Investigating Thermoregulatory Responses of *Rhabdomys pumilio* at High Wet-bulb Temperatures

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Investigating Thermoregulatory Responses of *Rhabdomys pumilio* at High Wet-bulb Temperatures

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General Abstract

As the Anthropocene continues to be characterised by ever rising temperature highs, increasingly sporadic and extreme climatic events, and their accompanying mass mortality events, climate scientists now warn that the continued climate destabilisation may for the first time in recorded history prevent terrestrial homeothermic endotherms from being able to thermoregulate. Having evolved to maintain body temperatures (T_bs) well above their ambient (T_a) conditions, these endotherms have evolved a suite of adaptations to a colder environment to allow for homeothermic thermoregulation to occur. However, by the same vein, having evolved in a colder climate to stay warm also has led to a reduced capacity to prevent heat stress when temperature conditions are elevated. In absence of behavioural counter measures, the only physiological means available to thermoregulation to prevent such heat stress is that of evaporative water loss (EWL). If still inefficient, the endotherm will risk becoming hyperthermic.

Literature has established that excessive heat exposure or reduced evaporative cooling capacities strain or retard thermoregulatory processes. If an endotherm experiences severe heat exposure, the rate at which passive heating is experienced will increase. Should an endotherm be in a humid environment, evaporative cooling efficiency is reduced. As a result, these two abiotic factors are therefore known to contribute towards heat storage, and therefore thermal stress. However, should both factors occur in the same environment, the heat stress effects are compounded, creating an environment dangerous for thermoregulating endotherms. For this reason, authors have emphasised that future wet-bulb (T_w) conditions may pose a penultimate threat to thermoregulating endotherms.

Being a measure of coldest temperature attainable as a result of evaporative cooling, T_w has been proposed to represent a lower temperature thermal limit to endothermic thermoregulation. Once reaching a 2°C differential below an endotherms T_b ($T_b – T_w = 2^\circ C$), T_w is believed to impede the evaporative cooling process and, consequentially, commit endotherms to becoming hyperthermic. Considering that most mammals defend constant T_bs within the range of predicted T_w maxima increases that future T_w conditions may pose a significant threat to mammalian thermoregulation. However, to date, despite being well supported in the literature, empirical investigations into how extreme T_w conditions will affect endothermic thermoregulation are scant. Therefore, understanding of the proposed inhibitory nature of extreme T_w conditions is lacking. Considering that such conditions are to have a profound effect of endothermic survival, this dearth in understanding could prove fatal. As such, this thesis sought to provide evidence on how extreme T_w conditions may affect thermoregulatory processes in homeothermic endotherms.

By measuring the rates of heat storage in a small obligate homeothermic mammal, the Four-striped grass mouse (*Rhabdomys pumilio*, n = 21), an investigation into how extreme T_w conditions approaching an endotherms T_b may affect its thermoregulatory responses, was conducted. Using a combination of the push open-flow respirometry design and intraperitoneally injected, temperature sensitive passively integrated transponder tags, a fine scale resolution dataset was generated by measuring the shifts in $T_{b,}$ metabolic rate, and EWL in response to prolonged static T_w heat stress. In doing so, an understanding of how the rates of heat storage differed between T_w conditions at T_ws approaching the endothermic T_b was attained. Consequentially, an understanding of how T_w heat stress will affect thermoregulatory processes while approaching the proposed T_w limit was drawn.

Wet-bulb temperature was found to enforce a lower limit on thermoregulatory processes. However, this occurred at a significantly lower $T_b – T_w$ differential than the 2˚C reported in literature. In the grass mice, thermoregulatory inhibition occurred at 33˚C ($T_b – T_w \approx 4$˚C) rather than at the predicted T_w of 35˚C ($T_b – T_w = 2$˚C). At this point, 50% of the mice needed to be removed from the experiment within the first 110 minutes of the trial to avoid lethal hyperthermia. By contrast, at the lower T_ws used (T_w = 30˚C – 32˚C), mice were largely able to be tolerate the level of heat exposure for the trial duration of 220 minutes, with insignificant differences being detected in all measured indicators of hyperthermic stress between these condition ($p > 0.05$ for all treatment metrics). While exposed to the 33˚C T_w, not only were the rates of heat storage significantly higher than in other treatments ($p < 0.01$), but also the rates of EWL used despite the reliance on facultative hyperthermia ($p = 0.01$). These findings indicated not only that T_ws can enforce a lower limit on thermoregulatory processes, but also that the threat these conditions may pose is likely underestimated. However, as T_w is comprised of both heat and humidity components, of which both occur heterogeneously in the natural world, it was also key to understand how the interplay between heat and humidity conditions may differ the effect of T_w on thermoregulatory processes.

Literature currently champions humidity as the key driver to thermoregulatory inhibition in terrestrial endotherms owing to its inverse relationship with evaporative cooling. However, it was found that the primary driver towards T_w induced hyperthermia was not the inhibition of evaporative cooling, but rather the extreme T_as associated with high T_w exposure. When exposing the mice to identical T_ws while using different humidities, the T_bs that mice defended were significantly different ($p = 0.001$). Individuals defended T_bs ~1˚C higher when exposed to a lower humidity and higher T_a ($T_b = 38.11 \pm 0.455$˚C) than when exposed to a higher humidity and lower T_a ($T_b = 37.02 \pm 0.870$˚C). Similarly, the treatment combination of low humidity and high T_a resulted in significantly higher rates of EWL ($p = 0.002$). As such, the results suggest that the primary risk factor to homeothermic mammal thermoregulation regarding elevated wet-bulb temperatures is less likely to be the inhibition of heat as a result of increased humidity limiting evaporative cooling, but rather high T_as driving heat storage beyond the capacities of evaporative cooling to prevent hyperthermia.

Collectively, this thesis found that T_w conditions do certainly pose a threat to thermoregulatory processes in homeothermic endotherms, however this threat is mainly driven by the T_a conditions associated with T_w heat stress. As such, considering the comparatively slower elevations in extreme T_w conditions versus the extreme and sporadic T_a elevations predicted to occur as a result of global warming, T_w as a threat is merely secondary to T_a conditions and the increasing extremes that future T_as are predicted to bring about.

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Declaration

I, **Oliver Hartley** (**s214 085 902**), hereby declare that the submission of the dissertation titled “**Investigating Thermoregulatory Responses of *Rhabdomys pumilio* at High Wet-bulb Temperatures**” for a **Masters of Zoology** is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

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Chapter One: General Introduction

Thermoregulation, Climate Change and Wet-bulb Temperature

Temperature plays a crucial role in biological functioning owing to its effects on chemical reaction rates (Gillooly et al., 2001) and macromolecular structures, such as proteins and enzymes (Daniel et al., 2010; Jaenicke, 1991; Tilton Jr et al., 1992). With an increase in temperature, chemical reaction rates increase in response to the increased molecular kinetic energy available (Boltzmann, 1872). As such, processes such as metabolism are stimulated, leading to an increase in resource use and conversion (Seymour, 1972). However, in biological systems, although such an effect would increase the amount of energy available to the organism in question, excessive thermal energy can also lead to physiologically disastrous effects. These include decreased cellular signalling capacity, decreased water economy efficiency (Tieleman and Williams, 1999), protein denaturing (Lepock, 2003), loss of active enzyme sites (Daniel et al., 2010), cytotoxicity, abnormal cellular functioning owing to membrane and organelle damage(Hazel, 1995), cell death once cells are exposed to temperatures of 40-41˚C (Daniel et al., 2010; Lepock, 2003), abnormal functioning of the central nervous system and ultimately, death (Bouchama and Knochel, 2002). For thermal specialists such as mammals, these effects enforce an upper thermal tolerance limit, whereby once overcome, the animal will become hyperthermic and risk suffering hyperthermic effects (Angilletta et al., 2010; Boyles et al., 2011).

For endothermic organisms that defend constant T_bs to maintain internal homeostasis and optimal physiological functioning (McNab, 1974), such as birds and mammals (Hoole et al., 2019; Lawrence, 2011), thermal homeostasis is usually maintained by triggered physiological or behavioural responses that offset potential T_b shifts (Sherwood et al., 2013). If the ambient temperature (T_a) is below their T_b, they will regulate their metabolism and passively offload excess heat to the atmosphere via conductive, convective and radiative processes (Sherwood et al., 2013). However, once T_a is equal to or greater than the T_b of the animal, then the animal must depend on evaporative cooling via evaporative water loss (EWL) by licking, sweating or panting (Sherwood et al., 2013). Following the second law of thermodynamics, however, EWL can only be used if the atmosphere is not saturated with water vapour, i.e. a system at dewpoint (T_{dp}; 100% relative humidity, RH; Sherwood and Huber, 2010). When T_{dp} is equivalent to an animals T_b, the capacity to lose heat is overrun owing to the inhibition of EWL, leading to heat loading and hyperthermia (Adolph, 1947).

Authors, however, warn that thermal loading will occur before T_{dp} is reached, such that once a theoretical 2˚C wet-bulb temperature (T_w) gradient between an animals T_b and the atmosphere is exceeded, EWL will be inefficient in countering thermal stress (Sherwood and Huber, 2010). Wet-bulb temperature measures the lowest attainable temperature an object can reach through evaporative cooling in the presence of an airstream and therefore, accounts for the interactive effect of T_a and RH on the cooling efficiency of EWL. Consequentially, T_w represents the lowest temperature an animal can cool through EWL. Considering that with anticipated global warming, the global moisture content will increase (Trenberth, 1998; Willett and Sherwood, 2012), ultimately increasing the T_w (Sherwood and Huber, 2010). This brings cause for concern, because the increase in T_w would imply a decreased thermal buffer zone would exist between the animal's body temperature and the point of uncontrolled heat storage.

Until recently, future ambient temperature increases have been predicted to be between 1.0-3.7˚C by 2100 compared to preindustrial conditions (Arias et al., 2021; Sherwood and Huber, 2010; Stocker et al., 2013). However, more recent evidence suggests that increases of between 1.7-4.8˚C are more likely (Brown and Caldeira, 2017). Such increases in average global T_a would induce T_w shifts from 25˚C currently to 29.8˚C, accompanied by an increase in maximum T_ws from an average of 35˚C to above 39.8˚C (Sherwood and Huber, 2010), thus approaching the upper limit of cellular heat tolerance. Considering that mammals thermoregulate their T_bs at temperatures near their lethal limit (Boyles et al., 2011) and that 80% of mammals maintain body temperature between 30-38˚C (Lovegrove, 2012) this implies that most mammals may be at significant risk of excessive heat storage under future climate scenarios. Under such conditions, many animals, including humans, have been noted to suffer from heat-related mortality (Fey et al., 2015; McKechnie et al., 2021; Welbergen et al., 2008; Welbergen et al., 2014). The Australian grey-headed flying fox *Pteropus poliocephalus* and the black flying fox *P. alecto*, for example, accounted for over 3000 heat-related mortalities in 2002 after prolonged exposure to 42˚C T_as

(Welbergen et al., 2008), with 24 500 similar deaths being recorded in *P. poliocephalus* over 19 heatwave events since 1994 (Jones et al., 2009; McKechnie and Wolf, 2019; Welbergen et al., 2008).

Sherwood and Huber (2010) however, warn that a significantly greater threat often not considered, yet potentially more dangerous, is the effect of T_w on thermoregulation inhibition. To date, the global T_w has been relatively uniform, currently averaging at 26˚C, with tropical and equatorial regions presenting T_w maxima around 1˚C and 2˚C above others (Sherwood and Huber, 2010). However, with the predicted increase in T_a and the consequential increase in the global ambient moisture content, T_ws are expected to increase near 35˚C in several regions (Pal and Eltahir, 2016; Raymond et al., 2020; Sherwood and Huber, 2010). Such extreme T_w conditions have already been experienced around the Prussian Gulf in Dhahran with a dewpoint of 35˚C in 2003 (Burt, 2007). Notably, this is the theoretical limit beyond which humans (T_b = 37˚C) are unable to off-load excess body heat and heat loading is inevitable (Akihiko et al., 2014; Brown and Caldeira, 2017; McKechnie and Wolf, 2010; Oleson et al., 2015; Sherwood and Huber, 2010), however in Pakistan in 2015, even T_w exposure of 33˚C lead to the deaths of over 2500 people (Wehner et al., 2016). Considering that this would exceed the theoretical limit of most mammalian tolerance limits, future climate change would therefore risk most mammals not only to suffering hyperthermic heat stress but is also set to prevent them from efficiently cooling to prevent succumbing to hyperthermia. However, to date, rigorous scientific testing of T_w effects on endotherms remains limited to milk production (Armstrong, 1994; Bohmanova et al., 2007; Ravagnolo et al., 2000), with little empirical investigation assessing endothermic thermoregulatory responses to the proposed limitations of T_w (Vecellio et al., 2022). Therefore, although highly cited (Im et al., 2017; Pal and Eltahir, 2016; Raymond et al., 2017; Raymond et al., 2020; Sherwood and Huber, 2010), the true nature of T_w heat stress remains largely unknown.

Study Species

Taxonomy

The study model selected for this study was the generalist species, the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784). However, owing to the contention around the classification of this species (Castiglia et al., 2012), a note on the taxonomy of this rodent seems pertinent. On account of there being several cryptic species within the *Rhabdomys* complex, the classification of *R. pumilio* has seen several changes since its initial description (Castiglia et al., 2012; Du Toit et al., 2012; Meester et al., 1986; Monadjem et al., 2015; Musser and Carleton, 2005; Rambau et al., 2003; Roberts, 1951).

Rhabdomys pumilio belongs to the Arvicanthini rat clade, the richest assemblage of savanna rats (Castiglia et al., 2012). Initially thought to be monophyletic, the wide diversity of phenotypic expression within the *Rhabdomys* clade was first proposed to be as a result of the clade housing as many as 20 subspecies (Meester et al., 1986; Roberts, 1951). More recently, using mtDNA analysis, Rambau et al., (2003) proposed the division of the complex into two distinct species such that *R. pumilio* and *R. dilectus* would be discernible based on habitat preference. This division found *R. pumilio* to inhabited drier, more arid areas whereas *R. dilectus* inhabited wetter, mesic areas. Musser and Carleton (2005) supported this division, but further argued that *R. dilectus* house two subspecies such that it comprised of *R. d. dilectus* as the base clade and *R. d. chakae* as the sister clade. However, following closer analysis by two independent studies in 2012, both the xeric *R. pumilio* clade and the mesic *R. dilectus* were found to both house several species, not just subspecies as previously thought to occur in the *R. dilectus*. In the case of *R. dilectus*, Castiglia et al. (2012) supported the recognition of the two *R. dilectus* subspecies. Regarding *R. pumilio*, Du Toit et al. (2012) postulated that shifts in vegetation that had occurred since the evolution of the *Rhabdomys* complex would have led to possible speciation within the clade in response to altered habitat conditions. Following deep mitochondrial analysis, coupled with ecosystemic niche modelling, it was found that the apparent generalist capacity of *R. pumilio* was rather owed to the presence of several cryptic species that in absence of molecular analysis would prove indistinguishable. As such, Du Toit et al. (2012) proposed the recognition of four distinct species that were initially grouped into the *R. pumilio* classification on account of distinct differences in mtDNA lineages and habitat preferences with limited boundary interaction between them (Figure 1.1). These species prepositions were namely *R. pumilio, R. intermedius*, *R. bechuanae* and the *R. dilectus* clade. The current recognised composition of the *Rhabdomys* clade follows that as proposed by Monadjem et al. (2015) in which the findings by Du Toit et al. (2012) is supported, however owing to one known instance of sympatry between the *R. d. dilectus* and *R. d. chakae*, these two clades have been elevated to species level such that *Rhabdomys* Tomas (1916) currently supports five distinct species. However, irrespective of these species inhabiting distinct biomes across southern Africa and presenting minimal boundary overlap, Du Toit et al. (2019) still recommend treating this groups as a species complex. As such, this study will follow this proposal. However, validation that of *R. pumilio* was indeed the species used will also be provided below based on habitat preferences.

Habitat Preferences

Rhabdomys Thomas (1916) as a complex presents a generalist taxa known to inhabit a wide variety of habitats, including grassland, marshes, deserts, semi-deserts and forests across southern Africa, to the base of the Democratic Republic of Congo (Figure 1.1; Coetzee and van der Straeten, 2008; Du Toit et al., 2012; Ganem et al., 2020; Kingdon, 1974; Schradin and Pillay, 2004; Skinner and Chimimba, 2005). However, following the biogeographical analysis of the complex by Du Toit et al. (2012), it was discovered that this apparent generalist capacity was in fact owed to the complex harbouring of several cryptic species, each with its own habitat preferences and climactic limitations. Therefore, although there appeared to be a wide tolerance of the species to a range of climactic conditions, it is owed to distinct species occupying specific environmental niches.

Following the results from Du Toit et al. (2012), *R. bechuanae*, for example, is limited to the arid conditions of the Nama-Karoo, arid Savanna and Desert biomes, showing a distinct preference for arid conditions whereby summer rainfall increases result in reduced abundance of this taxa (Figure 1.1; Castiglia et al., 2012; Du Toit et al., 2012; Monadjem et al., 2015). *Rhabdomys chakae*, by contrast, shows to be limited to the moist conditions of the Grassland biomes of southern Africa, again being rainfall dependant (Figure 1.1; Castiglia et al., 2012; Du Toit et al., 2012; Monadjem et al., 2015). In the case of *R. dilectus*, its preference for montane and sub-montane grassland limits its distribution to the moist conditions provided in these biomes (Figure 1.1; Castiglia et al., 2012; Du Toit et al., 2012; Monadjem et al., 2015). However, *R. intermedius*, on the other hand shows a negative association with summer rainfall and prefers the Nama-Karoo biome region between the coastal *R. pumilio* and the arid *R. bechuanae* taxa, making it endemic to the region (Figure 1.1; Castiglia et al., 2012; Du Toit et al., 2012; Monadjem et al., 2015). In the case of the basal *R. pumilio* clade, there is a strong affinity for the Fynbos and Succulent Karoo biomes along the Southern and western coasts of South Africa where this taxa shows to by limited primarily by altitude, with a preference for high temperature and winter rainfall regions (Figure 1.1; Castiglia et al., 2012; Du Toit et al., 2012; Monadjem et al., 2015). As such, it is evident that as a clade, the *Rhabdomys* complex does present a wide tolerance of environmental conditions, but *R. pumilio* alone does not encompass all of these conditions, making it rather a coastal specialist. Nevertheless, considering that karyotypically the taxa show little variation, and can only be distinguished following deep molecular analysis, and that it is recommended to treat the genus as a complex, irrespective of the newly found specialisation of *R. pumilio*, this thesis will consider this species capacity for thermal tolerance to be that of the generalist it is proposed to be.

Should the biome specificity of the recognised *R. pumilio* division of the complex be considered then it is safe to assume that this study did indeed rely on *R. pumilio*. Considering that all sampling was conducted on a population of *Rhabdomys* sp. in Gqeberha (formally Port Elizabeth), and that this basal clade is shown to be endemic to the southern and western coast of South Africa between Gqeberha and Fort Beaufort in the Eastern Cape Province and the mouth of the Orange river in the Northern Cape (Figure 1.1; Du Toit et al., 2012; Monadjem et al., 2015), it is therefore discernible that the species used in this study is indeed *R. pumilio*.

Habits

As a complex, *Rhabdomys* spp. have long been found to be among the few diurnally crepuscular rodents (Haim and Izhaki, 1995; Roll et al., 2006) whereby they are active throughout the day (Choate, 1972; Christian, 1977) while presenting their peak activity bouts at sunrise and sunset (Dewsbury and Dawson, 1979; Lovegrove, 2009; Schradin, 2006; Schumann et al., 2005). During this time period, individuals are known to spend most of their time individually foraging omnivorously on seeds, fruits, *Pinus* bark, plant stems and insects based on what is present at the time of year (Curtis and Perrin, 1979; Monadjem et al., 2015; Schradin, 2006; Schradin and Pillay, 2004; Skinner et al., 2005). Along with foraging, individuals will also spend significant durations of time patrolling their respective territories, with males displaying greater investment into this practice than females (Schradin, 2006; Schradin and Pillay, 2004).

