THE EFFECT OF CLIMATE WARMING ON THE METABOLIC PHYSIOLOGY AND ACTIVITY OF DEER MICE *(PEROMYSCUS MANICULATUS)*

THE EFFECT OF CLIMATE WARMING ON THE METABOLIC PHYSIOLOGY AND ACTIVITY OF DEER MICE *(PEROMYSCUS MANICULATUS)*

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LAY ABSTRACT

Global climate warming is a predominant issue in today's world and is causing dramatic changes in animals' ecology, overall health, and physiology. However, relatively few studies have investigated the impacts of increasingly high temperatures on endothermic species. My thesis focuses on the physiological and activity changes that occur with heat exposure and acclimation when endotherms transition into summer environmental temperatures. To address this question, I exposed North American deer mice to environmentally realistic summer temperatures and examined several physiological changes. My thesis contributes to understanding how climate warming impacts and will continue to impact endothermic animals.

ABSTRACT

As global temperatures continue to rise due to the effects of climate change, the ability of endotherms to property thermoregulate and maintain normal body temperature (T_b) may be challenged. The effects of warming summer temperatures on activity and thermoregulatory physiology in small mammals remain poorly understood. Using deer mice (*Peromyscus maniculatus*), a nocturnal species that maintains high activity in the wild allows us to investigate whether ecologically realistic summer ambient temperatures (T_a) will impact the physiology and activity of these animals. With exposure to warming temperatures up to 38°C, animals had a strong T_b dysregulation that culminated in a complete reversal of the diel pattern of T_b variation during the late weeks of the summer warming acclimation. During this, T_b surged to extreme highs (~40°C) during daytime heat but extreme lows (~34°C) at cooler nighttime temperatures. The decrease in nighttime T_b was associated with a decrease in thermogenic capacity and decreased mass and uncoupling protein (UCP1) content of brown adipose tissue. Activity, body mass and food consumption were also reduced, and water consumption was increased in later stages of summer warming. However, there was an increased capacity for heat dissipation through evaporative water loss (EWL) and a lower baseline metabolic rate typically of heat acclimation. Therefore, we propose that thermoregulatory trade-offs associated with heat acclimatization may limit nighttime activity, impacting the ability of nocturnal mammals to perform behaviours important for fitness in the wild.

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honest and gave your opinion while being supportive in the best way possible. I'm truly honoured to have worked with you, and I look forward to all the success that will come your way.

I'd like to leave this off with some sayings that my father would remind me of often, 1) be afraid of no one 2) keep your bullshit meter on high for those who want what is yours, 3) if you're on time you are late, and lastly, 4) be kind.

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LIST OF ABBREVIATIONS AND SYMBOLS

T_a: Ambient temperature

T_b: Body temperatures

TNZ: Thermoneutral zone

UCT: Upper critical temperature

LCT: Lower critical temperature

EWL: Evaporative water loss

f_R: Breathing frequency

O_2: Oxygen

CO_2: Carbon dioxide

iBAT: Interscapular brown adipose tissue

UCP1: Uncoupled protein 1

CS: Citrate synthase

COX: cytochrome c oxidase

HOAD: hydroxyacyl CoA dehydrogenase

LDH: lactate dehydrogenase

SEM: Standard error of the mean

ANOVA: Analysis of variance

V: Voltage

A: Amps

DECLARATION OF ACADEMIC ACHIEVEMENT

This thesis is organized in sandwich format, as recommended, and approved by members of my supervisory committee and approved by McMaster university. It consists of three chapters. Chapter 1 is an overview of the background material. Chapter 2 is a manuscript submitted to the Journal of Experimental Biology. Chapter 3 is an overview of the major findings of this thesis and how these findings relate to current knowledge, and the suggested future direction of research in this field.

CHAPTER 1:

GENERAL INTRODUCTION

CHAPTER 2:

Thermoregulatory Trade-Offs Underlie the Effects of Warming Summer Temperatures on Deer Mice

Authors: Luke D. Flewwelling, Oliver H. Wearing, Emily J. Garett, Graham R. Scott

Comments: The majority of data in this study was collected by L.D.F., under the supervision of G.R.S. O.H.W. performed and taught surgical procedures and E.J.G assisted in enzyme and protein quantification.

CHAPTER 3:

GENERAL DISCUSSION

CHAPTER 1: GENERAL INTRODUCTION

As global temperatures continue to rise due to the increasing effects of anthropogenic influences (Cook et al., 2013), these high ambient temperatures (T_a) are impacting the ecology, distribution, health, and physiology of many species (Mitchell et al., 2018; Pörtner and Knust, 2007). The effects of climate warming on the physiology of ectotherms such as insects (Kingsolver et al., 2011; Volney and Fleming, 2000) and marine and freshwater fishes (Ficke et al., 2007; Poloczanska et al., 2013) have been more widely studied than those in endotherms. Effects on ectotherms are well understood as T_a has a direct impact on their body temperature (T_b), metabolic rate, and ability to remain active (Deutsch et al., 2008). The literature on endotherms that exists tends to focus on the effects of T_a on large mammals (Hetem et al., 2014; McCain and King, 2014), and tropical animals (Lovegrove et al., 2014; McKechnie and Wolf, 2019; Tewksbury et al., 2008). Few studies focus on small temperate mammals such as mice (Bonebrake et al., 2020; McKechnie and Wolf, 2019). This is especially the case with nocturnal species, as they may be overlooked since they are not active during the most extreme changes in daytime high temperatures. However, it is unknown whether nocturnal animals are protected from the most significant effects of the high T_a during the day and/or if the negative effects of daytime heat will persist into the active nighttime phase.

In general, warming T_a can limit the ability to dissipate body heat generated by metabolism, as there is less of a temperature gradient between the animal and its environment. This tends to be accentuated in larger endotherms with a lower surface area to volume (SA/V) ratio, in which heat dissipation is a greater challenge. At rest, high T_a could threaten the ability of endotherms to maintain T_b, normally maintained within a relatively narrow range. Increases in T_b (hyperthermia) can be quite dangerous in endotherms, causing a range of physiological problems, such as protein damage, increased inflammatory response, apoptosis, hypoxia, DNA damage in germ cells, oxidative stress, mitochondrial dysfunction, liver damage, cerebral ischemia, embryonic

death (Speakman and Król, 2010), and can be fatal within a short amount of time (Yan et al., 2006). Indeed, the high T_a experienced by some tropical endotherms in recent years has pushed resting T_b upwards towards dangerous and even lethal temperatures (Danner et al., 2021; McKechnie and Wolf, 2019). However, less severe increases in T_a could reduce activity to minimize heat production through biochemical reactions. Therefore, endotherms may be forced to reduce activity to avoid increases in T_b (even when T_a are still low enough to maintain T_b at rest).

The impacts of environmentally relevant heat exposure on activity and energetics remain largely untested for many species (Levesque et al., 2016). This is especially true of many species of small nocturnal mammals. Studies have suggested that climate warming might have a greater negative impact on diurnal species, especially obligate diurnal species. Nocturnal species may have potential benefits as warming might increase nighttime temperatures into more favourable ranges allowing for increased locomotor performance, but whether this is indeed the case in small nocturnal species has received little empirical examination (Bonebrake et al., 2020; McCain and King, 2014). My thesis seeks to gain further insight into whether warming temperatures could impact the metabolic physiology of endotherms at rest and during activity by studying the North American deer mouse (*Peromyscus maniculatus*).

1.1 Impacts of warming temperature on animals

Changing temperatures in the ocean, land surface, and air are the most dominant consequence of recent climate change (Trenberth et al., 2007) and the main factor driving biotic responses (Oswald and Arnold, 2012). By the end of the 21st century, average global temperatures are predicted to increase further by ~1-5°C, resulting in dramatic changes and could even cause the disappearance of some of our current climates and ecosystems (Easterling et al., 1997; Williams et al., 2007). Many of these disappearing and changing climates are due to increasing global temperatures, decreasing seasonal and diurnal temperature variation, and changes in precipitation causing environmental conditions to rapidly transform (Easterling et al., 1997; Williams et al., 2007). These

changes will impact individuals in various ways, but species' traits that will directly impact the experienced environmental conditions are some of the most important to determine how species may be affected. Climate change is also predicted to have different impacts across latitudes, as higher latitudes are predicted to have a greater future increase in average T_a than lower latitudes (Hansen et al., 2006).

Species at higher latitudes typically have broad thermal tolerance (Sunday et al., 2019a; Sunday et al., 2019b) and are living in climates that are sometimes cooler than their physiological optima. Therefore, increasing T_a with climate warming during the daytime heat in more temperate conditions may enhance the performance and fitness of certain species as there may be less of an energetic demand on thermogenesis. However, this may not be the case in all species, and some endotherms at high latitudes may be more vulnerable to more extensive temperature changes in the future because of the adaptations they possess to minimize heat loss (McCain and King, 2014; Moritz et al., 2008; Oswald and Arnold, 2012). Conversely, inhabitants at lower latitudes and close to the equator tend to have a greater heat tolerance as that where the average T_a is higher (Spooner et al., 2018; Sunday et al., 2019a). However, due to the lower seasonal variability in T_a, and higher baseline T_a, animals at the equator may be more sensitive to changes in temperature (Spooner et al., 2018). Furthermore, they may already be living in conditions close to their optimal temperature or thermal limit (Deutsch et al., 2008; Oswald and Arnold, 2012; Tewksbury et al., 2008), and frequently show signs of hyperthermia (Lovegrove et al., 2014). Ultimately, organisms with the greatest risk of species extinction from rapid climate change are those with a low tolerance for warming, limited capacity for acclimation, and reduced dispersal capacity (Deutsch et al., 2008; Williams et al., 2007). Most terrestrial organisms with these characteristics are found in the tropics, where biodiversity is greatest, and many occupy changing and disappearing climates (Williams et al., 2007). Consequently, the most heat-tolerant species (those in the tropics and at low latitudes) may be the most at risk from a small increase in T_a due to climate warming (McCain and King, 2014; Sherwood and Huber, 2010). However, while our understanding of the effects of climate warming is improving for some endotherms,

we do not have a macroscale view of species' potential thermal tolerance across a global temperature gradient (Diamond, 2017).

Future changes to our climate and to thermally stressed animals may promote the formation of novel species associations and other ecological changes, whereas the drastic changes of some extant climates increases the risk of extinction for species with narrow geographic or climatic distributions and disruption of existing communities (Hobbs et al., 2006; Williams et al., 2007). While many animals respond negatively to climate warming with population extirpation, range contractions and decreases in population size (McCain and King, 2014; Moritz et al., 2008), individual species may increase ranges, growth rates and population size as warming environmental conditions may be favoured (McCain and King, 2014; Moritz et al., 2008). However, as each species will respond individualistically to this, it is difficult to predict how these climate changes will impact future species range shifts, extinction risks, biome shifts, altered disturbance regimes, and other ecological responses (Clark et al., 2001; Thomas et al., 2004; Williams et al., 2007). Metanalyses of more than 1700 species indicate that responses to rising temperature in the past has led to range shifts of 6.1 km per decade toward the poles on average (Parmesan and Yohe, 2003; Root et al., 2005). However, dispersal limitations may cause responses of some species to lag behind climate change, promoting the disequilibria between species distributions and climate, therefore furthering the impacts of climate warming on species. Impacts of climate change can be broadly categorized into either direct physiological effects (e.g. thermoregulation) or indirect biotically-mediated effects (e.g. resource availability; Oswald and Arnold, 2012). By studying the thermoregulatory physiology of animals, we can understand the plasticity, acclimation, and limiting factors associated with direct physiological effects that may be involved in the current and future biotic and abiotic impacts of climate warming.

1.2 Thermoregulatory Physiology

Thermoregulation is based on the heat balance equation (below), where heat dissipation must equal heat load and heat production for an endotherm to maintain a

stable T_b. Adjustments to heat dissipation and heat production are achieved through behavioural and physiological changes. The preoptic anterior area of the hypothalamus is used to detect the temperature of the brain and integrate afferent information from thermoreceptors in various other areas in the body, such as the spinal cord, internal organs, vasculature, and skin (Wendt et al., 2007). The hypothalamus can then initiate proper thermoregulatory responses in effector tissues to compensate for changes in temperature and thus maintain T_b within a narrow setpoint (Wendt et al., 2007).

Heat load + Heat production = Heat dissipation

Heat dissipation is a key component of the heat balance equation, and there are several factors that contribute to heat dissipation – radiation, evaporation, convection, and conduction. The importance and contribution of these factors to heat dissipation vary when exposed to various external conditions and influences, as well as morphological and physiological variations in species and individuals. For example, when experiencing high wind speeds, there is an increase in heat dissipation through convection and EWL, which can be beneficial at high T_a as metabolically expensive processes such as sweating may not be necessary to dissipate heat (Chappell and Holsclaw, 1984). Animals can also use morphological changes such as postural shifts to modify their surface area to volume (SA/V) ratio to maximize or minimize heat loss from radiation, conduction, and convection. There can also be modifications in blood flow to key sites of heat dissipation and thermoregulation such as the tail, ears and eyes. Given the high SA/V ratio and extensive vascularization near the skin's surface, blood flow can increase significantly in these areas when heat dissipation is necessary (Gordon, 2012; Hill and Veghte, 1976; Škop et al., 2020). The tails are especially important for control of heat loss as they contain arteriovenous anastomoses, allowing increased blood perfusion to the tail for heat dissipation (Gordon, 2012). While the ears of mice and rats do not have active control of blood flow, a very high SA/V ratio still allows for rapid heat exchange with the environment (Gordon, 1993).

The mechanisms of evaporation vary depending on species, for example, rodents use salivation and spreading saliva over the cutaneous surface or fur as well as panting compared to sweating (Morrison and Nakamura, 2011). As EWL has high energy cost and entails physiological alterations in osmotic stability, food must be available to fuel energetic demand, and water must stabilize this balance (Nilsson et al., 2016). However, when individuals have food and water shortages, they can reduce the high energetic costs associated with cooling mechanisms and enter a state of hyperthermia (Nilsson et al., 2016). However, during hyperthermia, evaporative cooling is the main mechanism of heat dissipation; in some species, EWL mechanisms can increase by as much as 80% (Sugenoya et al., 1998). However, the capacity to cope with high T_a is relatively limited, in part due to EWL being the principal mechanism used to dissipate heat in rodents (Mitchell et al., 2018; Tattersall et al., 2012). Indeed, recent studies of desert species of *Peromyscus* and other cricetids suggest that they have a limited capacity for evaporative cooling, potentially because saliva spreading is a far less effective means of evaporative cooling than panting (Ramirez et al., 2022). While cooling mechanism of vasodilation and EWL are normally described in separate literature, they are both broadly related, as vasodilation provides heat and blood plasma used as the fluid for EWL to occur through sweating, salivation, saliva sweating, and panting (Mota-Rojas et al., 2021; Nagashima, 2006).

Thermogenesis is an important mechanism for survival and normal physiological function in mammals. In cold environments, animals will increase heat production by shivering and non-shivering thermogenesis (van der Lans et al., 2013; Van Sant and Hammond, 2008) and reduce heat loss by decreasing thermal conductance (Haim, 1982; Pohl, 1965). In placental mammals, seasonal changes in thermogenic capacity are driven by modifications in the brown adipose tissue (BAT; Van Sant and Hammond, 2008). The BAT and skeletal muscle play an important role in regulating temperatures, though the relative contribution of each changes with age and size of the animal (Bal et al., 2017). BAT generates heat through uncoupling aerobic respiration from ATP production due to UCP1 and in small rodents can contribute up to 70% of thermogenic capacity

(McClelland et al., 2017; Nespolo et al., 1999). Muscle-based shivering thermogenesis also increases when acclimated to colder conditions by increasing succinate dehydrogenase activity, mitochondrial content, and vascularization (Bal et al., 2017)., though the capacity to increase shivering thermogenesis is significantly more limited than that of non-shivering thermogenesis (Coulson et al., 2021). These physiological changes to thermogenesis are important for proper thermoregulation and the fitness of species, especially those experiencing cold T_a.

