

## RESEARCH ARTICLE

# Thermal performance curve of endurance running at high temperatures in deer mice

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## ABSTRACT

The impacts of warming temperatures associated with climate change on performance are poorly understood in most mammals. Thermal performance curves are a valuable means of examining the effects of temperature on performance traits, but they have rarely been used in endotherms. Here, we examined the thermal performance curve of endurance running capacity at high temperatures in the deer mouse (*Peromyscus maniculatus*). Endurance capacity was measured using an incremental speed test on a treadmill, and subcutaneous temperature in the abdominal region was measured as a proxy for body temperature ( $T_b$ ). Endurance time at 20°C was repeatable but varied appreciably across individuals, and was unaffected by sex or body mass. Endurance capacity was maintained across a broad range of ambient temperatures ( $T_a$ ) but was reduced above 35°C.  $T_b$  during running varied with  $T_a$ , and reductions in endurance were associated with  $T_b$  greater than 40°C when  $T_a$  was above 35°C. At the high  $T_a$  that limited endurance running capacity (but not at lower  $T_a$ ),  $T_b$  tended to rise throughout running trials with increases in running speed. Metabolic and thermoregulatory measurements at rest showed that  $T_b$ , evaporative water loss and breathing frequency increased at  $T_a$  of 36°C and above. Therefore, the upper threshold temperatures at which endurance capacity is impaired are similar to those inducing heat responses at rest in this species. These findings help discern the mechanisms by which deer mice are impacted by warming temperatures, and provide a general approach for examining thermal breadth of performance in small mammals.

**KEY WORDS:** Exercise, Metabolism, Thermoneutral zone, Hyperthermia

## INTRODUCTION

Climate change is having pervasive impacts on animals. Warming temperatures associated with climate change are causing population declines in many species, and the ranges of many species are shifting towards higher latitudes and elevations (Moritz et al., 2008; Myers et al., 2009; Rowe et al., 2015). To help predict the future impacts of climate change, there is growing demand for mechanistic explanations for how climate warming affects organismal physiology, health and performance (Evans et al., 2015; Huey et al., 2012; Pörtner and Knust, 2007; Seebacher and Franklin, 2012). Among mammals, however, much of the evidence for climate-driven phenotypic changes has been phenological in nature

(e.g. changes in reproductive timing, emergence from hibernation, etc.) (Boutin and Lane, 2014; Inouye, 2022). Although there have been great strides in understanding the physiological impacts of warming temperatures and heat waves in some mammalian species (Hetem et al., 2014; McKechnie and Wolf, 2019), the physiological impacts of climate warming remain largely unknown for many others, and the general mechanisms for how climate warming impacts mammalian physiology and performance remain unresolved (Levesque and Marshall, 2021; Levesque et al., 2016; Mitchell et al., 2018).

Thermal performance curves (TPCs) are a valuable means of examining the impacts of temperature on organismal performance. Performance traits are often defined as the ecologically important tasks that animals carry out, such as locomotion or feeding. When TPCs are used to describe the effects of temperature on the full capacity to carry out a performance trait (e.g. maximum locomotory speed or endurance), they can help delineate the breadth of temperatures over which an animal can perform (Angilleta, 2009; Huey and Kingsolver, 1993; Rezende and Bozinovic, 2019; Schulte et al., 2011). Indeed, TPCs of performance capacities have been used extensively to examine the impacts of rising temperatures with climate change on many ectotherms (Deutsch et al., 2015; Eliason et al., 2011; Rezende and Bozinovic, 2019). TPCs have been used much less frequently to examine the impacts of rising temperature in endotherms (Glass and Harrison, 2022; Levesque and Marshall, 2021). More often, predictions for the effects of high temperature on mammals and birds have been based on the classic Scholander–Irving model of thermoregulation, which depicts how resting metabolic rate changes across ambient temperatures. However, the critical temperatures at which metabolism and other thermoregulatory traits change under resting conditions may not reflect the thermal limits of performance or tolerance (Boyles et al., 2019; Levesque and Marshall, 2021; Mitchell et al., 2018). Indeed, limits in the capacity to dissipate body heat at high temperatures may constrain the ability of endotherms to carry out tasks supported by high rates of aerobic metabolism (e.g. locomotion, reproduction) (Bao et al., 2020; Speakman and Król, 2010; Tapper et al., 2020; Zhao et al., 2020), and the temperatures at which this occurs may not necessarily alter resting metabolism. Unfortunately, few studies of endotherms have examined how performance capacities vary across a range of warm temperatures, or how effects of high temperature on performance relate to changes in metabolism and thermoregulation at rest (Levesque and Marshall, 2021).

The deer mouse (*Peromyscus maniculatus*) is a useful model for understanding the effects of temperature on performance capacities in small endotherms. Deer mice are nocturnal habitat generalists that are found in a broad range of thermal environments across North America, from extremely cold environments at high latitude in the Canadian Yukon and high elevation in the Rocky Mountains, to hot environments in the deserts of southwestern USA (Bedford and Hoekstra, 2015). However, some deer mouse populations are

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declining in parts of southern Canada and midwestern USA, where they are being displaced by the white-footed mouse (*Peromyscus leucopus*) at the northern extent of its range (Fiset et al., 2015; Myers et al., 2009, 2005; Sarah et al., 2016). Climate change is believed to be contributing to the decline of some deer mouse populations, but the physiological and/or ecological bases for these declines are poorly understood. We therefore sought to understand the potential impacts of high temperature on this species by determining the TPC of endurance running capacity. Running endurance is a performance trait that may have important fitness implications, as it supports voluntary activity, foraging and many other important locomotory behaviours (Davidson and Morris, 2001; Meek et al., 2009; Rehmeier et al., 2004; Sears et al., 2006). We also examined the effects of temperature on resting metabolism and thermoregulation, to understand whether the high temperatures that impair running performance have any physiological effects at rest.

## MATERIALS AND METHODS

### Captive deer mouse population

We studied *Peromyscus maniculatus* (J. A. Wagner 1845) raised in captivity under warm conditions to control for the potential effects of developmental conditions on endurance capacity. Wild deer mice were live-trapped near Kearney (Buffalo County, NE, USA; 40°49′48.0″N, 99°05′02.6″W). Mice were then transported to McMaster University (Hamilton, ON, Canada), where they were bred in captivity to produce first-generation lab progeny. These first-generation mice were raised to adulthood (6–18 months of age) at ~25°C with a 12 h:12 h light:dark photoperiod, and were housed in standard mouse cages (containing 7090 Teklad Sani-Chips® animal bedding; Envigo, Indianapolis, IN, USA) with unlimited access to water and standard rodent chow (Teklad 22/5 Rodent Diet formula 8640; Envigo). Animal husbandry conditions and experimental protocols were approved by the McMaster University Animal Research Ethics Board according to guidelines from the Canadian Council on Animal Care.