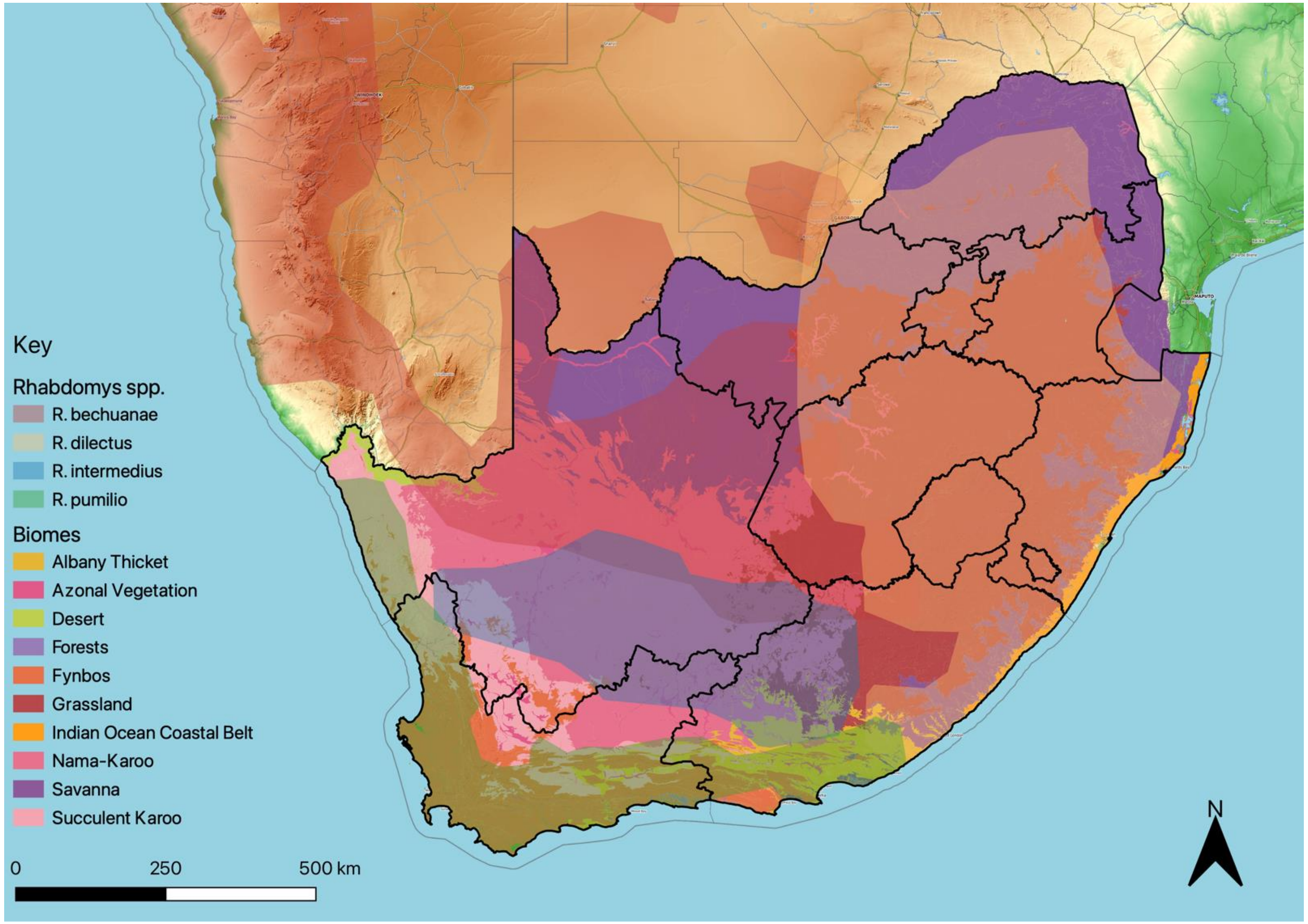

Figure 1.1 Distributional map of taxa in the *Rhabdomys* spp. complex based on the distributions provided by Du Toit et al. (2019). The distributional polygons are laid over the biomes of South Africa as described by Mucina and Rutherford (2006).

Socially, the behaviours presented by the *Rhabdomys* sp. complex have been found to be highly flexible and dependant on reproductive competition, and environmental conditions (Schoepf and Schradin, 2012; Schradin et al., 2012). Initially having been thought to be a solely solitary species that only interacted for breeding purposes (Willan, 1982), *Rhabdomys* have been found to rather display two distinctly different social behaviours. In the more mesic habitats, such as grasslands, individuals have been found to occupy distinct territories where 2 – 4 breeding females will present little overlap with one another, but will overlap with a single dominant male, following the initial understanding of the social organisation of this species (Choate, 1972; Perrin, 1980; Willan and Meester, 1989). By contrast, more recent investigations have found that in arid habitats, such as the succulent karoo, individuals are known to rather be philopatric in nature where up to 30 adults will live collectively in nesting groups consisting of one dominant male, up to four breeding females and their respective offspring, all sharing a collective territory and defending it aggressively from other groups (Schradin and Pillay, 2004). Although seeming strange, Schradin and Pillay (2005) suggest that this difference is owed to habitat saturation occurring in the more arid environments, whereas resource limitation may drive high intraspecific competition in the more mesic population, leading to greater benefit being found in solitary in nature.

Physiology

As a species complex, *Rhabdomys* has been well studied regarding its behavioural traits (Perrin, 1980; Scantlebury et al., 2006; Schoepf and Schradin, 2012; Schradin and Pillay, 2005; Schradin and Pillay, 2006; Schradin et al., 2010; Yuen et al., 2016), but comparatively less has been done on its physiological traits (Haim, 1987; Haim and Fairall, 1986; Haim and Fourie, 1980; Haim et al., 1998; Welman, 2012) with little understanding being present on its physiological responses to elevated heat stress. This complex is known to be obligatorily homeothermic in nature (Couture, 1980; Scantlebury et al., 2006), defending a constant T_b of 34.6˚C-38.5˚C by 15 days of age (Couture, 1980; Haim and Fourie, 1980), with more recent assessments showing the T_b of 36.8˚C during the day and 36.6˚C at night (Lovegrove, 2009). The thermal neutral zone of this species, as initially described by Haim and Fourie (1980), appears to be limited rather to a thermal neutral point at 32˚C whereby

Rhabdomys will maintain a BMR of 0.8-0.9 ml O_2 g^{-1} hr^{-1} (Haim and Fourie, 1980; Welman et al., 2022) in summer and will increase to 1.5 ml O_2 g^{-1} hr^{-1} by winter (Welman et al., 2022).

Considering the diurnal nature of the species, *Rhabdomys* sp. have been found to engage in postural adjustment and avoidance behaviours in the warmest parts of the day (Christian, 1977). This is typical of diurnal species whereby they will lay flat against the earth in shaded areas to maximise heat loss to their respective environment (Schradin, 2006). However, when hot, *Rhabdomys* sp. will rely on pulmocutaneous evaporative water loss (EWL) to maintain thermal homeostasis. Interestingly, their EWL responses to heat stress have shown to be curvilinear in nature at increasing RH such that once being exposed to RH above 40%, Christian (1978) found that these mice are capable of increasing EWL use irrespective of increased RH conditions. Granted this treatment was conducted under 24˚C T_a exposure and would therefore be well below the thermal neutral point of the complex, however no other studies on the thermoregulatory capacity of this complex under heat stress have been conducted.

Study Site

This study was conducted in the Nelson Mandela University nature reserve, in Gqeberha, South Africa (Figure 1.2). This reserve is characterised the endangered St. Francis Dune Thicket vegetation type whereby a matrix of thicket bush clumps can be found dispersed within a matrix of asteraceous Dune Fynbos, and will be found to occur from 0 – 221 meters above sea level (Grobler et al., 2018). The region generally experiences 397 – 868 mm of aseasonal rainfall throughout the year, and may experience up to 3 days of frost in the winter time (Grobler et al., 2018).

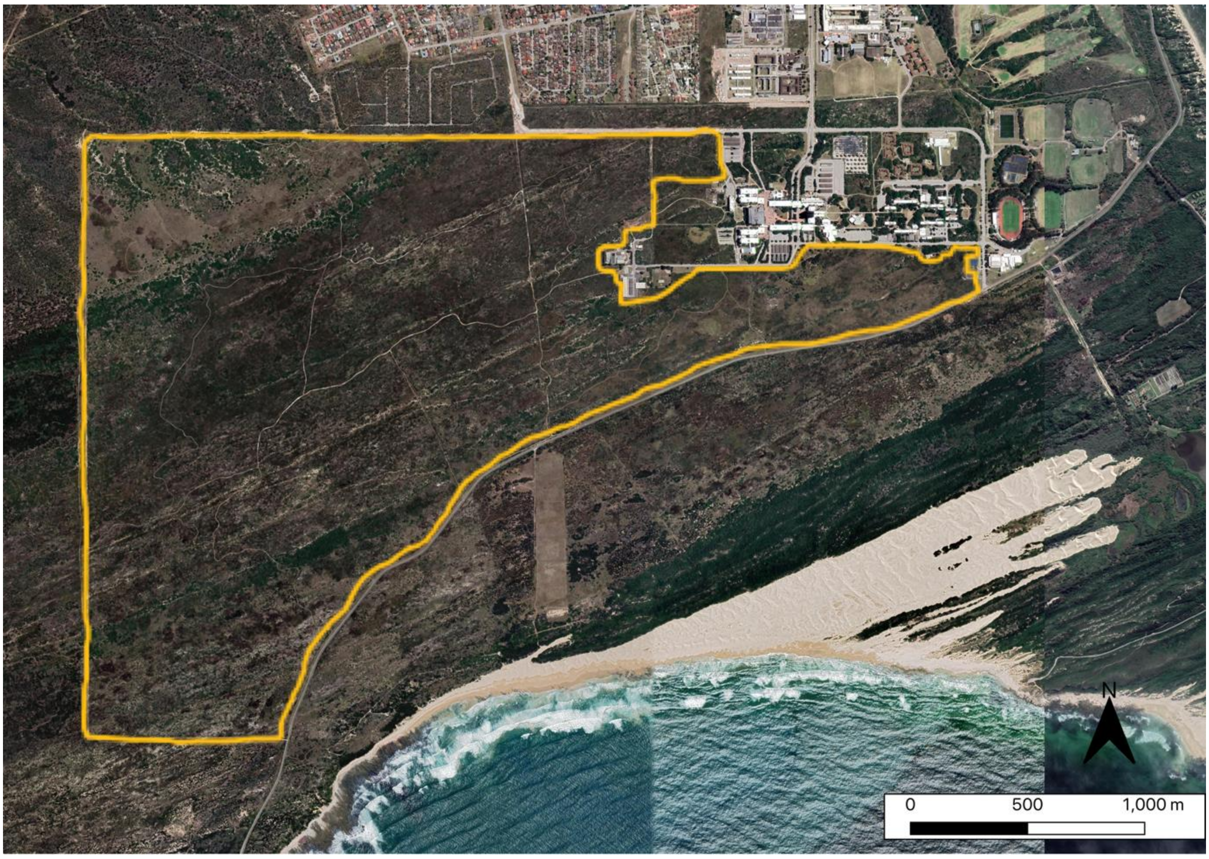

Figure 1.2. Area map of Nelson Mandela University Nature Reserve demarcated in yellow.

The reserve is known to experience average T_as between 14.5˚C – 21.9˚C throughout the year. The coldest period of the year occurs between June and July (Figure 1.3) during the winter months where T_as will average between 14.5˚C – 14.7˚C, respectively, while varying between 8.2˚C – 20.5˚C (Table 1.1). This winter season is also accompanied with the regions driest season (Figure 1.3) where the average RHs for June and July are 68.4% and 70.8% respectively while presenting ranges of 41.4 – 91.1% (Table 1.1). Nonetheless, the humidity in this reserve remains highly stable throughout the year with the mean RH ranging from 68.4-77.8% throughout the year (Figure 1.3)

Table 1.1 Meteorological data of the Nelson Mandela Nature Reserve for the years 2014 – 2019 as provided by the South African National Weather Service. Ambient temperature (T_a; ˚C), relative humidity (RH; %) and wet-bulb temperature (T_w; ˚C) data are provided, along with the accompanying minimum and maximum readings for each respective month.

Month	Ta (˚C)	Min-Max (˚C)	RH (%)	Min-Max (%)	Tw (˚C)	Min-Max (˚C)
January	21.9	17.7 - 26.2	75.7	55.4 - 91.9	18.8	12.4 - 25.1
February	21.7	17.3 - 26.1	77.8	57.0 - 92.6	18.8	12.2 - 25.1
March	20.7	16.2 - 25.2	77.2	54.8 - 92.9	17.8	11.0 - 24.3
April	18.6	13.7 - 23.5	77.1	53.5 - 93.1	15.8	8.6 - 22.6
May	17.0	11.4 - 22.5	75.6	50.8 - 92.7	14.1	6.4 - 21.6
June	14.7	8.2 - 21.2	68.4	41.4 - 90.1	11.1	2.9 – 20.0
July	14.5	8.4 - 20.5	70.8	44.0 - 91.1	11.2	3.3 - 19.4
August	15.0	9.6 - 20.5	74.5	49.7 - 92.2	12.1	4.8 - 19.5
September	16.2	11.3 - 21.1	75.6	53.0 – 92.0	13.4	6.5 – 20.0
October	17.4	12.9 - 21.9	75.4	54.6 - 92.2	14.5	8.1 - 20.9
November	17.9	13.6 - 22.3	76.0	55.9 - 93.1	15.1	8.8 - 21.4
December	20.4	15.9 - 24.8	76.2	55.2 - 92.5	17.4	10.8 - 23.8

The warmest time of year in the reserve follows that of the austral summertime period such that peak T_as are recorded between January and March (Figure 1.3). During this time period, T_as reach 20.7 – 21.9˚C with ranges of 16.2 – 26.2˚C being recorded throughout the day (Table 1.1). Summer also correlates with the majority of the more humid time of year where RH is 75.7 – 77.8% between January and March (Figure 1.3), with ranges of 54.8 - 92.9% being recorded (Table 1.1).

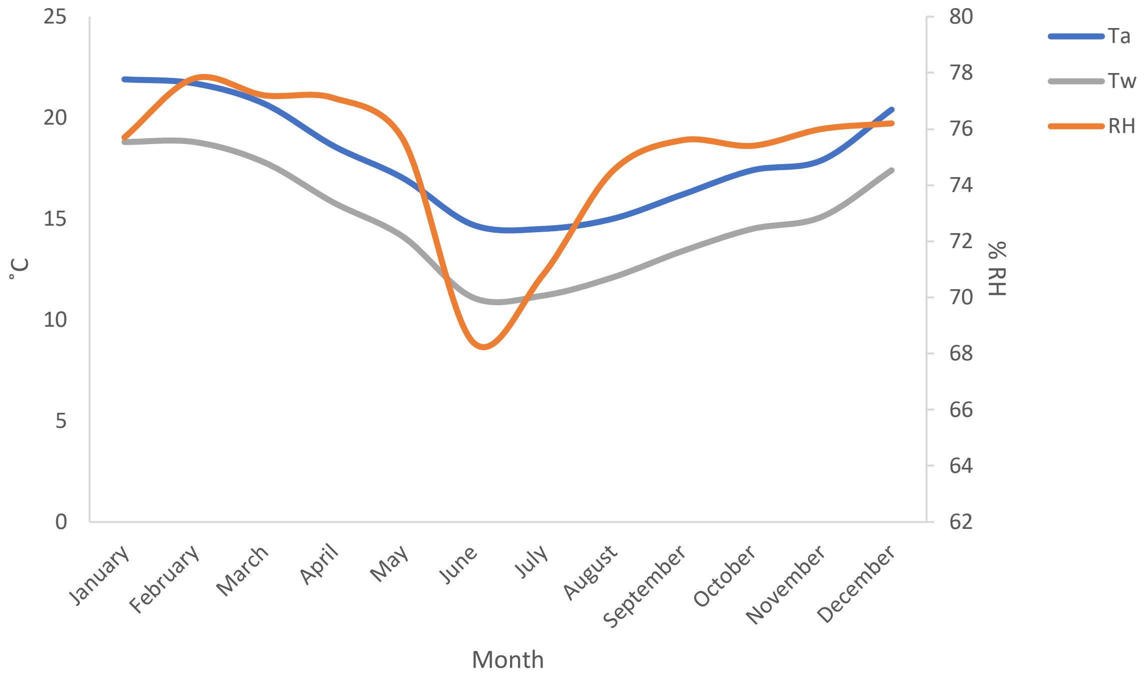

Figure 1.3 Atmospheric data for the Nelson Mandela Nature University Nature Reserve for the years 2014-2019. Provided are the ambient temperature (T_a) indicated by the blue line, relative humidity (RH) indicated by the orange line, and wet-bulb temperature (T_w) indicated by the grey line. The data were provided courtesy of the South African National Weather Service.

The T_w conditions in the reserve mimic those of the T_a conditions being presented (Figure 1.3), such that peaks and troughs in T_w conditions correlate with the shifts in T_a and RH collectively (Figure 1.3). Wet-bub conditions in the reserve range between 11.1˚C and 18.8˚C with the lowest T_w conditions being found in winter following the decrease in both heat and humidity conditions commensurate with the area, while the highest T_w measured correlates with the summertime (Figure 1.3). The average winter T_w conditions for at the study site vary between 11.1˚C and 12.1˚C between June and August, with ranges of 2.9˚C and 20.0˚C being recorded throughout the day (Table 1.1). In summer, average T_w conditions are 17.8 – 18.8˚C between January and March while ranges of 11.0˚C to 25.1˚C are found to occur throughout the day (Table 1.1).

All meteorological data was provided curtesy of the South African National Weather Service from data site 0035209B1. This site is at 33.99˚S, 25.62˚ E at 60 m above sea level and is only 5 km away from the Nelson Mandela University Nature Reserve. As such these data are indicative of the conditions experienced within the nature reserve. All data presented in this section are data for 2014 – 2019.

Rationale and Motivation

Aims

In response to the lack of information on how future humid heat stress, in particular T_w shifts as the global T_w conditions tend towards homeothermic endothermic T_bs, this thesis sought to investigate the effect of high T_ws on the thermoregulatory capacity of grass mice, to determine the thermal gradient required to avoid hyperthermia. Thus, these mice serve as a model species to test the proposed minimum thermal gradient of 2˚C between the animal and the ambient temperature ($T_b - T_w$), needed to allow mammals to dissipate excess heat from their body. As such, the key aims of this thesis were to scrutinise the proposed T_w thermal limit of 2˚C < T_b to establish whether the thermal limit does exist and whether literature is currently over- or underestimating the thermal tolerance of endotherms when exposed to high T_w conditions. Furthermore, on account of T_w being comprised of heat and humidity components whereby both are known to affect the capacities of endotherms to prevent heat stress, the second key aim sought to understand whether T_w heat stress would be equitable between different heat and humidity conditions. As such, the data chapters presented in this thesis aim to address these key aims respectively.

Key Objectives, Research Questions and Hypotheses

Chapter Two: Comparing the rate of heat storage at wet-bulb temperatures approaching the normothermic body temperature in *Rhabdomys pumilio*

Objective: To empirically investigate the theoretical limitation imposed on thermoregulation in homeothermic endotherms when exposed to extreme wet-bulb temperature, as proposed by Sherwood and Huber (2010).

Research Question: Can extreme wet-bulb temperature conditions enforce a limit on thermoregulation?

H1: As predicted by Sherwood and Huber (2010), wet-bulb temperature conditions will render thermoregulatory processes redundant once 2˚C below the grass mice’s body temperature, committing them to pathological hyperthermia beyond this point.

Chapter Three: An investigation into how different heat and humidity combinations affect thermoregulatory processes under identical wet-bulb temperature conditions

Objective: To investigate how different heat and humidity combinations affect wet-bulb temperature induced heat stress.

Research Question: Is wet-bulb temperature heat stress equitable between identical wet-bulb temperatures consisting of different heat and humidity combinations?

H1: Wet-bulb temperature conditions comprising of greater humidity components will induce greater heat stress in the grass mice, as cautioned by Lovegrove (2003).

Research Licence and Ethics

All sampling collections over both years of field work required by this thesis were conducted in the Nelson Mandela University Nature Reserve under the authorisation of the Eastern Cape Department of Economic Development, Environmental Affairs and Tourism, as stipulated by permit number CRO 91/19CR. The experimental nature of this thesis was conducted under the guidelines and approval of the Nelson Mandela University Animal Research Ethics committee under the permit number A19-SCI-ZOO-002.

Thesis Outline

Following the **General Introduction** above, this thesis is comprised of two data chapters and then followed by a synthesis and conclusions chapter. The format of the two data chapters has followed the layout and requirements as set forth by the Journal of Thermal Biology, to allow for ease of submission of these chapters for scientific publication. As such, there is some overlap of information and ideas used between these two chapters. The references section of this thesis is also written to adhere to the requirements of the Journal of Thermal Biology. The plagiarism report for this thesis can be found in Appendix I.

In **Chapter 2**, I address the implications and limitations of the thermal limit that has been proposed in literature, yet not studied. This chapter refers to data collected in the austral winter of 2019 using push open flow respirometry to measure the physiological responses of 11 male Four-striped grass mice at static T_b – T_w gradients approaching their normothermic T_b of 36.7˚C. This chapter aimed to empirically investigate the proposed limitation that future T_w maxima may have on homeothermic endothermic mammals by using the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784) as a case study.

In **Chapter 3** I build on the findings of Chapter 2, investigating how T_w heat stress on thermoregulating endotherms may differ when under identical T_w conditions but under different heat and humidity component. This chapter used data collected from 10 male Four-striped grass mice in the austral summer of 2020 using push open flow respirometry. By exposing the mice to static T_w conditions of 31˚C, but using RHs of either 70% or 90% and adjusting the T_a constituent as required, the nature of the threat that T_w may pose between different environments is then discussed.