While there are many traits that could determine how animals will be affected by climate change, traits that directly impact the environmental conditions experienced by the individual may be the most important. The mammalian response to climate change is mediated predominantly by body size, with larger mammals being more likely to respond to climate change. Large body size has been correlated to higher extinction risk while burrowing and hibernating is known to reduce this risk (Cardillo et al., 2005; McCain and King, 2014). While large mammals are unable to dissipate T_b as easily due to their low SA/V ratio, they are typically more mobile and can vary their environmental location more, whereas small mammals use microclimates to moderate environmental conditions (Angert et al., 2011; Cardillo et al., 2005; McCain and King, 2014). Small species are less likely to be effected by climate warming than large mammals (McCain and King, 2014), but still vulnerable (Oswald and Arnold, 2012). Due to Bergmann's rule, we would also expect that with warmer temperatures, smaller mammals that have a greater SA/V ratio would be favoured during times of heat stress (Ashton et al., 2000; Teplitsky and Millien, 2014). However, the evidence that an animal's body mass would evolve at the same rate as climate warming would change over time is weak (Teplitsky and Millien, 2014). The effect of body size is very similar to that of the SA/V ratio, as decreases in body size can increase an animal's SA/V ratio and therefore increases heat dissipation. Size also impacts the TNZ as it often becomes larger with increasing body size, with some larger endotherms having ~20°C+ range of temperatures within the TNZ, compared to only ~2°C in some small rodents species (Chappell, 1984; Heldmaier, 1989; Hock and Roberts, 1966; Parker, 1988; Porter and Kearney, 2009; Riek and Geiser, 2013;

Scholander et al., 1950). Small mammals also have a narrower range of tolerable T_a above their TNZ compared to below. For example, some *Peromyscus* species maintains T_b as low as 10°C but are unable to maintain T_b at temperatures slightly above their upper critical temperature (UCT) of approximately 36°C (Gordon, 2012; Hock and Roberts, 1966; Musser and Shoemaker, 1965). Therefore, when experiencing changes in T_a, small mammals need to increase their metabolic rate to a greater extent to maintain heat loss/production than larger endotherms (Riek and Geiser, 2013).

Activity also plays an important role in how climate warming will impact species. When animals remain active during high T_a, they must cope with the combination of high internal heat production from metabolic processes as well as the heat load being added from the environment. Heat production as a by-product of metabolic processes can vary dramatically based on energy demands (Speakman and Król, 2010), and activity requires large amounts of energy and therefore has high heat production. Fatigue generated by exercise is an inhibitory stimulus to the hypothalamus in response to hyperthermia where it decreases resistance to exercise that functions as a heat-dissipating mechanism (Nybo, 2008). Flexibility in activity times can be one factor that allows for a selection of environmental conditions which are optimal (McCain and King, 2014). To avoid increases in T_b, animals are expected to reduce metabolic activity at high T_a to ensure that capacities for heat dissipation are not exceeded. Hence, at high T_a, animals such as rodents reduce levels of activity as a strategy to limit heat production (Bacigalupe et al., 2003; Ruperto et al., 2022; Speakman and Król, 2010; Terrien et al., 2011). This constraint on the ability to dissipate heat has led to the 'heat dissipation limitation' hypothesis, which is the prevalent theory for explaining limitations on energy expenditure at high T_a (Speakman and Król, 2010). This theory suggests that metabolically costly activities are constrained by the capacity to dissipate heat produced by metabolism (Speakman and Król, 2010; Weiner, 1989; Weiner, 1992). This was initially supported in studies of lactating mammals who decreased milk production at high T_a (Speakman and Król, 2010), and has also been supported by studies in tree swallows, where foraging and provisioning for offspring during summer heat was improved by increasing heat

dissipation by removing insulating feathers (Tapper et al., 2020). To avoid damage or death by the high T_a, highly exothermic activities, which are often key for reproduction, locomotion, foraging, lactation, and ultimately survival, are expected to be reduced (Bacigalupe et al., 2003; Speakman and Król, 2010).

1.3 Plasticity and Heat Acclimation

Individual organisms will react to changes in their environment through changes in their performance, whether positive or negative, through acclimation, adaptation, dispersal, or behavioural changes. Plasticity in these traits and phenotypic plasticity in general will help mitigate the adverse impacts of climate change (DeWitt et al., 1998) but are unlikely to completely offset the predicted decreased fitness, especially in those species impacted most by climate warming. It is especially important to understand the impact of thermal plasticity in the context of climate change, as the ability of a species or population to mount beneficial plastic response to T_a variation may be essential until long-term microevolutionary improvements in heat tolerance occur, if they arise at all (Fragata et al., 2016; Vázquez et al., 2017). Plastic responses occur both physiologically and behaviourally as patterns of activity can vary to optimize energy use (Hut et al., 2012; Ruperto et al., 2022). Thermal plasticity can result in physiological changes that can be beneficial when exposed to warm temperatures for prolonged periods of time. However, beneficial plasticity in response to one condition may be detrimental in other conditions, referred to as the "information reliability limit" (DeWitt et al., 1998).

Heat acclimation has been shown to augment the capacity for heat dissipation, improve the ability to maintain a normal T_b at high T_a (Horowitz, 2001), and can improve the animal's ability to remain active during subsequent thermal stress (Sareh et al., 2011). Specific physiological changes will vary depending on species. For example, rodents have a limited ability to cool themselves through sweating compared to humans, but will increase their heat dissipation predominantly though saliva spreading. However, many animals including mice will have lower resting heart rate, T_b, and T_b thresholds for heat dissipation, while also increasing the UCT and heat dissipation capacity when exposed to subsequent high T_a after heat acclimation (Horowitz, 2001; Sareh et al., 2011; Sunday et

al., 2019b). Heat acclimation depends greatly on having adequate water to replace water lost by evaporative cooling (Périard et al., 2015) and may play an important role in determining locations suitable for species to survive during high T_a exposure. Heat acclimation also results in changes in fluid balance such as reduced electrolyte loss and increased total body water (Jacobs et al., 2020; Mitchell et al., 2018; Périard et al., 2015). Through this, mammals can produce large volumes of sweat with lower salt content, allowing optimal cooling while maintaining ionic and osmotic balance. There is also an increase in heat shock proteins during heat exposure and an increase in baseline levels post acclimation (Horowitz, 2001; Sareh et al., 2011), aiding with maintenance of cellular homeostasis. Interestingly, these physiological and cellular responses noted in mice and other animals resemble that of heat acclimation in humans (Sareh et al., 2011). However, acclimation to a single environmental temperature may be detrimental at dissimilar temperatures. While heat acclimation improves the ability to cope with future heat exposure, it can also reduce thermogenic capacity (Guo et al., 2020; Kuroshima et al., 1982) and thus reduce the ability to cope with cooler T_a. This suggests that thermoregulatory trade-offs underlie the effects of heat acclimation on animals and while beneficial during times of high T_a, can limit the ability to maintain important fitness at cooler T_a.

1.4 Behavioural thermoregulation and implications of diel niche

There are many ways in which animals can avoid changes in environmental conditions and reduce energy expenditure. Animals that are capable of seasonal or daily heterothermy can escape extreme environmental fluctuations and reduce energy expenditure (Liow et al., 2009). However, not all animals can do so and therefore, animals must have a variety of ways in which to cope with environmental conditions. Since small mammals are typically unable to change their range distribution to the same extent as large animals, they therefore are often more likely to use burrows and vegetation to avoid temperature fluctuations (Cardillo et al., 2005; McCain and King, 2014) which is also found in many *Peromyscus* species (Hu and Hoekstra, 2017; McCain and King,

2014). Burrows are energetically costly to dig and can create an environment prevalent to risk of hypoxia and hypercapnia. However, they provide an area that can protect animals against predation and limit the effects of environmental conditions such as humidity, weather, and temperature fluctuations, reducing energy expenditure from thermoregulation. While the degree of protection depends largely on the burrow's size, depth and substrate, smaller and shallow burrows provide only partial protection from summer daytime temperatures (Hayward, 1965; Hu and Hoekstra, 2017).

Another way that animals can avoid exposure to extreme temperatures is through nocturnal behaviour. Nocturnal species may be somewhat protected from high daytime heat exposure as they are not active during the greatest daytime T_a (Bonebrake et al., 2020; McCain and King, 2014), and protection from the most extreme temperatures can further increase in species living in burrows (Hansell and Hansell, 2005; Hu and Hoekstra, 2017). Climate warming may even be advantageous to some nocturnal species as increases in nighttime T_a may move temperatures into a more favourable range for activity (Bonebrake et al., 2020; McCain and King, 2014). However, nocturnal species have been found to have narrower TNZ on average than diurnal species (Shkolnik and Borut, 1969), which may make them more vulnerable to any extreme temperatures they do experience. For most nocturnal species, it remains unclear whether they will be protected from the most significant effects of the high T_a during the day and/or if daytime heat may have negative effects that persist into the nighttime active phase.

1.5 Range shifts

The most significant latitudinal range shifts have occurred in areas that have experienced the most warming, hence why populations at high latitudes are, and will continue to be affected dramatically by climate change (Rosenzweig et al., 2008). The deer mouse is a great model species to examine in this context, as it has colonized a vast array of habitats across North America (Bedford and Hoekstra, 2015). In recent years, however, deer mice have shifted their distribution further northward in some parts of North America, with their southern range limit having shifted northward from the mid-

western USA to Quebec and Ontario, in many locations replaced by the congeneric white-footed mouse (*P. leucopus*) (Fiset et al., 2015; Myers et al., 2005; Myers et al., 2009; Roy-Dufresne et al., 2013; Walsh et al., 2016). In some locations such as Michigan and Ontario, *P. maniculatus* populations have declined five-fold between 1883-1981, and 40% from 1931-2003 (Myers et al., 2005; Myers et al., 2009). The effects of warming on thermoregulatory physiology may play a role in these range shifts but could involve species differences in the effects of warming temperatures during hot summers or cold winters. Displacement of deer mice by white-footed mice at high latitudes could result from species differences in their ability to dissipate body heat and the resulting effects of summer heat on thermoregulation and/or activity. Alternatively, the northern range of white-footed mice could be limited by their thermogenic capacity to maintain T_b in cold winters, such that the species is moving north and displacing deer mice as winter temperatures rise (Roy-Dufresne et al., 2013). These potential physiological limitations have not been studied previously and may help better predict the impacts of future climate change on many species of endotherms.

1.6 Aims and objective

This thesis aimed to determine the impacts and trade-offs associated with ecologically realistic summer warming on deer mice's metabolic physiology and activity. We hypothesized that exposure to warming diel temperature cycles would disrupt thermoregulation and thus impair activity levels due to thermoregulatory trade-offs associated with heat acclimation. We hope that this information will allow for greater understanding of how future warming and extreme heat events may impact endothermic species and underlie latitudinal range shifts.

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CHAPTER 2:

THERMOREGULATORY TRADE-OFFS UNDERLIE THE EFFECTS OF WARMING SUMMER TEMPERATURES ON DEER MICE

2.1 ABSTRACT

Climate warming could challenge the ability of endotherms to thermoregulate and maintain normal body temperature (T_b), but the effects of warming summer temperatures on activity and thermoregulatory physiology in small mammals remain poorly understood. We examined this issue in deer mice (*Peromyscus maniculatus*), a nocturnal species that maintains high activity in the wild. Mice were exposed in the lab to simulated seasonal warming, in which an environmentally realistic diel cycle of ambient temperature (T_a) was gradually warmed from spring conditions to summer conditions (controls were maintained in spring conditions). Activity (voluntary wheel running) and T_b (implanted bio-loggers) were measured throughout, and indices of thermoregulatory physiology (thermoneutral zone, thermogenic capacity) were assessed after exposure. In control mice, activity was almost entirely restricted to the nighttime, and T_b fluctuated ~1.7°C between daytime lows and nighttime highs. Activity, body mass and food consumption were reduced and water consumption was increased in later stages of summer warming. This was associated with strong T_b dysregulation that culminated in a complete reversal of the diel pattern of T_b variation, with T_b surging to extreme highs (~40°C) during daytime heat but extreme lows (~34°C) at cooler nighttime temperatures. The latter was partly caused by a reduced ability to generate body heat, as reflected by decreased thermogenic capacity and decreased mass and uncoupling protein (UCP1) content of brown adipose tissue. Therefore, thermoregulatory trade-offs associated with heat acclimatization may limit nighttime activity, impacting the ability of nocturnal mammals to perform behaviours important for fitness in the wild.

Key words: Climate change, Hyperthermia, Thermal plasticity, Thermoregulation, Metabolism.

2.2 INTRODUCTION

As global temperatures continue to rise due to the effects of climate change, there is a growing need to understand how warming ambient temperatures (T_a) impact the physiology, health, and distribution of animal species (Mitchell et al., 2018; Pörtner and Knust, 2007). In endotherms, warming T_a can limit body heat dissipation and threaten their ability to maintain body temperature (T_b). Although species can vary in the magnitude of T_b variation considered normal, excessive increases in T_b (hyperthermia) can cause a range of cellular and physiological problems that lead to pathology and death (Speakman and Król, 2010; Yan et al., 2006). Indeed, the high T_a recently experienced by some tropical and desert endotherms has increased T_b towards dangerous and even lethal temperatures (Danner et al., 2021; McKechnie and Wolf, 2019). Less severe increases in T_a can constrain locomotor activity, reproduction, and various other important performance traits, likely due to limits to metabolic heat production (Bao et al., 2020; Speakman and Król, 2010; Tapper et al., 2020b). However, while our understanding of the effects of climate warming is improving for some endotherms, such as tropical and desert endotherms (Lovegrove et al., 2014; McKechnie and Wolf, 2010; Tewksbury et al., 2008) and some large temperate mammals (Hetem et al., 2014; McCain and King, 2014), the impacts of climate warming remain largely unknown for many other species (Levesque et al., 2016).

Climate warming may present some distinct challenges for small temperate mammals. Small mammals tend to have relatively narrow thermoneutral zones (TNZ), defined as the range of T_a over which resting metabolic rate is lowest, bounded by lower (LCT) and upper critical temperatures (UCT). For example, small mammals such as rats and mice have TNZ between 28-32°C or a narrow portion of that range (Chappell, 1985; Heldmaier, 1989; Hock and Roberts, 1966; Parker, 1988; Porter and Kearney, 2009; Romanovsky et al., 2002). Considering the increasing summer temperatures in many temperate regions, some small mammals could potentially spend much of the daily cycle outside their TNZ – above it during hot daytime periods (necessitating increased heat dissipation, evaporative water loss, etc.) but still below it during cool nighttime periods (necessitating thermogenesis, heat retention, etc.) (Gordon, 2012; Mitchell et al., 2018;

Rosenmann and Morrison, 1974; Song et al., 1987; Van Sant and Hammond, 2008). The consequences of chronic exposure to such conditions are poorly understood. Chronic exposure to hot temperatures leads to well-known processes of heat acclimation, characterized by an improvement in heat dissipation capacity and heat tolerance (Glaser, 1949; Horowitz, 2001; Racinais et al., 2019; Sareh et al., 2011). By contrast, cold acclimation can increase thermogenic capacity and heat retention (Rezende et al., 2004; Stager et al., 2020; Van Sant and Hammond, 2008). Chronic exposure to daily cycles of hot and cool temperatures may present the possibility for a thermoregulatory trade-off, whereby plastic improvements for coping with daytime heat are associated with a reduced ability to cope with cool temperatures at night. This possibility has received relatively little attention but could have a significant role in the impacts of climate warming on small temperate endotherms. This issue may be significant for nocturnal species that need to remain active at night, particularly if they are unable to completely avoid exposure to high daytime T_a in burrows (Murray and Smith, 2012; Tracy and Walsberg, 2002). Indeed, if high daytime T_a leads to plastic adjustments that reduce tolerance of cool temperatures below the TNZ, nighttime activity could be impaired at temperatures that might otherwise be considered favourable (Bonebrake et al., 2020; McCain and King, 2014).

The deer mouse (*Peromyscus maniculatus*) is a useful species for understanding the potential impacts of climate warming on small temperate mammals. Deer mice are nocturnal and highly active, and they inhabit many different environments and habitats across North America (Bedford and Hoekstra, 2015). They build relatively short, shallow burrows that provide only partial protection from summer daytime temperatures (Hayward, 1965; Hu and Hoekstra, 2017). In recent years, the latitudinal distribution of deer mice has been shifting northward in Canada (southern Ontario and Quebec) and the mid-western USA, where they have often been displaced by the white-footed mouse (*P. leucopus*) (Fiset et al., 2015; Myers et al., 2005; Myers et al., 2009; Roy-Dufresne et al., 2013; Walsh et al., 2016). The potential role of the effects of summer warming on thermoregulatory physiology in these range shifts is poorly understood. To gain insight

into this issue, we examined how exposure to environmentally realistic patterns of summer warming in areas of decreasing deer mouse abundance impacts thermoregulation and activity. We hypothesized that exposure to warming diel temperature cycles, representative of the transition from spring to mid-summer, would disrupt thermoregulation and thus impair activity levels. We further hypothesized that these effects would arise from thermoregulatory trade-offs associated with heat acclimation.

2.3 MATERIALS AND METHODS

2.3.1 Animals and husbandry conditions

Deer mice were live-trapped in Sherman traps in prairie and farmed habitats near Kearney, Nebraska (Buffalo County, NE, USA, in the area surrounding GPS coordinates 40.830000, -99.084056). Mice were then transported to McMaster University (Hamilton, ON, Canada) and held in standard mouse cages (containing 7090 Teklad Sani-Chips® animal bedding; Envigo, Indianapolis, IN, USA) at ~25°C, 14 h:10 h light-dark photoperiod, and with unlimited access to water and rodent chow (Teklad 22/5 Rodent Diet formula 8640; Envigo). Seven independent families were bred in captivity to produce first-generation lab progeny, which were then raised to adulthood (~6-18 months of age) in the same conditions. 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.