### Implantation of thermosensitive passive transponders

Thermosensitive passive integrated transponder (PIT) tags (micro LifeChips with Bio-therm technology; Destron Fearing, Dallas, TX, USA) were implanted subcutaneously in the abdominal region (just ventral to the abdominal cavity) and were used as a proxy for body temperature ( $T_b$ ). This was achieved by anaesthetizing mice with isoflurane and then injecting the PIT tag using a sterile trocar (Destron Fearing). Following injection, mice were administered 2 mg ketoprofen  $\text{kg}^{-1}$  body mass for analgesia, aroused from anaesthesia, and observed until breathing frequency and reflexes had returned to normal. Mice were then returned to their cage and monitored for at least 7 days before they were used in endurance running tests or in respirometry measurements at rest.

### Endurance running tests

Thirty mice (13 females, 17 males) from 7 distinct families were used for measurements of endurance running capacity, using procedures similar to those used successfully in house mice (Meek et al., 2009). Mice were run on a rodent treadmill (compartment dimensions of 384 mm×51 mm×102 mm; Columbus Instruments, Columbus, OH, USA) at an uphill incline of 5 deg. Incurrent airflow was maintained at 1 l  $\text{min}^{-1}$ , which was confirmed in preliminary experiments to avoid any substantial decreases in  $\text{O}_2$  levels or increases in  $\text{CO}_2$  levels in the chamber during running tests. Ambient temperature ( $T_a$ ) in the treadmill chamber was controlled

(0.1°C hysteresis) using a temperature control system (TMP-REG, Loligo Systems, Viborg, Denmark), in which feedback from a temperature probe inside the chamber was used to control a heating fan placed 15 cm away and directed towards the air intake at the front of the treadmill. Running was conducted during daylight hours (10:00–18:00 h local time). For each run, mice were first allowed to rest in the chamber for 10 min in the dark, achieved by covering the treadmill, and this cover was then lifted shortly before the treadmill belt started moving so the mice were run in the light. Mice underwent a series of three training sessions at a  $T_a$  of 20°C, each separated by 24 h, to become accustomed to the treadmill environment before any measurements of endurance running capacity. In training session 1, mice were run at 10  $\text{m min}^{-1}$  for 15 min. In training session 2, mice were run at 10  $\text{m min}^{-1}$  for 1 min and then 14  $\text{m min}^{-1}$  for 14 min. In training session 3, mice were run at 10  $\text{m min}^{-1}$  for 1 min, 14  $\text{m min}^{-1}$  for 1 min, and finally at 18  $\text{m min}^{-1}$  for 13 min. After all training sessions were completed, mice were allowed ~48 h to rest before tests of endurance running capacity.

Three tests of endurance running capacity were performed for each mouse, each separated by a ~72 h recovery period. The first and second testing sessions were completed at a  $T_a$  of 20°C to assess variation and repeatability of endurance running capacity across individuals, and to establish a baseline for each mouse for examining the effects of different  $T_a$ . The third testing session was completed at one of six  $T_a$ : 15, 20, 25, 30, 35 and 38°C. Equal numbers of mice were randomly assigned to each  $T_a$ , such that there were five mice run at each  $T_a$ . The highest temperature of 38°C was chosen because preliminary tests found that mice did not run consistently at temperatures higher than this. For each testing session, mice were first allowed to rest in the chamber for 10 min in the dark. Mice were then subjected to incremental increases in treadmill speed: 10  $\text{m min}^{-1}$  for 2 min, 14  $\text{m min}^{-1}$  for 2 min, 18  $\text{m min}^{-1}$  for 2 min, 20  $\text{m min}^{-1}$  for 2 min and 21  $\text{m min}^{-1}$  for 2 min, increasing by 1  $\text{m min}^{-1}$  every 2 min thereafter.  $T_b$  was recorded at the end of each 2 min increment using a PIT tag reader (Global Pocket Reader Plus; Destron Fearing). As mice approached exhaustion, visual and audible cues (e.g. tapping on walls of the treadmill, bright light, etc.) were used to motivate them to run. Mice were considered to be exhausted when they were unable to run on the treadmill belt for 4 consecutive seconds. At this point, they were removed from the treadmill and placed into a dark chamber to recover for 15 min.

### Metabolic and thermoregulatory measurements at rest

To examine whether the high temperatures that impair running performance have any physiological effects at rest, mice were subjected to stepwise increases in temperature above their normal husbandry temperature of ~25°C. Effects of  $T_a$  on  $T_b$ , rates of  $\text{O}_2$  consumption ( $\dot{V}_{\text{O}_2}$ ) and evaporative water loss (EWL), and breathing frequency ( $f_R$ ) were measured in 14 mice (7 males, 7 females) from 5 distinct families. Measurements were made in a cylindrical respirometry chamber (Data Sciences International, St Paul, MN, USA) that was 500 ml in volume, and was connected to a sealed reference chamber via a pneumotachograph (for measuring  $f_R$  as described below). The respirometry chamber contained a metal platform that elevated mice above any faeces and urine that accumulated during the experiment. The entire respirometry apparatus was placed inside a Peltier-effect drop-in cabinet (Sable Systems International, Las Vegas, NV, USA) in which the  $T_a$  was controlled using a Peltier-effect temperature controller (PELT-5, Sable Systems International). Dry incurrent air (21%  $\text{O}_2$ , balance  $\text{N}_2$ )

was produced by mixing  $N_2$  and  $O_2$  from compressed gas cylinders using precision flow meters (Sierra Instruments, Monterey, CA, USA) and a mass flow controller (MFC-4, Sable Systems International). This incurrent air passed through a stainless-steel coil situated inside the Peltier cabinet in order to equilibrate with chamber temperature, and was then supplied to the chamber at a flow rate of  $600 \text{ ml min}^{-1}$ . A small video camera was placed inside the Peltier cabinet for monitoring the mice.