In **Chapter 4**, I concluded my thesis finding and contextualised my results within the broader literature. Here I also provide my recommendations for future research into this phenomenon.

Chapter Two: Comparing the Rates of Heat Storage at Wet-bulb Temperatures Approaching the Normothermic Body Temperature in *Rhabdomys pumilio*

Abstract

Wet-bulb temperature (T_w) provides a measure of how cold an object can become when experiencing its maximum rate of evaporative cooling within its respective environment. Considering that evaporative cooling is also the final countermeasure against heat stress for terrestrial endotherms, such as birds and mammals, T_w is also theorised to represent the lower limit of thermoregulation. To date literature informs that this limitation exists around 2˚C below an endotherms body temperature (T_b), however empirical investigations of this limitation are lacking. As future climate predictions inform that this 2˚C threshold will be exceeded for many mammalian species, this also implies that the threat that T_w may pose remains unknown. Therefore, using Four-striped grass mice (n = 11; *Rhabdomys pumilio* Sparrman 1784) as a model species, the thermoregulatory responses to static T_ws (T_w = 30-33˚C) approaching their T_bs (T_b = 36.8˚C) were recorded. Wet-bulb temperature was found to override thermoregulatory processes once reaching a $T_b – T_w$ differential of 4˚C, rather than the 2˚C currently accepted. At this point, not only were the rates of heat storage significantly greater than in the lower T_w trials ($p < 0.05$), but so were the rates of EWL as well ($p < 0.05$). These findings indicated that not only are the current safety margins being underestimated by at least 2˚C, but also that since evaporative cooling was not inhibited but rather stimulated, the current understanding of how T_w heat stress operates remains limited.

Introduction

Being able to effectively thermoregulate is vital for the survival of homeothermic endotherms, such as birds and mammals (Cowles and Dawson, 1951; Lovegrove, 2017; McNab, 1978; Rezende et al., 2020). Having evolved to maintain relatively stable body temperatures (T_bs) above their respective ambient temperatures (T_as), endotherms conventionally remove excess metabolic heat passively through convective, conductive and radiative means; collectively referred to as sensible heat loss (Calder, 1996; Jessen, 2001; Kleiber, 1961; McNab, 2002; Sherwood et al., 2013). However, should the rate at which heat is gained be greater than the rate at which sensible heat loss can operate, the only countermeasure available to endotherms is that of evaporative cooling; also known as latent heat loss (Sherwood, 2018; Sherwood et al., 2013). Physiologically this is achieved through the costly process of evaporative water loss (EWL; Cowles & Dawson 1951, Angilletta et al. 2010, Sherwood et al. 2012). Should this still be inefficient, heat storage will ensue, risking the endotherm becoming hyperthermic (Cowles & Dawson 1951, Angilletta et al. 2010, Sherwood et al. 2012).

Although minor hyperthermic bouts have been found to be beneficial in (Calderwood et al., 2005; Gerson et al., 2019; Sherwood et al., 2013; Singh and Hasday, 2013; Turner, 2020), prolonged hyperthermic exposure can be physiologically damaging. For example, minor bouts of prolonged hyperthermia can lead to decreased cellular functioning (Daniel et al., 2010; Hazel, 1995; Lepock, 2003), lowered water economic efficiencies (Tieleman and Williams, 1999) and hampered physiological functioning (Bouchama and Knochel, 2002). Under extreme or prolonged hyperthermic bouts, should T_bs of mammalian endotherms reach 40 – 41˚C, cell death may ensue (Daniel et al., 2010; Lepock, 2003) and if not countered efficiently, will lead to hyperthermic mortality. Although historically exposure to such environmental conditions has been infrequent and associated with extreme climatic events, authors now anticipate that in conjunction with global warming, future climate conditions may surpass the capacities of endotherms to thermoregulate, committing them to prolonged hyperthermic exposure (Ahima, 2020; Coffel et al., 2018; Kang and

Eltahir, 2018; Pal and Eltahir, 2016; Raymond et al., 2020; Sherwood and Huber, 2010).

To date, anthropogenically driven climate change has seen an increase of 1.1˚C in the mean terrestrial temperature since the pre-industrial era (1850-1900; Arias et al., 2021; Stocker et al., 2013), with estimates of as high as 2˚C being reported (Lindsey & Dahlman 2020) and further warmings of 1.7 – 4.7˚C being predicted to occur by the end of the century (Arias et al., 2021; Brown and Caldeira, 2017). Accompanying these thermal shifts, extreme climactic events have shown a marked increase in frequency, intensity, duration and sporadicity as a result (Coumou and Rahmstorf, 2012; Coumou and Robinson, 2013; Diffenbaugh et al., 2017; Hansen et al., 2012; Sippel and Otto, 2014). Heat waves in particular are expected to become 2 – 3 times more intense, and are expected to at least double in frequency across Europe, presenting up to 3 – 4 severe heat wave events per year (Lhotka et al., 2018). Areas such as Australia are expected to exhibit even greater shifts with increases of even little as 2˚C in the mean T_a, doubling the likelihood of experiencing heatwaves similar to those experienced in 2013 (King et al., 2017); the hottest recorded since 1972-1973, nineth warmest globally since 1880 (NOAA National Centers for Environmental Information, 2013; Special Climate Statement 43 – extreme heat in January 2013, 2013) and an anomaly that, in absence of global warming, would occur only every 12 300 years (Lewis and Karoly, 2014). Under such conditions, anomalous heat related mortality akin those experienced in Chicago 1995 (~ 679 dead; Kaiser et al. 2007), Europe 2003 (~ 70 000 dead; Robine et al. 2008) and Russia in 2010 (~ 55 000 dead; Barriopedro et al. 2011) would become more frequent.

Heat related mass mortality events (MMEs) are also expected to increase in response to the increased extreme climate events (Fey et al., 2015; McKechnie and Wolf, 2010; McKechnie et al., 2021). Having first been described in the 1980s (Fey et al., 2015), MMEs in relation to heat stress have shown a marked increase in occurrence over recent decades (Fey et al., 2015). Examples of the catastrophic effect that these sporadic events can bring about can readily be found in the ongoing crisis in the Australian flying-fox (*Pteropus sp.*) populations. With losses of ~ 30 000 over 19 extreme heat wave events between 1994 – 2007 across Australia (Welbergen et al., 2008), a further ~ 45 000 were lost in a single day in 2014 in south-east Queensland accounting for at least 50% of the local population in one of these species (Welbergen et al., 2014). In light of continued global warming, not only have these events become a regular occurrence in Australia, but are predicted to worsen (Ratnayake et al., 2019). South Africa has also recently reported is first occurrence of an MME following anomalous heat exposures of 45˚C in the Phongolo Nature Reserve, Kwa-Zulu Natal (McKechnie et al., 2021). Following surveying only 1.1% of the affected area, individuals from over 47 birds of different species, and 58 Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) were reported dead in relation to heat stress following a single days exposure to T_as of 43-45˚C (McKechnie et al., 2021). However, although increases T_a pose a readily accepted threat to future populations, authors warn that the a key driver behind these threats is linked to the combined effect of heat and humidity on thermoregulation (McKechnie et al., 2021; Raymond et al., 2017; Raymond et al., 2020; Sherwood, 2018).

The effect of humidity on the thermal physiology in endotherms has long been appreciated (Brunt, 1943; Candas et al., 1982; Haldane, 1905; Lin et al., 2005; Moyen et al., 2014; Walters et al., 2004). Under increasingly humid conditions, ambient moisture content is negatively correlated with endothermic evaporative cooling capacity when under heat stress (Luber and McGeehin, 2008; Moyen et al., 2014; Sherwood and Huber, 2010), retarding the efficiency of evaporative cooling. When combined with elevated temperatures, this limitation in latent heat loss has been found to exacerbate the effect of heat stress (Gerson et al., 2014; McKechnie et al., 2021). For this reason, the combined increases in humidity and temperature associated with climate change (Arias et al., 2021; Stocker et al., 2013) is expected to increase heat stress experienced by endotherms, and for the first time in recorded history, prevent endotherms from being able to thermoregulate through both exogenous and endogenous means (Pal and Eltahir, 2016; Raymond et al., 2020; Schär, 2016; Sherwood and Huber, 2010). As a result, recent literature has highlighted that future wet-bulb temperature conditions (T_w) may pose the penultimate threat to the thermal welfare of terrestrial endotherms (Coffel et al., 2018; Sherwood, 2018).

Being a measure of how cold an object can become under maximal rates of evaporative cooling, beyond which further cooling would not be possible (Rogers and Yau, 1989), T_w provides a thermal measure of end effect that the interaction between heat and humidity conditions has on evaporative cooling processes. Although being well used in the built environment, recent literature has proposed that owing to the limiting nature that heat and humidity have on evaporative cooling, T_ws can therefore also prove useful in a biological context (Li et al., 2017; Raymond et al., 2017; Raymond et al., 2020; Sherwood and Huber, 2010). By representing the lowest attainable temperature in response to evaporative cooling processes, and therefore the coolest temperature that endothermic organisms can attain when under heat stress, T_w conditions have therefore been adopted to indicate a lower limit to thermoregulatory processes (Sherwood and Huber, 2010). Following the seminal works Sherwood and Huber (2010), it was postulated that should a $T_b – T_w$ differential of 2˚C or less exists between an endotherm and their environmental T_w, the endotherm will not be able to cool down efficiently enough to prevent hyperthermic stress. Considering that over 80% of mammals maintain T_bs between 30.5˚C-37.9˚C (Lovegrove, 2012), and that T_w shifts have been predicted to reach as high as ~ 35˚C by the end of the century (Coffel et al., 2018; Pal and Eltahir, 2016), future T_w conditions are therefore set to risk over 80% of mammalian species to severe hyperthermic stress. As regional readings as high as 35˚C have already been reported in areas of South Asia, the Middle East and southwest North America (Raymond et al., 2020), the threat that T_w conditions may pose will already start to become an issue. However, despite the importance of such a metric in understanding the future impact of climate change, an empirical assessment into the proposed thermal boundary enforced by ambient T_w conditions is still lacking, leaving the severity and nature of the threat that T_w may pose relatively unknown. Therefore, this study sought to investigate the effect that T_w conditions at decreasing $T_b – T_w$ differentials would have on thermoregulatory processes in small homeothermic endotherms. In so doing this study sought to provide the first empirical assessment into the potentially limiting nature that T_w may present, allowing for an understanding of the threat that future T_w conditions may pose to terrestrial endotherms.

Methods and Materials

This study aimed to investigate how T_w conditions approaching an endotherms T_b would affect the rate of heat storage in small mammals. This was done by exposing obligatory homeothermic endothermic rodents, Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784), to static T_w conditions of 30-33˚C under constant relative humidity (RH) conditions of 70%. During this time period, shifts in T_b, metabolic rate (MR) and evaporative water loss (EWL) were continuously measured. From this data an understanding into the effect of elevated T_w heat stress on thermoregulatory capacity, endogenous heat production and thermoregulatory effort were drawn. It was hypothesised that the rate of heat storage and MR would increase as T_w conditions approached the T_b of the mice in question, while the rates of EWL would decrease.

Study Species, Animal Capture and Husbandry

Four-striped grass mice (henceforth grass mice) are philopatric (Schradin and Pillay, 2004), homeothermic endotherms (Couture, 1980; Scantlebury et al., 2006) that are endemic to the Fynbos and Succulent Karoo biomes in South Africa (Du Toit et al., 2019). Being diurnal-crepuscular, these mice are active throughout the day (Choate, 1972; Christian, 1977) while presenting peak activity periods at sunrise and sunset (Dewsbury and Dawson, 1979). Weighing between 40-80g at maturity (Schradin and Pillay, 2004), they are known to maintain body temperatures (T_bs) of ~ 36.5˚C (Couture, 1980; Lovegrove, 2009). The study population is reported to have a basal metabolic rate (BMR) of 0.27 ± 0.30 W during summer (Haim and Fourie, 1980) and 0.50 ± 0.03 W during winter (Welman, 2012).

Between July and August of 2019, adult grass mice were captured in the Nelson Mandela University Nature Reserve in Gqeberha (Port Elizabeth), South Africa (34˚00’23” S, 25˚39’51” E) using baited Sherman traps. Trapping occurred twice a day, correlating with the reported peak activity periods for the mice in question. All the traps were opened for three hours at a time, commencing one hour before dawn (ca. 05:00) and dusk (ca. 16:00), before being closed. Following closure, all caught individuals were transported to the nearby Nelson Mandela University Applied Ecophysiology Research Laboratory where they were sexed and weighed. Only non-pregnant adult individuals were considered for testing, however owing to the low number of non-pregnant females collected (n = 2), only males were used in this study (n = 11; M_b = 55.21 ± 9.118 g). All other individuals were released back at their site of capture.

The retained individuals were housed separately inside glass cubicles (300mm x 300mm x 297.5mm) that were each equipped with pine shavings to act as ground cover (DUVO$^+$ Wood Fibre; Laroy Group, Belgium) and a modified PVC pipe to provide a hiding area in order to reduce captivity stress. Once housed, all individuals were acclimated to captive conditions for a week (Animal Care and Use Committee, 1998) whereby a 12Hr:12Hr Light:Dark (LL:DD) cycle was implemented at a room temperature of 26.4 ± 0.73˚C before measurements began. While captive, individuals had *ad libitum* access to water and food comprising of Cavia Nature Guinea Pig Feed (Versele-Laga, Belgium) that was supplemented with sunflower seeds. Upon completion of measurements, all animals were monitored for a 24hr period before being released back into the wild at their site of capture. The average duration that individuals spent in captivity was 13 days (± 3 SD).

All experimental procedures were approved by the Nelson Mandela University Animal Research Ethics Committee (A19-SCI-ZOO-002) and animals were collected with approval by the Eastern Cape Department of Economic Development, Environmental Affairs and Tourism (permit CRO 91/19CR).

Experimental Design and Gaseous Exchange Measurements

A push open flow-through respirometry design, as described by Lighton (2008), was used for this investigation. Oxygen consumption (VO_2), carbon dioxide production (VCO_2) and evaporative water loss (EWL) were measured simultaneously at T_Ws of 30˚C-33˚C. The respirometer used in this investigation comprised of a modified 2.7L airtight container (295 mm x 230 mm x 68 mm; HPL832; Lock & Lock; Seoul, South Korea) filled with 200ml of vegetable oil to cover the base, thereby preventing any evaporation from urine and faecal matter obscuring EWL measurements. To prevent the study animal from coming into contact with the oil, the respirometer was equipped with a stainless-steel grid fitted with a plastic mesh top. The mesh size was large enough to allow urine and faeces to easily fall through and be trapped in the oil to prevent evaporation from them affecting the readings.

Mice were exposed to static T_w conditions at 1°C intervals between T_w 30˚C – 33˚C for a maximum of 3.5 hrs over a period of at least four days to allow for recovery following a particular T_w test. Based on the known normothermic T_b for the species, the chosen range of T_w corresponded to a T_b - T_w gradient of 7˚C - 4˚C respectively. A similar approach to the pioneer work of Ewing and Studier (1973) was used to generate the desired T_w. The dew point temperature (T_{dp}) of the incurrent airstream and the T_a of the respirometer were manipulated based on Antoine's equation for the determination of the water vapour pressure (WVP; Wood, 1970) and equation 1 from Stull (2011) to determine T_w from RH and T_a. The psychrometric measures used in this study are provided in Table 2.1 below.

Table 2.1 Psychrometric metrics required to generate wet-bulb temperature (T_w) conditions of 30-35˚C under constant relative humilities of 70%. Included are the ambient temperatures (T_as), dew-point temperatures (T_{dp}s), water vapour pressures (WVP), saturated WVP (WVP_s) and water vapour densities (WVDs).

T_w (˚C)	T_a (˚C)	T_d (˚C)	WVP (kPa)	WVP_s (kPa)	WVD (kg m^{-3})	WVD (µg ml^{-1})
30	34.73	28.46	3.88	5.55	0.028	27.725
31	35.80	29.48	4.12	5.89	0.029	29.338
32	36.87	30.50	4.37	6.24	0.031	31.011
33	37.95	31.53	4.63	6.62	0.033	32.742
34	39.02	32.55	4.91	7.02	0.035	34.603
35	40.10	33.59	5.20	7.43	0.037	36.52

The temperature of both the control and the animal respirometers was regulated by placing both respirometers inside of a modified ice box (60.33 cm x 29.85 cm x 33.00 cm; model 100 Quart Xtreme 5 Cooler; The Coleman Company Inc.; Kansas, United States of America) fitted with copper piping. The temperature of the ice box was regulated using a water pump (model FP-48; output: 2100 L Hr^{-1}; Guangdong BOYU Aquarium Industries Co.; Guangdong, China) and a waterbath (memmert WNB 45; operating temperatures of 10˚C-95˚C; Memmert GmbH + Co. KG; Schwabach, Germany) to circulate water that had been heated to the appropriate temperature through the copper piping circling the ice box's interior. The temperature of the waterbath was assessed using a precision mercury-in-glass thermometer traceable to NIST accuracy with a resolution of 0.1˚C.

Humidified air was flowed through the respirometer at a constant flow rate of 1.5 L min^{-1}. Using these flow rates, a washout rate of 1.67 min was achieved and found to be sufficient to maintain O_2 concentrations above 20.58%. A compressor was used to draw in atmospheric air from outside of the building, allowing for atmospheric concentrations of all gasses present in the incurrent airstream to exist. The flow of the incurrent airstream was regulated by a mass flow controller (model FMA 5400A/5500A; Omega Engineering Inc.; New Jersey, United States of America) to maintain a constant incurrent flow rate of 3 L min^{-1} into the respirometry system. The incurrent airstream was flown through two bubbler bottles submerged in a separate circulating water bath (18 L; operating temperatures of 5˚C above T_a – 100˚C; Scientific Manufacturing cc.; Cape Town, South Africa) being regulated with by digital temperature controller (TTM-J4; Toho Electronics Incorporated; Kanagawa, Japan) to humidify the airstream with water vapour at a known dew point temperature (T_{dp}) before being split equivalently into a control and an animal respirometry line. This ensured that both respirometers would receive equivalent amounts of air with the required water vapour density (WVD) to ensure the desired T_w would be achieved. The temperature of the water-bath was monitored using a mercury-in-glass thermometer of NIST standards with a resolution of 0.1˚C.

Once having flown through the respirometers, the WVD of the excurrent airstream was first measured using an RH-300 relative humidity meter (Sable Systems; Nevada, United States of America), before being subsampled from a downstream manifold. An SS-4 sub-sampler (Sable Systems) was pull subsampled air through the ensuing series of gas scrubbers and analysers at a constant rate of 200 ml min^{-1}, and was plumbed in at the terminal end of the analyser set up. To prevent any interference by the presence of water vapour in subsequent gaseous analyses (Lighton, 2008), all water vapour was scrubbed from the excurrent stream using a column of self-indicating Drierite (W. A. Hammond DRIERITE Co. LTD; Ohio, United States of America). A CO_2 gas analyser (CA-10A Carbon Dioxide Analyser; Sable

Systems) was then subsequently used to determine the fractional concentration of CO_2 (F_eCO_2) present in excurrent, before the airstream was scrubbed of all CO_2 using a column of self-indicating soda lime. On account of the CO_2 – soda lime reaction again generating minor quantities of water vapour (Lighton, 2008) the airstream was also then scrubbed again of water vapour. An O_2 gas analyser (FC-10A Oxygen Analyser; Sable Systems) was then used to determine the fractional concentrations of O_2 (F_eO_2) within the excurrent airstream before the air was expelled into the surrounding atmosphere.

All analysers used relied an analogue system to continuously determine their respective concentrations based on an electrical potential measure between 0 – 5V. To digitise the readings, all of analysers were connected to a personal computer via a UI-2 (Sable Systems) to interpret the analogue outputs and record them in a digital format using Sable System's data acquisition software, Expedata (v 1.7.22; Sable Systems). To ensure accurate readings, all analysers underwent calibration before the start of the study at flow rates concurrent to those to be used in the study, as described in Lighton (2008). For each analyser, the lower span calibration was set using pure N_2 gas, while the upper span for each was set using a known concentration of the respective gas. The CO_2 analyser used pure CO_2, the O_2 analyser used a mixture of helium and oxygen of known concentrations, and the RH meter was calibrated using a dewpoint generator (DG – 4; Sable Systems). On account of the high volumes of water vapour that the RH meter would be exposed to, further intermittent calibrations occurred to retain the analysers accuracy and account for potential drift.