2.3.2 Experimental treatment groups

Adult first-generation lab mice were separated into two experimental groups: one subjected to a constant diel temperature cycle representative of late spring, and one subjected to warming diel temperature cycles representative of the transition from spring to mid-summer. To inform the temperatures used in each experimental group, we measured environmental temperatures at a site near Lincoln, Nebraska (Lancaster County, NE, USA, at GPS coordinates 40.74950247590457, -96.81771341527244) in 2020. Deer mice were abundant at this site until recently, but the site is now populated primarily by white-footed mice (Jay F. Storz, University of Nebraska, personal communication),

potentially due to range shifts resulting from climate change (Myers et al., 2005; Roy-Dufresne et al., 2013). An iButton temperature logger (DS1922L, Embedded Data Systems, Lawrenceburg, KY, USA) was deployed at ground level under shelter of a large wood pile, representing mouse microhabitat with strong trapping success, and temperature was recorded every 2 hours. The temperature data acquired were used to determine average daytime high and average nighttime low for each week over the period of summer warming from May to July (Fig. 1A), and these values were used to inform the exposure temperatures used in the two experimental groups. The 'spring control' group was exposed to diel temperature variation representative of late May, with temperatures of 27.5°C during the daytime (07:30 to 21:30 local time) and 13°C during the nighttime (21:30 to 07:30 local time) for eight weeks (10 males, 12 females) (top panel in Fig. 1B). The 'summer warming' group was exposed to temperatures of 27.5°C in the daytime and 13°C during the nighttime for one week, after which the daytime and nighttime temperatures were increased each week by 1.5°C and 1.0°C respectively for weeks 2 through 8 (9 males, 5 females) (bottom panel in Fig. 1B).

Shortly before exposures began, mice were implanted with temperature loggers and/or thermosensitive passive integrated transponder (PIT) tags (as described below) to provide subcutaneous temperature measurements that we used as a proxy for T_b. After full recovery from these implantations, mice were moved in pairs into standard mouse cages that contained in-cage running wheels (Starr life Sciences, Oakmont, PA, USA), and cages were moved into a rodent incubator with automated temperature and photoperiod control (model RIS28SSD, Powers Scientific, Pipersville, PA, USA). Mice were first held at standard holding temperature (25°C) for at least one week to become accustomed to the incubator and running wheel. Mice were then subjected to diel temperature cycles as described above. The transition from daytime to nighttime temperatures began at lights on/off, taking approximately 10 minutes. In mice implanted with temperature loggers (see below), T_b was measured every 10 min. From these data, we determined the average value at each time of day across the last 5 days of each week for each mouse. We also determined the daytime average T_b, the nighttime average T_b,

the nighttime minimum T_b, and the average magnitude of daily T_b variation for each week for each mouse. Wheel rotations were measured every hour using digital counters, recorded using a GoPro camera set, and a red LED light was used to enable visualization during the dark phase. We thus determined the average of total daily wheel rotations as an index of daily activity for each week. Food and water consumption over 24 h were measured 4 to 7 days of each week (which required that we weigh and replace food and water at 14:30 local time), and the average daily value for each week is reported here. Body mass was measured on days 3 and 7 of each week, concurrent with cage changes (at 14:30 local time), and the average value for each week is reported here. Otherwise, husbandry conditions were the same as those described above. After completing the 8-week temperature exposures, mice were used for respirometry measurements before being sampled for tissues, as described below.

2.3.3 Implantation of temperature loggers and PIT tags

A subset of mice in the spring control group (7 males, 3 females) and summer warming group (5 males, 5 females) were implanted with temperature loggers to facilitate the continuous recording of T_b during exposures (DST nano-T, Star-Oddi, Garðabær, Iceland). Anesthesia was induced with 3% isoflurane in O_2, and a surgical plane of anaesthesia was maintained with 1.5-2% isoflurane. The ventral surface of the neck was shaved and disinfected with iodine solution, and the mouse was transferred to a heating pad to maintain T_b. A 2-3 cm incision was made along the midline of the neck, the skin was carefully separated by blunt dissection from the underlying muscle to access the subcutaneous interscapular region, and the logger was inserted between the scapulae. The incision was then closed using an interrupted subcuticular suture (6-0 Vicryl). These mice were also implanted with thermosensitive PIT tags shortly after logger implantation (same location as described below), and the animal was removed from anaesthesia, and recovered in a new clean cage (Envigo Teklad 7070C Certified Diamond Dry) that was warmed with a heating pad. The surgery took approximately 20 minutes to complete. Carprofen (5 mg/kg in saline solution) was given by subcutaneous injection during surgery as well as 24 and 48 h after surgery for analgesia. Mice were closely monitored

for seven days post-surgery before experimental treatments began, by which time the incision site had healed well, and mice had resumed their normal behaviour.

All remaining mice were implanted with thermosensitive PIT tags (micro LifeChips with Bio-therm technology; Destron Fearing, Dallas, TX, USA) to facilitate individual identification and the measurement of T_b during respirometry (see below). Mice were lightly anaesthetized using 2% isoflurane and given a subcutaneous injection of 2 mg/kg ketoprofen in saline for analgesia. The fur on the left side of the abdomen was cleaned using 70% ethanol, and the PIT tag was injected subcutaneously into the abdominal region near the leg using a sterile trocar (Destron Fearing). Mice were then removed from anesthesia and returned to their cage to recover. The implantation took approximately 5 minutes to complete, and mice were monitored for seven days to ensure complete recovery before experimental treatments.

2.3.4 Respirometry measurements

We conducted two sets of respirometry measurements in each treatment group at the completion of the 8-week exposures. The first set of measurements were made in all mice, in which we examined the metabolic and thermoregulatory responses to acute warming at rest, using respirometry and whole-animal plethysmography techniques we have used in previous studies (Ivy and Scott, 2017a; Ivy and Scott, 2017b). Measurements were made in a cylindrical respirometry chamber (from Data Sciences International, St. Paul, MN, USA) that was ~700 ml in volume and was connected to a sealed reference chamber via a pneumotachograph (for measuring breathing frequency 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, Las Vegas, NV, USA) in which temperature was controlled using a Peltier-effect temperature controller (PELT-5, Sable Systems). Dry incurrent air flowing at 600 ml min^{-1}, controlled using precision flow meters (Sierra Instruments, Monterey, CA, USA) and a mass flow controller (MFC-4, Sable Systems), was first passed through a stainless-steel coil inside the Peltier cabinet and then supplied to the respirometry chamber. Gas composition was

measured continuously in both incurrent and excurrent airflows subsampled at 200 ml min^{-1}. The O_2 fraction of incurrent air (which was dry and free of CO_2) was measured using a galvanic fuel cell O_2 analyzer (FC-10, Sable Systems). For excurrent air, water vapour pressure was measured using a thin-film capacitive water vapour analyzer (RH-300, Sable Systems), the gas stream was then dried with pre-baked Drierite, O_2 fraction was measured as above, and CO_2 fraction was measured using an infrared CO_2 analyzer (CA-10, Sable Systems). Measurements of O_2 and CO_2 fractions and water vapour were used to calculate rates of O_2 consumption and evaporative water loss using the appropriate equations with a known incurrent flowrate as described previously (Lighton, 2008). The ambient temperature (T_a) in the chamber was continuously recorded with a thermocouple (TC-2000, Sable Systems). Breathing frequency was measured using whole-body plethysmography from pressure oscillations in the animal chamber relative to the reference chamber using a differential pressure transducer (DP103, Validyne, Northridge, CA, USA). T_b was measured every minute using a PIT tag reader (Global Pocket Reader, Destron Fearing, Dallas, TX, USA). Data were acquired using a PowerLab 16/32 and LabChart 8 Pro software (ADInstruments, Colorado Springs, CO, USA).

The measurements of metabolic and thermoregulatory responses to acute warming began by placing mice in the chamber, with T_a initially set to 26°C. Mice were allowed to adjust for at least 60 min until stable patterns of O_2 consumption, water loss, and breathing were observed. Mice were then kept at 26°C for an additional 20 min, followed by exposure to stepwise increases in temperature of 2°C every 20 min, ending with 20 min at 40°C. Measurements of O_2 consumption rate (VO_2), evaporative water loss (EWL), breathing frequency (f_R), and T_b were determined using the last 10 min at each T_a and when responses had reached a steady state. After measurements were completed, mice were returned to their home cage in the appropriate chronic temperature exposure, held for two more days, and either used for a second set of measurements or sampled (see below).

The second set of measurements were made in a subset of mice in the spring control group (7 males, 3 females) and summer warming group (5 males, 2 females) to determine thermogenic capacity using similar methods to those we have used previously (Tate et al., 2017). These measurements were conducted ~48 h after the first set of measurements above. Mice were placed in a respirometry chamber (~600 ml) inside a temperature-controlled cabinet held at -5°C. Heliox (21% O_2, 79% He) passed through a stainless-steel coil within the cabinet and was then delivered to the metabolic chamber at 1000 ml min^{-1}, with incurrent flowrate controlled using precision flow meters (Sierra Instruments) and a mass flow controller (MFC-2, Sable Systems). Mice were exposed to these conditions for ~15 min and O_2 consumption rate was measured throughout as described above. Thermogenic capacity was defined as the maximal O_2 consumption rate achieved over a 30 s period during the trial, which generally occurred after ~2-6 min in the chamber. T_b was measured before and at the end of the trial, confirming that T_b was reduced by at least 5°C at the end of the trial.

2.3.5 Euthanasia and sampling

Euthanasia was performed with an overdose of gaseous isoflurane followed by decapitation. Blood samples were collected, haemoglobin content was measured using Drabkin's reagent (according to the instructions from the manufacturer, Sigma-Aldrich, Oakville, Ontario, Canada), and blood was centrifuged in capillary tubes at 12700*g* for 5 min to measure haematocrit. Interscapular brown adipose tissue (iBAT) and one gastrocnemius muscle were dissected, weighed, frozen in liquid N_2, and stored at -80°C. The entire lungs, heart ventricles, liver, both kidneys, stomach, and small intestine were dissected and weighed.

2.3.6 Western blotting

Protein content of uncoupling protein 1 (UCP1) and citrate synthase (CS) were measured in iBAT by western blotting as previously described (Hayward et al., 2022; Robertson et al., 2019). Tissues were first powdered using a liquid N_2-cooled mortar and pestle, and ~20 mg of powdered tissue was homogenized with a PowerGen 125

homogenizer (Thermo Fisher Scientific, Mississauga, ON, Canada) in cold RIPA buffer containing 150 mM NaCl, 50 mM Tris, 1% Triton X-100, 0.5% deoxycholic acid, 0.l% SDS, and 1 tablet/10ml of cOmplete™, Mini Protease Inhibitor Cocktail (Sigma-Aldrich, Oakville, ON, Canada) at pH 8.0. Samples were homogenized 3 times for 30 s each, interspersed by periods of at least 3 min on ice between each homogenization. Samples were then centrifuged at 10 000 g at 4°C for 20 min and the supernatant was retained. Proteins in the supernatant were denatured at 95°C for 5 min in Laemmli buffer containing 10% 2-mercaptoethanol (Bio-Rad, Mississauga, ON, Canada). Total protein concentrations were determined using a detergent compatible (DC) protein assay kit (Bio-Rad). 2 μg of total protein was loaded into each lane of pre-cast 12% sodium dodecyl sulfate-polyacrylamide gels (Bio-Rad) and separated for ~30 min at 100 V, followed by ~45 min at 150 V using a Mini-Protein Tetra system (Bio-Rad). Proteins were then transferred from the gel to polyvinylidene difluoride (PVDF) membranes for 7 min at 25 V and 2.5 A using a Trans Blot Turbo Transfer System (Bio-Rad). Membranes were cut at roughly 40 kDa between the expected location of protein bands for UCP1 (30 kDa) and CS (52 kDa). Membranes were blocked overnight at 4 °C using 1% bovine serum albumen (BSA; Sigma-Aldrich) in phosphate-buffered saline containing Tween-20 (PBST; 1.5 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, 145.5 mM NaCl, 0.05% Tween-20 at pH 7.4). Membranes were then incubated for 1 h at 4°C with rabbit IgG primary antibodies in PBST containing 1% BSA. The bottom half of each membrane was incubated with primary antibody against UCP1 (1:500 dilution; UCP11-A, Alpha Diagnostics International, San Antonio, TX, USA) and the top half with primary antibody against CS (1:500 dilution; PA5-22126, Invitrogen, Thermo Fisher Scientific). Membranes were then rinsed 3 times with PBST for 10 min each, and then incubated at 4°C for 1 h with goat anti-rabbit IgG secondary antibody conjugated with horseradish peroxidase (1:5000 dilution; 31466, Invitrogen, Thermo Fisher Scientific). Membranes were developed in Clarity ECL Substrate (Bio-Rad) and band intensity was detected by chemiluminescence using a ChemiDoc MP Imaging System and Image Lab Software (Bio-Rad). A common protein sample was included in each gel to account for any variation in membrane protein

transfer efficiency, and target protein abundance in all other samples were determined relative to this common sample. Target protein abundance in each sample was also normalized to total protein, determined by Coomassie blue staining. Protein abundance data reported here are expressed relative to the mean value in the spring control group.

2.3.7 Enzyme activity assays

Gastrocnemius muscle was powdered using a liquid N_2-cooled mortar and pestle, and ~20 mg of powdered muscle was homogenized using a glass tissue grinder in 20 volumes of homogenization buffer (100 mM KH_2PO_4, 5 mM ethylenediaminetetraacetic acid, and 0.1% Triton-X-100, at pH 7.2). Cytochrome c oxidase (COX) and 3-hydroxyacyl-CoA dehydrogenase (HOAD) activities were assayed shortly after homogenization, and citrate synthase (CS) and lactate dehydrogenase (LDH) activities were measured after storage of homogenate at -80°C. Activity was assayed at 37°C by measuring the change in absorbance over time (CS, 412 nm; COX, 550 nm; HOAD and LDH, 340 nm) under the following conditions (in mM unless otherwise stated): CS, 40 Tris, 0.5 oxaloacetate, 0.23 acetyl-CoA, 0.1 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), pH 8.0; COX, 100 KH_2PO_4, 0.15 reduced cytochrome c, pH 7.0; HOAD, 100 triethylamine hydrochloride, 0.1 acetoacetyl-CoA, 0.28 NADH, pH 7.0; LDH, 40 Tris, 0.28 NADH, 3 pyruvate, pH 7.4. All enzyme assays were run in triplicate. Enzyme activities were determined by subtracting the control rate (measured without key substrate) from the rates measured in the presence of all substrates. Preliminary experiments verified that substrate concentrations were saturating.

2.3.8 Statistics

Linear models were performed using the lme4 package (Bates et al., 2015) in RStudio (1.4.1717) (RStudio-Team, 2021). We tested for fixed effects of experimental treatment group, sex, week, and individual/cage (to account for repeated measures) for daytime average T_b, nighttime average T_b, nighttime minimum T_b, daily T_b variation, daily wheel rotations, food and water consumption, and body mass. For the more frequent measures of T_b (measured every 10 min) and wheel running (measured every hour), we

tested for fixed effects of sex, week, time of day, and individual/cage within each treatment group. For measurements of the metabolic and thermoregulatory responses to acute warming, we tested for fixed effects of experimental treatment group, sex, T_a, and individual. For the measurements of thermogenic capacity and of organs/tissues, we tested for fixed effects of experimental treatment group and sex. For the statistical analyses of O_2 consumption rate, EWL, and organ/tissue masses, body mass was also included as a covariate and tests were carried out on the absolute values of these traits; however, these data are reported relative to body mass as is conventional in the literature. The full results of the linear models are reported in Tables S1-S5 and the salient findings are reported in the Results. When fixed effects or interactions were significant, we usually used the package emmeans (version 1.7.4) to make post-hoc pairwise comparisons using the Tukey method (the only exception was that we did not perform post-hoc comparisons for the repeated measurements of T_b and wheel running made throughout the day, due to the large volume of data). Values are reported as mean ± SEM, and $P < 0.05$ was considered to be significant.

2.4 RESULTS

2.4.1 Summer warming led to a dysregulation of body temperature

There was a strong diel cycle in T_b in the spring control group (Fig. 2A), in which T_b rose during the nighttime active phase to temperatures that were ~1°C greater on average than T_b during the daytime inactive phase (main effect of time of day, $P<0.001$). There was a transient rise in T_b at ~14:30 local time when all health checks, cage changes, and measurements were done (see Materials and Methods for details). There were some significant differences in T_b between weeks (main effect of week, $P<0.001$), but the absolute variation in T_b between weeks was generally modest, and the overall pattern of daily T_b variation was similar across weeks (week×time, $P=0.999$). Females had greater T_b than males (main effect of sex, $P<0.001$), but sexes did not exhibit different diel cycles in T_b (sex×time×week, $P=0.999$).