Mice were placed in the chamber at a  $T_a$  of  $26^\circ\text{C}$  and were given at least 60 min to adjust before measurements began, until they were noticeably relaxed with stable water loss and breathing pattern. Mice were then held for an additional 20 min at  $26^\circ\text{C}$ , after which they were exposed to stepwise increases in  $T_a$  of  $2^\circ\text{C}$  every 20 min until  $40^\circ\text{C}$ . Measurements were made during periods of inactivity in the last 10 min at each  $T_a$ . Incurrent flow rate was measured using a precision flow meter (Alicat Scientific, Tucson, AZ, USA). Incurrent and excurrent air flows were subsampled at  $200 \text{ ml min}^{-1}$ ; incurrent air was continuously analysed for  $O_2$  fraction (FC-10, Sable Systems International), and excurrent air was analysed for water vapour pressure (RH-300, Sable Systems International) and was then dried with pre-baked Drierite and analysed for  $O_2$  and  $CO_2$  fraction (FC-10 and CA-10, Sable Systems International). These data were used to calculate  $\dot{V}_{O_2}$  and EWL rate, expressed in volumes at standard temperature and pressure (STP) using established equations (equations 10.6 and 10.9 from Lighton, 2008).  $f_R$  was determined by measuring breathing-induced flows across the pneumotachograph using a differential pressure transducer (Validyne DP45, Cancoppas, Mississauga, ON, Canada) and carrier demodulator (Validyne CD15, Cancoppas).  $T_a$  was continuously recorded with a thermocouple connected to a thermocouple temperature meter (TC-2000, Sable Systems International). All the above data were acquired and analysed using a PowerLab 16/32 and Labchart 8 Pro software (ADInstruments, Colorado Springs, CO, USA).  $T_b$  was recorded using the PIT tag reader as above. Rectal temperature was measured using a rectal probe (RET-3-ISO, Physitemp) at the beginning and end of the experiment, when additional PIT tag measurements of  $T_b$  were also taken, in order to examine the relationship between subcutaneous temperature and rectal temperature.

### Statistics

Statistical analyses were performed in R (version 4.2.0) unless otherwise stated (<http://www.R-project.org/>). We calculated the repeatability of endurance running capacity between endurance trials 1 and 2 using the rptR package in R (Stoffel et al., 2017). Repeatability was calculated controlling for the fixed effect of sex and the random effect of family, standard error was quantified with 1000 bootstrap iterations, and  $P$ -values were calculated using likelihood ratio tests as described by the developer (Stoffel et al., 2017). Linear mixed-effects models were performed using the lme4 package in R (Bates et al., 2015), including mouse family as a random effect in all models. We tested for the effects of sex and body mass (covariate) on maximum endurance time at  $20^\circ\text{C}$  (i.e. the greater endurance time value from trials 1 and 2). We tested for the effects of sex,  $T_a$ ,  $T_a \times \text{sex}$  and body mass on endurance time, mean  $T_b$  and maximum  $T_b$  in trial 3. To account for inter-individual variation in baseline endurance capacity, we also included maximum endurance time at  $20^\circ\text{C}$  as an additional covariate for tests of endurance time in trial 3. We also tested for variation in body mass across groups for the mice used for endurance running measurements (i.e. tested for effects of sex,  $T_a$  and  $T_a \times \text{sex}$ ). We tested for the effects of sex,  $T_a$ ,  $T_a \times \text{sex}$  and body mass on

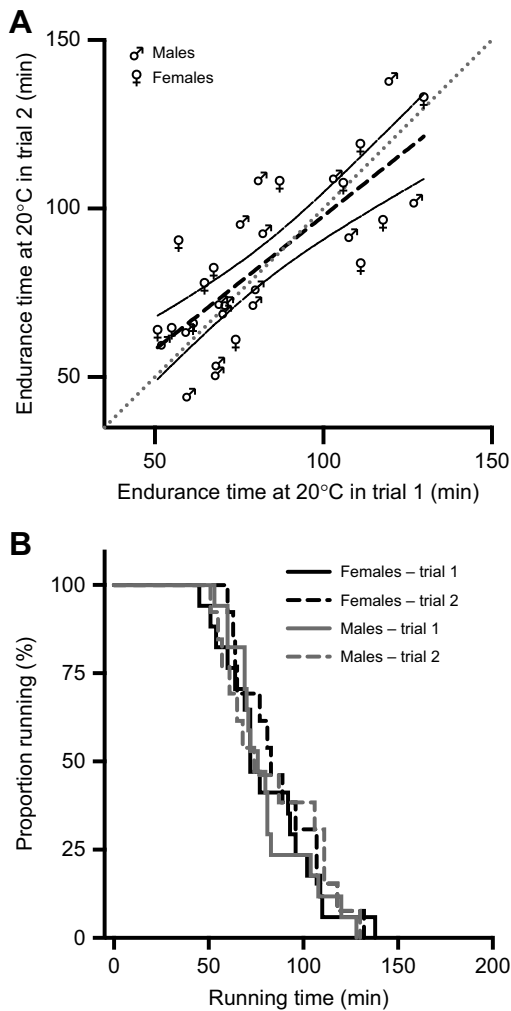
measurements at rest during respirometry, but we included individual as an additional random factor (nested within family) to account for repeated measurements. These analyses of respirometry measurements were carried out on absolute values of traits that were not normalized to body mass (because body mass was included as a covariate). We used the lmerTest package to generate ANOVA tables containing  $F$ - and  $P$ -values for fixed effects and interactions (Kuznetsova et al., 2017) (Tables 1 and 2). When effects of  $T_a$  were significant in the above models, we used the package emmeans (version 1.7.4; <https://CRAN.R-project.org/package=emmeans>) to make pairwise comparisons between all  $T_a$  using the Tukey method. Survival analysis was also used to examine running endurance, in which we plotted 'survival curves' of the proportion of mice still running over time, and carried out log rank tests to compare survival curves between groups using the survival package in R (<https://CRAN.R-project.org/package=survival>). Generalized additive mixed models were used to test for effects of  $T_a$ , sex and  $T_a \times \text{sex}$  on  $T_b$  over time in endurance trial 3 using the gam function in the mgcv package in R (Wood, 2017). Different  $T_a$  were allowed to have distinct smoothing curves, and smoothing curves could also vary by the random effect of individual (fs smooth term). Linear regressions were used to assess the relationship between subcutaneous temperature and rectal temperature measurements during respirometry (using Prism version 9.3; GraphPad Software, San Diego, CA, USA), and we used linear mixed-effects models to compare the difference between these measurements before versus after the respirometry trial (including sex and  $\text{sex} \times \text{measurement time}$  as fixed effects, and individual nested within family as random factors). Smoothing spline fitting of the relationship between relative endurance time and mean running  $T_b$  was carried out in Prism using 3 knots. Data are reported as  $\text{means} \pm \text{s.e.m.}$  and/or individual values.  $P < 0.05$  was considered to be significant.

