expenditure.

Relationship between DEE and RMR

PAL of striped mice decreased with increasing RMR, suggesting that RMR constituted an increasing fraction of DEE when DEE increased. Striped mice have a low PAL of 1.29, which resembles only 43.2% of the value predicted by the allometric equation for eutherian mammals (Degen and Kam 1995). In degus, occupying a semi-arid habitat, a similarly low PAL (1.6 which was 67% of the expected value) was reported during summer (Bozinovic et al. 2004). These values are lower than the postulated limit of four times RMR (Drent and Daan 1980) and the mean of 3.4 reported in a study including 62 small mammal species (Speakman 2000). These results suggest that striped mice, and likely other small mammals occupying semi-arid habitats, display lower levels of physical activity than small mammals living in other habitat types. This behaviour probably represents an adaptive strategy in a food-restricted environment. Further, when RMR increased, striped mice reduced their physical activity level, such that the overall increase in energy expenditure is rather moderate. Such a buffering function of adjustments in activity resembles a physiological strategy to cope with harsh environments, such as semi-arid habitats.

Which factors explain seasonal changes in DEE?

Surprisingly, although we found that striped mice were less active in the dry season, which could be an adaptive strategy to reduce energy expenditure, an individual's activity level (and distance travelled) did not significantly relate to DEE. Similarly, no relationship between DEE and measures of physical activity has been found in Hadza hunter-gatherers, free-living birds and captive mammals (Perrigo 1987; Jönsson et al. 1996; Pontzer et al. 2015; Edwards et al. 2017). Our results suggest that reductions in RMR and reduced thermoregulatory costs are the main mechanisms by which striped mice reduced DEE during the dry season. However, we cannot exclude the possibility that reduced activity also functions to reduce energy expenditure because it is possible that our proxies of activity from behavioural observations were not sufficiently accurate to estimate energy expenditure due to physical activity. We found that PAL, a widely used indirect estimate of the energetic cost of activity (Speakman 2000; Goran 2005; Simmen et al. 2010), influenced DEE, and that this effect was dependent on T_a. PAL and DEE were positively correlated at low, but not at high T_a, indicating that increases in activity co-occurred with increases in DEE at low T_a. One explanation for this finding is that the lower T_a gets, the more energy the individual spends, and therefore, it also has to increase its activity, for example, time spent foraging, to meet its energy demands.

Reductions in RMR during the dry season enabled striped mice to reduce their DEE. Similar decreases in RMR when T_a increases have been reported across a wide range of organisms (Lynch 1973; Tomasi and Mitchell 1996; Lovegrove 2003), including striped mice (Rimbach et al. 2017), where this decrease is driven by simultaneous changes in food availability (Rimbach et al. 2018). In

addition, thermoregulatory costs in endotherms decrease as T_a increase. Thermoregulatory costs are high for small mammals because of their large surface-to-volume-ratio and high thermal conductance (Degen and Kam 1995). T_{DEE} was significantly higher in the dry season and below the thermoneutral zone of striped mice both during the dry and the moist season, such that overall thermal stress can be assumed to be lower in summer than in winter. In striped mice living in the Succulent Karoo, reductions of thermoregulatory costs in summer, implied here by increased T_a, are linked to decreases in DEE. Thus, the temporal coincidence of low food availability with high T_a seems to enable striped mice to cope with this aspect of environmental harshness by passively reducing DEE.

Individuals living in more productive habitats typically have a higher maintenance metabolism than individuals of the same species living in less productive habitats (Mueller and Diamond 2001; Bozinovic et al. 2009). Animals living in food-restricted environments can additionally save energy via different mechanisms. They can actively reduce their RMR (Merkt and Taylor 1994; Ostrowski et al. 2006) and behavioural activity (Bacigalupe et al. 2003) and/or passively save energy if at the same time costs for thermoregulation decrease as T_a increases (Lynch 1973; Tomasi and Mitchell 1996; Lovegrove 2003). For example, reduced activity is linked to the conservation of energy when the thermoregulatory costs of being active at high/low temperatures outweigh benefits gained from foraging. Thus, for animals living in environments where thermal stress (very high or very low temperatures) co-occurs with food restriction, adjustments of energy expenditure will be more challenging, and additional strategies to avoid thermal stress might be needed. Consequently, the effects of climate change might be especially detrimental in environments where several environmental stressors (e.g. decreased food availability in the cold season) temporally co-occur (Rymer et al. 2016).

Conclusions

To be able to cope with environmental harshness, such as droughts, animals need to be able to rely on different physiological and behavioural mechanisms (Rymer et al. 2016). Collectively, our results indicate that striped mice reduce energy expenditure during the food-restricted dry season by several mechanisms, especially reductions in RMR and to a lower degree reduced physical activity. These reductions are further aided by reduced thermoregulatory costs due to higher T_a during the food scarce period. Our results indicate multiple mechanisms of how animals can avoid entering allostatic overload when faced with seasonal food shortages. This study demonstrates that it can be important to consider several environmental factors and whether they simultaneously increase or decrease in intensity (here shown on the example of food availability and T_a) when trying to understand how animals cope with the harshness of their environment. Thus, animals exposed to increased harshness due to climate change might be able to employ different coping mechanisms which must be studied at the same time, and it will be species-specific at which level resilience to change will collapse.

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Author contributions RR, CS, NP, SB conceived the idea and designed methodology; RR and MG collected the data; RR and AZ analysed the blood samples, RR analysed the data; RR, CS and NP led the writing of the manuscript. All authors contributed to the drafts and gave final approval for publication.

Compliance with ethical standards

Conflict of interest No competing interests are declared.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Animals were captured and handled following protocols approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC 2014/40/B).

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