In contrast, there were major changes in the diel cycle of T_b between weeks in the summer warming group (Fig. 2B), which drove a significant main effect of week

($P<0.001$) and a significant week×time interaction ($P<0.001$). Daytime T_b started increasing at week 4, and the increase extended across the daytime phase in weeks 6-8 (Fig. 2B). Surprisingly, there were also pronounced drops in T_b during the hours after the transition to cooler nighttime temperatures in weeks 6-8 (albeit with some T_b recovery as the nighttime progressed), even though nighttime T_a was greater in weeks 6-8 than in week 1. As a result, there was a complete reversal in the diel T_b cycle in weeks 6-8, with much greater T_b during the daytime inactive phase than during the nighttime active phase. Sex had a main effect on T_b in the summer warming group ($P<0.001$), but sex did not alter the strong changes in the diel T_b cycle between weeks (sex×week×time, $P=0.999$).

The appreciable differences in the diel T_b cycle between the spring control group and the summer warming group were reflected in average and minimum measures of T_b and in daily T_b variation (Fig. 3). There were significant effects of treatment group (all $P\leq0.001$) and significant treatment×week interactions ($P<0.001$, $P=0.033$, $P<0.001$, $P<0.001$, respectively) for average daytime T_b, average nighttime T_b, minimum nighttime T_b, and the average magnitude of daily T_b variation. Average daytime T_b did not change between weeks in the spring control group, but it increased progressively in the summer warming group from week three onwards (Fig. 3A). Average nighttime T_b and minimum nighttime T_b were also stable across weeks in the spring control group but decreased significantly in weeks 8 and 7-8 in the summer warming group (Fig. 3B,C). The magnitude of daily T_b variation was stable at <3°C in the spring control group but increased appreciably in weeks 6-8 in the summer warming group (Fig. 3D). Females tended to have greater average daytime T_b (sex effect, $P<0.001$), average and minimum nighttime T_b ($P=0.009$ and 0.033), and average magnitude of daily T_b variation ($P=0.010$) than males, but the pattern of variation between treatments was similar between sexes (treatment×week×sex, all $P\geq0.480$) (Fig. S1).

2.4.2 Summer warming reduced activity and body mass

Voluntary wheel running, which was used as a metric of routine activity, was almost exclusively restricted to the nighttime active phase (time of day effects, $P<0.001$)

(Fig. 4). Wheel running was somewhat variable between cages (each of which contained two individuals of the same sex), and part of this variation was explained by females running more than males (sex effects, $P<0.001$) (Fig. S1), as previously observed in house mice (Meek et al., 2009). There was a significant effect of week on wheel running in the spring control group ($P=0.019$), with the highest levels in week 5, but the diel pattern of variation in wheel running was stable (week×time, $P=0.999$) (Fig. 4A). There was also a significant effect of week on wheel running in the summer warming group ($P<0.001$) but the pattern of variation was quite different, with highest levels in weeks 3-5 but lowest levels in week 8 (Fig. 4B). There was also a significant week×time interaction in the summer warming group ($P<0.001$), likely driven by week 8, in which levels of activity were lowest in the early hours after the transition to cooler nighttime T_a but increased thereafter (Fig. 4B). This variation in hourly measures of wheel running was reflected in measures of total daily wheel running (Fig. 5), for which there was a significant effect of treatment group ($P=0.004$) and a significant treatment×week interaction ($P=0.0094$). Indeed, the summer warming group had 89% greater total daily wheel running than the spring control group in week 3, but by week 8, wheel running had fallen to 48% of the week 1 level (Fig. 5). Although there was a significant effect of sex on total daily wheel running ($P=0.003$), sex did not alter the effects of the treatment (treatment×sex and treatment×week×sex, all $P\geq0.854$) (Fig. S1).

Summer warming also led to a decrease in body mass (Fig. 6A). There was a significant main effect of treatment ($P<0.001$) and a significant treatment×week interaction ($P<0.001$) for body mass, which was stable over time in the spring control group but decreased in weeks 7 and 8 in the summer warming group (Fig. 6A). Variation in body mass coincided with variation in food consumption (Fig. 6B), as reflected by a significant treatment×week interaction ($P=0.0076$), with the summer warming group consuming ~21% less food than the spring control group in week 8. The summer warming group also exhibited different patterns of water consumption than the spring control group (treatment effect, $P<0.001$; treatment×week, $P=0.008$), increasing water consumption in weeks 6-8 by up to 80% (Fig. 6C). Females and males differed in body

mass, food consumption, and water consumption in each treatment group, but sex did not alter the different effects of treatment on body mass over time (treatment×week×sex, all $P \geq 0.1011$) (Fig. S3).

2.4.3 Summer warming led to plastic adjustments in thermoregulatory physiology

The patterns of variation during the 8-week treatments showed that mice in the summer warming group may be heat stressed during the daytime but less able to maintain T_b and activity at cooler nighttime temperatures. We hypothesized that this pattern of variation could be explained by plastic responses to daytime heat that come at the cost of reducing the ability to cope with cold. We tested this hypothesis by examining the metabolic and thermoregulatory responses to acute heat exposure at rest, as well as thermogenic capacity during acute cold exposure.

Measurements of metabolism and thermoregulatory physiology during acute warming revealed several differences between treatment groups (Fig. 7). In general, O_2 consumption rate (VO_2), evaporative water loss (EWL), breathing frequency (f_R), and T_b were lowest at intermediate T_a (~28-34°C depending on the trait) and increased as T_a reached or approached 40°C (T_a effect, $P<0.001$ for all). However, the summer warming group had lower VO_2, EWL, f_R, and T_b from 28-38°C (treatment effects, $P<0.001$ for all), but converged with the spring control group at 40°C (treatment×T_a, $P<0.001$ for EWL and $P=0.001$ for T_b). Females had slightly warmer T_b than males, but males had slightly higher VO_2 and f_R than females (sex effects, all $P \leq 0.004$) (Fig. S4).

Thermogenic capacity, measured as the maximal rate of O_2 consumption during acute exposure to -5°C in heliox (i.e., thermogenic VO_2max), was reduced by summer warming (Fig. 8A). There was a significant treatment effect on thermogenic capacity ($P=0.029$), in which mice in the summer warming group had capacities only ~47% on average of those in the spring control group, but there was no significant effect of sex on this trait ($P=0.166$). This variation in thermogenic capacity between treatment groups was associated with parallel variation in the mass of the interscapular brown adipose tissue (iBAT), a key thermogenic tissue in small mammals (treatment effect, $P<0.001$) (Fig.

8B). Not only was the iBAT smaller in the summer warming group, it also contained lower abundance of uncoupling protein 1 (UCP1; treatment effect, $P<0.001$) (Fig. 8C). The abundance of citrate synthase (CS, a common marker of mitochondrial volume) was similar between treatment groups (treatment effect, $P=0.407$), such that the summer warming group had a lower ratio of UCP1 to CS ($P=0.003$) (Table 1, Fig. S5). However, there were no effects of treatment on the mass or activities of metabolic enzymes (CS, COX, HOAD or LDH) in the gastrocnemius (Table 1). The mass of the kidneys was reduced slightly in the summer warming group (treatment effect, $P=0.012$), but treatment had no significant effects on the masses of the lungs, heart ventricles, liver, stomach, or small intestine (Table 1). Blood haemoglobin content and mean cell haemoglobin content were increased in the summer warming group (treatment effects, $P=0.013$ and 0.003) with no change in haematocrit (Table 1).

2.5 DISCUSSION

The physiological impacts of chronic exposure to warming temperatures are poorly understood in many endothermic species (Levesque et al., 2016; Mitchell et al., 2018). Here, we show that seasonal warming simulating the transition from spring to mid-summer leads to disruptions in thermoregulation that may have detrimental health impacts on deer mice. Summer warming led to a dysregulation of T_b that culminated in a complete reversal of the diel pattern of T_b variation, with T_b rising to extreme highs during the day and dropping to extreme lows at night (Figs. 2,3). This reduced ability to maintain T_b at cool nighttime temperatures was associated with reductions in thermogenic capacity and the mass and UCP1 content of iBAT (Fig. 8), which likely arose as part of a heat acclimation response to summer warming. These adjustments likely constrained normal behaviour during the nighttime active phase, in association with reductions in voluntary wheel running, food consumption, and body mass (Figs. 4-6). These findings suggest that thermoregulatory trade-offs underlie the effects of warming summer temperatures on small nocturnal mammals, in which acclimatization to daytime heat exposure may limit the ability to remain active and perform behaviours important for fitness at cooler nighttime temperatures.

The exposure of deer mice in the summer warming group to daytime heat was likely a strong stimulus for heat acclimation. Daytime T_a rose to 35-38°C in weeks 6-8 (Fig. 1), which appears to be hotter than the TNZ of deer mice (Brower and Cade, 1966; McNab and Morrison, 1963) (Fig. 7). This was associated with pronounced increases in daytime T_b (Figs. 2,3), consistent with previous results in domestic mice and rats exposed to prolonged heat stress (Kuroshima et al., 1982; Sareh et al., 2011). Summer warming also led to physiological adjustments that are typical of heat acclimation in other species, such as reductions in resting metabolic rate, which can be associated with reductions in metabolic heat generation (Guo et al., 2020; Shido et al., 1994) (Fig. 7). It is possible that heat acclimation induced other changes to help deer mice cope with high daytime temperatures, including increased capacities for evaporative heat dissipation (Horowitz, 2001; Sareh et al., 2011). However, the ability of such mechanisms to attenuate the rise in T_b during the day may be limited, based on our observation that daytime T_b always exceeded T_a (Figs. 2,3). Indeed, recent studies of desert species of *Peromyscus* and other cricetids suggest that they have a limited capacity for evaporative cooling, potentially because saliva spreading (the dominant means of EWL in rodents) is a far less effective means of evaporative cooling than panting (Ramirez et al., 2022). Therefore, the later stages of summer warming likely imposed significant heat stress on deer mice.

Daytime heat exposure likely contributed to the pronounced drop in T_b at cooler nighttime temperatures (Figs. 2,3). Deer mice exhibit natural diel variation in T_b like many other nocturnal rodents, characterized by higher T_b during the nighttime active phase (Beaudry and McClelland, 2010; Weinert and Waterhouse, 1998). However, the diel pattern of T_b variation was reversed in the later stages of summer warming, when the transition to cool nighttime temperatures led to a transient period of hypothermia (T_b ~34°C). In domestic mice acclimated to 25°C, a single acute bout of heat exposure followed by a return to cooler T_a is known to induce hypothermia, and the magnitude and duration of hypothermia depends on how high T_b rises during heat exposure (Leon et al., 2005; Wilkinson et al., 1988). How such effects of acute heat exposure might change after prolonged heat acclimation is unclear, but acclimation to warm temperatures within

the thermoneutral zone (30°C) hinders recovery rather than improving it (Leon et al., 2005). Therefore, the pronounced drop in T_b at night in the later stages of summer warming can be partly attributed to the residual effects of heat exposure the previous day, but may also be accentuated by plastic responses to prolonged heat exposure.

Prolonged exposure to daytime heat reduced thermogenic capacity (Fig. 8), which may have contributed to the poor ability of deer mice in the summer warming group to maintain T_b at cooler nighttime temperatures. This observation is consistent with previous findings in various other small mammals, in which heat acclimation has been shown to reduce thermogenic capacity (Guo et al., 2020; Kuroshima et al., 1982). The reduction in thermogenic capacity observed here was associated with a reduction in the mass and UCP1 content of brown adipose tissue (Fig. 8), the primary site of non-shivering thermogenesis, consistent with previous findings in other species (Kuroshima et al., 1982; Saha et al., 2000; Zhang et al., 2012). Whether shivering capacities were also affected by summer warming is unclear, but there were no significant effects on metabolic capacities in the gastrocnemius – a large hindlimb muscle involved in shivering and locomotion (Table 1). Nevertheless, these findings support our hypothesis that heat acclimation induces plastic adjustments that limited the ability of deer mice to defend T_b at T_a below the TNZ.

The prolonged reduction in nighttime T_b likely constrained normal behaviour and contributed to reducing activity levels in deer mice (Figs. 4,5). Running activity was restricted to the nighttime when T_a was cooler. Findings of previous studies suggest that running activity was probably not impaired by the direct effects of increases in nighttime T_a, which rose to 18-20°C in weeks 6-8 of summer warming. Firstly, in previous studies of deer mice, total distance and time of voluntary wheel running did not vary between ambient temperatures of 3°C, 10°C, and 25°C (Chappell et al., 2004). Similarly, voluntary wheel running is only modestly affected by chronic exposure to constant temperatures from ~20-30°C in house mice (Fregly et al., 1957; Vaanholt et al., 2007). Secondly, forced endurance capacity is maintained from ~15-30°C in deer mice, and performance is not impaired until T_a is increased above 35°C (Eizenga et al., 2022). It is

more likely that decreases in running activity during the later stages of summer warming resulted from the prolonged decrease in nighttime T_b, as reductions in T_b are known to reduce locomotor performance (Rojas et al., 2012). It is also possible that reductions in T_b reduced the motivation to run and be active.

The reduction in body mass in the later stages of summer warming represents another potential adverse health impact of daytime heat exposure (Fig. 6). This reduction in body mass could have been caused by the concurrent decrease in food consumption in the summer warming group, which occurred even though mice had unlimited access to food. Indeed, prolonged heat exposure has frequently been observed to decrease food consumption and/or body mass in several species (Dikstein et al., 1970; Guo et al., 2020; Harikai et al., 2003; Saha et al., 2000; Sareh et al., 2011; Shido et al., 1994; Vaanholt et al., 2007; Yamauchi et al., 1983). However, the drop in food consumption may have also been a response to decreased metabolic demands associated with reductions in running activity, and may not have contributed to the fall in body mass. It is instead possible that prolonged increases in evaporative water loss led to desiccation, if the observed increases in water consumption (Fig. 6) were insufficient to meet increases in water demands, such that total body water and body mass were reduced. This might have contributed to the observed variation in haematology (Table 1), which could be explained by reductions in plasma volume concurrent with reductions in erythrocyte volume that increased mean cell haemoglobin content. Either way, reductions in body mass might be expected to reduce the ability of deer mice to invest resources into growth and reproduction, which could have corresponding impacts on fitness in the wild.

The effects of warming temperatures observed here could have important implications for how climate change is impacting deer mice in the wild. Deer mouse populations have declined in several regions of North America, where they are being displaced by white-footed mice (Fiset et al., 2015; Myers et al., 2005; Myers et al., 2009; Roy-Dufresne et al., 2013; Walsh et al., 2016). This is likely true for the deer mouse population studied here, which are from a region of Nebraska adjacent to sites where deer mice used to be abundant but are now scarce. Our data here suggest that the current

summer temperatures in this region of Nebraska lead to a dysregulation of T_b, a reduced ability to remain active, and a loss of body mass, and it is possible that these impacts could be accentuated by future increases in summer temperature as climate change intensifies. The effects of summer heat might be expected to reduce fitness if they decrease the ability to forage and avoid predators or impair growth and reproduction. Such detrimental impacts could explain why deer mice have been unable to outcompete white-footed mice in some of the hotter parts of their range. The thermoregulatory trade-offs that underlie the observed effects of summer heat are likely to be relevant to many other small nocturnal mammals, and may help explain the underlying causes for why the latitudinal distributions of many species are shifting northward in response to climate change

2.6 TABLES AND FIGURES

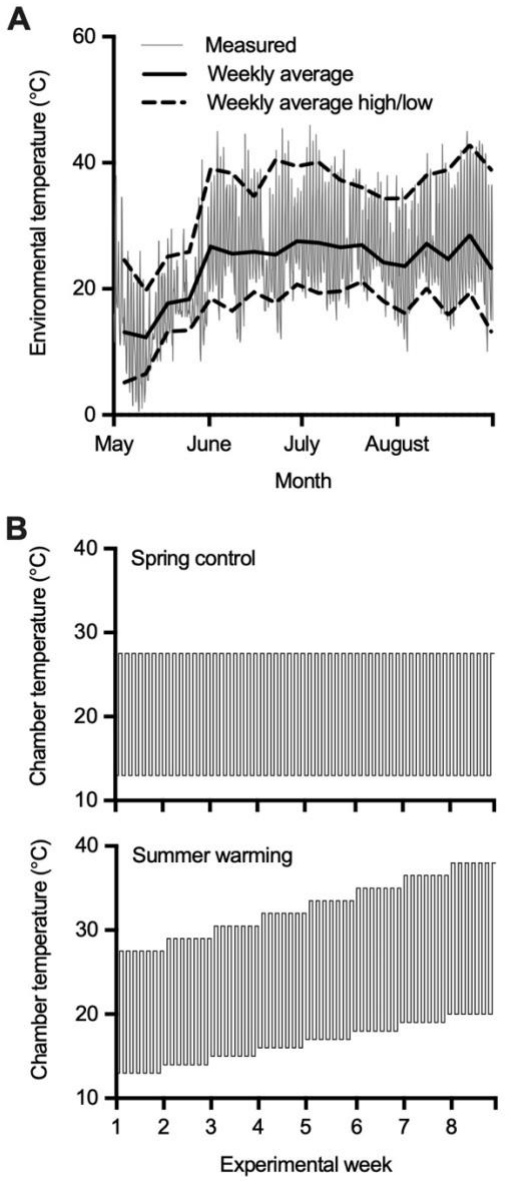

Fig. 1. Experimental treatment groups were modelled from realistic patterns of diel temperature variation and summer warming in the native environment of deer mice. (A) Environmental temperatures in Nebraska at a site where deer mice were once abundant but have been largely displaced by white-footed mice. Temperature was recorded every 2 h at ground level under shelter in microhabitat where there is typically strong trapping success of for *Peromyscus* mice. (B) Patterns of temperature variation used in the current study in the 'spring control group' (top panel) and the 'summer warming group' (bottom panel). See Materials and Methods for additional details.