## RESULTS

### Endurance running capacity

Endurance time measured at  $20^\circ\text{C}$  was repeatable but varied appreciably across individuals (Fig. 1). Endurance times ranged from 45 to 139 min and were highly repeatable within individuals, with a repeatability estimate ( $\pm \text{s.e.}$ ) of  $0.647 \pm 0.159$  ( $P < 0.0001$ ). Mice ran for the same duration on average between trials 1 and 2, as reflected by the regression of endurance times in trial 2 versus trial 1, for which the 95% confidence intervals included the line of equality (Fig. 1A). There was also a strong overlap in curves of the proportion of mice still running over time (Fig. 1B), with no significant variation across trials and sexes (log-rank test,  $\chi^2 = 0.50$ ,  $P = 0.90$ ). Maximum endurance time at  $20^\circ\text{C}$  (i.e. the greater endurance time value from trials 1 and 2) was unaffected by sex or body mass (Table 1). Body mass was  $21.5 \pm 0.9 \text{ g}$  ( $\text{mean} \pm \text{s.e.m.}$ ) overall and did not differ significantly between sexes (Table 1). These results suggest that inter-individual variation is substantial and needs to be accounted for when examining the effects of  $T_a$  on endurance running capacity.

Endurance time was strongly affected by  $T_a$  (Fig. 2, Table 1). This was supported by the highly significant effect of  $T_a$  on endurance time in trial 3 ( $T_a$  effect,  $P = 0.0020$ ). Endurance times were greatest on average at a  $T_a$  of  $25^\circ\text{C}$  ( $130.9 \pm 19.8 \text{ min}$ ;  $\text{mean} \pm \text{s.e.m.}$ ) followed by  $20^\circ\text{C}$  ( $109.4 \pm 15.2 \text{ min}$ ), and there were no pairwise differences in endurance time from  $15^\circ\text{C}$  to  $35^\circ\text{C}$  (Fig. 2A). Endurance time at  $38^\circ\text{C}$  ( $32.2 \pm 7.2 \text{ min}$ ) was significantly lower than that at both 20 and  $25^\circ\text{C}$  in pairwise *post hoc* tests between temperatures (Fig. 2A). Inter-individual variation in endurance running capacity was accounted for by including



**Fig. 1. Endurance running times were repeatable and varied between individuals.** (A) Symbols represent individual values for female and male deer mice, dashed black lines represent the linear regression ( $r^2=0.623$ ;  $P<0.0001$ ) and 95% confidence intervals, and the dotted grey line represents the line of equality. (B) Survival curves of the proportion running over time show a strong overlap between trials and sexes.

maximum endurance time at 20°C as a covariate in statistical models, and had a significant effect on endurance times in trial 3 ( $P=0.0044$ ). Neither sex nor body mass had any significant effects on endurance time in trial 3 (sex effect,  $P=0.200$ ; body mass effect,  $P=0.992$ ), and sex did not alter the effects of  $T_a$  on endurance time

( $T_a \times \text{sex}$ ,  $P=0.940$ ). There was no significant variation in body mass across  $T_a$  groups (Table 1).

$T_a$  also had strong effects on both the mean and maximum  $T_b$  during endurance running ( $T_a$  effects,  $P<0.0001$ ) (Table 1). Temperature was measured remotely from PIT tags implanted subcutaneously in the abdominal region as a proxy for  $T_b$  (see Materials and Methods). Mean running  $T_b$  was lowest at 20°C and increased progressively with increasing  $T_a$ , from an average of 37.1°C at a  $T_a$  of 20°C to 41.0°C at a  $T_a$  of 38°C (Fig. 2B). Maximum running  $T_b$  was  $\sim 0.4$ – $0.7^\circ\text{C}$  greater on average than mean  $T_b$ , but it exhibited a very similar pattern of variation with increasing  $T_a$  to that of mean  $T_b$  (Fig. 2C). Sex had no effect on mean  $T_b$  or maximum  $T_b$ , or how they varied between  $T_a$  (sex effects,  $P \geq 0.846$ ;  $T_a \times \text{sex}$ ,  $P \geq 0.239$ ; Table 1). At  $T_a$  from 15°C to 35°C,  $T_b$  tended to increase early in the endurance running trial to values that then remained relatively stable throughout the run (Fig. 3A). In most individuals,  $T_b$  varied by 0.5–1.0°C or less after the first 10–20 min of running, and no individuals exhibited continuous increases in  $T_b$  over time (Fig. S1). As a result, the time at which maximum running  $T_b$  was reached varied between individuals from early to late in the endurance running trial at  $T_a$  from 15°C to 35°C (Fig. S1). However, at a  $T_a$  of 38°C,  $T_b$  tended to increase throughout the endurance running trial (Fig. 3A), and 4 of 5 individuals reached their maximum running  $T_b$  at the point of exhaustion or within the last 5 min leading up to exhaustion (Fig. S1). Although average  $T_b$  appeared to change and/or become more variable at longer running times in some  $T_a$  groups (Fig. 3A), this was often coincident with reductions in the proportion of mice still running (Fig. 3B). The proportion of mice still running over time differed across  $T_a$  groups (log-rank test,  $\chi^2=49.7$ ,  $P<0.0001$ ) (Fig. 3B), as expected from the variation in endurance times (Fig. 2A). Increases in running  $T_b$  above  $\sim 40^\circ\text{C}$  appeared to be the approximate threshold for reductions in running endurance, as reflected by plots of relative endurance time (endurance in trial 3 relative to maximum endurance at 20°C) versus mean running  $T_b$  (Fig. 4).