Bev-A-Line IV tubing (Thermoplastic Processes In.; New Jersey, United States of America) was used throughout to minimize any absorption of water vapour altering the incurrent and measured WVDs. All flow controllers were calibrated using a soap-bubble flow meter. To prevent condensation forming within the tubing, and thereby altering the vapour densities and negatively affecting readings, the T_a of the room housing the experimental setup was maintained at least 2˚C above that of the T_{dp} of the air entering the respirometer

Respirometery and Body Temperature Measurements

The respirometer temperature was continuously monitored using two iButtons (Model DS1922L; Thermochron, Sydney, Australia) placed on opposite chamber walls inside the respirometer. Body temperature measurements were recorded using temperature sensitive BioTherm13 PIT (Passive Integrated Transponder) tags (13mm x 2.12mm, FDX-B 134.2 kHz; Biomark Inc., Bois, United States of America) that were injected into the abdominal cavity of mice upon their initial housing. Body temperature readings were measured every ~ 30 s using an HPR Plus PIT tag reader (Biomark Inc), that was connected to the personal computer allowing for real-time monitoring of T_b during experimentation.

Experimental Procedure

Food was removed six hours prior to measurements to ensure that all animals were in a post-absorptive state during measurements. The animals were weighed using a digital scale (WTC-2000; Radwag Balances and Scales; Radom, Poland) and allowed to settle in respirometers for approximately 30 minutes before measurements commenced. While settling, they were left in a darkened, secluded room and fresh air was flowed through the respirometer to prevent hypoxia. This seclusion period served to calm animals after handling, thereby minimizing the potential of handling stress biasing the results, as was observed in preliminary trials. During this time, baseline measurements were collected, and the O_2 analyser was spanned. After 30 minutes had elapsed, the study animal was then placed inside of the modified ice box and the airstream entering the series of analysers was manually changed to that of the excurrent air from the animal's respirometer. To prevent hyperthermic lethality, experiments were terminated when an individual's T_b reached 40˚C. However, following the death of an individual whose T_b did not exceed 39.6˚C this margin was further extended to such that trials were also terminated if an individual was unable to cool below 39˚C within an hour of reaching this point.

Data Analyses

On account of minor temporal differences that exist between gas-exchange traces when collecting respirometric measures (Lighton, 2008), all collected traces were lag-corrected against their respective WVD trace to ensure temporal synchronicity when later undergoing data extraction in Expedata. Following this process, each trace then underwent drift correction using the intermittent baseline measurements, so as to account for any bias as a result of analytical drift. The data for F_eO_2, F_eCO_2 and WVD (F_eH_2O), were extracted every 10 min, using a 5 min average, centred around the corresponding 10 min mark.

Following Lighton (2008), the rate of oxygen consumption (V_{O_2}), carbon dioxide production (V_{CO_2}) and EWL (V_{H_2O}) were determined using equations 2.1 - 2.3. To correct for the effect that presence of water vapour has on the excurrent flow rates (FR_e), all measured flow rates were corrected (FR_c) using equation 2.4, as detailed in Lighton (2008). Barometric pressure (BP) was assumed to be 101.325 kPa while WVP was measures by the O2 analyser, and the incurrent flow rates (FR_i) of both respirometers was set to 1.5 L min^{-1}. The incurrent fractional concentrations of O2 (F_iO_2) and CO2 (F_iCO_2) were assumed to be at atmospheric levels (20.95% and 0.4 ppm respectively).

$$V_{O_2} = FR_i(F_iO_2) - FR''_c(F''_eO_2) \qquad \text{equation 2.1}$$

$$V_{CO_2} = FR'_c(F'_eCO_2) - FR_i(F_iCO_2) \qquad \text{equation 2.2}$$

$$V_{H_2O} = FR_c(F_eH_2O) - FR_i(F_iH_2O) \qquad \text{equation 2.3}$$

$$FR_c = FR_e(BP - WVP)/BP \qquad \text{equation 2.4}$$

Equations 2.5 and 2.6 were used to determine the respective excurrent flow rates FR'_c and FR''_c following humidity and CO2 scrubbing respectively, while equations 2.7 and 2.8 were used to determine excurrent fractional concentrations of CO2 (F'_eCO_2) and O2 (F''_eO_2) respectively, again taken from Lighton (2008).

$$FR'_c = FR_c(1 - F_eH_2O) \qquad \text{equation 2.5}$$

$$FR''_c = FR'_c(1 - F'_eCO_2) \qquad \text{equation 2.6}$$

$$F''_eO_2 = F_eO_2/(1 - F'_eCO_2) \qquad \text{equation 2.7}$$

$$F'_eCO_2 = F_eCO_2/(1 - F_eH_2O) \qquad \text{equation 2.8}$$

To determine water vapour density (WVD) equation 10.10 from Lighton (2008) was used (equation 2.9), where T represents T_a in Kelvin and R_w represents the gas constant for water vapour (461.5 J kg^{-1} K^{-1}).

$$WVD = WVP/(T \times R_w) \qquad \text{equation 2.9}$$

Using the V_{O_2} and V_{CO_2} data, the metabolic rate (MR; W) was calculated the respiratory exchange quotient (RQ; V_{CO_2}/V_{O_2}) and used the thermal equivalence data in Table 4.2 of Withers (1992) to convert MR to a measure of energy (Joules). However, owing to O_2 analyser instability issues, presumably caused by the unstable local electrical power grid, the RQ was rather assumed to be the mean RQ of the stable O_2 readings (0.67 ± 0.22) following the methods of Gerson et al. (2014). Using the calculated mean RQ, metabolism was then able to be calculated using the more stable V_{CO_2} readings. To determine the corresponding T_bs for each time period over which the response variables were extracted, the mean T_b expressed over the corresponding 5-minute time period was used. The change in M_b was determined by assuming a linear rate of change between the initial to final M_b. This allowed for the calculation of each corresponding M_b over each of the respective 10-minute time intervals.

Statistical Analyses

All statistical analyses were conducted in the R programming environment (R Core Team, 2019) using R studio (RStudio Team, 2016) as an IDE. A simple linear regression analysis was used to determine the rate of change over time for each physiological response variable (T_b, MR or EWL) under each experimental condition. Using the *segmented* R package (Muggeo, 2008), a piecewise regression analysis was then conducted on these regressions at an individual level for each T_w response to identify any break points, that would indicate a change in the slope of the relationship. Mixed effects models were used to test for differences in T_b, MR or EWL at different experimental conditions, while accounting for the potential effect of individual specific differences. As such, individual specific differences were treated as fixed effects. For all significant mixed effect models, a Tukey post-hoc test was conducted using the *emmeans* R package (Lenth, 2019) to undertake pairwise comparisons to determine specific differences in the response of mice between T_w treatments. A p-value of 95% was required to infer statistical significance.

To ensure that the data met the assumptions of linear mixed-effects models, similar protocols to those described by Levesque et al. (2014) and Pinheiro and Bates (2000) were used. Following the construction of each model, the assumptions of (1) homogeneity of variance and (2) normal distribution of residuals were visually checked using qq-plots and histograms, and then numerically checked using a Levene's test for homogeneity of variance and a Shapiro-Wilks test for normality respectively.

Results

This study found that T_w had a significant effect on the thermoregulatory processes in grass mice. A total of 44 trials were completed using the above methodology, equating to 11 complete sets. During these trials a total of 4, 5, 6 and 9 mice had to be removed owing to surpassing the safety limit to prevent lethal hyperthermia for the 30˚C, 31˚C, 32˚C and 33˚C T_w heat stress trials respectively (Figure 2.1).

The Effect of Wet-bulb Temperature on Body Temperature

Wet-bulb temperature had a significant effect on the rates of heat storage in grass mice. Visual inspections of the data indicated that the change in T_b over time occurred in two phases (Figure 2.1). Therefore, a segmented analysis was conducted to determine if and at which points there was a change of the rates of heat storage over time. The analysis found a single break point, dividing the T_b responses into an initial heat storage and a subsequent elevated homeothermic phase. The model that best explained the observed variation in the time taken to reach the break point observed (BP) included both T_w and M_b (AICc = 388.5, w_i = 0.999; Table 2.2).

However, while M_b was found to be a significant factor in the overall model ($F_{1,9.3459}$ = 7.6137, p = 0.0214), T_w was not ($F_{3,26.9886}$ = 1.6857, p = 0.19). Body mass was also negatively correlated with BP between M_b 40 - 80g (r = -0.418, t = -2.8388, df = 38, p = 0.0072; Figure 2.2). The mean amount of time taken to reach BP was 69.58 ± 42.009 min (Figure 2.1) between all T_ws. The trial specific BPs are presented in Table 2.4.

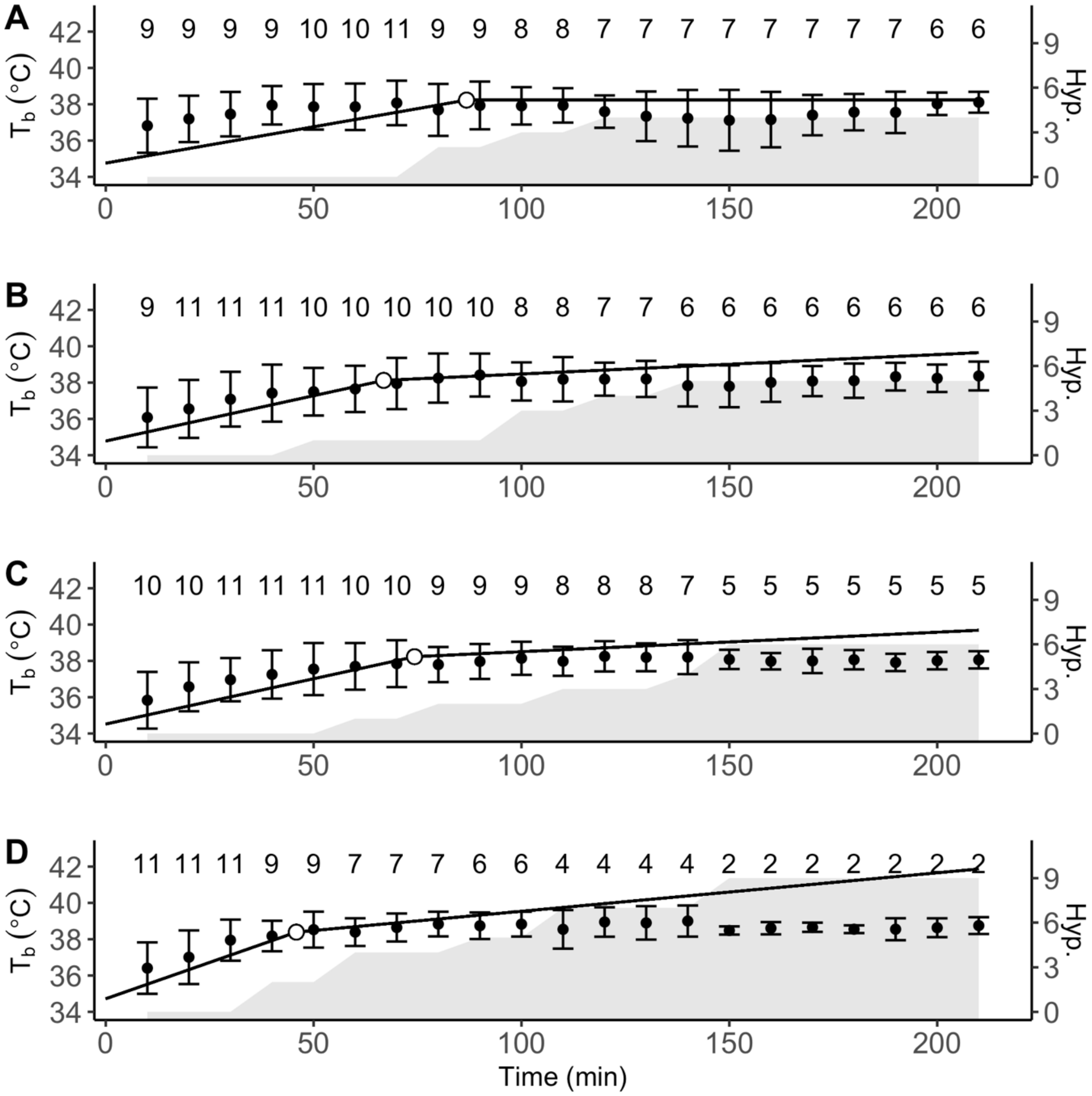

Figure 2.1. Body temperatures (T_bs) of wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) exposed to static extreme wet-bulb temperatures (T_ws) of **A**. 30˚C, **B**. 31˚C, **C**. 32˚C and **D**. 33˚C under 70% relative humidity conditions. The mean T_bs are represented by the filled-in circles and the

accompanying ± SD are represented by the overlayed error bars. The number of observations used to calculate the means are provided above. The number of mice removed to prevent lethal hyperthermia is indicated by the shaded area behind each graph. The rates of heat storage before and after the break points are indicated by the solid lines and the break point is indicated by the hollow circle.

During the heat storage phase, the mean rates of heat storage (β_{T_b}) observed were 0.04 ± 0.031˚C min^{-1} at T_w = 30˚C, 0.05 ± 0.022˚C min^{-1} at T_w = 31˚C, 0.05 ± 0.030˚C min^{-1} at T_w = 32˚C and 0.08 ± 0.032˚C min^{-1} at T_w = 33 (Table 2.4). The model that best explained this variation in β_{T_b} before BP was the null model (AICc = -163.1, w_i = 0.997; Table 2.2). Although this model was found to be significant (t = 8.564, df = 10, $p < 0.0001$), accounting for the effect of T_w also presented a significant descriptor of the variation observed ($F_{3,30} = 7.6378$, $p = 0.0006$; Table 2.2). The Tukey post-hoc pairwise comparison found that the β_{T_b} at 33˚C T_w was greater than all other T_ws, while no other differences could be found (Figure 2.1).

Table 2.2 Linear mixed-effects models describing the significant relationships between prolonged exposure to extreme static wet-bulb temperatures (T_w) and body temperature (T_b) in wild-caught African Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784).

Fixed Factors	**Random Factor**	***df***	***logLik***	***AICc***	**∆*AICc***	***w_i***
Break Point						
$T_w + M_b$	1|ID	7	-185.524	388.5	0.00	0.999
T_w	1|ID	6	-194.025	402.5	13.97	0.001
T_b At BP						
Null	1|ID	3	-48.697	104	0.00	0.956
M_b	1|ID	4	-50.909	111	6.92	0.030
T_w	1|ID	6	-49.061	112.6	8.55	0.013
β_{T_b} before BP						
Null	1|ID	3	84.863	-163.1	0.00	0.997
T_w	1|ID	6	82.771	-151.3	11.85	0.003
β_{T_b} after BP						
Null	1|ID	3	109.722	-212.8	0.00	1.000

Following the heat storage phase, all the mice attempted to defend an elevated T_b once reaching their respective BPs (Figure 2.1). The observed variability in this elevated T_b was best described by the null model (AICc = 104, w_i = 0.956; Table 2.2). This model was also found to be the only significant predictor of the variability in the observed elevated T_bs (t = 164.8, df =10.0543, $p < 0.0001$). The mean defended T_b at BP was 38.24 ± 0.924 ˚C. The mean trial specific defended T_bs can be found in Table 2.4.

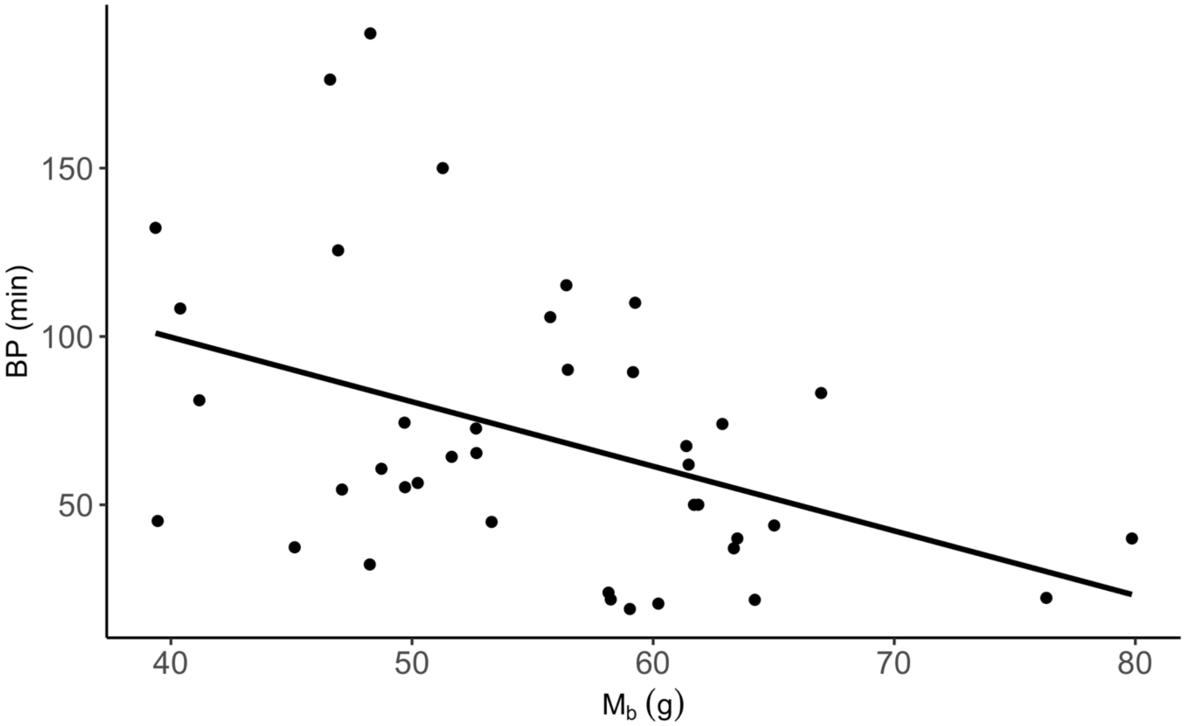

Figure 2.2 Scatterplot of the points of inflection (BP) in the body temperature (T_b) responses of wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) against their respective body masses (M_bs) at BP when experiencing static wet-bulb temperatures between 30-33˚C.

The success in maintaining their elevated homeothermic states under the different T_ws was determined by the β_{T_b} after BP. The mice experienced β_{T_b}s of 0.00 ± 0.013˚C min^{-1} at T_w = 30˚C, 0.01 ± 0.008˚C min^{-1} at T_w = 31˚C, 0.01 ± 0.011˚C min^{-1} at T_w = 32˚C and 0.02 ± 0.023˚C min^{-1} at T_w = 33˚C (Table 2.4). The variation in these observed β_{T_b} after BP was best described by the null model (AICc = -212.8, w_i = 1.000;

Table 2.2). This too was also the only significant predictor of the observed variability in (t = 3.454, df = 9.0025, p = 0.0072).

Table 2.3 Linear regression models describing the significant relationships between body temperature (T_b) and prolonged exposure to extreme static wet-bulb temperatures (T_w) in wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784).

Parameter	**Regression Equation**
Break Point	$BP = T_w - 1.884 \times M_b + 189$
T_b At BP	T_b at BP = 1 + 38.27
Rate of Change before BP	β_{T_b} before BP = 1 + 0.05278 β_{T_b} before BP = T_w + 0.03555
Rate of Change after BP	β_{T_b} after BP = 1 + 0.01106

Table 2.4. Data summary of the calculated metrics regarding the effect that wet-bulb temperature of 30 - 33˚C had on the thermoregulatory processes of wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784). This table details the points of inflection (BP) for the body temperature (T_b), and evaporative water loss (EWL) data. The values of the mean T_b and EWL at their respective BPs, as well as the rates of change (β) in T_b and EWL both before and after their BPs are also provided. The *F* statistics (F_{stat}), degrees of freedom (df) and *p* values (p_{value}) for each of the respective metrics can also be found here. The mean MRs measured at the T_b BP are also provided for each T_w.

T_b	30 ˚C	± SD	31 ˚C	± SD	32 ˚C	± SD	33 ˚C	± SD	F_{stat}	df	p_{value}	
BP (min)	86.78	58.680	66.85	28.889	74.27	38.397	45.84	25.644	2.17	3,27.53	0.11	-
T_b At BP (˚C)	38.23	1.030	38.12	1.086	38.23	0.882	38.39	0.776	0.67	3,27.29	0.58	-
β_{T_b} Before BP (˚C min^{-1})	0.04	0.031	0.05	0.022	0.05	0.030	0.08	0.032	6.31	3,27.71	0.002	**
β_{T_b} After BP (˚C min^{-1})	0.00	0.013	0.01	0.008	0.01	0.011	0.02	0.023	5.07	3,26.76	0.007	**
MR												
MR (W)	0.82	0.222	0.8	0.191	0.82	0.186	0.82	0.073	0.0276	3,25.86	0.99	-
EWL												
BP (min)	74.91	40.426	70.26	31.833	64.84	23.821	68.12	26.279	0.19	3,27.20	0.90	-
EWL At BP (mg min^{-1})	5.41	2.208	6.64	2.765	6.84	2.918	8.37	4.078	4.54	3,26.12	0.01	**
β_{EWL} Before BP (mg min^{-2})	0.10	0.069	0.13	0.072	0.12	0.070	0.15	0.063	1.21	3,26.42	0.33	-
β_{EWL} After BP (mg min^{-2})	0.00	0.024	0.01	0.047	0.02	0.060	0.00	0.071	0.68	3,26.35	0.57	-

The Effect of Wet-bulb Temperature on Metabolic Rate

Wet-bulb temperature did not affect the MRs of grass mice (Figure 2.3). Although being correlated with the duration of exposure (r = 0.6756, t = 5.6493, df = 38, $p < 0.0001$; Figure 2.3) and presenting a mean gradient of – 0.0001 W min^{-1}, when comparing the slope of MR over time at an individual level, this was found (p > 0.05 for all individuals). As such, to compare for the effect of T_w on MR, the corresponding MR with the BP for T_b for each mouse was used. The model found to best describe the variation observed in MR included M_b as a factor (AICc = -29.6, w_i =0.728; Table 2.5), which contributed significantly to the observed variability ($F_{1,11.466} = 19.933$, $p = 0.0009$) and was also correlated with MR at BP (r = 0.6756, t = 5.6493, df = 38, $p < 0.0001$; Figure 2.4). The observed variability in MR was also found to be well described by the null model (AICc = -27.6, w_i = 0.272; Table 2.5), indicating that random variability also contributed significantly to the variability in MR at BP (t = 17.25, df = 9.54924, $p < 0.0001$).