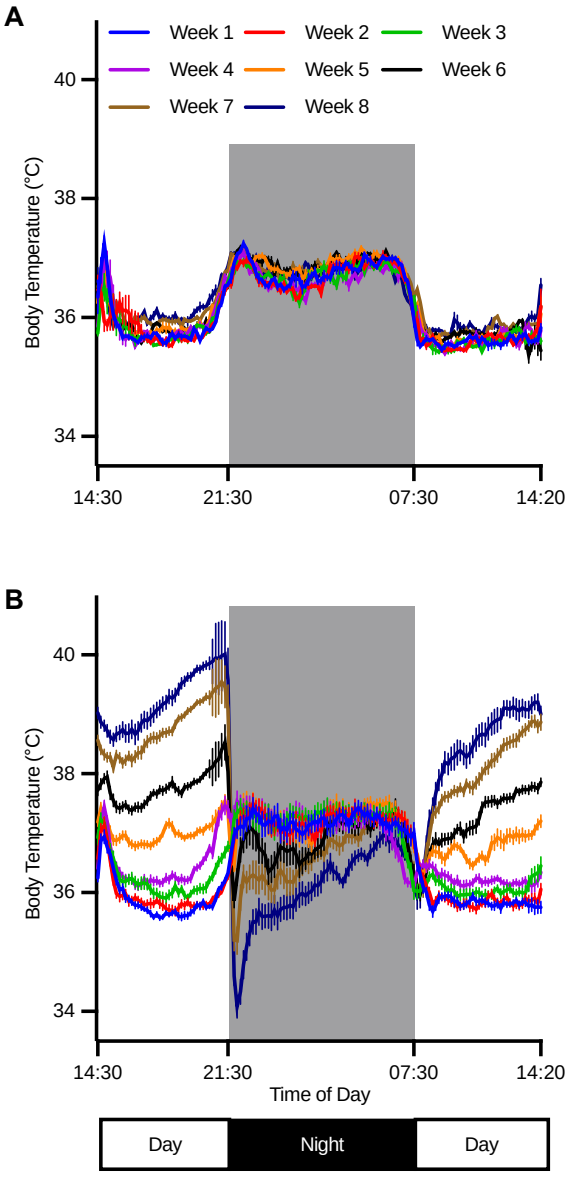
A
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Body Temperature (°C)
40
38
36
34
14:30
21:30
07:30
14:20
B
Body Temperature (°C)
40
38
36
34
14:30
21:30
07:30
14:20
Time of Day
Day
Night
Day

Fig. 2. Summer warming altered the diel cycle of body temperature (T_b). T_b was measured every 10 min using temperature loggers implanted subcutaneously in the interscapular area. The average diel T_b cycle is shown for each week as mean ± SEM in (A) the spring control group (N=10) and (B) the summer warming group (N=10). The grey shaded area reflects the dark nighttime phase when mice are active, and the white areas reflects the light daytime phase of inactivity. The daytime and nighttime temperatures in each week are shown in Fig. 1.

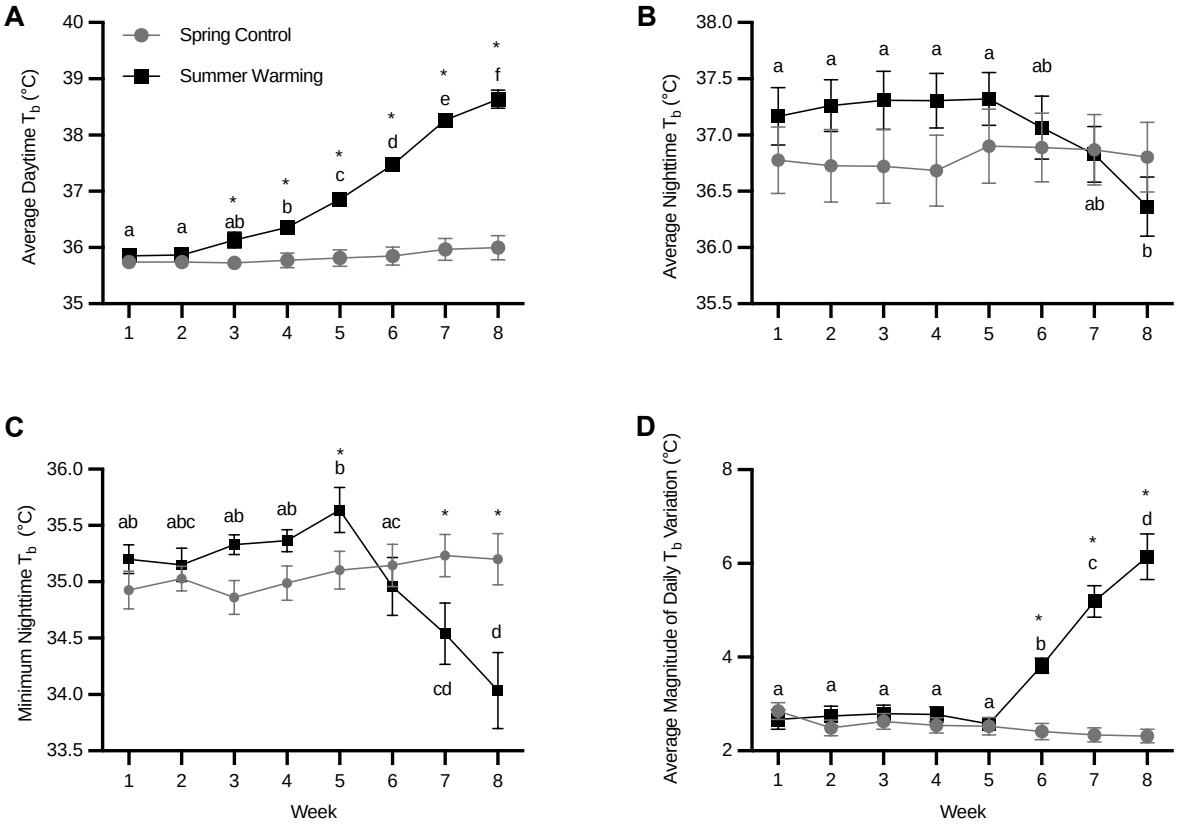
A
Spring Control
Summer Warming
Average Daytime T_b (°C)
B
Average Nighttime T_b (°C)
C
Minimum Nighttime T_b (°C)
Week
D
Average Magnitude of Daily T_b Variation (°C)
Week

Fig. 3. Summer warming altered average body temperature (T_b) and T_b variation. (A) Average daytime T_b, (B) average nighttime T_b, (C) minimum nighttime T_b, and (D) the magnitude of daily T_b variation in the spring control group (grey circles; N=10) and the summer warming group (black squares; N=10), shown as mean ± SEM. * Represents a significant pairwise difference between treatments within each week ($P<0.05$). Within each treatment group, weeks not sharing a letter are significantly different ($P<0.05$) (there were no significant differences between weeks in the spring control group). The daytime and nighttime temperatures in each week are shown in Fig. 1.

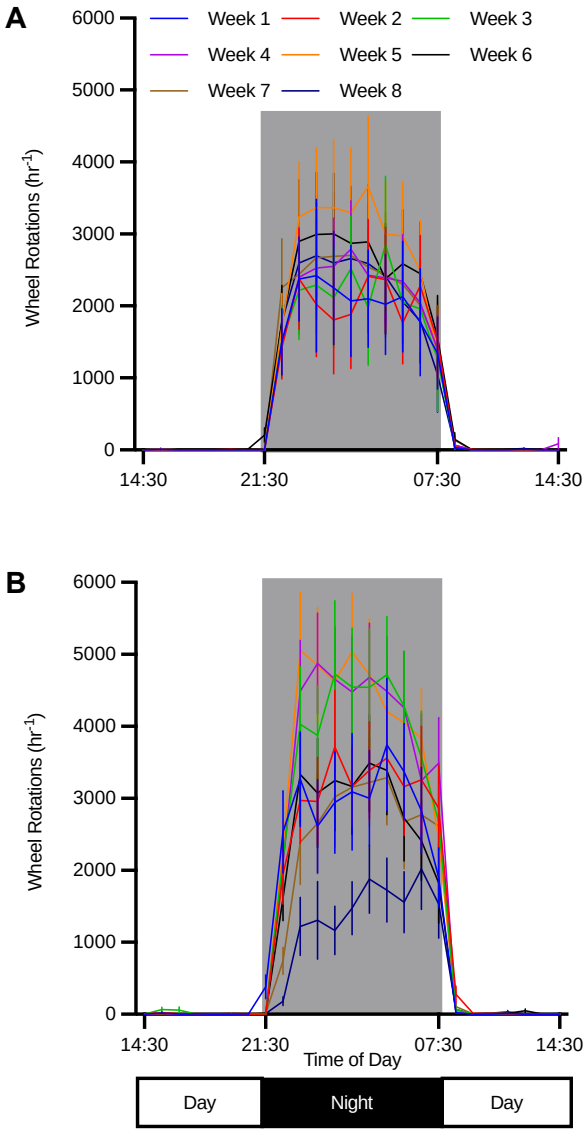
A
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Wheel Rotations (hr-1)
6000
5000
4000
3000
2000
1000
0
14:30
21:30
07:30
14:30
B
Wheel Rotations (hr-1)
Time of Day
Day
Night
Day

Fig. 4. Summer warming altered wheel running across the daily cycle. The number of wheel revolutions was measured every hour for each cage (each of which contained two mice). The average diel cycle of wheel running is shown as mean ± SEM in (A) the spring control group (N=8 cages) and (B) the summer warming group (N=5 cages). The grey shaded area reflects the dark nighttime phase when mice are active, and the white areas reflects the light daytime phase of inactivity. The daytime and nighttime temperatures in each week are shown in Fig. 1.

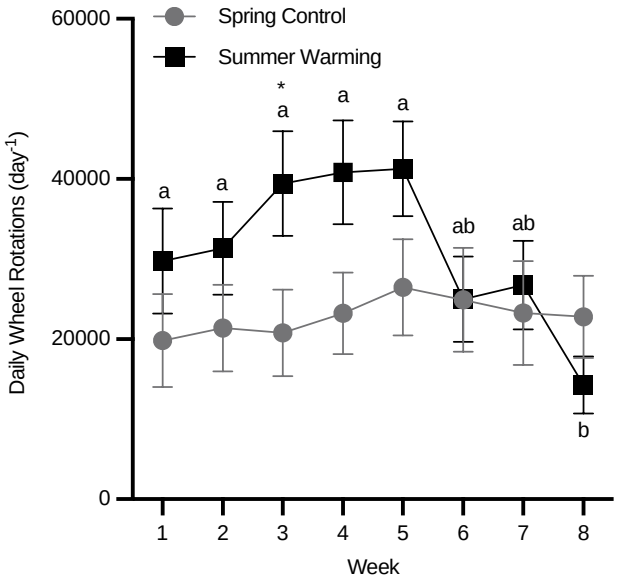
Spring Control
Summer Warming
Daily Wheel Rotations (day-1)
60000
40000
20000
0
1
2
3
4
5
6
7
8
Week
a
a
*
a
a
a
ab
ab
b

Fig. 5. Summer warming reduced total daily wheel running. Average daily wheel rotations were measured each week for the spring control group (grey circles; N=8) and the summer warming group (black squares; N=5), and data are shown as mean ± SEM. * Represents a significant pairwise difference between treatments within each week ($P<0.05$). Within each treatment group, weeks not sharing a letter are significantly different ($P<0.05$) (there were no significant differences between weeks in the spring control group). The daytime and nighttime temperatures in each week are shown in Fig. 1.

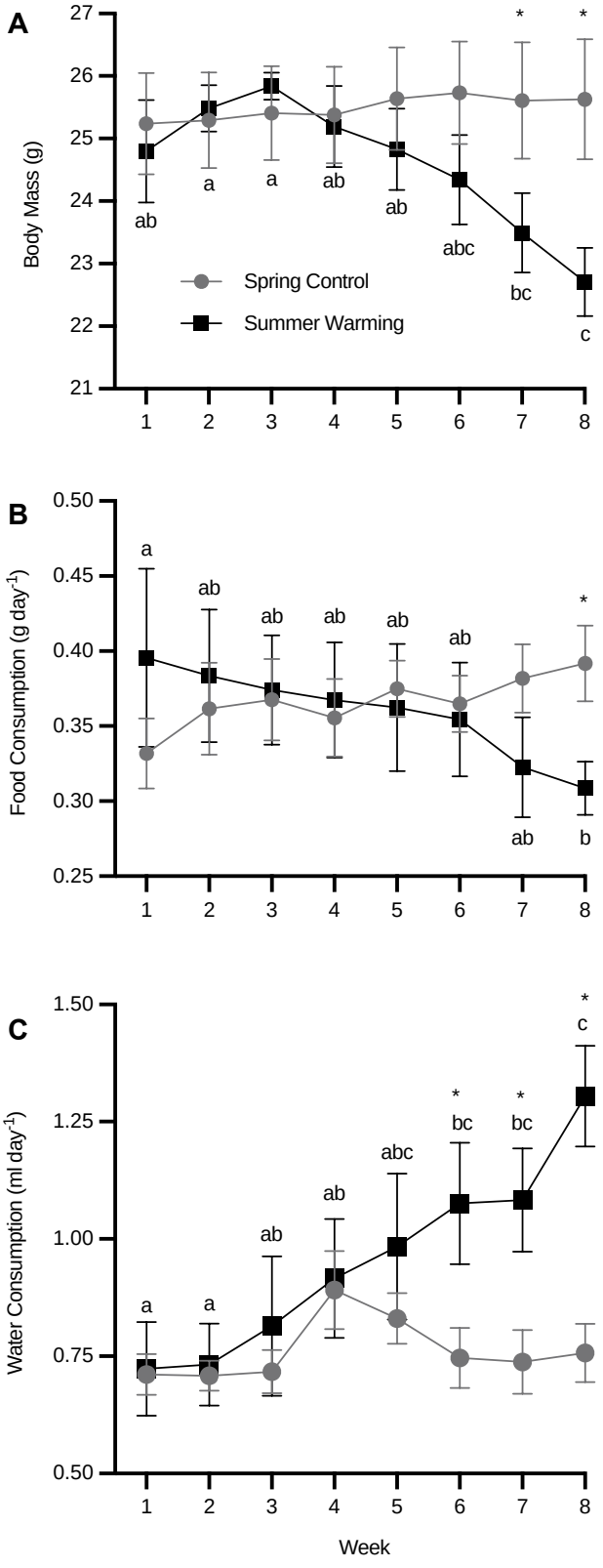
A
Body Mass (g)
27
26
25
24
23
22
21
ab
a
a
ab
ab
abc
bc
c
*
*
Spring Control
Summer Warming
1
2
3
4
5
6
7
8
B
Food Consumption (g day-1)
0.50
0.45
0.40
0.35
0.30
0.25
a
ab
ab
ab
ab
ab
ab
b
*
C
Water Consumption (ml day-1)
1.50
1.25
1.00
0.75
0.50
a
a
ab
ab
abc
bc
bc
c
*
*
*
Week

Fig. 6. Summer warming reduced body mass (A) and daily food consumption (B), and increased daily water consumption (C). Data for the spring control group (grey circles; N=16, 8, and 8, respectively) and the summer warming group (black squares; N=10, 5, and 5) are shown as mean ± SEM. * Represents a significant pairwise difference between treatments within each week ($P<0.05$). Within each treatment group, weeks not sharing a letter are significantly different ($P<0.05$) (there were no significant differences between weeks in the spring control group). The daytime and nighttime temperatures in each week are shown in Fig. 1.