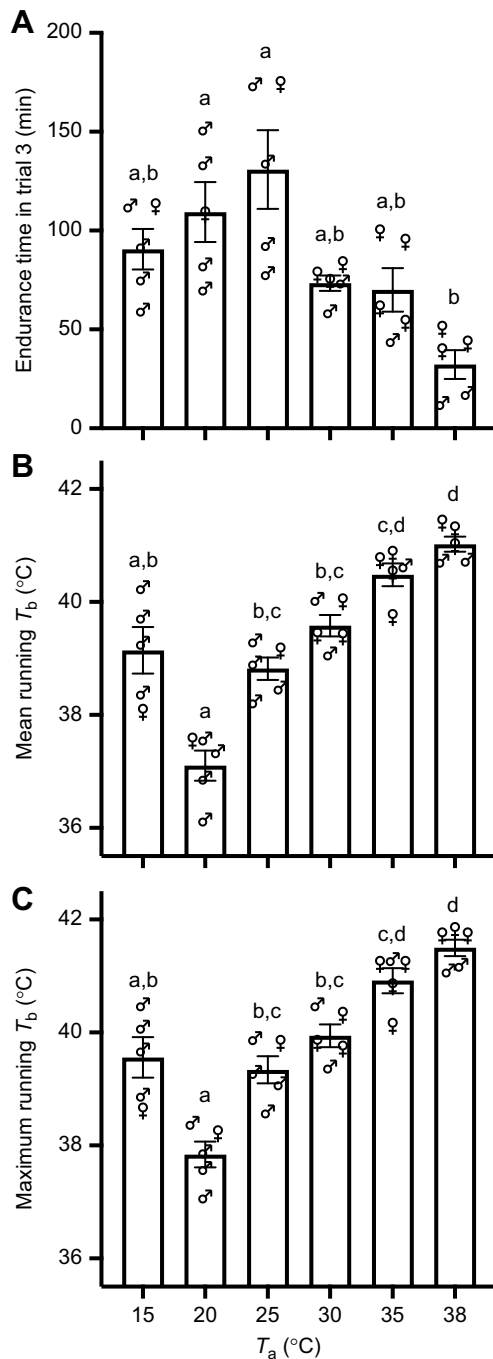
### Metabolic and thermoregulatory responses to acute warming at rest

There were strong effects of  $T_a$  on  $T_b$  ( $P<0.0001$ ),  $\dot{V}_{O_2}$  ( $T_a$  effect,  $P=0.025$ ), EWL ( $P<0.0001$ ) and  $f_R$  ( $P<0.0001$ ) (Fig. 5, Table 2). Values tended to be lowest between  $\sim 28$  and  $32^\circ\text{C}$  or a portion thereof.  $T_b$  increased significantly at and above a  $T_a$  of  $36^\circ\text{C}$ , and the difference between  $T_b$  and  $T_a$  was reduced to  $\sim 0.7^\circ\text{C}$  at a  $T_a$  of  $40^\circ\text{C}$  (Fig. 5A). Coinciding with this increase in  $T_b$  were significant increases in EWL (Fig. 5C) and  $f_R$  (Fig. 5D), suggesting that resting deer mice exhibit thermoregulatory responses to heat at a  $T_a$  of  $36^\circ\text{C}$ .

**Table 1. Statistical results for linear mixed-effects models of data collected during endurance running**

Trait	F-/P-value	$T_a$	Sex	$T_a \times \text{sex}$	$M_b$	Max. endurance
Max. endurance	F	–	0.399	–	0.041	–
	P	–	0.533	–	0.841	–
Endurance trial 3	F	6.36	1.79	0.238	<0.001	10.95
	P	<b>0.0020</b>	0.200	0.940	0.992	<b>0.0044</b>
Mean $T_b$	F	16.30	<0.001	1.37	2.23	–
	P	<b>&lt;0.0001</b>	0.997	0.299	0.159	–
Maximum $T_b$	F	17.62	0.039	1.56	1.30	–
	P	<b>&lt;0.0001</b>	0.846	0.239	0.271	–
$M_b$	F	0.147	0.424	0.283	–	–
	P	0.976	0.529	0.912	–	–

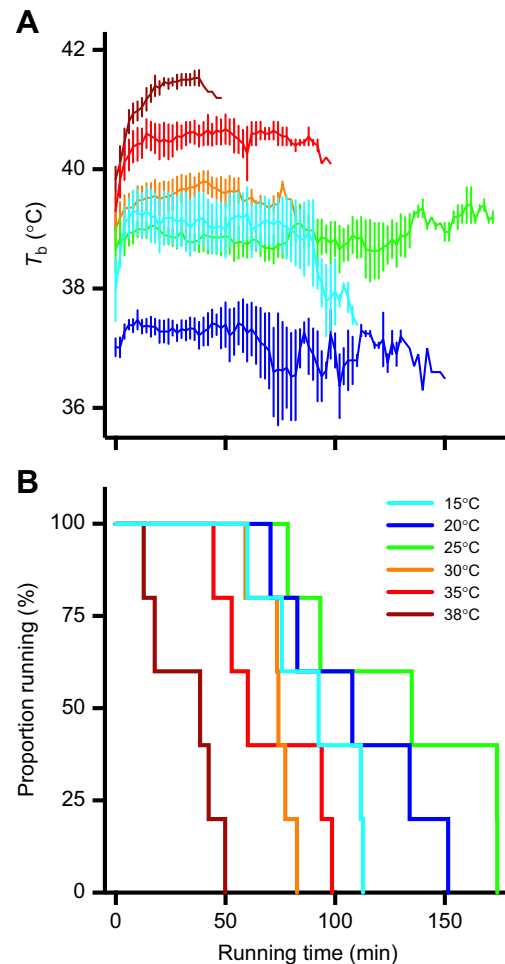
F- and P-values are given for fixed effects and interactions: ambient temperature ( $T_a$ ), sex,  $T_a \times \text{sex}$  interaction, body mass ( $M_b$ ), maximum endurance time at 20°C (the greater of the endurance times from trials 1 and 2), endurance time in trial 3, and mean and maximum body temperature ( $T_b$ ) measurements during trial 3. Bold indicates significance.



**Fig. 2. Endurance running capacity was reduced at high ambient temperature.** (A) Endurance running times were determined across a range of ambient temperatures ( $T_a$ ) in trial 3. (B,C) Temperature was measured using thermosensitive PIT tags implanted subcutaneously in the abdominal region as a proxy for body temperature ( $T_b$ ); mean (B) and maximum (C) values are shown. Bars indicate means $\pm$ s.e.m. and symbols represent individual values for males ( $\sigma$ ) and females ( $\varphi$ ) ( $n=5$  for each  $T_a$ ). Data were analysed statistically using linear mixed-effects models (Table 1) followed by pairwise comparisons between all  $T_a$  using the Tukey method. Different letters indicate significant pairwise differences between temperatures ( $P<0.05$ ).

The body mass of the mice used for these resting measurements was  $23.0\pm 1.5$  g (mean $\pm$ s.e.m.).