Table 2.5 Linear mixed-effects models describing the significant relationships between prolonged exposure to extreme static wet-bulb temperatures (T_W) and metabolic rate (MR) in wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784).

Fixed Factors	**Random Factor**	***df***	***logLik***	***AICc***	***ΔAICc***	w_i
MR at BP						
M_b	1|ID	4	19.365	-29.6	0.00	0.728
Null	1|ID	3	17.143	-27.6	1.97	0.272

Table 2.6 Linear regression models describing the significant relationships between metabolic rate (MR) and prolonged exposure to extreme static wet-bulb temperatures (T_W) in wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784).

Parameter	**Regression Equation**
MR at BP	$MR = 0.01399 \times M_b + 0.04226$

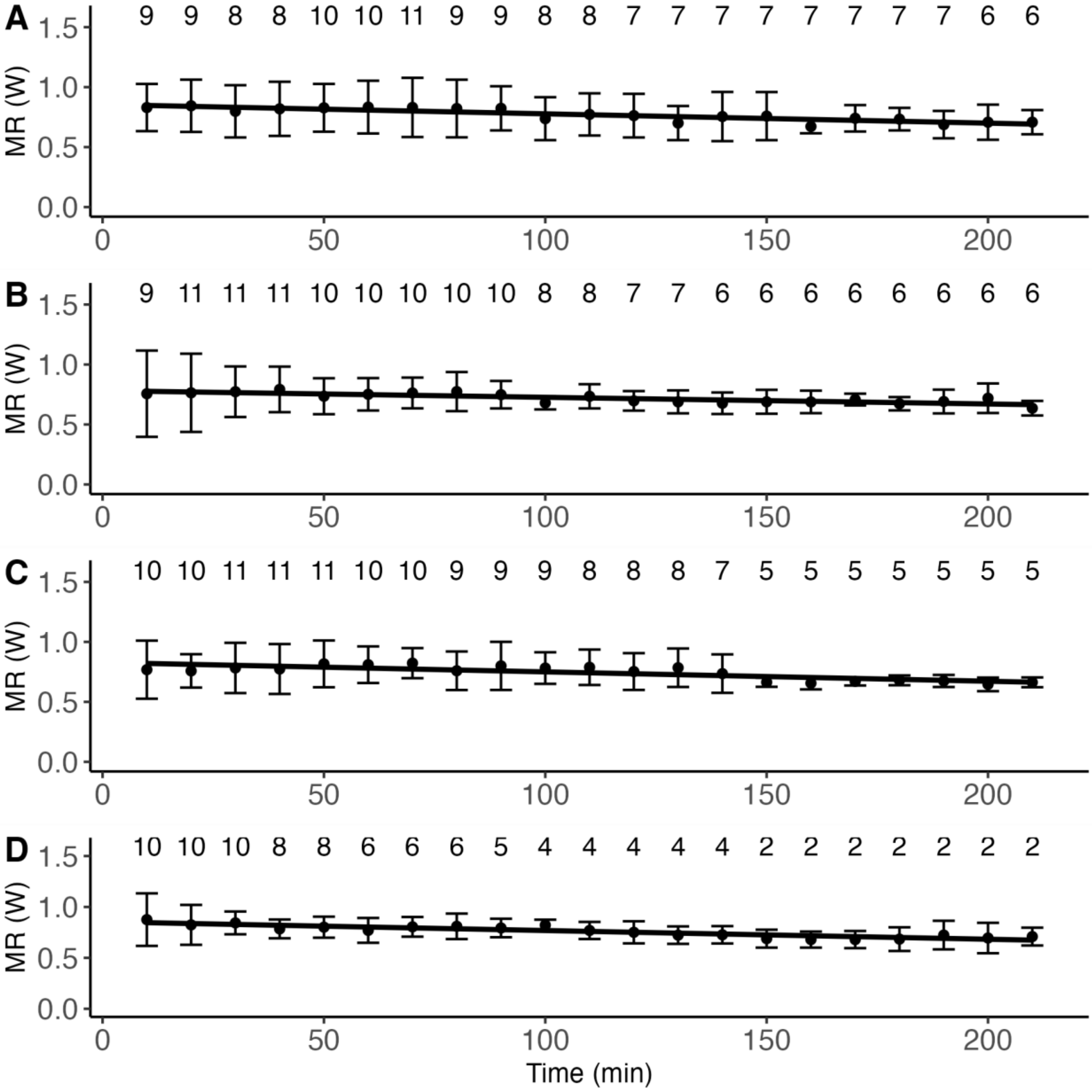

Figure 2.3. Metabolic rates (MRs) of wild-caught Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) exposed to static extreme wet-bulb temperatures (T_Ws) of **A**. 30˚C, **B**. 31˚C, **C**. 32˚C and **D**. 33˚C under 70% relative humidity conditions. The mean MRs are presented by the filled-in circles and the accompanying ± SD are represented by the overlayed error bars. The number of observations used to calculate the means are provided above. The corresponding rates of change are indicated by the solid lines.

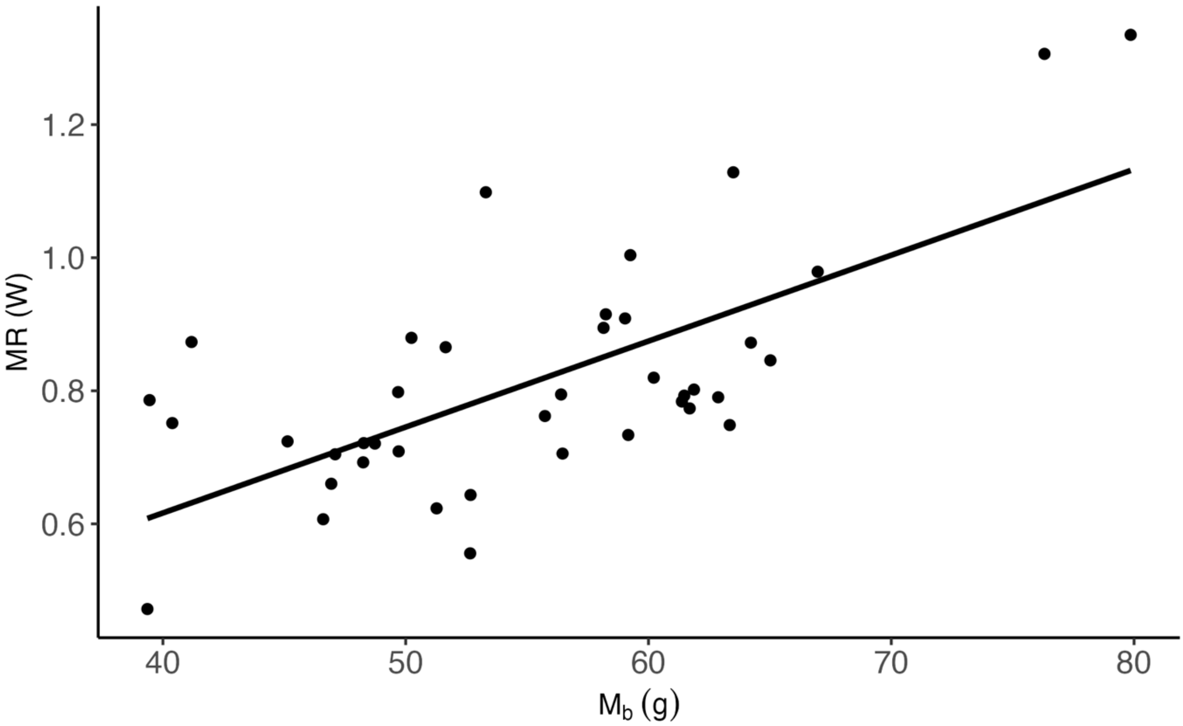

Figure 2.4 Scatterplot of the mean metabolic rates (MRs) of wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) against their respective body masses (M_bs) when experiencing static wet-bulb temperatures between 30-33˚C.

The Effect of Wet-bulb Temperature on Evaporative Water Loss

Wet-bulb temperature had a significant effect on the rates of EWL in grass mice. Visual inspections of the data indicated that the change in EWL over time occurred in two phases (Figure 2.5). Therefore, a segmented piecewise regression analysis was conducted to determine if and at which points there was a change in the slope of the rates of change in EWL. A single BP was found. The model that best described the observed variation in the time taken to reach the BP in the mice included T_w as a fixed effect (AICc = 372.5, $w_i = 0.564$; Table 2.7), however the ability of this model to explain the variability observed was insignificant ($F_{3,27.247} = 0.1891$, $p = 0.90$). As such, the mean BP in the EWL trace over time occurred at 69.84 ± 30.832 min. The BPs for the individual T_ws are available in Table 2.4.

The initial incremental phase in EWL showed to be positively correlated with the duration of time of T_w exposure (Figure 2.5). The measured rates of change in EWL (β_{EWL}) in this initial phase were found to be 0.10 ± 0.069 mg min^{-2} at T_w = 30˚C, 0.13 ± 0.072 mg min^{-2} at T_w = 31˚C 0.12 ± 0.070 mg min^{-2} at T_w = 32˚C and 0.15 ± 0.063 mg min^{-2} at T_w = 33˚C (Table 2.3). The model that best described the variation observed in β_{EWL} was the null model (AICc = -88.7, $w_i = 0.832$; Table 2.7), with the random effect playing a significant role in the observed variation (t = 9.7, df = 9.2232, $p < 0.0001$). Interestingly, when including M_b as a factor, this too presented a significant descriptor of the observed variation in β_{EWL} ($F_{1,10.564} = 13.115$, $p = 0.0043$; Table 2.7). Furthermore, M_b positively correlated with the initial β_{EWL}(r = 0.5115, t = 3.6694, df = 38, $p = 0.0007$; Figure 2.6). The mean β_{EWL} measured at 0.12 ± 0.069 mg min^{-2}. The means for each T_w are provided in Table 2.4.

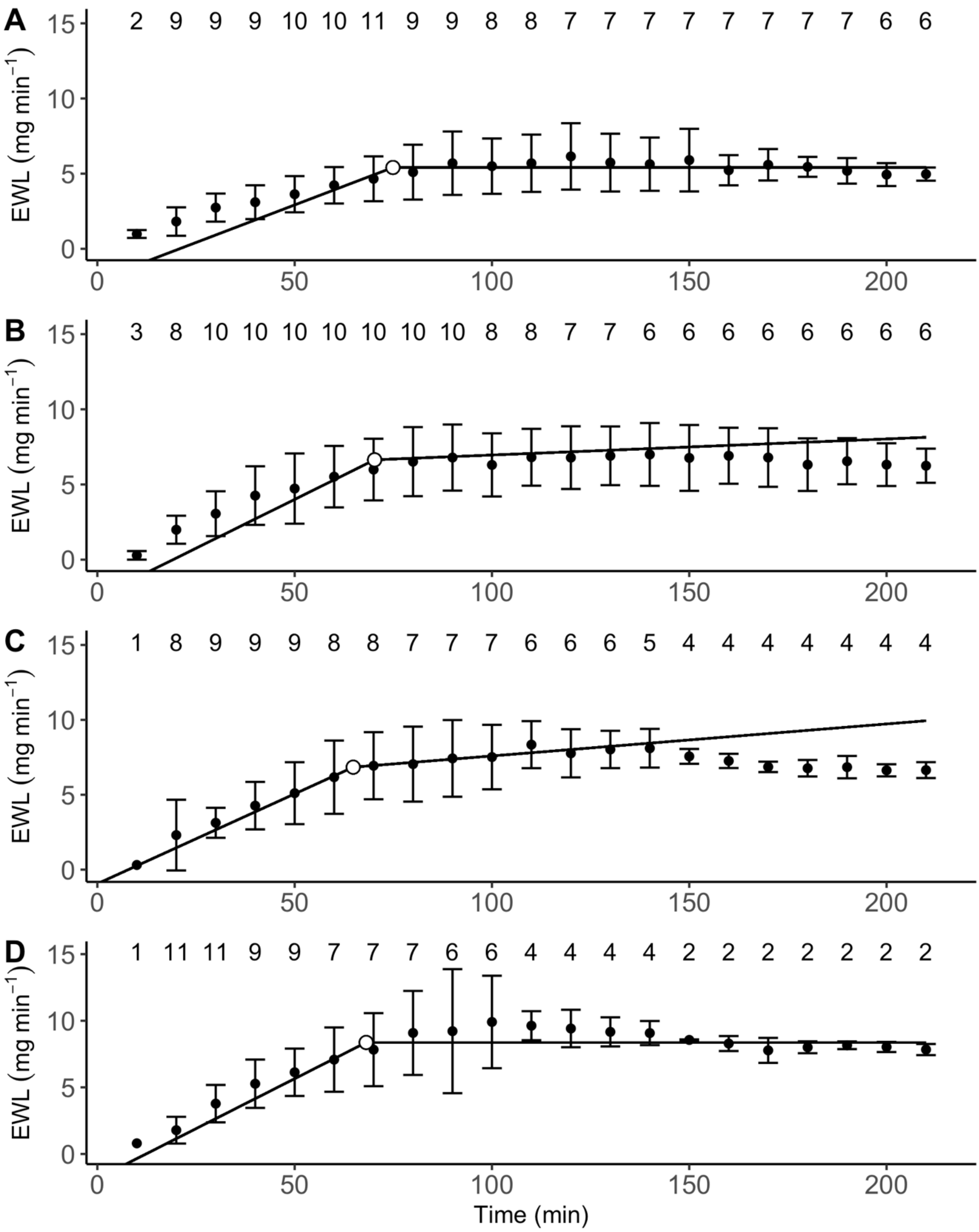

Figure 2.5. Rates of evaporative water loss (EWL) of wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) exposed to static extreme wet-bulb temperatures (T_Ws) of **A**. 30˚C, **B**. 31˚C, **C**. 32˚C and **D**. 33˚C under 70% relative humidity conditions. The mean rates of EWL are represented by the filled-in

circles and the accompanying ± SD are represented by the overlayed error bars. The number of observations used to calculate the means are provided above. The rates of EWL before and after the break points are indicated by the solid lines and the break point is indicated by the hollow circle.

Table 2.7 Linear mixed-effects models describing the significant relationships between prolonged exposure to extreme static wet-bulb temperatures (T_w) and evaporative water loss (EWL) in wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784).

Fixed Factors	**Random Factor**	***df***	***logLik***	***AICc***	**Δ*AICc***	w_i
Break Point						
T_w	1|ID	6	-178.983	372.5	0.00	0.564
T_w + M_b	1|ID	7	-177.766	373	0.52	0.434
M_b	1|ID	4	-187.846	384.8	12.32	0.001
Null	1|ID	3	-189.198	385.1	12.55	0.001
EWL At BP						
T_w	1|ID	6	-88.75	192	0.00	0.932
T_w + M_b	1|ID	7	-90.206	197.9	5.87	0.050
Null	1|ID	3	-96.73	200.1	8.08	0.016
M_b	1|ID	4	-97.777	204.7	12.65	0.002
Mean EWL After BP						
T_w	1|ID	6	-82.807	180.2	0.00	0.938
T_w + M_b	1|ID	7	-84.313	186.1	5.97	0.047
Null	1|ID	3	-91.01	188.7	8.53	0.013
M_b	1|ID	4	-92.174	193.5	13.33	0.001
β_{EWL} ***Before BP***						
Null	1|ID	3	47.664	-88.7	0.00	0.832
M_b	1|ID	4	47.303	-85.5	3.20	0.168
β_{EWL} ***After BP***						
Null	1|ID	3	64.029	-121.4	0.00	0.999
M_b	1|ID	4	58.412	-107.7	13.71	0.001
Maximum EWL						
T_w + M_b	1|ID	7	-85.032	187.6	0.00	0.983
M_b	1|ID	4	-93.295	195.7	8.17	0.017

Table 2.8 Linear regression models describing the significant relationships between evaporative water loss (EWL) and prolonged exposure to extreme static wet-bulb temperatures (T_w) in wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784).

Parameter	Regression Equation
EWL At BP	$EWL = T_w + 5.413$ $EWL = T_w + 0.04508 \times M_b + 2.962$ $EWL = 1 + 6.872$
Mean EWL After BP	$\mu EWL = T_w + 5.146$ $\mu EWL = T_w + 0.05312 \times M_b + 2.257$ $\mu EWL = 1 + 6.561$
Rate of Change before BP	$\beta_{EWL} = 1 + 0.123$ $\beta_{EWL} = 0.003924 \times M_b - 0.09424$
Maximum EWL	$EWL_{max} = T_w + 0.05015 \times M_b + 3.517$

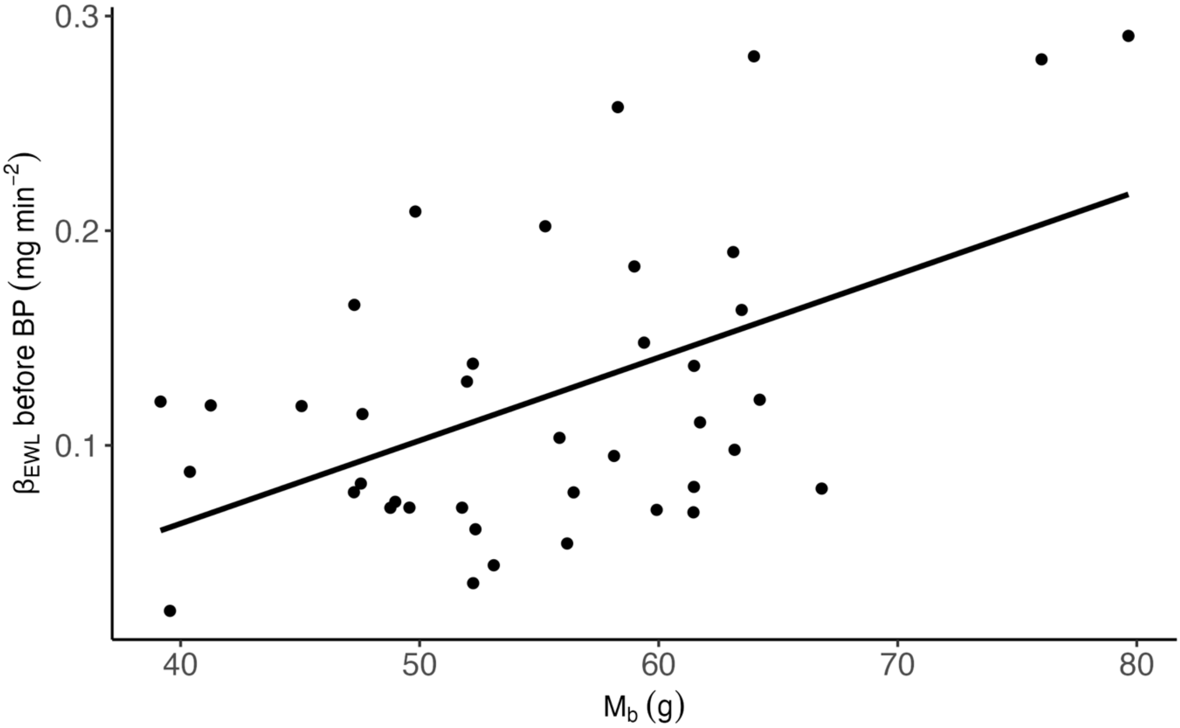

Figure 2.6 Scatterplot of the initial rate of increase in evaporative water loss (β_{EWL}) of wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) against their respective body masses (M_bs) when experiencing static wet-bulb temperatures between 30-33˚C.