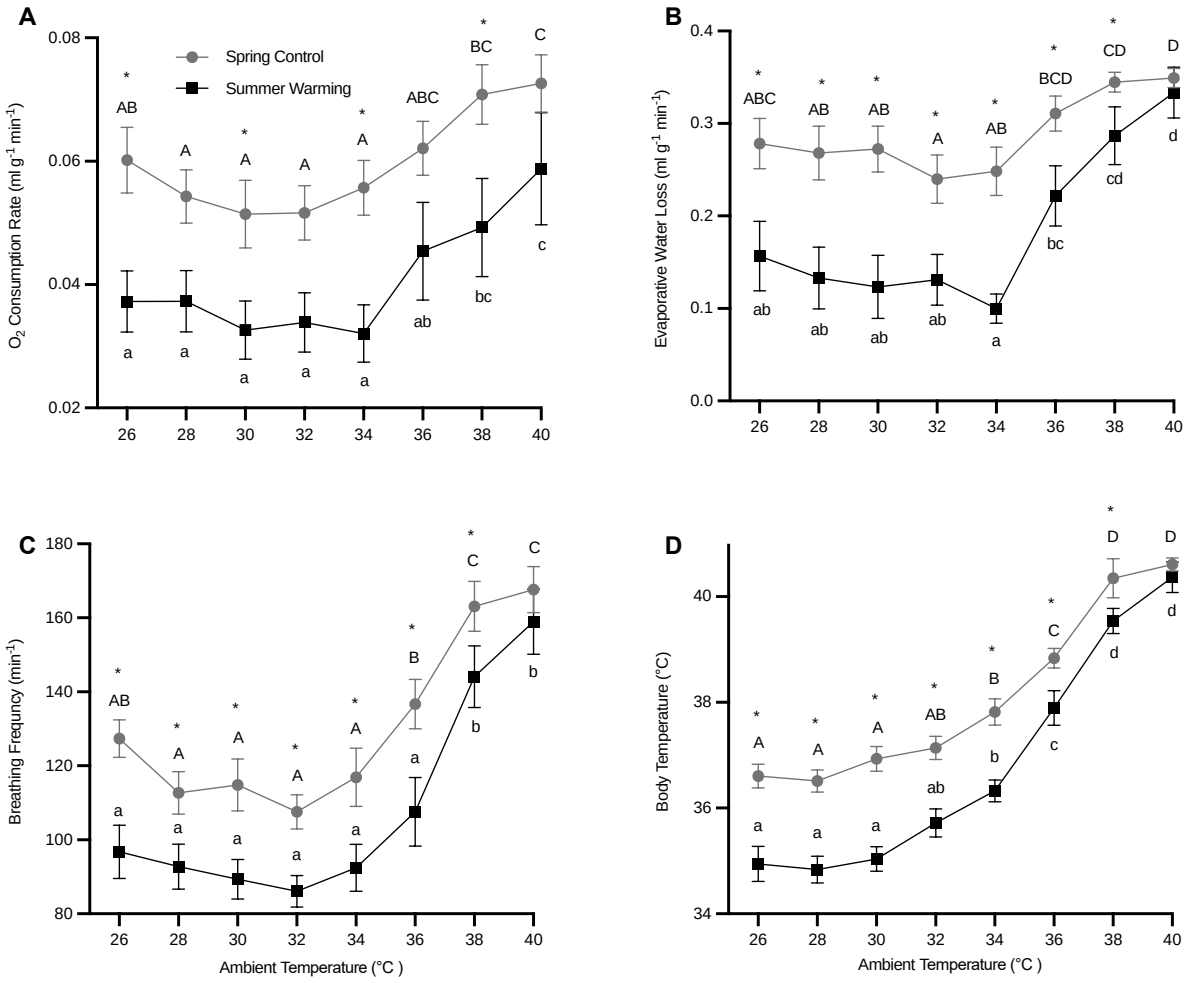
A
B
C
D
Spring Control
Summer Warming
O_2 Consumption Rate (ml g^{-1} min^{-1})
Evaporative Water Loss (ml g^{-1} min^{-1})
Breathing Frequncy (min^{-1})
Body Temperature (°C)
Ambient Temperature (°C)
Ambient Temperature (°C)

Fig. 7. Summer warming reduced resting O_2 consumption rate (A), evaporative water loss (B), breathing frequency (C), and body temperature (D) across a range of ambient temperatures. Data for the spring control group (grey circles) and the summer warming group (black squares) are shown as mean ± SEM. N=22 for spring control group and N=13 for the summer warming group, except for one individual in each group for which technical issues precluded breathing frequency measurements at a subset of ambient temperatures (26˚C and 38°C in the spring control group and 36°C in the summer warming group). * Represents a significant pairwise differences between treatment groups within each ambient temperature ($P<0.05$). Within each treatment group, ambient temperatures not sharing a letter are significantly different ($P<0.05$).

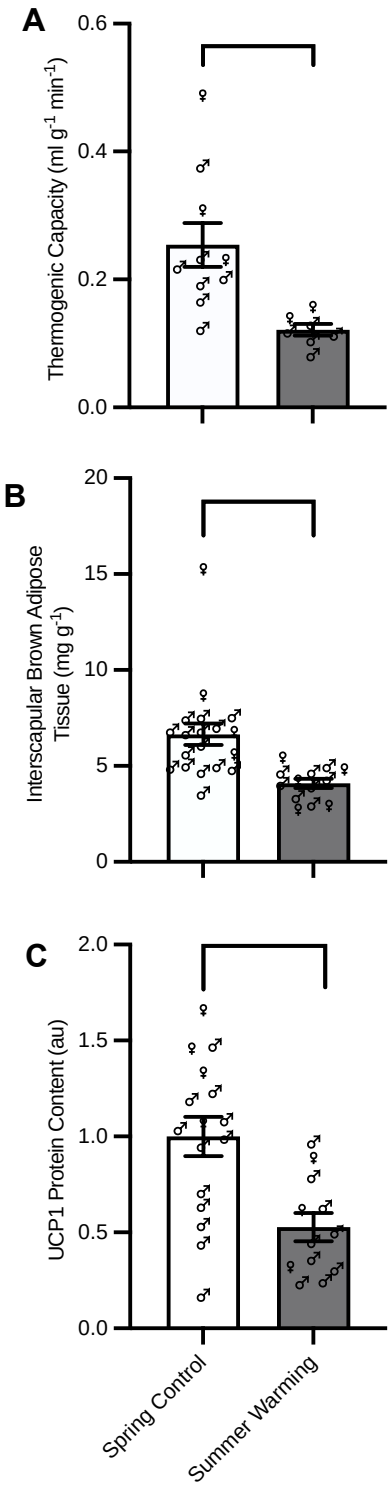
A
Thermogenic Capacity (ml g-1 min-1)
0.6
0.4
0.2
0.0
B
Interscapular Brown Adipose Tissue (mg g-1)
20
15
10
5
0
C
UCP1 Protein Content (au)
2.0
1.5
1.0
0.5
0.0
Spring Control
Summer Warming

Fig. 8. Summer warming reduced thermogenic capacity and induced phenotypic changes in thermogenic tissues. (A) Thermogenic capacity (measured as the maximal rate of O_2 consumption during acute cold exposure), (B) mass of interscapular brown adipose tissue (iBAT; in units mg per g body mass), and (C) the abundance of uncoupling protein 1 (UCP1) in iBAT (in arbitrary units, au, expressed relative to the average value in the spring control group) are shown with bars indicating means ± SEM and symbols representing individual values for males (♂) and females (♀). * Represents a significant difference between treatment groups ($P<0.05$).

Table 1. Tissue phenotypes.

Phenotype	Spring control group	Summer warming group
iBAT CS content (au)	1.00 ± 0.05 (20)	0.91 ± 0.09 (12)
iBAT UCP1/CS contents (au)	1.00 ± 0.07 (20)	0.62 ± 0.08* (12)
Gastrocnemius mass (mg g^{-1})	4.37 ± 0.23 (20)	4.31 ± 0.11 (13)
Gastrocnemius CS activity (μmol g^{-1} min^{-1})	21.55 ± 1.86 (19)	14.76 ± 3.24 (11)
Gastrocnemius COX activity (μmol g^{-1} min^{-1})	22.35 ± 1.28 (19)	24.61 ± 2.07 (11)
Gastrocnemius HOAD activity (μmol g^{-1} min^{-1})	25.78 ± 1.75 (19)	23.66 ± 1.52 (11)
Gastrocnemius LDH activity (μmol g^{-1} min^{-1})	376.08 ± 27.17 (19)	392.57 ± 34.50 (11)
Diaphragm mass (mg g^{-1})	3.57 ± 0.15 (20)	3.34 ± 0.16 (13)
Heart ventricle mass (mg g^{-1})	5.15 ± 0.15 (20)	4.90 ± 0.12 (13)
Lung mass (mg g^{-1})	7.23 ± 0.28 (20)	6.94 ± 0.30 (13)
Liver mass (mg g^{-1})	40.10 ± 2.00 (20)	38.38 ± 1.65 (13)
Stomach mass (mg g^{-1})	15.21 ± 0.51 (20)	13.43 ± 1.17 (13)
Small intestine mass (mg g^{-1})	18.95 ± 0.95 (20)	16.99 ± 1.24 (13)
Kidney mass (mg g^{-1})	12.39 ± 0.33 (20)	11.27 ± 0.40* (13)
Haematocrit (%)	46.57 ± 1.13 (20)	44.20 ± 1.63 (13)
Blood haemoglobin content (g/dl)	12.83 ± 0.81 (19)	14.98 ± 0.72* (13)
Mean cell haemoglobin content (g/dl)	27.8 ± 1.86 (19)	33.81 ± 1.62* (13)

Enzyme activities (μmol substrate per g tissue per min), iBAT CS content (au, arbitrary units, expressed relative to the average value in the spring control group), tissue masses (mg per g body mass), and haematological traits are shown as mean ± SEM (N). COX, cytochrome c oxidase; CS, citrate synthase; HOAD, hydroxyacyl-CoA dehydrogenase; LDH, lactate dehydrogenase iBAT; interscapular brown adipose tissue. * Represents a significant effect of treatment.

2.7 SUPPLEMENTARY INFORMATION

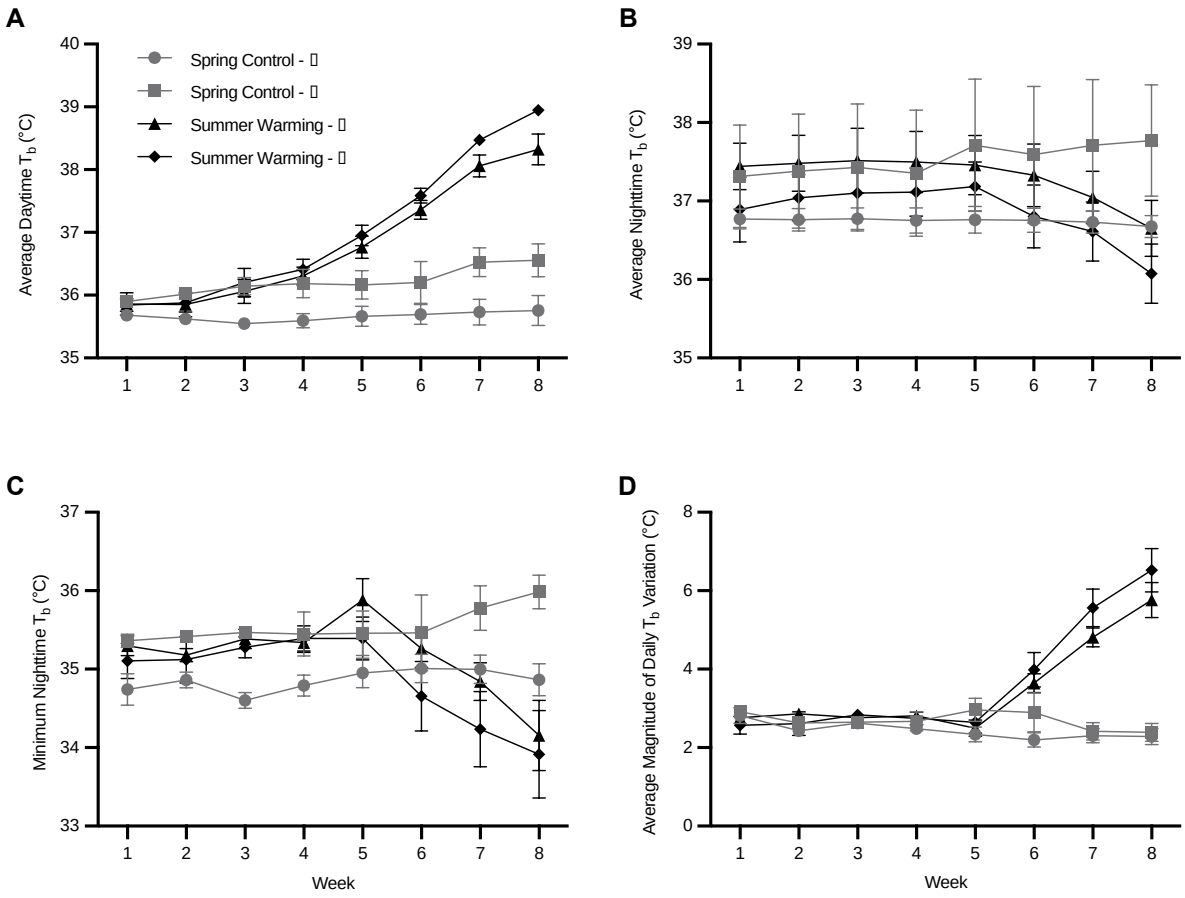
A
B
C
D
Spring Control - □
Spring Control - ▯
Summer Warming - △
Summer Warming - ◇
Average Daytime T_b (°C)
Average Nighttime T_b (°C)
Minimum Nighttime T_b (°C)
Average Magnitude of Daily T_b Variation (°C)
Week
Week

Fig S1. Females (♀) had greater average body temperature (T_b) and T_b variation than males (♂) overall across weeks within both treatment groups. (A) Average daytime T_b, (B) average nighttime T_b, (C) minimum nighttime T_b, and (D) the magnitude of daily T_b variation in the spring control males (N=7) and females (N=3) and in the summer warming males (N=5) and females (N=5), shown as mean ± SEM.

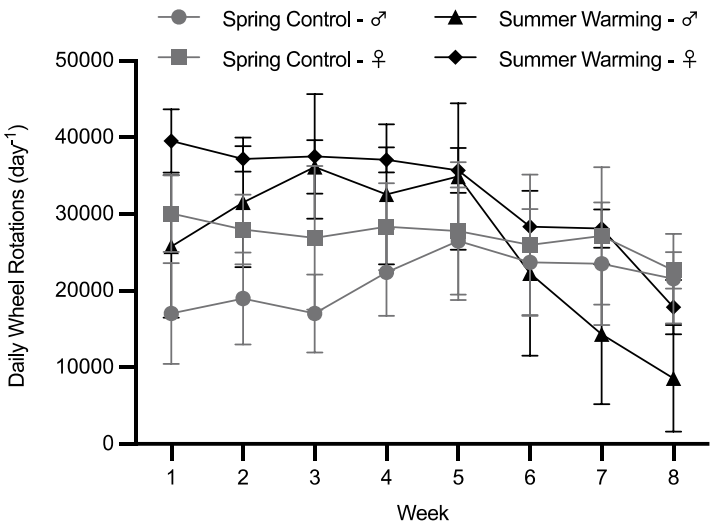
Spring Control - ♂
Summer Warming - ♂
Spring Control - ♀
Summer Warming - ♀
Daily Wheel Rotations (day-1)
50000
40000
30000
20000
10000
0
1
2
3
4
5
6
7
8
Week

Fig S2. Females (♀) had higher total daily wheel running activity than males (♂) overall across weeks within both treatment groups. Average daily wheel rotations were measured each week for spring control males (N=7) and females (N=3) and for summer warming males (N=5) and females (N=5), and are shown as mean ± SEM.