We also examined the relationship between subcutaneous temperature measured in the abdominal region with PIT tags

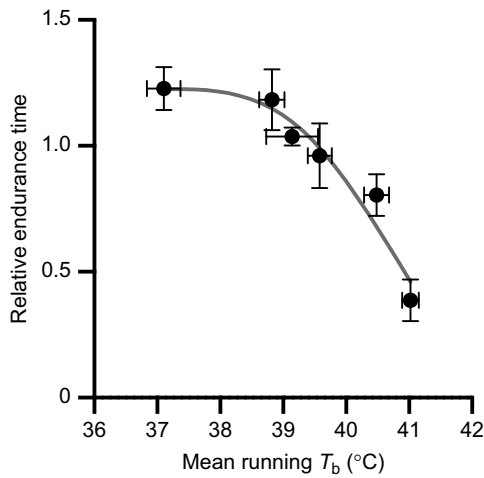


**Fig. 3.  $T_b$  throughout endurance running trials at different  $T_a$ .** (A)  $T_b$  was measured as in Fig. 2, every 2 min during running trial 3. Data are means $\pm$ s.e.m. for each  $T_a$  ( $n=5$  for each  $T_a$ ; data for individual mice are shown in Fig. S1). Generalized additive mixed models showed that there were significant effects of  $T_a$  ( $F=6.23$ ;  $P<0.0001$ ) but not sex ( $F=2.53$ ;  $P=0.112$ ) or  $T_a\times$ sex ( $F=0.692$ ;  $P=0.630$ ) on  $T_b$  over time. (B) The proportion of mice running decreased over time as individuals reached exhaustion.

(which was used throughout as a proxy for  $T_b$ ) and rectal temperature. This was achieved by taking each measurement concurrently both before and immediately after measuring the responses to acute warming (Fig. 5). Subcutaneous temperature was strongly correlated and was  $\sim 0.5$ – $1.0^\circ\text{C}$  cooler than rectal temperature on average (Fig. 6A). However, this difference was lower after the acute warming measurements (Fig. 6B), suggesting that subcutaneous temperature approached rectal temperature as the animals warmed up.

## DISCUSSION

The impacts of warming temperatures on performance are poorly understood in most endothermic species (Levesque and Marshall, 2021; Levesque et al., 2016; Mitchell et al., 2018). We addressed this knowledge gap by examining the TPC of endurance running capacity at high temperatures in the deer mouse. Endurance capacity was well maintained across a broad range of  $T_a$  from  $15^\circ\text{C}$  to  $30^\circ\text{C}$ , with the highest average value observed at  $25^\circ\text{C}$ , but endurance capacity was reduced above  $35^\circ\text{C}$  (Fig. 2).  $T_b$  during running varied with  $T_a$  (Fig. 2), and running endurance was reduced when  $T_b$  increased beyond  $40^\circ\text{C}$  when  $T_a$  was above  $35^\circ\text{C}$  (Fig. 4).  $T_b$  in

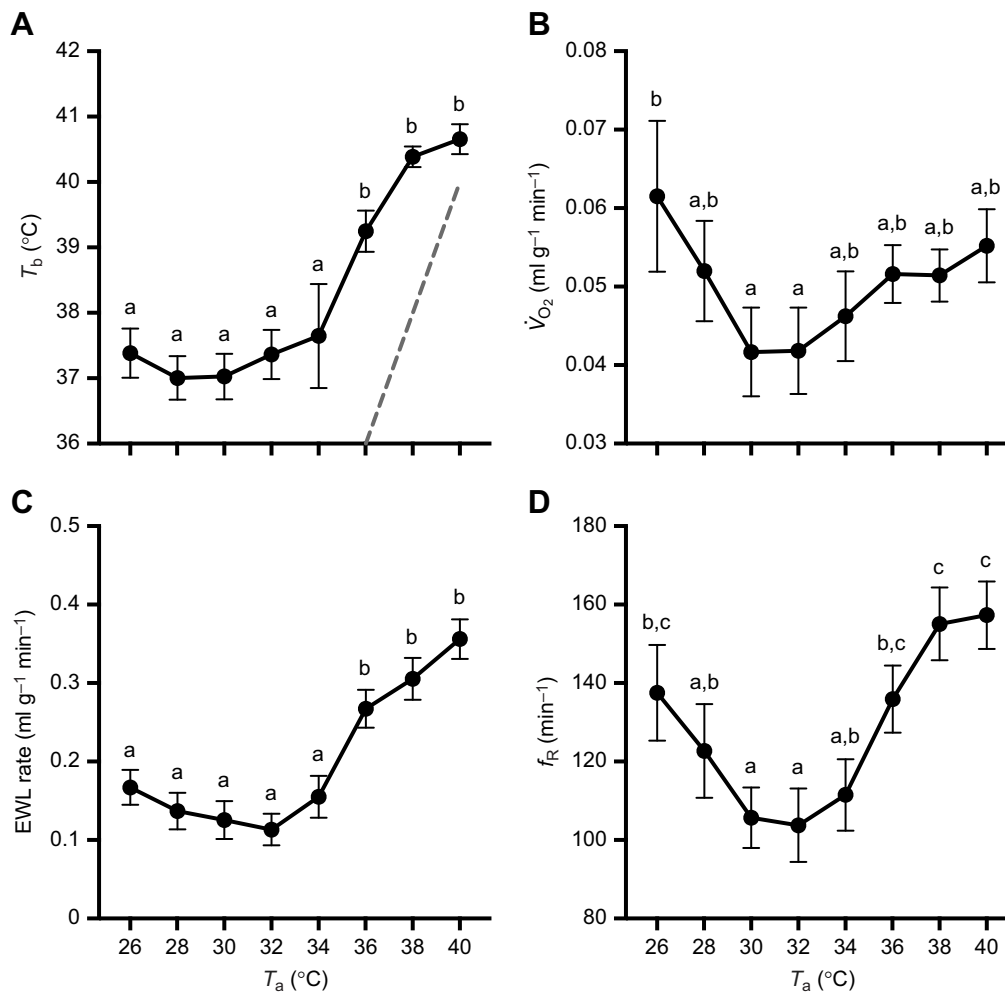


**Fig. 4. Endurance running capacity was reduced at high  $T_b$ .** Relative endurance time was calculated as the endurance in trial 3 relative to maximum endurance at 20°C. Symbols indicate means  $\pm$  s.e.m. and the grey line represents a smoothing spline fit of the data, used to illustrate the general pattern of variation without choosing a regression model or making any assumptions about the underlying relationships.

resting mice was increased at  $T_a$  at and above 36°C, coinciding with increases in EWL and  $f_R$  (Fig. 5). Therefore, deer mice maintain running performance across a broad range of  $T_a$ , and the upper

threshold temperatures at which performance is impaired are similar to those inducing heat responses at rest.