The rates of EWL at the BP differed significantly between T_ws. The model that best described the variation only included T_w as a factor (AICc = -88.75, w_i = 0.932; Table 2.7). This was also found to contribute significantly to the variability in the rates of EWL at BP ($F_{3,26.123}$ = 4.5411, p = 0.01088; Figure 2.5). The Tukey post-hoc analysis showed that significant variability only existed between the rates of EWL observed at T_w 30˚C and T_w = 33˚C and no other T_w pairs. When accounting for M_b in conjunction with T_w, this too was found to be a significant predictor of the variability in the rates of EWL at BP, with T_w still contributing significantly ($F_{3,26.707}$ = 4.1006, p = 0.0162), but M_b not ($F_{1,13.566}$ = 0.3214, p = 0.58). The mean rates of EWL at BP were 5.41 ± 2.208 mg min^{-1} at T_w = 30˚C, 6.64 ± 2.765 mg min^{-1} at T_w = 31˚C, 6.84 ± 2.918 mg min^{-1} at T_w = 32˚C and 8.37 ± 4.078 mg min^{-1} at T_w = 33˚C (Table 2.4)

Wet-bulb temperature did not have an effect on the second phase of the EWL response in grass mice. Rather, the observed variability in the β_{EWL} after BP was best described by the null model (AICc = -121.4, w_i = 0.999; Table 2.7), however this did not have a significant effect on the observed variability (t = 0.535, df = 9.853795, p = 0.61). The second-best descriptor of the variability in β_{EWL}included only M_b as a factor (AICc = -107.7, w_i = 0.001; Table 2.7). This too did not have a significant effect ($F_{1,13.142}$ = 0.2196, p = 0.647). The mean β_{EWL} after BP measured as 0.008 ± 0.0096 mg min^{-2}. The βEWL for each T_w are provided in Table 2.4.

Wet-bulb temperature did have an effect on the maximum rates of EWL (EWL_{max}). The model that best described the observed variability in EWL_{max} included both T_w and M_b as predictors (AICc = 187.6, w_i = 0.983; Table 2.7) with T_w contributing significantly to the observed variability ($F_{3,26.845}$ = 5.1600, p = 0.0060) while M_b did not ($F_{1,13.980}$ = 0.5204, p = 0.48). The Tukey post-hoc analysis found that between T_ws the only significant differences occurred between T_w = 30˚C and T_w = 33˚C and no other T_w pairs. The rates of EWL_{max} were 6.24 ± 1.976 mg min^{-1} at T_w = 30˚C, 7.29 ± 2.461 mg min^{-1} at T_w = 31˚C, 7.89 ± 2.320 mg min^{-1} at T_w = 32˚C and 8.07 ± 3.832 mg min^{-1} at T_w = 33˚C (Table 2.4).

Discussion

This study aimed to investigate the potential threat that future T_w conditions may pose to thermoregulating endothermic organisms. By exposing grass mice to several T_w trials imitating ambient conditions that promoted T_b – T_w gradients of 6.5˚C, 5.5˚C, 4.5˚C and 3.5˚C (T_w exposures of 30˚C, 31˚C, 32˚C and 33˚C respectively) for a period of 3.5 hours per trial, this investigation sought to provide insights into how future T_w conditions may affect thermoregulatory processes in small homeothermic endotherms. Wet-bulb temperature was found to exert a significant effect on each mouse’s thermoregulatory capacity, with key differences being detected between the high stress T_w test (33˚C T_w) and other lower stress treatments. Interestingly, these effects remained limited to the T_b and EWL responses of the study animals while MR was found to be unaffected across the T_w regimes, only having been influenced by the duration of exposure to each T_w and not the T_ws in question.

The Thermoregulatory Effects of Extreme Static Wet-bulb Temperature Exposure in Rhabdomys pumilio

When heat stressed, the only physiological means to offload excess heat efficiently is to rely on evaporative cooling. However, some species are also known to engage in facultative hyperthermia (Gerson et al., 2019), tolerating T_b elevations of up to 4˚C (Maclean, 1996), and in extreme cases engaging in daily torpor or seasonal hibernation (Gerson et al., 2019; McKechnie and Lovegrove, 2002; Ruf and Geiser, 2015). This is done to both maintain the passive thermoregulatory pathways to offload excess heat to their surrounding environment by maintaining their $T_b > T_a$ (Dawson and Bartholomew, 1968; Gerson et al., 2019), and therefore reduce the cost of thermoregulation by reducing the reliance up EWL to thermoregulate. All of the grass mice were found to defend a set elevated T_b of 38.2˚C, at least 1.6˚C above their normothermic T_bs (Couture, 1980; Haim and Fourie, 1980; Lovegrove, 2009) across all T_w trials. Since all of the T_a conditions were at least 2˚C above their thermal neutral point of 32˚C (Haim and Fourie, 1980) this finding is therefore not surprising and is in accordance with prior findings for this species (Haim and Fourie, 1980), with similar being noted in other homeothermic murids such as the Libyan jird (*Meriones libycus*; defended a T_b of 41.9˚C, 3.7˚C above their reported T_b; Alagaili et al. 2017). Granted, this elevation may be been caused by handling stress as seen in the Eastern Broad-toothed Field Mouse (*Apodemus mystacinus*; Zevgolis et al. 2022), the meadow vole (*Microtus pennsylvanicus*; Fletcher & Boonstra 2006) and potentially a prior study on the grass mice (Couture, 1980) where handling lead to the animals experiencing elevated T_bs (Mertens et al., 2019; Zethof et al., 1994). However, considering that in all of the trials the initial T_bs of the mice of 36.3˚C were within the range of their reported normothermic T_bs of 34.6 ˚C – 36.8˚C (Haim and Fourie, 1980; Lovegrove, 2009) makes this is unlikely. Rather, considering that the defended T_b was consistent across all T_w exposures where T_as differed by up to 3.2˚C between trials (Table 2.1) and that all of the T_as were above the thermal neutral point of this species suggests that the use of an elevated homeothermic state was deliberate, making this response facultative rather than pathological (Gerson et al., 2019).

Interestingly, the defended T_b is similar to the reported acrophasic T_b for this species (Haim et al., 1998). Known to occur at night when the mice are least active as an adaptation to diurnality to prevent hyperthermia occurring during the day while most active (Haim et al., 1998), this response also may have been a result of diurnal cues as that all of the trials were conducted in darkness. However, being poor thermoregulators in colder environments (Haim and Fourie, 1980), *R*. *pumilio*'s acrophase will generally occur during the coldest period of the night, typically around 3 hours after sunset (Haim et al., 1998). This elevation in T_b is likely a by-product of their diurnal adaptation to counter their low insulation and high thermal conductance through their enhanced capacity for non-shivering thermogenesis in response to the colder night time conditions (Haim et al., 1998). As such, the observed T_b elevations are not likely in response to diurnal cues stimulating T_b elevations. Not only were the elevated homeothermic T_bs attained within 69 minutes of exposure, an hour and a half earlier than if it were a circadian response, but all test conditions were above the T_{crit} for this species and therefore also preventing the observed response being a countermeasure to cold stress. As such it despite the similarity to the acrophasic T_b of this species, the observe T_b elevation was more likely purely in response to the heat stress being experienced.

Regarding the effect of extreme T_w conditions on metabolic processes, being chemical conversions in biological material, metabolic rates (MR) are well known to be affected by temperature shifts (Dillon et al., 2010) being stimulated by temperature elevations as a result of Arrhenius upcycling (Geiser and McMurchie, 1985; Withers, 1992). Such observations have been found not only to occur in species such as the multimammate mouse (*Praomus natalensis;* Haim & Fourie 1980), but in *R. pumilio* as well (Haim and Fourie, 1980). Thus, it was hypothesised that in response to different T_w exposures, the MRs of the mice would respond accordingly. Since the T_as between for the least and most stressful trials were 34.8˚C and 37.8˚C, the absence of this effect was surprising. Therefore, one may be tempted to conclude that T_w heat stress does not affect MR in this species, as has been found to occur in other humid heat stress studies on White mice (*Mus musculus*; Ewing and Studier 1973), Deer mice (*Peromyscus maniculatus*; Edwards & Haines 1978), Red Kaluta (*Dasykaluta rosamondae*; Withers and Cooper 2014) and Rhesus monkeys (*Macaca mulatta*;

Walters et al. 2004). However, considering that when under dry heat stress at T_as of 34°C, akin to the least stressful T_w trials T_a component, previous studies on the grass mice presented MRs of ~1 ml O_2 g^{-1} hr^{-1} (Haim and Fourie, 1980), it is apparent that the mice did in fact respond to the heat stress applied. The observed values across all of trials were at least 2.718 ml O_2 g^{-1} hr^{-1}, more than twice the previously measured value, implying that the mice not only were affected by the heat stress in question but were also producing at least twice as much endogenous heat. Presumably this observed elevation in MR was in response to Arrhenius upcycling, however, if this were the case the differences in T_as would have also stimulated different MRs between the treatments as a result of the different T_a conditions (Lovegrove et al., 2013). Rather, what appears to have occurred is metabolic upcycling in response to the shift in the thermoregulatory set-point as outlined above, thereby requiring the mice to elevate their MRs to produce sufficient heat to maintain their facultative hyperthermic states.

Regarding the responses of EWL, it was initially hypothesised that at greater T_w exposure there would be a reduction in EWL because of the elevated WVPs concomitant with elevated T_w conditions. It is well established in literature that as WVP gradients decrease between an animal’s site of evaporative water loss and their environment, there is also a reduction in evaporative cooling as a result (Ewing and Studier, 1973; Gerson et al., 2014; Lasiewski et al., 1966; Powers, 1992; Withers and Cooper, 2014) as seen in the California Ground Squirrel, (Baudinette, 1972), White mice (*Mus musculus*; Ewing and Studier 1973) and Deer mice (*Peromyscus maniculatus*; Edwards & Haines 1978) among other endotherms (Christian, 1978; Lasiewski et al., 1966). This therefore makes the findings regarding EWL use by the mice seem anomalous, for not only were they able to increasingly rely on EWL to thermoregulate, but despite the significant differences in WVPs noted between the trial conditions (Table 2.1), at the peak stress trial of 33°C the mice were able to use and sustain a significantly greater rate of EWL to thermoregulate. This may have been due to the resulting experimental set up. Firstly, having maintained a constant RH between all the trials would imply that although WVPs might have been greater at higher T_ws, in each trial category there was still a constant 30% of the ambient environment for the mice to exploit for evaporative cooling. Further, as the capacity for an environment to contain water vapour scales positively with T_a conditions (Arias et al., 2021; Trenberth, 1998; Trenberth, 2011), the increased T_a required to elevate the T_w conditions may have retained conduciveness to evaporative cooling processes rather. As such, further study into how different RH combinations of T_w stress affect thermoregulatory processes is required to better understand the proposed inhibitive nature of T_w on evaporative cooling. Nevertheless, the absence of an effect of humidity on evaporative cooling processes does indicate the positive scaling of EWL with increased heat stress, agreeing the current understanding of EWL use in current literature (McNab, 2002; Tattersall et al., 2012; Withers, 1992).

Collectively, the above evidence for the effect of T_w on the physiological responses of the grass mice strongly suggests that the conditions were indeed stressful to the mice in question. Furthermore, it appears that the thermoregulatory responses of the mice were geared towards exploiting their capacities for facultative hyperthermia. This to be achieved by elevating their MRs to at least twice that of their reported BMRs to increase their metabolic heat production, and thereby elevating their T_bs to a hyperthermic set points. In doing so the costs of thermoregulation were reduced and ensured that a positive gradient was retained between their T_bs and the surrounding T_as. The mice then relied on their EWL capacities to then regulate this elevated hyperthermic state, augmenting the use of EWL in response to the T_a around them. The lack of response from the differing T_w conditions, while being under constant RH exposure suggests that this response also occurred in absence of the effect of humid heat stress.

Wet-bulb Temperature as a Thermal Limit

Sherwood and Huber (2010) argued that a minimum gradient of 2˚C between an animal's T_b and the ambient T_w is required to allow for the efficient offload excess metabolic heat from its body to the surrounding environment. As such, it was hypothesised that the onset of the T_w would occur at T_ws of 34 ˚C for this species. Given not only the death of an individual at 33 ˚C during the preliminary phase of this study, but also the high rate of heat storage occurring at a T_b - T_w gradient of 3.5˚C, the findings of this study not only support the existence of the thermal boundary, but also suggest that this boundary exists at a lower T_w, therefore at a greater $T_b - T_w$ differential. When exposed to T_ws below 33˚C, all the mice were able to rely on facultative hyperthermia sufficiently to allow at least five individuals to complete each trial (Figure 2.1). Conversely, when exposed to T_w = 33˚C most of the mice quickly reached the predetermined safety margin (n = 9), with less than 50% of individuals safely persisting past 100 minutes of exposure (Figure 2.1). Furthermore, the significantly greater βT_b after reaching the hyperthermic set-point despite the significantly greater rates of EWL detected in the 33˚C T_w trial indicates a critical shift in the response of mice at a $T_b - T_w$ gradient of 4˚C. As such this suggests that a $T_b - T_w$ gradient of 4˚C was the point where their thermoregulatory capacity was overcome and where pathological hyperthermia became the dominant state. Granted, this study did not investigate T_ws up to the proposed 2˚C differential, however as thermoregulatory efforts at the T_w of 33˚C were already ineffective, coupled with the loss of an individual in the pilot study for this thesis at T_w = 33˚C, further and potentially harmful tests were deemed unnecessary. Therefore, the results of the present study suggest the T_w limit does exists, as proposed by proposed by Sherwood and Huber (2010), but rather that for this species at least it exists at lower T_b-T_w gradient of 4˚C, not the T_b-T_w gradient of 2˚C initially thought.

The 2˚C difference between the proposed and the realised thermal limit may be as a result of several reasons. Firstly, unlike in the initial development of the T_w limitation theory that used human thermoregulatory capacities as a theoretical framework (Sherwood and Huber, 2010), this study relied on a small mammal. Humans are thought to be highly efficient thermoregulators when exposed to heat stress (Bligh, 1967; McKechnie and Wolf, 2019). They are able to survive T_a exposures exceeding 110˚C (Blagden, 1775; Murray, 1966) and exploit EWL rates upwards of 3.5 L hr^{-1} (Armstrong et al., 1986; Eichna et al., 1945), with unofficial reporting's of rates of EWL as high as 5.6 L hr^{-1} (Kenney et al., 2004), making them anomalous to the majority of other mammals. Small mammals, in contrast, are known to have limited capacities to counter high heat stress through evaporative cooling as a result of limitations imposed by their fur and size (Hoole et al., 2019; Schmidt-Nielsen, 1975), resulting in a much reduced capacity to counter thermal stress by comparison. However, despite these significant differences in study model, a recent physiological investigation into human responses to extreme T_w heat stress also concurs that T_w tolerance is far lower than predicted (Vecellio et al., 2022).

Secondly, when assessing how the initial calculation was performed, it was reasoned that once the ambient T_w reaches an organisms skin temperature (T_{skin}), T_w will then inhibit cooling below this point, thus committing an endotherm to hyperthermic stress (Sherwood and Huber, 2010). However, as cutaneous heat loss also requires a thermal gradient sufficient enough to exist between T_{skin} and the environment to allow sufficient cooling to occur to prevent heat storage (McNab, 2002; Rajek et al., 2000), a lower T_w should therefore be expected. Furthermore, when considering the potential heat stress experienced at the proposed T_w limit of 35˚C, the T_a component of these conditions exceed the T_bs of most mammals, which would commit them to hyperthermia irrespective of T_w limitation. For example, a T_w of 35˚C requires a T_a of 40.1˚C if the RH conditions are 70%. Over 80% of mammal defend T_b below this, with 80% defending T_b of 37.9˚C (Lovegrove, 2012). Therefore, once accounting for the required gradient between the T_{skin} and the ambient thermal environment, it is evident that a lower thermal limit would therefore exist. Granted, in the case of small mammals, whereby proxy of their small size, the gradient between their T_{skin} and their core temperature is minimal. Therefore, it is likely that in this case the limitation occurred not on the T_{skin} heat transfer but rather the T_b heat transfer. However, this merely further echoes the need for a lowered T_w safety margin to be realised.

Lastly, as T_ws measure the lowest temperature attainable when under the maximum rate of evaporative cooling, to be able to reach and fully exploit the maximum allowable rates of EWL in their respective environment would require that the endotherm is exposed to gale force winds while being covered in a film of water (Sherwood & Huber 2010). Considering that an endotherms capacity to counter heat stress is limited to its capacity to dissipate heat (Speakman and Król, 2010), and that in a natural setting, the likelihood of experiencing maximal evaporative cooling is minimal, it is again not surprising that a lower limit would exist under extreme T_w conditions. Furthermore, considering that that globally wind speeds are declining at a rate of – 0.014 m s^{-1} a^{-1} (meters per second per annum) congruous with global warming (McVicar et al., 2012), the potential to experience such conditions is even less likely in future. Therefore, although the predicted T_w limit was 2˚C, heat storage should begin at lower T_ws than predicted. Considering that there was relatively lowered airflow in the respirometer used by virtue of pressure shifts from the Bev-a-Line tubing to the respirometer, the findings of the limit in this study are also likely more indicative of the actual limitations imposed by extreme T_w conditions.

When assessing for the cause of the thermal limit imposed by T_w, the initial understanding was that T_w would impose a limitation on evaporative cooling by enforcing a lower thermal boundary enforced by the laws of physics below which cooling would be unattainable as a result of the nature in which T_w operates (Sherwood and Huber, 2010). What is interesting in these findings, however, is that the way the thermal limit is imposed differed from that originally thought. In none of the cases recorded at the measured T_w limit (T_w = 33˚C) were any of the individuals able to cool down sufficiently to allow T_w from preventing further cooling as a result of T_w being 33˚C (Figure 2.1). Instead, all animals not only engaged in facultative hyperthermia, reducing the cost of thermoregulation, but once exposed to the measured T_w boundary, the defence of the higher T_b was no longer possible. Combining these data with the fact that despite EWL being stimulated even at the limit itself, the mice were still unable to prevent breaching the set safety margin (Figure 2.3), alludes to the possibility that the nature of the threat imposed by T_w is not as a result of the inhibitory nature to cooling below a certain point, but is rather as a result of the effects of that extreme T_as have of inducing heat storage beyond evaporative cooling capacities. As such, the limit may instead be enforced not by T_w directly, but rather indirectly by its relative constituents. However, to truly understand how heat and humidity interact under extreme T_w conditions would require further investigate and is beyond the scope of the present study.

Broader Perspectives

Although an increase of 2°C in the recommended safety margin may seem minimal, the effects are far reaching. In the case of the percentage of mammals at risk of succumbing to pathological hyperthermia, the realisation of a lower limit informs that significantly more species are at risk under future T_w climate conditions. By overlaying the two T_w limits on the frequency distribution of mammalian T_bs in Lovegrove (2012), under a T_w climate of 35°C the number of species at risk of lethal hyperthermic exposure shifts from ~60% to over 90% *in leu* of the T_w limit found in this study (Figure 2.7). Considering that the greatest T_w heat wave reported to date was in Dhahran in 2003, reaching a T_w of 35˚C (Burt, 2007; Samenow, 2015), and that areas in horn of Africa, coastal Eastern India and the coastal South West United States of America have already recorded T_ws exceeding 35˚C (Pal and Eltahir, 2016; Raymond et al., 2020), with the highest recording as of 2020 being in Ras Al Khaimah reaching 36.3˚C (Raymond et al., 2020), also suggests that exposure to these extreme conditions may not be so car fetched. Further, although Sherwood and Huber (2010) initially theorised that such extreme maxima may only become more regular with a further 8˚C increase in global temperatures and would occur potentially come the end of the century, suggests that not only is the limitation being underestimated but the time initially thought until extreme T_w conditions are to be a key concern seems likely to occur sooner than anticipated. What's more is that the nature in which extreme T_w operate may further exacerbate the threat that these conditions impose.

Wet-bulb temperature heat wave conditions are known to be highly stable in nature (Sherwood and Huber, 2010). Unlike conventional heat waves, they tend to be long lived, lasting up to four weeks in duration, and the difference between their maxima and minima within a day vary by ± 1˚C, with similar variation occurring between consecutive days (Sherwood and Huber, 2010). Under normal daily fluctuates in temperature, animals are required to defend their T_b across a flux of increasing and then decreasing environmental temperatures. By contrast, during a prolong heat wave animals are required to invest large amounts of body water, which may not be sustainable, particularly for smaller endotherms. When exposed to severe heat stress, not only do endotherms require water resources to be replenished, but also will require a prolonged period following hyperthermic exposure to recover physiologically (Quinn et al., 2015; Wilkinson et al., 1988). Needing to defend a constant T_b across a series of extreme T_w conditions may lead to hampered physiological functioning and ultimately increase the potential that an endotherm will be unable to counter the coming thermal stress in absence of behavioural countermeasures. Combining this with the extended duration in which the animals will be required to defend a constant, albeit elevated, T_b, the duration in which T_w heat waves occur may further risk an endotherms capacity for physiological functioning by voiding the required recovery period, leading to even greater risk of unavoidable hyperthermia. This would also imply that collectively despite the potential for endotherms to survive the peak T_w heat stress, they would also need to possess sufficient physiological stamina to do this continually with minimal time for recovery. As such, although a static limit of 4˚C below an endotherms T_b was detected in this study, it is highly likely that the tolerance limitation of endotherms in a natural capacity is lower still.