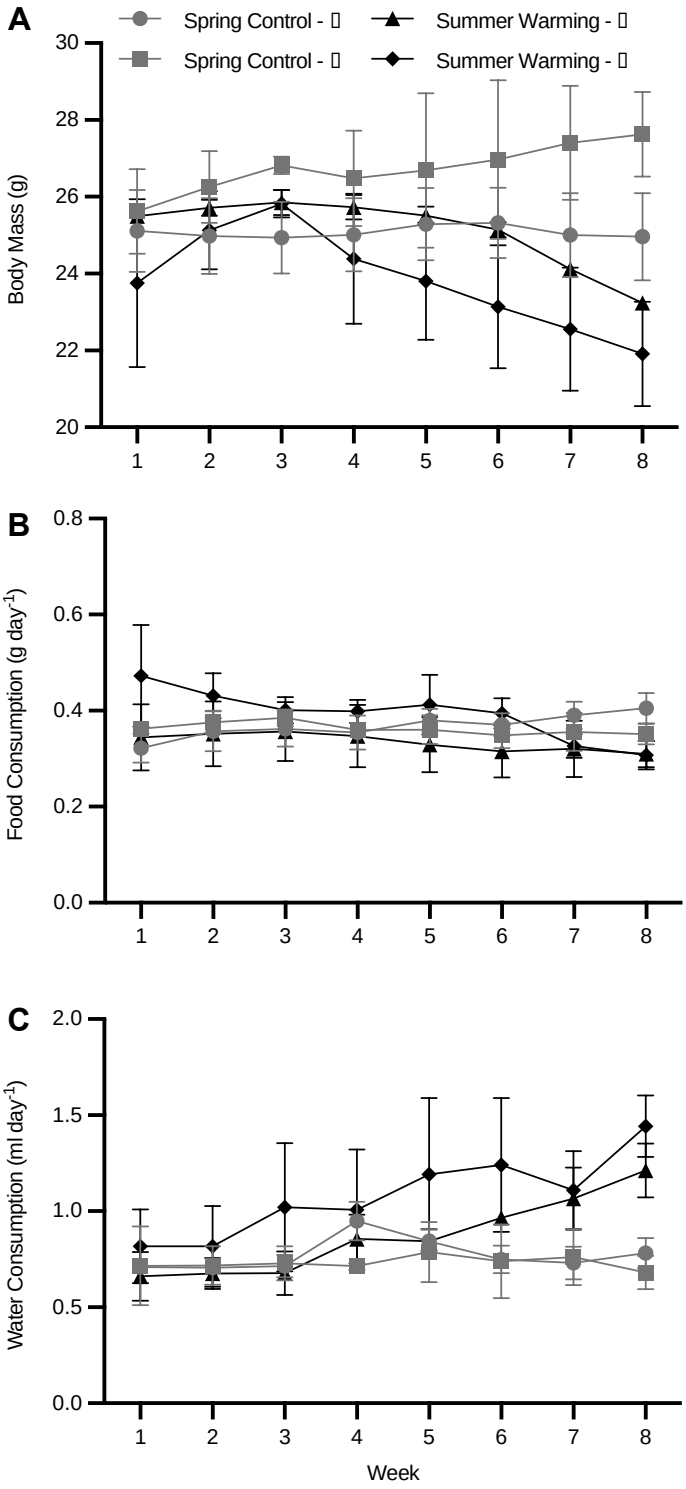
A
Spring Control - ♀
Summer Warming - ♀
Spring Control - ♂
Summer Warming - ♂
Body Mass (g)
30
28
26
24
22
20
1
2
3
4
5
6
7
8
B
Food Consumption (g day-1)
0.8
0.6
0.4
0.2
0.0
C
Water Consumption (ml day-1)
2.0
1.5
1.0
0.5
0.0
Week

Fig S3. Body mass (A), similar daily food consumption (B), and increased daily water consumption (C) in females (♀) and males (♂). Data for the spring control males (N=12, 6, 6, respectively) and females (4, 2, 2, respectively) and for the summer warming males (6, 3, 3, respectively) and females (4, 2, 2, respectively) are shown as mean ± SEM.

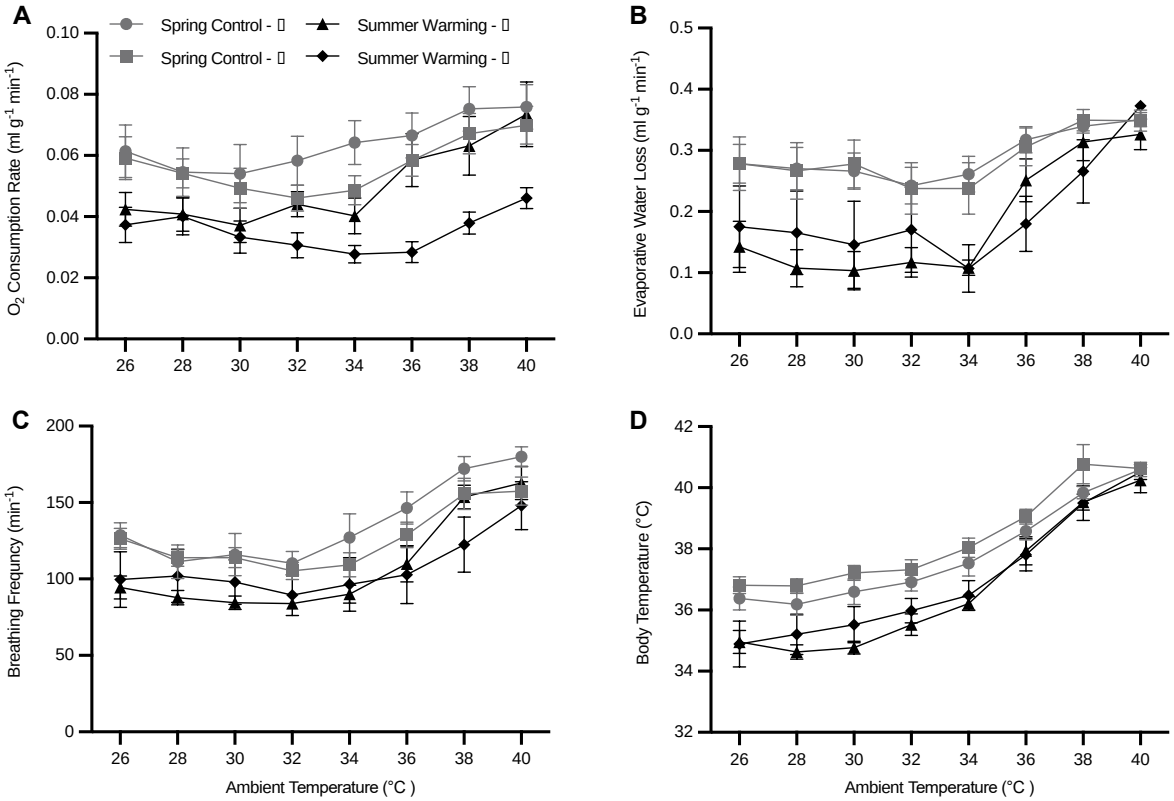
A
Spring Control - □
Summer Warming - □
Spring Control - □
Summer Warming - □
O2 Consumption Rate (ml g-1 min-1)
0.00
0.02
0.04
0.06
0.08
0.10
26
28
30
32
34
36
38
40
B
Evaporative Water Loss (ml g-1 min-1)
0.0
0.1
0.2
0.3
0.4
0.5
C
Breathing Frequncy (min-1)
0
50
100
150
200
Ambient Temperature (°C)
D
Body Temperature (°C)
32
34
36
38
40
42
Ambient Temperature (°C)

Fig S4. Resting O_2 consumption rate (A), evaporative water loss rate (B), breathing frequency (C), and body temperature (D) across ambient temperatures in females (♀) and males (♂). Data for the **s**pring control males (N=10) and females (N=12) and for the summer warming males (N=9) and females (N=4) are shown as mean ± SEM.

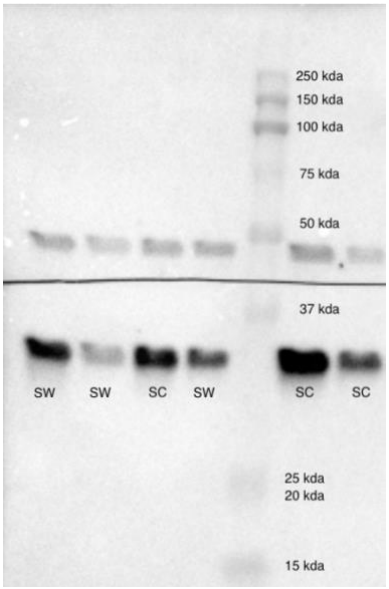
250 kda
150 kda
100 kda
75 kda
50 kda
37 kda
SW
SW
SC
SW
SC
SC
25 kda
20 kda
15 kda

Fig. S5. Representative western blot for uncoupling protein 1 (UCP1) and citrate synthase (CS). A protein ladder was loaded into lane 5 and the weights of each band are as follows from top to bottom: 250, 150, 100, 75, 50, 37, 25, 20, and 15 kDa. Remaining lanes show representative bands for the spring control group (lanes 1, 3, and 6) and the summer warming group (lanes 2, 4, and 7). The horizontal line shows where the membrane was cut between the 50 kDa and 37 kDa bands of the protein ladder to facilitate separate staining of UCP1 in the bottom half (showing the expected bands at 30 kDa) and CS in the top half (showing the expected bands at 52 kDa).

Supplemental Tables

Table S1. Statistical results for linear models of body temperature and wheel running data collected throughout the daily cycle

Trait	Time	Week	Sex	Time×Week	Week×Sex	Time×Week×Sex
SC T_b	$F_{143,9209}$=49.39 **P<0.001**	$F_{7,9209}$=28.11 **P<0.001**	$F_{1,9209}$=123.61 **P<0.001**	$F_{1001,9209}$=0.49 P=0.999	$F_{7,9209}$=14.18 **P<0.001**	$F_{1001,9209}$=0.22 P=0.999
SW T_b	$F_{143,9209}$=8.16 **P<0.001**	$F_{7,9209}$=729.34 **P<0.001**	$F_{1,9209}$=21.69 **P<0.001**	$F_{1001,9209}$=10.98 **P<0.001**	$F_{7,9209}$=9.04 **P<0.001**	$F_{1001,9209}$=0.43 P=0.999
SC WR	$F_{23,1146}$=105.26 **P<0.001**	$F_{7,1146}$=2.41 **P=0.01862**	$F_{1,1146}$=19.72 **P<0.001**	$F_{161,1146}$=0.34 P=0.999	$F_{7,1146}$=1.48 P=0.17087	$F_{161,1146}$=0.22 P=0.999
SW WR	$F_{23,1146}$=108.422 **P<0.001**	$F_{7,1146}$=20.08 **P<0.001**	$F_{1,1146}$=10.08 **P<0.001**	$F_{161,1146}$=1.51 **P<0.001**	$F_{7,1146}$=1.58 P=0.1380	$F_{161,1146}$=0.36 P=0.999

SC, Spring control group; SW, Summer warming group; T_b, body temperature; WR, wheel running. Individual or cage were also included as factors as appropriate to account for repeated measurements and had a significant effect in each model (P<0.001).

Table S2. Statistical results for linear models of average daily body temperature (T_b) and T_b variation

Trait	TR	Week	Sex	TR×Sex	TR×Week	TR×Week×Sex
Average daytime T_b	$F_{1,122}$=746.54 **P<0.001**	$F_{7,122}$=108.44 **P<0.001**	$F_{1,122}$=75.95 **P<0.001**	$F_{7,122}$= 21.3 **P<0.001**	$F_{7,122}$=74.13 **P<0.001**	$F_{7,122}$=0.19 P=0.986
Average night-time T_b	$F_{1,122}$=10.78 **P=0.001**	$F_{7,122}$=1.73 P=0.109	$F_{1,122}$=6.98 **P=0.009**	$F_{7,122}$=116.97 **P<0.001**	$F_{7,122}$= 2.28 **P=0.032**	$F_{7,122}$=0.17 P=0.990
Minimum T_b	$F_{1,122}$=0.14 **P<0.001**	$F_{7,122}$=2.97 **P<0.001**	$F_{1,122}$=3.08 **P=0.033**	$F_{7,122}$=26.85 **P<0.001**	$F_{7,122}$= 5.74 **P<0.001**	$F_{7,122}$=0.32 P=0.849
Daily T_b variation	$F_{1,122}$=173.51 **P<0.001**	$F_{7,122}$=28.83 **P<0.001**	$F_{1,122}$=6.91 **P=0.009**	$F_{7,122}$=5.52 **P=0.020**	$F_{7,122}$=42.02 **P<0.001**	$F_{7,122}$=0.94 P=0.480

TR, treatment; T_b, body temperature. Individual was included as a factor to account for repeated measurements and had a significant effect in each model (P<0.001).

Table S3. Statistical results for linear models of daily activity, body mass, food consumption, and water consumption

Trait	TR	Week	Sex	TR×Sex	TR×Week	TR×Week×Sex
Daily WR	$F_{1,63}$=455.1 **P=0.004**	$F_{7,63}$=66.11 P=0.094	$F_{1,63}$=46.30 **P=0.003**	$F_{1,72}$=0.034 P=0.853	$F_{7,63}$=31.92 **P=0.009**	$F_{7,63}$=0.12 P=0.961
Body mass	$F_{1,63}$=71.36 **P<0.001**	$F_{7,63}$=5.46 **P<0.001**	$F_{1,63}$=9.23 **P=0.003**	$F_{1,72}$=10.66 **P=0.002**	$F_{7,63}$=14.75 **P<0.001**	$F_{7,63}$=1.80 P=0.101
Food consumption	$F_{1,63}$=0.83 P=0.363	$F_{7,63}$=0.2187 P=0.979	$F_{1,63}$=5.3875 **P<0.001**	$F_{1,72}$=3.384 P=0.070	$F_{7,63}$= 3.07 **P= 0.008**	$F_{7,63}$= 0.28 P= 0.958
Water consumption	$F_{1,63}$=47.43 **P<0.001**	$F_{7,63}$=6.04 **P<0.001**	$F_{1,63}$=5.61 **P=0.021**	$F_{1,72}$=7.42 **P=0.008**	$F_{7,63}$=6.17 **P<0.001**	$F_{7,63}$=0.l6 P=0.683

TR, treatment; WR, wheel rotations. Cage was included as a factor to account for repeated measurements and had a significant effect in each model (P<0.001).

Table S4. Statistical results for linear models of metabolic and thermoregulatory responses to acute warming at rest

Trait	TR	T_a	Sex	TR×T_a	T_a×Sex	TR×T_a×Sex
VO_2	$F_{1,216}$=455.09 **P<0.001**	$F_{7,216}$=66.10 **P<0.001**	$F_{1,216}$=46.30 **P<0.001**	$F_{7,216}$=31.916 P=0.548	$F_{7,216}$=31.91 **P=0.045**	$F_{7,216}$=0.12 P=0.127
EWL	$F_{1,217}$=128.16 **P<0.001**	$F_{7,217}$=23.36 **P<0.001**	$F_{1,217}$=0.10 P=0.750	$F_{7,217}$=4.23 **P<0.001**	$F_{7,217}$=0.59 P=0.759	$F_{7,217}$=0.83 P=0.558
f_R	$F_{1,214}$=86.52 **P<0.001**	$F_{7,214}$=55.09 **P<0.001**	$F_{1,214}$=8.41 **P=0.004**	$F_{7,214}$=0.96 P=0.45816	$F_{7,214}$=2.33 P=0.026	$F_{7,214}$=0.57 P=0.774
T_b	$F_{1,214}$=155.28 **P<0.001**	$F_{7,214}$=169.84 **P<0.001**	$F_{1,214}$=16.41 **P<0.001**	$F_{7,214}$=3.49 **P=0.001**	$F_{7,214}$=0.44 P=0.873	$F_{7,214}$=0.43 P=0.879

TR, treatment; T_a, ambient temperature; VO_2, O_2 consumption rate; EWL, evaporative water loss; f_R, breathing frequency; T_b, body temperature. Individual was included as a factor to account for repeated measurements and had a significant effect in each model (P<0.001).

Table S5. Statistical results for linear models of thermogenic capacity and tissue masses

Trait	TR	Sex	TR×Sex
Gastrocnemius mass	$F_{1,28}$=0.11	$F_{1,28}$=2.36	$F_{1,28}$=0.28
	P=0.733	P=0.135	P=0.596
iBAT mass	$F_{1,27}$=16.7	$F_{1,27}$=3.74	$F_{1,27}$=5.77
	P<0.001	P=0.063	**P=0.023**
Diaphragm mass	$F_{1,28}$=1.18	$F_{1,28}$=2.36	$F_{1,28}$=1.06
	P=0.286	P=0.135	P=0.312
Heart ventricle mass	$F_{1,28}$=1.42	$F_{1,28}$=1.65	$F_{1,28}$=8.99
	P=0.243	P=0.209	**P=0.006**
Lung mass	$F_{1,28}$=0.21	$F_{1,28}$=1.77	$F_{1,28}$=1.20
	P=0.650	P=0.194	P=0.282
Liver mass	$F_{1,28}$=0.22	$F_{1,28}$=0.15	$F_{1,28}$=0.01
	P=0.644	P=0.703	P=0.999
Stomach mass	$F_{1,28}$=2.65	$F_{1,28}$=0.83	$F_{1,28}$=4.08
	P=0.115	P=0.369	P=0.053
Small intestine mass	$F_{1,28}$=1.31	$F_{1,28}$=2.83	$F_{1,28}$=1.90
	P=0.262	P=0.104	P=0.179
Kidney mass	$F_{1,28}$=4.83	$F_{1,28}$=1.70	$F_{1,28}$=0.13
	P=0.036	P=0.202	P=0.717
Haematocrit	$F_{1,28}$=1.54	$F_{1,28}$=7.77	$F_{1,28}$=0.36
	P=0.225	**P=0.009**	P=0.553
Blood haemoglobin content	$F_{1,27}$=6.56	$F_{1,27}$=0.07	$F_{1,27}$=0.79
	P=0.016	P=0.786	P=0.381
Mean cell haemoglobin content	$F_{1,27}$=10.49	$F_{1,27}$=1.99	$F_{1,27}$=1.53
	P=0.003	P=0.169	P=0.227
Interscapular brown adipose tissue (iBAT) protein contents			
UCP1	$F_{1,24}$=15.20	$F_{1,24}$=5.75	$F_{1,24}$=2.02
	P<0.001	**P=0.025**	P=0.167
CS	$F_{1,24}$=0.71	$F_{1,24}$=3.56	$F_{1,24}$=0.81
	P=0.407	P=0.071	P=0.377
UCP1/CS	$F_{1,24}$=10.85	$F_{1,24}$=1.72	$F_{1,24}$=5.07
	P=0.003	P=0.201	**P=0.034**
Gastrocnemius enzyme activities			
COX	$F_{1,25}$=0.90	$F_{1,25}$=0.10	$F_{1,25}$=0.69
	P=0.350	P=0.750	P=0.411
HOAD	$F_{1,25}$=0.53	$F_{1,25}$=0.98	$F_{1,25}$=0.003
	P=0.475	P=0.333	P=0.959
CS	$F_{1,25}$=0.56	$F_{1,25}$=1.95	$F_{1,25}$=0.62
	P=0.464	P=0.178	P=0.440

LDH	$F_{1,25}$=0.07 P=0.799	$F_{1,25}$=2.56 P=0.123	$F_{1,25}$=0.63 P=0.434

TR, treatment; iBAT, interscapular brown adipose tissue; UCP1, uncoupling protein; 1 CS, Citrate synthase; COX, Cytochrome c oxidase; HOAD, hydroxyacyl CoA dehydrogenase; LDH, lactate dehydrogenase. Individual was included as a factor to account for repeated measurements and had a significant effect in each model (P<0.001).