The thermal breadth of endurance running capacity was associated with some variation in  $T_b$ , in which performance was maintained up to a  $T_b$  of  $\sim$ 39–40°C (Fig. 2). The exact  $T_a$  at which running performance was impaired appeared to be between 35 and 38°C, based on the fact that there was a significant pairwise difference from 20°C and 25°C at 38°C but not at 35°C. The upper core temperature limit for running endurance may be slightly higher than observed here, because our proxy measure of  $T_b$  (subcutaneous temperature in the abdominal region) is expected to be slightly lower than core temperature (Lovegrove et al., 1991). However, this difference is often minimal in small mammals because internal temperature gradients are modest (Wacker et al., 2012), as supported by our findings here (Fig. 6). Furthermore, our observation that subcutaneous temperature approached rectal temperature as the animals warmed up (Fig. 6) suggests that the magnitude of this difference may have been very small during running. Deer mice appear to have similar or greater thermal breadth for running endurance than domestic house mice, based on observations that endurance is reduced at 34°C relative to 24°C in Swiss strain mice (Wanner et al., 2014). Reproductive performance of female Swiss mice is sensitive to similar  $T_a$  ( $\sim$ 33°C) to those that impair running endurance (Zhao et al., 2020), but it remains unclear whether the temperatures that impair running performance also impair reproduction or other performance traits in deer mice.



**Fig. 5. Thermoregulatory curves showing the effects of  $T_a$  at rest.**

(A)  $T_b$  (grey dashed line represents the line of equality), (B)  $O_2$  consumption rate ( $\dot{V}_{O_2}$ ), (C) rate of evaporative water loss (EWL) and (D) breathing frequency ( $f_R$ ) are shown as raw means  $\pm$  s.e.m. ( $n=14$ ; with 7 males and 7 females pooled) against  $T_a$  to illustrate general trends.  $T_b$  was measured as in Fig. 2. Data were analysed statistically using linear mixed-effects models (Table 2) followed by pairwise comparisons between all  $T_a$  using the Tukey method.  $\dot{V}_{O_2}$  and EWL rate are shown relative to body mass here, but absolute rates were used for statistical analyses and are shown in Fig. S2. Different letters indicate significant pairwise differences between temperatures ( $P<0.05$ ).

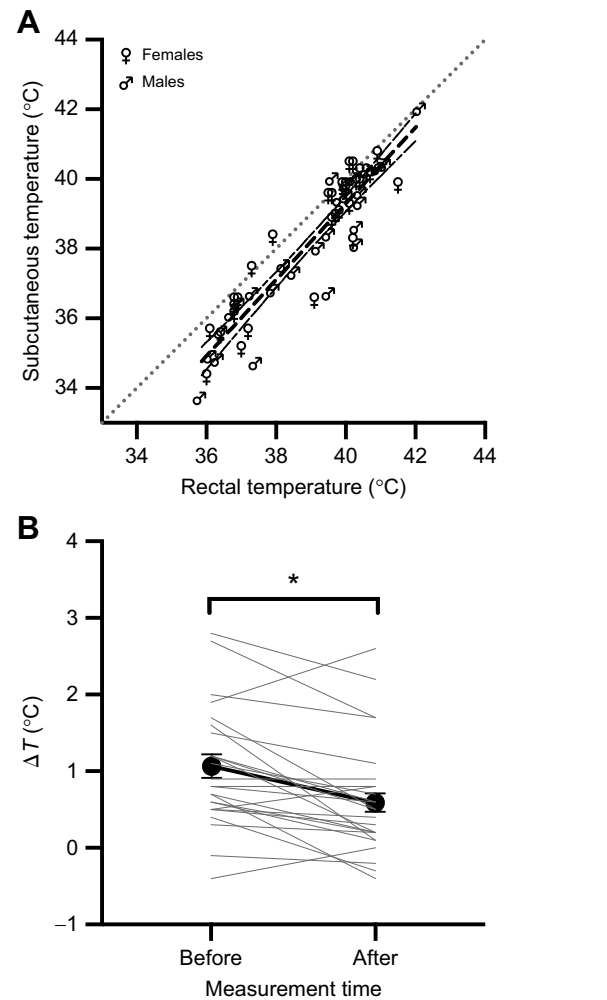
**Table 2. Statistical results for linear mixed-effects models of metabolic and thermoregulatory data collected at rest**

Trait	F-/P-value	$T_a$	Sex	$T_a \times \text{sex}$	$M_b$
$T_b$	F	20.57	0.487	0.412	0.399
	P	<b>&lt;0.0001</b>	0.521	0.892	0.541
$\dot{V}_{O_2}$	F	2.44	0.832	0.478	5.36
	P	<b>0.025</b>	0.398	0.848	<b>0.041</b>
EWL	F	19.74	1.372	0.446	4.89
	P	<b>&lt;0.0001</b>	0.266	0.871	<b>0.049</b>
$f_R$	F	9.028	0.015	0.576	2.17
	P	<b>&lt;0.0001</b>	0.907	0.773	0.169

F- and P-values are given for fixed effects and interactions: ambient temperature ( $T_a$ ), sex,  $T_a \times \text{sex}$  interaction, body mass ( $M_b$ ), body temperature ( $T_b$ ),  $O_2$  consumption rate ( $\dot{V}_{O_2}$ ), evaporative water loss rate (EWL) and breathing frequency ( $f_R$ ). Individual was included as a random factor in each model to account for repeated measurements. Bold indicates significance.

$T_b$  changed little over time with increases in running speed during the endurance test in most individuals at  $T_a$  from 15°C to 35°C (Fig. 3; Fig. S1). This finding in deer mice is consistent with previous work in Swiss mice, in which  $T_b$  did not vary with running speed at 24°C or 34°C (Wanner et al., 2014). Therefore, across the range of warm  $T_a$  that do not limit endurance capacity, deer mice may allow  $T_b$  to rise initially, but they appear capable of maintaining  $T_b$  thereafter as running intensity and metabolic heat production increase until exhaustion. At 38°C, however,  $T_b$  tended to rise over time and 4 out of 5 individuals reached their maximum running  $T_b$  at or near exhaustion (Fig. S1).  $T_b$  increases during intense metabolic activity when metabolic heat production exceeds heat dissipation, a difference that can arise when high  $T_a$  constrains heat dissipation (Speakman and Król, 2010). In larger mammals, for example,  $T_b$  rises progressively over time with increases in running intensity at high  $T_a$  (Francesconi et al., 1982; González-Alonso et al., 1999; Marlin et al., 1999; Mora-Rodríguez et al., 2008). Our findings here suggest that similar patterns of  $T_b$  variation are exhibited by deer mice at the high  $T_a$  that limit endurance running capacity. However, the magnitude of this  $T_b$  rise was somewhat modest considering the small difference between  $T_b$  measured during running and  $T_b$  measured at rest. For example, at a  $T_a$  of 38°C, average resting  $T_b$  was 40.4°C (Fig. 5A) and maximum running  $T_b$  was 41.5°C (Fig. 2C). This may explain why evidence of heat impacts occurred at similar  $T_a$  at rest (increased EWL and  $f_R$  at  $T_a \geq 36^\circ\text{C}$ ) and during running (reduced endurance at  $T_a > 35^\circ\text{C}$ ).