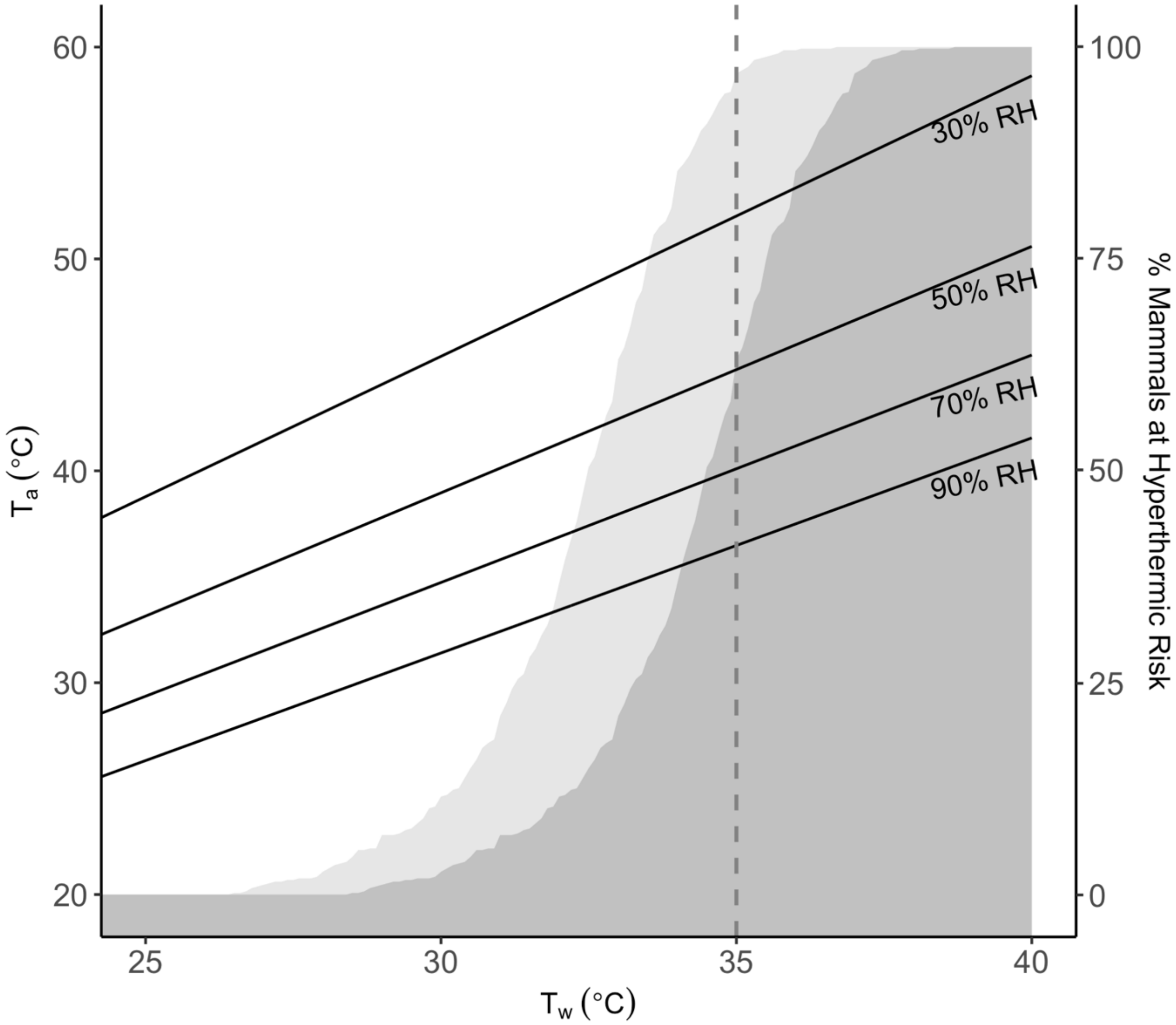

Figure 2.7. Psychrometric chart providing the wet-bulb temperature (T_w) to dry-bulb temperature (T_s) combinations for various relative humidity (RH) components. The cumulative percentage of mammals at risk of T_w induced hyperthermia are

overlayed. The darker area indicates the % of mammals at risk under the initial 2˚C body temperature (T_b) – T_w differential, while the lighter area indicates the % of mammals at risk under the measured minimum of 4˚C T_b – T_w differential. A vertical reference line is included at T_w 35˚C. Data of mammalian T_b frequencies used to determine the percentages of mammals at hyperthermic risk were extracted from Lovegrove (2012).

Conclusion

The current study aimed to provide empirical insight into the limiting capacity of T_w exposure to understand if and where its proposed limitation would occur. It was found that T_w can indeed pose a significant threat to thermoregulating endotherms, despite the use of thermoregulatory counter measures. Furthermore, the T_w limit was found not to be enforced as a result of limitation placed on evaporative cooling, but rather despite the increased reliance on EWL, the rate of heat loss was insufficient to prevent significant heat storage. The T_w limit was also found to be significantly lower than literature suggested such that under the testing conditions, a T_b – T_w differential of at least 4˚C was required before committing endotherms to pathological hyperthermia, at least in the grass mice used in this study. However, as extreme T_w heat waves events are prolonged and would require endotherms to prevent becoming hyperthermic for significant periods of time with minimal rest periods between hyperthermic bouts, the safety margins regarding T_w heat stress are likely lower still. Collectively, this study ultimately indicates that T_w conditions do possess the capacity to overcome thermoregulatory processes, the true threat posed by T_w events lies not its inhibitive capacity, but rather the stability in which these conditions will operate from.

Chapter Three: An Investigation into the Influence of Heat and Humidity Combinations on Thermoregulatory Processes Under Identical Wet-bulb Temperature Conditions

Abstract

Wet-bulb temperature (T_w) represents the lowest temperature to which an object can be cooled by the evaporation of water. Thus, it is an important consideration regarding an animal's ability to thermoregulate and off-load excess body heat as global warming advances. Similar T_w can be attained from different combinations of environmental temperatures and humidity levels, and it is not yet known if or how this may affect a mammal's ability to defend normothermia. Therefore, using ten male Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) the thermoregulatory responses to static T_ws of 31˚C using 70% and 90% relative humidity (RH) conditions were investigated. Heat stress was found to be the greatest driver towards hyperthermic stress, while the effect of humidity was only ancillary to this provided that the thermal conditions were above the animal's thermal tolerance limit. As such, although extreme T_ws may prove dangerous as heat and humidity conditions elevate in response to global warming, this study presents that the threat posed by extreme heat exposure is still of greater concern.

Introduction

Maintaining a stable body temperature (T_b) is vital to mammalian survival (Ahima, 2020; Angilletta et al., 2010; Lovegrove, 2017). However, this is entirely reliant on an animals ability to mitigate between the amount of heat being gained by, and lost from, their bodies (Kenney and Zeman, 2002; Renaudeau, 2005; Sherwood et al., 2013; Snyder and Nestler, 1990). In the case of heat stress, should the rate of heat gained exceed the rate of heat being lost, the animal will store heat and risk succumbing to hyperthermic stress and the physiologically deleterious effects associated with it (Daniel et al., 2010; Hazel, 1995; Lepock, 2003; Tieleman and Williams, 1999). As the world continues to warm, climate scientists and physiologists warn that future environmental conditions may for the first time, become intolerable to homeothermic endotherms, such as birds and mammals (Ahima, 2020; Coffel et al., 2018; Pal and Eltahir, 2016; Raymond et al., 2020; Sherwood and Huber, 2010; Willett and Sherwood, 2012).

Thermoregulatory processes undertaken by an animal are geared towards regulating its internal thermal environment through behavioural (Lopes and Bicca-Marques, 2017; Terrien, 2011; Willems and Armitage, 1975) or physiological means (Angilletta et al., 2010; Huey and Bennett, 1990; Sherwood et al., 2013; Tattersall et al., 2012). By mitigating between all sources of thermal input, namely convective (C), conductive (K), radiative (R) and metabolic heat sources (M), and sources of heat loss, namely C, K, R and evaporative cooling (E) through evaporative water loss (EWL), animals are able to regulate their respective internal thermal environments within a thermal preferendum (Angilletta et al., 2010; Tattersall et al., 2012; Withers, 1992). In the case of mammals and other homeothermic endotherms, this preferendum falls within a narrow temperature range, referred to as their T_b (McNab, 1974; Speakman and Król, 2010). By modelling the causes of thermal flux, an understanding of how thermal perturbations will impact an animals heat storage (S) can be achieved, as seem in as seen in equations 3.1 (Bligh & Johnson 1973, and Blatteis et al. 2001). Should $S > 0$, the animal will experience heat storage and conversely so should $S < 0$. In the case of birds and mammals, by endeavouring to maintain S~ 0 they ensure that a stable T_b can be maintained through thermoregulatory responses (McNab 1974, Kurz 2008, Sherwood et al. 2012). However, two key shifts are well established to affect the efficiency of these thermoregulatory processes. Namely, these are increases in heat stress or elevations in ambient humidity conditions (Angilletta et al., 2010; Lovegrove et al., 2013; Tattersall et al., 2012)

$$S = \pm C \pm K \pm R + M - E$$

The effect of heat stress in thermoregulatory processes and its effects on physiological well-being have long been appreciated in literature (Armstrong, 1994; Collins, 1973; Speakman and Król, 2010; Withers, 1992). Owing to the direct relationship between temperature and physicochemical functioning, temperature plays a key role in biotic survival (Angilletta et al., 2010; Tattersall et al., 2012; Withers, 1992). For thermal specialists such as mammals, this is even more so (Tattersall et al., 2012). Shifts in heat exposure not only alter the thermal stress experienced by the individual, therefore increasing S, but as maintaining a stable T_b relies on catalytic metabolic processes of which heat is a by-product, they are therefore also vulnerable to Arrhenius up-cycling (Angilletta et al., 2010; Lovegrove et al., 2013). Under elevated temperature stress, their metabolic rates are passively stimulated in response to excess heat energy increasing metabolic processes, and therefore further compounding the effects of heat stress on the mammal in question (Angilletta et al., 2010; Boyles et al., 2011). However, heat is not the only stressor of thermoregulatory process in endothermic organisms.

Unlike the effect of heat, that of humidity on thermoregulatory processes, although being well accepted in literature, is far less studied (van Dyk et al., 2019; Withers and Cooper, 2014). When under heat stress, an endotherms final physiological countermeasure to maintain normothermia is to rely on evaporative cooling via EWL (Angilletta et al., 2010; Boyles et al., 2011; Sherwood et al., 2013). This comes as a result of the reversal of the passive heat transportation driving $S > 0$. Being a physical conversion in the state of matter from a liquid to a gas, evaporative cooling rates are governed by physical laws of partial pressure and saturation pressures (Lasiewski et al., 1966; McNab, 2002; Powers, 1992; Withers, 1992; Withers and Cooper, 2014). As a result, evaporative processes are negatively correlated with the surrounding water vapour pressures of an animals environment (Ewing and Studier, 1973; Gerson et al., 2014; Lasiewski et al., 1966; Powers, 1992). In cases of extreme heat stress and mortality, greater threats are associated with the elevations in ambient water vapour following extreme heat events than with heating events alone (McKechnie et al., 2021; Raymond et al., 2017). For this reason, many authors now warn that the concomitant effect of the predicted increases in heat and humidity will drive future heat stress (Ahima, 2020; Kang and Eltahir, 2018; Li et al., 2017; Matthews et al., 2017; Pal and Eltahir, 2016; Raymond et al., 2020; Schär, 2016; Sherwood and Huber, 2010). Key amount these concerns is the effect that the shifts in heat and humidity will have on the global wet-bulb temperature (Coffel et al., 2018; Raymond et al., 2020; Sherwood, 2018; Sherwood and Huber, 2010).

Wet-bulb temperature is the measure of how cold an object can become when experiencing its maximum rate of evaporative cooling (Rogers and Yau, 1989; Sherwood and Huber, 2010). As such, it measures the resulting temperature when measuring the effect of S and T_a, while accounting for heat loss limitation of E as a result of ambient humidity conditions. For this reason, T_w has also been found to be of key biological importance when an endotherm if physiologically stressed. Once reaching at least 4˚C below an animals T_b, T_w conditions have been found to impose a lower thermal barrier (Vecellio et al., 2022; Chapter 2). Considering that future T_w heat wave events are long lived and that T_w maxima are set to exceed the lower tolerance limit of 80% of mammals brings cause for concern (Sherwood & Huber 2010, Raymond et al. 2020, Chapter 2). However, although T_w maxima may be relatively universal between habitats (Sherwood and Huber, 2010), the heat and humidity make up of environment is more heterogenous in nature. For example, the initially proposed T_w limit of of 35˚C can be achieved using a heat and humidity combination of 40.10˚C and 70% RH respectively or 36.48˚C and 90% RH respectively (Table 3.1). Similarly, current global T_w maxima of 26˚C (Sherwood and Huber, 2010) can be achieved under a combination of 29.36˚C and 70% RH, or 26.2˚C and 90% RH (Table 3.1), or future predicted shifts exceeding 31˚C T_w conditions can be achieved using 35.80˚C and 70% RH or 32.42˚C and 90% RH (Table 3.1). As studies into T_w stress are limited to livestock, with only two studies investigating the limitation of T_w, it still remains unclear as to whether the absolute limitation of T_w is in fact absolute. As such, the rigidity of the proposed T_w between environments remains uncertain.

By exposing a known obligate homeothermic generalist, the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784; *sensu* Chapter 2), this study aimed to quantify the effect that identical T_w conditions have on thermoregulatory processes when using different heat and humidity combinations. Owing to the dual effect that elevated humidity conditions are known to have on thermoregulatory processes, such that EWL is retarded while Arrhenius effects elevate metabolic heat production, it was predicted that T_w conditions with a greater humidity component would present a greater threat to thermoregulatory processes than drier, albeit warmer conditions.

Table 3.1. Heat and humidity combinations required to generate identical wet-bulb temperatures (T_ws) between 26-35˚C while using 70% and 90% relative humidities (RHs). Corresponding ambient temperatures (T_as) are provided in ˚C under each RH column.

T_w (˚C)	Relative Humidity (%)	
	70%	**90%**
26	30.43	27.34
27	31.51	28.35
28	32.58	29.37
29	33.66	30.39
30	34.73	31.40
31	35.80	32.42
32	36.88	33.43
33	37.95	34.45
34	39.02	35.46
35	40.10	36.48

Methods and Materials

Study Species, Animal Capture and Husbandry

Ten male Four-striped grass mice (M_b = 59.34 ± 8.767 g) were caught in the Nelson Mandela University Nature Reserve in Gqeberha (previously Port Elizabeth), South Africa (34˚00’44” S, 25˚39’21” E; Welman et al. 2022) between January and March 2020. A similar trapping procedure to that described in Chapter 2 of this thesis was used. Briefly, Sherman traps were baited with a mixture of rolled oats and peanut butter and traps were opened for three hours, starting at dawn (ca. 05:00). All capture grass mice were collected and transported to the Nelson Mandela University Applied Ecophysiology Research Laboratory for further processing, while non-target species were released. Once in the laboratory, the mice were sexed and weighed. Only adult males were kept, and all other individuals were released at their capture site. The same husbandry protocol as described in Chapter 2 of this thesis was followed, however the animal housing area was kept at a higher temperature of 30.9 ± 0.89˚C for this time period.

The average ambient temperature (T_a) and relative humidity (RH) experienced in the nature reserve during the time of sampling was 21.8˚C and 79% respectively. Ambient temperatures were found to fluctuate between 17.6 – 26.1 ˚C and recorded a maximum and minimum of 40.2 ˚C and 9.8˚C respectively. Relative humidity readings during this time fluctuated between 58 – 94%, with a maximum and minimum recording of 100% and 19% being reported. The total rainfall recorded was 111.6 mm. All meteorological data was provided by the South African Weather Service. Further information on the vegetation and historical meteorological patterns of the site can be found in Chapter 1.

Permission to collect animals was granted by the Eastern Cape Department of Economic Development, Environmental Affairs and Tourism (permit CRO 91/19CR). All experimental protocols used in the study were approved by the Nelson Mandela University Animal Research Ethics Committee (A19-SCI-ZOO-002).

Respirometry and Body Temperature Measurements

Following a similar experimental design as described in Chapter 2, two iButtons (Model DS1922L; Thermochron, Sydney, Australia) were placed on opposite side walls within respirometers to record the internal air temperature at 1 min intervals. A temperature-sensitive passive integrated transponder (PIT) tag (BioTherm13 PIT tags; 13mm x 2.12mm, FDX-B 134.2 kHz; Biomark Inc., Bois, United States of America) was injected into the abdominal cavity of each mouse after its initial inspection following capture. This allowed for continuous monitoring of the individual's core temperature using an HPR Plus PIT tag reader (Biomark Inc.) that was connected via Bluetooth to the laboratory computer. Body temperatures were recorded at 30s intervals.

Gaseous Exchange Measures

A push open flow-through respirometry setup, as described by Lighton (2008) was used. The changes in the fractional concentrations of oxygen (F_eO_2) and carbon dioxide (F_eCO_2), and water vapour density (WVD; F_eH_2O) were measured; device details are provided below. At the start of each trial, the individual to be measured was placed inside the respirometer comprised of a modified, airtight 2.7 L plastic container (Lock & Lock; Seoul, South Korea). To prevent any urine and faecal matter obscuring WVD readings, the respirometer was also fitted with a stainless steel, wireframe stage placed above 200 ml of vegetable oil.

While undergoing a treatment, the respirometer was housed inside a modified cooler box (60.33 cm x 29.85 cm x 33.00 cm; model 100 Quart Xtreme 5 Cooler; The Coleman Company Inc.; Kansas, United States of America) fitted with rows of copper tubing that were connected to a circulating waterbath (Memmert WNB 45; operating temperatures of 10˚C-95˚C; Memmert GmbH + Co. KG; Schwabach, Germany). To regulate the internal temperature of the modified cooler box, the water from the water bath was circulated through the copper tubing using an aquarium pump (model FP-48; Guangdong BOYU Aquarium Industries Co.; Guangdong, China). Temperature regulation of the cooler box was initiated at least 3 hours prior to the commencement of each trial to ensure that the desired temperature was achieved and maintained. The T_a inside the ice box was monitored using a precision mercury-in-glass thermometer with a resolution of 0.1˚C traceable to NIST standards.

An industrial air compressor supplied atmospheric air from outside of the building for the use in respirometry measurements. The airstream was directed through a series of two bubbler bottles submerged inside of a circulating water bath (18 L; operating temperatures of 5˚C above T_a – 100˚C; Scientific Manufacturing cc.; Cape Town, South Africa) before being split into two separate airstreams, one to be used as a reference airstream for baseline measurements and one to supply the respirometer. The submergence of the bubbler bottles allowed for both visual confirmation that no leaks were present from the bubblers, as well as ensuring that the airstream was saturated at the required dewpoint temperatures (T_{dp}) before entering the respirometer. The temperature of the water bath was by digital temperature controller (TTM-J4; Toho Electronics Incorporated; Kanagawa, Japan) and then monitored using a precision mercury-in-glass thermometer traceable to NIST standards with a resolution of 0.1˚C. The flow rates in the system were regulated using mass flow controllers (model FMA 5400A/5500A; Omega Engineering Inc., United States of America). The incurrent airstream was restricted a flow rate of 3 L min^{-1}, while the split airstreams were both regulated at 1.5 L min^{-1}. This ensured a washout rate of 1.67 min while maintaining O_2 concentrations of above 20.53% and avoiding excessive moisture build up within the respirometer. The mass flow controllers were calibrated using a bubble flow meter.

The excurrent air from the animal respirometry, or the reference airstream, was alternatively flowed through a water vapour analyser (RH-300; Sable Systems; Nevada, United States of America) and then dried using two columns of self-indicating Drierite (W A Hammond Drierite CO LTD ; Ohio, United States of America). Thereafter, the air was subsampled at a rate of 200 ml min^{-1} using an SS-4 sub-sampler (Sable Systems) and sequentially flowed through a CO_2 analyser (CA-10A Carbon Dioxide Analyser; Sable Systems) and an O_2 analyser (FC-10A Oxygen Analyser; Sable Systems). Columns of soda lime and Drierite were used to remove CO_2 and any remaining moisture from the airstream before the air entered the O_2 analyser. A Universal Interface unit (UI-2, Sable Systems) was used to convert the analogue outputs from the respective analysers to a digital form to be recorded on a personal computer, at 1 s intervals using Expedata data acquisition software(v 1.7.22; Sable Systems).