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CHAPTER 3: GENERAL DISCUSSION

With increasing T_a from climate warming, animals across the globe are being thermally stressed. In endotherms, climate warming has the potential to challenge their ability to maintain T_b and, consequently, their ability to maintain energy levels, activity, and body mass. Exposure to high T_a at various latitudes can have many detrimental effects on small mammals, which can be fatal at extremes (Danner et al., 2021; McKechnie and Wolf, 2019). I sought to investigate how deer mice cope with ecologically relevant increases in temperature from the spring to mid-summer, focusing on changes in T_b and activity and corresponding physiological changes. The primary objective of my thesis was to gain further insight into how exposure to warming temperatures in areas of decreasing population abundance impacts the metabolic physiology and activity of endotherms. The overarching hypothesis is that exposure to warming diel temperature cycles, representative of the transition from spring to mid-summer, would disrupt thermoregulation and thus impair activity levels.

3.1 Summary of Key Findings

I have shown that transitioning to ecologically realistic summer temperatures results in changes in deer mouse physiology similar to heat acclimation but may have detrimental health impacts when exposed to cool nighttime temperatures. Specifically, dysregulation of T_b at T_a greater than the TNZ resulted in the reversal of the diel pattern of T_b, leading to increased T_b during the day and decreased T_b at the cooler nighttime T_a. This was accompanied by a reduction in thermogenic capacity, mass and UCP1 content of the iBAT, likely due to heat acclimation in response to the summer warming daytime T_a. These adjustments resulted in changes in behaviour during the active phase by reducing voluntary activity, food consumption, and body mass. These findings suggest that thermoregulatory trade-offs occur during warming summer temperatures in deer mice to increase fitness at high T_a, while still being constrained by physiological limitations when exposed to cooler nighttime T_a. Animals that have been extirpated from their previous

range are valuable models to study as they provide us with further insight into the physiological changes that occur in ecologically relevant areas that will be impacted further by climate warming.

High T_a can affect animals in a variety of ways, perhaps the most significant being the impacts on T_b, which can result in many other physiological consequences. The daytime heat exposure in this study is suggested to contribute to the decrease in T_b at the cooler nighttime T_a. Studies have previously shown that heat exposure which increases T_b results in a decrease in T_b once removed from the high T_a (Leon et al., 2005; Wilkinson et al., 1988). We suggested this decline in nighttime T_b is likely to be attributed to the residual and plastic responses of heat exposure from the previous day and the decrease in thermogenic capacity due to a reduction in mass and UPC1 content of the iBAT. After the initial drop in T_b, the recovery and slow increase in nighttime T_b demonstrates that one potential source of heat during rewarming is locomotion, though it is unlikely to fulfill all thermogenic needs of the animal (Gordon and Yang, 1997; Ruperto et al., 2022; Vaanholt et al., 2007). While limited after heat acclimation, shivering and non-shivering thermogenesis is another potential heat source to increase T_b back to baseline levels.

We demonstrated that nocturnal species that experience high daytime T_a also have reduced and delayed nighttime activity, likely caused by the decrease in nighttime T_b which has been demonstrated previously (Weinert and Waterhouse, 1998). This decrease in activity following high T_a has been noted previously in marsupials and rodents, where locomotor performance is impaired at low T_b (Meek et al., 2009; Rojas et al., 2012) and where heat stress causes delayed arousal the following day in mice (Fregly et al., 1957; Leon et al., 2005). Temperature-dependent activity may be explained in part by the effect of low temperatures on neural transmission, tetanic tension, and muscle power output (Cullingham et al., 1960; Rojas et al., 2012). The decrease in the voluntary activity seen in this study also coincides with a daytime T_a similar to and greater than the T_a at which deer mice reduce their maximal endurance capacity (>35°C; Eizenga et al., 2022). How these limiting temperatures may impact other performance traits related to fitness remains

largely untested and may provide valuable mechanistic information to understand how climate change impacts animals in the wild.

Changes in resource acquisition and consumption can have many implications on the health and fitness of individuals. Therefore, another adverse effect of high heat exposure was decreased body mass and food consumption. It is common for many stressors, including acute or chronic heat, to result in decreases in food consumption and therefore result in a decrease in body mass (Dikstein et al., 1970; Harikai et al., 2003; Sareh et al., 2011; Shido et al., 1994; Vaanholt et al., 2007; Yamauchi et al., 1983). There was also an increase in water consumption, as mice had access to ad libitum drinking water, likely to compensate for the increased demand placed on evaporative cooling during exposure to high T_a and during heat acclimation (Périard et al., 2015).

Chronic exposure to high temperatures leads to heat acclimation, characterized by improved heat dissipation capacity and tolerance (Glaser, 1949; Horowitz, 2001; Racinais et al., 2019; Sareh et al., 2011). The physiological adjustments seen in this study are typical of heat acclimation in other species, such as reductions in metabolic rate, heat production (Guo et al., 2020; Shido et al., 1994), and increased capacities for evaporative heat dissipation (Horowitz, 2001; Sareh et al., 2011). These findings also showed that the upper-temperature threshold that induces a heat response prior or after acclimation is similar to those at which endurance capacity is impaired (Eizenga et al., 2022). However, heat acclimation reduced thermogenic capacity, which is consistent with previous findings in other small mammals (Guo et al., 2020; Kuroshima et al., 1982), and likely contributed to the poor ability of deer mice in the summer warming group to maintain T_b at cooler nighttime T_a.

3.2 Prospective future research

Previous literature has supported aspects of my research; however, the novelty of this study has led to new questions that warrant future examination. While not measured in this study, additional impacts of heat acclimation include decreased heart rate (Horowitz, 2001), changes in fluid balances (i.e. reduced electrolyte loss), increased total

body water (Mitchell et al., 2018; Périard et al., 2015), and increased baseline levels of heat shock proteins, allowing for further improvements during future heat stress (Horowitz, 2001; Sareh et al., 2011). These modifications during heat acclimation play an important role in the ability of animals to tolerate and survive changes in their environment and, therefore should be addressed in deer mice in the future. During heat acclimation, the improvement in heat tolerance comes at the expense of thermogenesis as it is less important and may need to be suppressed to limit heat production (Kuroshima, 1993; Kuroshima and Yahata, 1979; Saha et al., 2000). A previous study on rats showed this decrease might be caused by a reduction in the metabolic activity of the BAT through an increase in the saturated phospholipid fatty acid level, while reducing the monosaturated fatty acid ratio (Saha et al., 2000). This area remains largely unexplored in warm acclimation conditions with other species and may explain why there was a decreased thermogenic capacity unrelated to the reduction in UCP1 content. Another possibility for the reduction in thermal capacity could be interpreted as a result of thermal damage to homeostatic sites such as the central nervous system or as an adaptive thermoregulatory survival mechanism to prevent intestinal damage and increase survivability, as noted previously in mice (Wilkinson et al., 1988). Whether the T_a used in the present study was high enough to result in thermal damage to the central nervous system is unknown but still clearly warm enough to have lasting physiological impacts.

Muscles play an important role in locomotion and are also useful for shivering thermogenesis. Though there was no difference between treatments in the enzymatic activity within the gastrocnemius muscle, it is unclear whether shivering thermogenesis was negatively impacted by heat acclimation which could have contributed to the decrease in thermogenic capacity. This could be explored by characterizing the relative contribution of shivering and non-shivering thermogenesis through blocking neuromuscular transmission with curariform drugs to prevent shivering thermogenesis (Alexander and Williams, 1968), or through muscle electromyograms, though typically measured in larger species (Hohtola, 2004). Previous studies have noted that while non-shivering thermogenesis is able to increase its contribution to thermogenic capacity, after

cold acclimation, shivering thermogenesis is much more limited in its ability to change with acclimations (Coulson et al., 2021). Therefore, we may still not expect for shivering thermogenesis to vary dramatically with heat acclimation. Interestingly, while the ability of muscles to acclimate to temperatures is limited (Bennett, 1985), heat acclimation in humans, which often has similar physiological and cellular changes to mice (Sareh et al., 2011), has shown an increase in muscle force production when exposed to subsequent future high T_a (Périard et al., 2015). However, this increase in muscle force may be a result of higher T_b increasing the rate processes of muscles and allowing for greater muscle contractions to occur and not due to physiological modifications (Bennett, 1984; Bennett, 1985). Species differ in their ability to create maximal forces at cool temperatures, which upon further study, could provide another mechanistic view of limitations on locomotory ability at a variety of temperatures and other environmental conditions. While species such as deer mice are obligate nocturnal animals, many species can alter activity times, which may allow for increased muscle use and, therefore, locomotion. While this may be associated with more trade-offs to cope with high daytime T_a, it could provide animals with increased locomotory ability.

While resource consumption has been studied often, the mechanism responsible for changes in resource consumption is largely not specified. This study's decrease in food consumption and body mass were associated with indirect effects of heat exposure caused by stress. However, it is possible that the heat stress in the late weeks of the study caused a disorder, that limited food consumption, such as gastric ulcers and even mild bleeding, causing petechial hemorrhagic spots to form in the stomach, reducing body mass (Harikai et al., 2003). Additionally, while water consumption increased with increasing T_a, dehydration may have occurred in the summer warming acclimation, which could also explain the observed variation in haematology (as mentioned in chapter 2). Dehydration has been noted to induce hypothermia in hamsters (Ibuka and Fukumura, 1997) and may have contributed to the hypothermia seen in the later weeks of the summer warming acclimation. Therefore, determining how food and water consumption and changes in body mass occur with summer warming and how it may be compounded

during climate warming plays an important role in determining locations suitable for species to survive during high T_a exposure.

Acclimation to temperature extremes is important for determining physiological limits, however, as noted in this thesis, there are trade-offs associated with this acclimation. Therefore, studying the transitions between environmental extremes such as summer to winter acclimation is also important to determine how these trade-offs and physiological limiting factors change. While there are many studies on the acclimation to cold T_a in rodents (Haim, 1982; Haim and Fourie, 1980; Ukropec et al., 2006), there are few that highlight the transition from high summertime temperature acclimation into the transition of cold/winter temperature acclimation, especially in our current changing climate. This may be an important aspect to study as there is the potential to be lasting effects of heat acclimation and the reduced thermogenic capacity to persist into some of the cool winter months. The transition between seasons has dramatic impacts on animals, and if they are unable make the necessary physiological adjustments, there may be serious detriments in the ability to maintain physiological homeostasis and activity in the cooler months until thermogenesis capacity increases again.

Studying the differences between species and the physiological factors that may contribute to or limit range shifts is critical to understand how species will interact and react to climate warming on a broad scale. As mentioned, deer mice populations have declined in some regions and are being replaced by white-footed mice. Displacement could result from species differences in the effects of summer T_a on thermoregulation and/or activity, as deer mice may differ in their ability to maintain heat dissipation, T_b, and levels of activity at high T_a relative to white footed mice. This could require deer mice to shift their distribution away from southern locations. Alternatively, the northern range of white-footed mice could be limited by their thermogenic capacity to maintain T_b in cold winters, such that the species is moving north and displacing deer mice as winters warm (Roy-Dufresne et al., 2013). Additionally, the mice studied here come from a part of the Great Plains and are genetically distinct from populations from colder temperatures at northern latitudes (high elevation) and from southern desert populations. (Natarajan et

al., 2015). Comparisons between genetically distinct populations would be beneficial to determine physiological limitations and how potential species interactions may conflict and force further range displacement.

3.3 Physiological impacts of climate change

There are serious implications for future climate warming. As global temperatures continue to rise, species will likely become further thermally stressed, and highly exothermic activities important for fitness may become more limited. This has been noted previously in many species such as rodents and birds, where activity and ability to forage are reduced (Murray and Smith, 2012; Tapper et al., 2020a). Additionally, when rodents experience high T_a, they have demonstrated reduced lactation, leading to reduced pup size, increased mortality (Zhao et al., 2020), and a reduced upper lethal temperature by up to 6°C, making individuals more sensitive to high T_a (Speakman and Król, 2010; Zhao et al., 2020). In birds, it has also been shown that thermal constraints reduce provisioning rates and can lead to lower body masses (Tapper et al., 2020a; Tapper et al., 2020b) This suggests that future increases in T_a will impact the breeding success of many mammals and may have compounding effects with other physiological changes from high T_a exposure.

While thermal plasticity can be beneficial for species experiencing a range of T_a, temperatures above the TNZ result in decreased activity in deer mice and other rodents compared to cooler T_a, even when activity is not during the daytime high T_a (Ruperto et al., 2022). During activity there are increases in metabolic rate and heat accumulation within the body, compromising animals' thermal equilibrium (Speakman, 1999; Terrien et al., 2011). Therefore, the lower activity observed at higher T_a could be a strategy to limit heat production and avoid overheating (Speakman and Król, 2010). A study on the desert woodrat also found a strong relationship between T_a and the onset and duration of activity (Murray and Smith, 2012). As T_a increased, there were fewer and shorter bouts of activity and animals were constrained during intense summer months (Murray and Smith, 2012). Animals therefore face a trade-off between remaining in a more thermally safe area of a

den or burrow and emerging to obtain resources. Further, activity has been shown to decrease to a greater extent at high T_a when food was given ad libitum compared to restricted, demonstrating a trade-off between food acquisition and thermoregulation and how activity has a low capacity to adapt to temperature variations (Ruperto et al., 2022). However, the energetic costs of avoiding overheating and maintaining activity levels may not be sustainable for long durations by introducing food deprivation, as the animal may also have trouble maintaining energy balance and expend more energy than is acquired (McNab, 2012; Ruperto et al., 2022).

While nocturnal species may be less likely to face as great of a threat from high environmental T_a, there could be circumstances where animals are forced to experience these temperatures as a result of predation or various other stressors and, subsequently, undergo increased heat production from locomotion and stress. Therefore, this may result in a thermal challenge and limit their endurance capacity. This has similarly been demonstrated in deer mice exposed to high T_a, where performance is impaired at $T_a >$ 35°C, and that T_a largely determines T_b during activity at high T_a (Eizenga et al., 2022). However, the high daytime T_a can still impact nocturnal species that are not active during the highest T_a as shown in this study. The reduction in T_b at night can cause hypothermia following a period of heat stress, with the magnitude and duration of hypothermia correlated with heat stress severity (Leon et al., 2005; Wilkinson et al., 1988). This reduction in T_b reduces locomotion (Weinert and Waterhouse, 1998) and causes delays in arousal in nocturnal species (Fregly et al., 1957; Leon et al., 2005).

3.4 Conclusion

Overall, these findings suggest that seasonal warming from the spring to mid-summer leads to acclimatization to warming temperatures but disrupts thermoregulation, which can have negative impacts on the health and fitness of deer mice. I showed dysregulation of T_b when exposed to high daytime temperatures, which persist into their nighttime active phase and decrease activity and body mass. Overall, this indicates that warm summer temperatures are already impacting temperate endotherms. Continued

increases in average T_a and extreme heat events may have detrimental impacts that can threaten individuals' ability to remain active and may reduce reproductive success. Future research on species from various latitudes and acclimation to other seasonal conditions should be explored to provide further insight into how species are likely to respond to climate warming.

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