$T_b$  appeared to be elevated during running trials at 15°C compared with those at 20°C (Figs 2 and 3). One potential explanation for this finding is that exposure to any novel running temperature might have elevated  $T_b$ , whether above or below the 20°C temperature that mice were accustomed to from the training runs and from endurance trials 1 and 2. However, an alternative explanation is that mice running at 15°C were also experiencing cold-induced thermogenesis. The thermoneutral zone of deer mice was ~28–32°C (Fig. 5), consistent with previous findings (Brower and Cade, 1966; Hayward, 1965a; McNab and Morrison, 1963). Exposure of deer mice to temperatures of 15°C is known to increase  $\dot{V}_{O_2}$  to support thermogenesis in the cold (Brower and Cade, 1966; Chappell, 1985; Hayward, 1965a). In our endurance running protocol, mice were allowed to rest in the treadmill chamber at the target  $T_a$  for 10 min before running began, which would have provided ample time to mount a thermogenic response (Meyer et al., 2010). Once running subsequently began, some of the combined heat production from thermogenesis and locomotion may have been retained and thus led to the observed increase in running  $T_b$ .

**Fig. 6. Subcutaneous temperature was slightly less than rectal**

**temperature.** Subcutaneous temperature and rectal temperature were measured concurrently at rest before and immediately after the metabolic and thermoregulatory measurements shown in Fig. 5. (A) Subcutaneous versus rectal temperature. Symbols represent individual values for males and females, dashed black lines represent the linear regression ( $R^2=0.866$ ;  $P<0.0001$ ) and 95% confidence intervals, and the dotted grey line represents the line of equality. (B) The difference between rectal temperature and subcutaneous temperature ( $\Delta T$ ) was reduced at the end of the respirometry experiment (shortly after exposure to high  $T_a$ ), as reflected by linear mixed-effects models showing a significant effect of measurement time ( $F=19.80$ ;  $P=0.0001$ ) but not sex ( $F=2.20$ ;  $P=0.148$ ) or measurement $\times$ sex ( $F=2.35$ ;  $P=0.137$ ). Symbols indicate means $\pm$ s.e.m. and grey lines represent data for individual mice. \*Significant pairwise difference using the Tukey method ( $P<0.05$ ).

Exposure to much colder temperatures (0°C or -16°C) has been observed to reduce running performance in deer mice, likely due to competing demands for  $O_2$  between locomotion and thermogenesis (Chappell and Hammond, 2004), but the more modest demands for thermogenesis at 15°C did not appear to reduce running endurance here.

Deer mice examined here were found to maintain running endurance across a broad range of  $T_a$ , but the thermal breadth of running performance could vary in different populations from different parts of the native range. The mice studied here come from a Great Plains clade within the *P. maniculatus* species complex, and are genetically distinct from populations from colder temperatures at northern latitudes or high elevation and from southern desert

populations (Natarajan et al., 2015). Responses to and tolerance of cold and hot temperatures vary between populations (Hayward, 1965a; McNab and Morrison, 1963), and at least some of this variation likely arises from thermal plasticity (acclimatization, developmental plasticity, etc.) in response to distinct habitat temperatures across the broad range of the species. Local adaptation to distinct habitat temperatures likely also plays a role, based on recent evidence of selection along thermal clines in *Peromyscus* mice (Garcia-Elfring et al., 2019). The potential for thermal adaptation of running performance remains unclear but endurance capacity was highly repeatable and varied between individuals (Fig. 1), suggesting that selection could act on heritable variation in running performance in different thermal environments (Meek et al., 2009).

Our findings may have important implications for understanding the effects of warming temperatures associated with climate change. Running performance supports many locomotory behaviours that have important fitness consequences (Davidson and Morris, 2001; Meek et al., 2009; Rehmeier et al., 2004; Sears et al., 2006), so detrimental effects of warming temperatures on running performance might be expected to impact wild populations. However, the thermal breadth of endurance running capacity during acute changes in temperature was relatively wide in the Great Plains population of deer mice studied here, and endurance was not reduced until  $T_a$  exceeded 35°C. Deer mice are nocturnal and current environmental temperatures during the night-time active phase are rarely that high across much of their range (Hayward, 1965b; Morris and Kendeigh, 1981; Vose et al., 2017). Furthermore, thermal plasticity and thermal adaptation could lessen the direct impacts of high temperatures on running performance in hotter regions. Nevertheless, direct effects of high temperatures may limit important activities that deer mice carry out during the daytime, such as lactation associated with reproduction (Zhao et al., 2020). Daytime heat exposure could also have detrimental physiological effects that persist into cooler night-time periods and constrain locomotor activity, as examined in our companion paper (Flewwelling et al., 2023). Such effects of high daytime heat exposure may be an important issue in nocturnal rodents such as deer mice that have shallow burrows (Hu and Hoekstra, 2017), which do not provide full protection from the heat and can still experience high temperatures (Murray and Smith, 2012; Tracy and Walsberg, 2002). Understanding the effects of temperature on important performance traits is key to understanding the breadth of temperatures over which animals can perform, and to discern the mechanisms by which climate change is impacting animals in the wild.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: G.R.S.; Methodology: M.R.E., L.D.F., G.R.S.; Validation: M.R.E., L.D.F., G.R.S.; Formal analysis: M.R.E., G.R.S.; Investigation: M.R.E., L.D.F., T.W.; Resources: G.R.S.; Writing - original draft: M.R.E., G.R.S.; Writing - review & editing: M.R.E., L.D.F., T.W., G.R.S.; Supervision: G.R.S.; Funding acquisition: G.R.S.

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#### Data availability

The data that support the findings of this study are publicly available in the Figshare repository at <https://doi.org/10.6084/m9.figshare.22148084.v1>.

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