Experimental Procedure

All the mice were subjected to both experimental conditions, namely the 70% and 90% trials under continual T_w exposures of 31˚C. The required ambient conditions were generated using a combination of T_as with the moisture level at either 70% RH or 90% RH. The necessary temperature and humidity levels required to achieve each of these conditions was determined using Equation 1 from Stull (2011) together with Antoine’s equation (Wood, 1970) to determine the required T_a and T_{dp} respectively. Details for the psychrometric settings can be found in Table 3.1

At the start of each trial, individuals were inserted into the respirometer and weighed using a digital scale (WTC-2000; Radwag Balances and Scales; Radom, Poland). They were then left in a warm and darkened room for 30 min with room air flowing through the respirometer. This was done to allow the animal time to recover from being handled, but also served as time to span the O_2 analyser and collect the initial baseline measurements using the reference airstream. Thereafter, the animal respirometer was plumbed into the respirometry system and placed inside the modified cooler box. Here the individual was left for a period of 3 hours and 30 min during which time their T_b, and data to determine their EWL and MR, were continuously recorded, after which a base line measure was again collected, and the animal was removed. If the individual was found to express potentially lethal hyperthermic stress during the sampling time period, sampling was terminated prematurely to ensure the preservation of each individual. Such conditions required that the individual expressed a T_b > 40˚C or showed to be unable of maintaining a T_b < 39˚C for at least one hour. Following the termination of each treatment exposure, the respirometer was weighed again before the individual was returned to its housing. This allowed for the determination of the mass of water lost during the trial.

All experimental protocol were conducted in a heated room to ensure that vapour condensation within the respirometry setup did not occur, therefore preventing condensation altering the incurrent and excurrent WVDs. This was achieved by ensuring that the T_a within the room remained at least 2˚C above that of the required T_{dp} for each treatment. This was monitored using a mercury-in-glass thermometer with a 1˚C resolution.

Data Analysis

Following the methodology used in Chapter 2 of this thesis, all gaseous exchange measures were collected and extracted using Expedata. To ensure synchronicity between all gas-exchange traces, lag corrections were made against the WVD trace. All traces then underwent drift correction using the baseline measurements taken to allow for potential experimental drift. The data collected for the F_eO_2, F_eCO_2 and F_eH_2O were then extracted using the 5 min average value of these measures centred around 10 min intervals. Equations 2.1 – 2.9, as detailed in Chapter 2, were used to convert these values into oxygen consumption (V_{O_2}), carbon dioxide production (V_{CO_2}) and EWL (V_{H_2O}). To determine the MR (W), the respiratory exchange quotient (RQ; V_{CO_2}/V_{O_2}) was used in conjunction with the thermal equivalence values from Table 4.2 in Withers (1992) to provide MR as a measure of energy (Joules).

Owing to challenges presented while collecting the F_eO_2 data, following the methods described by Gerson et al. (2014), V_{CO_2} data was used to determine V_{O_2} by assuming an RQ of 0.71 since the all mice were in a post-absorptive state upon initiating each trial. This was done following analyser instability as a result of electrical supply issues resulting from failure to the national electricity grid during the sampling period. The mean T_b was determined as the mean T_b expressed over the corresponding 5 min time period from which the gaseous exchange measures were extracted. The M_b was determined using a linear regression of the animal's starting and end weight.

Statistical Analysis

All statistical analyses were conducted in the R programming environment (R Core Team, 2019) using R studio (RStudio Team, 2016) as an IDE. To model the responses of the each of the physiological response variables (T_b, MR and EWL), a simple linear regression analysis was conducted. Owing to deviations of T_b and EWL from the assumptions of linearity, a segmented analysis was rather conducted on these two variable using the *segmented* R package (Muggeo, 2008). This allowed for a piece-wise linear regression analysis to identify for shifts in the slope of the responses for these two variables over time, as well as the identification of potential breakpoints in the data to indicate the point of this change. To test for differences between the trial conditions, repeated measure mixed effects models were constructed while accounting for individual specific differences in the measured responses. Consequentially, individual identification was used as a fixed effect in the model generation. The assumptions for the mixed effects models were assessed using the protocol described in Chapter 2. Tukey post-hoc analyses were conducted on all significant model findings to provide a pairwise comparison between the specific treatments using the *emmeans* R package (Lenth, 2019). A p-value of 95% was required to infer statistical significance.

Results

This study found that the differences in environmental compositions under identical T_w exposure did indeed affect the rates of heat storage of grass mice. A total of 20 trials were conducted using the above methodology and provided a total of 10 complete trial sets. During these trials four animals were removed from the 70% RH tests, while one was removed from the 90% RH tests owing to surpassing the safety margin enforced to prevent lethal hyperthermia (Figure 3.1).

The Effect of Differing Relative Humidities on Heat Storage Under Identical Wet-bulb Temperature Exposure

Differences in the heat and humidity compositions of identical T_ws significantly affected on the rates of heat storage in grass mice. Visual inspections of the data suggested that the change in T_b over time occurred in two phases (Figure 3.1). As such, a segmented analysis was conducted to investigate whether a point of inflection (BP) in the relationship between T_b and the duration of exposure existed. A single BP was observed, dividing the T_b responses into an initial period of heat storage followed by a period of homeothermic defence. The mean BPs occurred at 67.50 ± 25.549 min in the 70% RH trial and at 67.65 ± 55.619 min in the 90% RH trial. The model that best explained this variation in BP included both the test conditions and M_b (AICc = 292.8, w_i = 0.565; Table 3.2), however neither the test conditions ($F_{2,26}$ = 0.0700, p = 0.93) nor M_b ($F_{1,26}$ = 2.4084, p = 0.13) had a significant effect.

The rates of heat storage (β_{T_b}) during the initial heating phase did not differ as a result of differing heat and humidity combinations under identical T_ws. The model that best explained the observed variation in these initial β_{T_b}s was the null model (AICc = -86.8, w_i = 0.999; Table 3.2) from which random scatter contributed significantly to the observed variation (t = 4.677, df = 9, p = 0.0012). The mean β_{T_b} of the initial heat storage phase was 0.05 ± 0.014 ˚C min^{-1}. The test-specific initial β_{T_b}s are provided in Table 3.4.

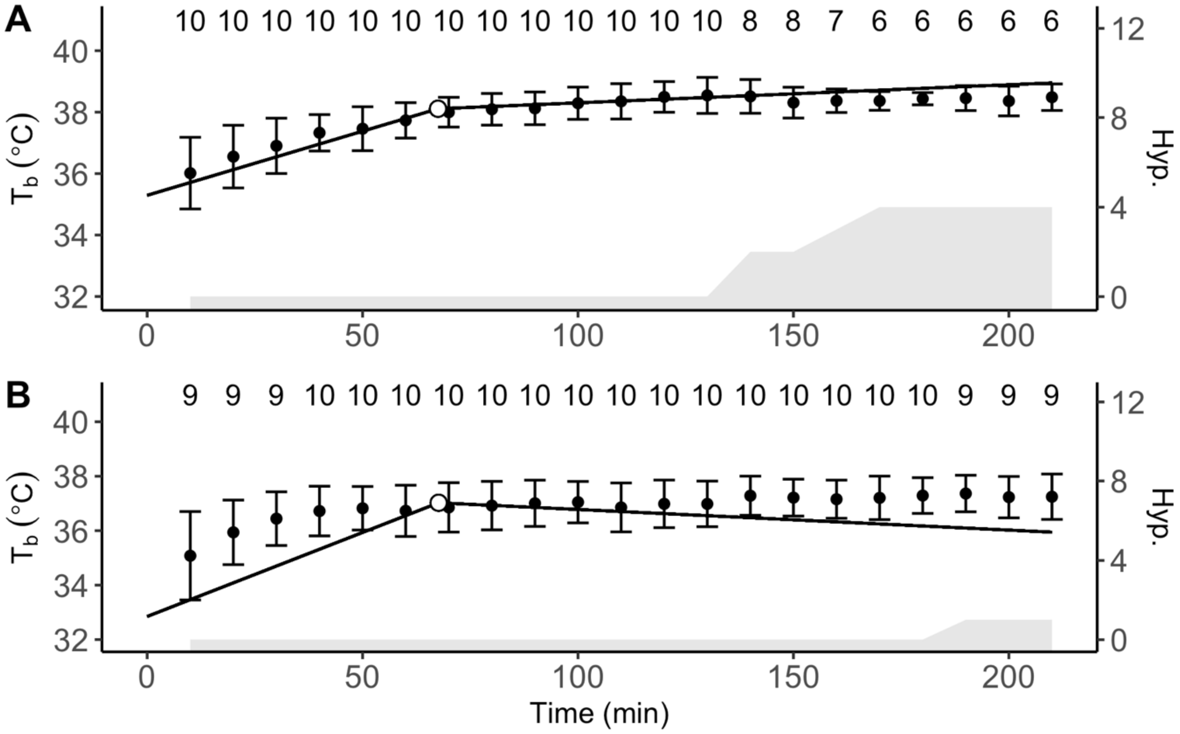

Figure 3.1 Body temperatures (T_bs) of wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) exposed to a static wet-bulb temperatures of 31 °C generated using **A**. 70% and **B**. 90% RHs. The closed circles represent the mean T_b at each time interval, with the error bars indicating the ± SD over the mean. The total number of individuals included in the calculation of mean values are provided above each point. The open circles indicate an inflection in the slope of the relationship of metabolism over time identified using a breakpoint analysis.

Following their initial heat storage phase, all the mice attempted to defend an elevated T_b once reaching their respective BPs (Figure 3.2). The mean defended T_bs were 38.11 ± 0.455 ˚C in the 70% RH tests and 37.02 ± 0.870˚C in the 90% RH tests (Table 3.4). The observed variability in these T_bs was best explained by the model only accounting for the test conditions (AICc = 68.7, w_i = 0.983) of which these conditions were a significant descriptor of the variation observed in T_bs being defended ($F_{2,18}$ = 15.685, p = 0.0001). Furthermore, once accounting for the effect of the test conditions on the T_bs being defended, the inclusion of M_b in the model also provided insight into the variability being observed (AICc = 77.3, w_i = 0.013). However, although the test conditions in this additive model still significantly contributed to the T_bs being defended ($F_{2,18.0206}$ = 15.6002, p = 0.0001), M_b did not ($F_{1,8.6811}$ = 0.1362, p = 0.72).

Table 3.2. Linear mixed-effects models describing the significant relationships between prolonged exposure to identical wet-bulb temperatures with different heat and humidity components, and body temperature (T_b) in the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784).

Fixed Factors	**Random Factor**	***df***	***logLik***	***AICc***	**△*AICc***	***w_i***
Break Point						
Test + M_b	1|ID	6	-138.596	292.8	0	0.565
Test	1|ID	5	-140.46	293.4	0.58	0.424
M_b	1|ID	4	-145.979	301.6	8.71	0.007
Null	1|ID	3	-147.838	302.6	9.76	0.004
T_b At BP						
Test	1|ID	5	-28.097	68.7	0	0.983
Test + M_b	1|ID	6	-30.836	77.3	8.63	0.013
Null	1|ID	3	-36.58	80.1	11.39	0.003
β_{T_b} Before BP						
Null	1|ID	3	46.867	-86.8	0	0.999
M_b	1|ID	4	41.231	-72.9	13.95	0.001
β_{T_b} After BP						
Null	1|ID	3	73.85	-140.8	0	0.999

The β_{T_b}s while the mice attempted to maintain a homeothermic T_b were not affected by the different heat and humidity conditions. The model that best explained the observed variation in β_{T_b}s during the elevated homeothermic phase was the null model (AICc = -140.8, w_i = 0.999; Table 3.2). However, the random variation detected did not significantly explain the observed variation (t = 0.274, df = 29, p = 0.79). The mean β_{T_b} was 0.00 ± 0.014˚C min^{-1}. The test-specific β_{T_b}s are provided in Table 3.4.

Table 3.3. Linear regression models describing the significant relationships between body temperature (T_b) and prolonged exposure to identical wet-bulb temperatures with different heat and humidity components in the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784).

Parameter	***Regression Equation***
T_b At BP	T_b = Test + 38.11 T_b = Test - 0.009112 x M_b + 38.65 T_b = 1 + 37.62
Rate of Change before BP	β_{T_b} = 1 + 0.05149
Rate of Change after BP	β_{T_b} = 1 + 0.0008957 β_{T_b} = 0.0003923 x M_b - 0.02215 β_{T_b} = Test + 0.005908 β_{T_b} = Test + 0.0004055 x M_b - 0.01787

The Effect of Differing Relative Humidities on Endogenous Heat Production Under Identical Wet-bulb Temperature Exposure

Metabolic rates were not affected by differing heat and humidity conditions while maintaining identical T_ws. Visual inspection of the data did not show a shift between MR and the duration of exposure to either test (Figure 3.5). This was confirmed following a linear regression analysis ($p > 0.05$ for both tests). As such, following the methods used in Chapter 2 to compare between test conditions, the MR that corresponded with each mouse's BP was used. The model that best described the MR at BP was the null model (AICc = -12.1, w_i = 0.977; Table 3.5). Here random variation contributed significantly to the observed variability (t_{stat} = 13.54, df = 9, $p <$ 0.0001). Once factoring in the effect of the test conditions, this too provided a viable description of the observed MR at BP (AICc = -3.6, w_i = 0.014; Table 3.5), however the contributions of the test conditions to the observed MR was insignificant ($F_{2,18}$ = 2.8134, p = 0.09).

Table 3.4 Statistical results acquired from the segmented analyses conducted on the thermoregulatory responses of grass mice in response to exposure to constant a 31˚C T_w under 70% RH and 90% RH conditions. Results are provided for the breakpoints (BP) in each variables mean trace, the value expressed for each thermoregulatory response at their respective BPs, as well as the rates of change in each response both before and after their respective BPs. The F_{stat}, df and p_{value} are also provided to indicate the comparison in responses between the respective RH treatments.

T_b	70%	± SD	90%	± SD	F_{stat}	df	p_{value}	
BP (min)	67.50	25.549	67.65	55.619	0.0001	1,18	0.99	-
T_b At BP (˚C)	38.11	0.455	37.02	0.870	26.76	1,9	0.001	***
β_{T_b} Before BP (˚C min^{-1})	0.04	0.023	0.06	0.071	0.72	1,18	0.41	-
β_{T_b} After BP (˚C min^{-1})	0.01	0.003	-0.01	0.030	2.15	1,9	0.18	-
MR								
Mean MR (W)	0.72	0.184	0.59	0.232	2.8134	2, 18	0.09	-
EWL								
BP (min)	76.62	41.173	77.04	57.314	0.0004	1,9	0.98	-
EWL At BP (mg min^{-1})	3.80	1.350	2.07	0.544	18.26	1,9	0.002	**
β_{EWL} Before BP (mg min^{-2})	0.07	0.059	0.05	0.043	0.73	1,18	0.40	-
β_{EWL} After BP (mg min^{-2})	0.00	0.013	0.01	0.038	0.54	1,8.9853	0.48	-

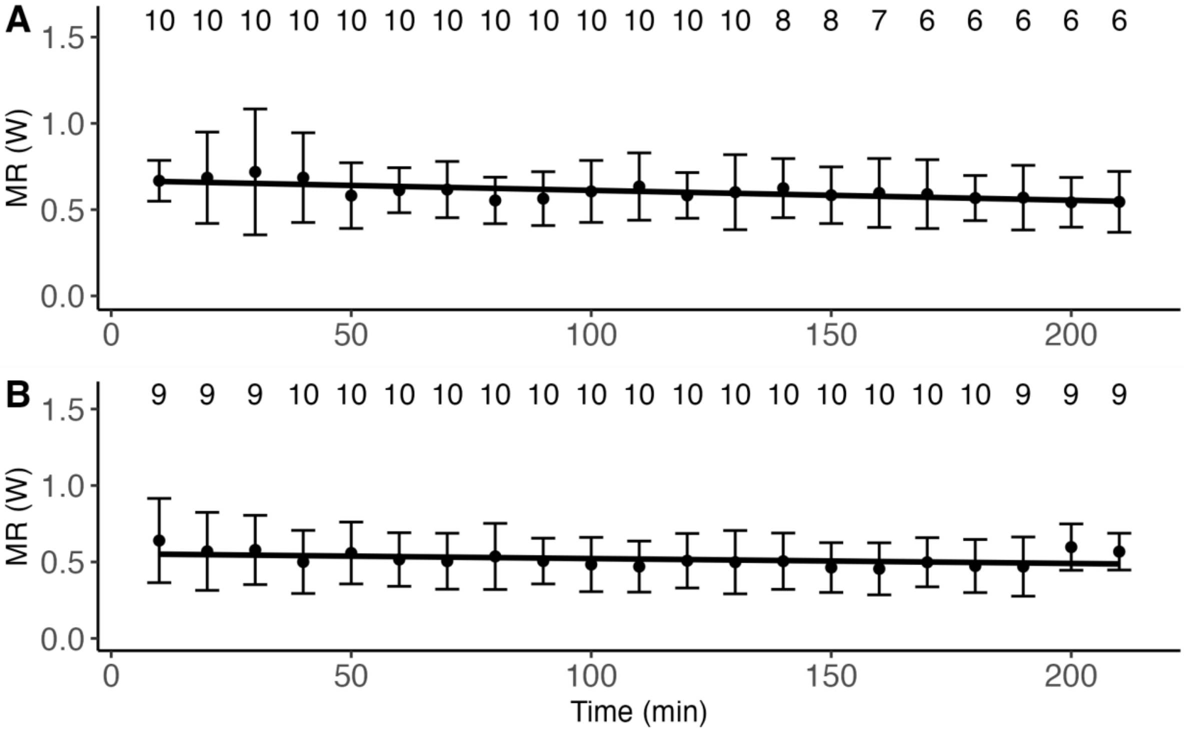

Figure 3.2 The metabolic rates (MRs) of wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) exposed to a static wet-bulb temperatures of 31 °C generated using **A**. 70% and **B**. 90% RHs. The closed circles represent the mean MR at each time interval, with the error bars indicating the ± SD over the mean. The total number of individuals included in the calculation of mean values are provided above each point. The solid line over the means indicates the rate of change in MR over time.

Table 3.5. Linear mixed-effects models describing the significant relationships between prolonged exposure to identical wet-bulb temperatures with different heat and humidity components, and metabolic rate (MR) in the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784)

Fixed Factors	**Random Factor**	***df***	***logLik***	***AICc***	***ΔAICc***	***w_i***
MR at BP						
Null	1|ID	3	9.515	-12.1	0	0.977
Test	1|ID	5	8.059	-3.6	8.49	0.014
M_b	1|ID	4	6.133	-2.7	9.44	0.009

Table 3.6. Linear regression models describing the significant relationships between metabolic rate (MR) and prolonged exposure to identical wet-bulb temperatures with different heat and humidity components in the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784).

Parameter	***Regression Equation***
MR at BP	MR = 1 + 0.6551

The Effect of Differing Relative Humidities on Evaporative Water Loss Under Identical Wet-bulb Temperature Exposure

Differing the heat and humidity to generate identical T_Ws had a significant effect on the rates of EWL in grass mice. Visual inspections of the data suggested that the temporal shift in EWL also occurred in two phases. Therefore, a segmented piecewise regression analysis was preformed to investigate whether a BP could be detected. A single breakpoint was detected, of which the observed variation was best explained by the test conditions (AICc = 300.7, w_i = 0.580; Table 3.7). However, the test conditions did not contribute significantly to the observed variation ($F_{2,17.736}$ = 0.0521, p = 0.95). The mean BP was 76.83 ± 0.297 min. The test-specific BPs are provided in Table 3.4.

The initial phase of the EWL response was characterised by an incremental shift in the rates of EWL (β_{EWL}) being expended (Figure 3.7). The initial increase in β_{EWL} was best explained by the null model (AICc = -86.8, w_i = 0.998; Table 3.7), following which random variability was found to contribute significantly to the rates being observed (t_{stat} = 6.746, df = 29, $p < 0.0001$). Body mass was also shown to provide some measure of the observed variability in the increased β_{EWL} (AICc = -74.0 w_i = 0.002; Table 3.7), however this did not contribute significantly to the observed β_{EWL} ($F_{1,28}$ = 1.9776, p = 0.1706). The mean rate of increase in EWL during this initial phase was 0.06 ± 0.014 mg min^{-2}. Test-specific rates are provided in Table 3.4

The initial incremental phase in EWL ended once the mice were expending 3.80 ± 1.350 mg min^{-1} in the 70% RH tests and 2.07 ± 0.544 mg min^{-1} in the 90% RH tests at their respective BPs (Table 3.4). The model that best described this variability included only the test conditions as a factor (AICc = 89.7, w_i = 0.970; Table 3.7) of which the test conditions showed to contribute significantly to this variation ($F_{2\ 17.152}$ = 15.001, p = 0.0002). Furthermore, when M_b into a model accounting for the test conditions, the variability in EWL at BP could also be explained. In this model the test conditions still significantly contributed to the observed EWL at BP ($F_{2,17.5727}$ = 15.3651, p = 0.0002), however M_b did not ($F_{1,8.6867}$ = 1.6099, p = 0.24).

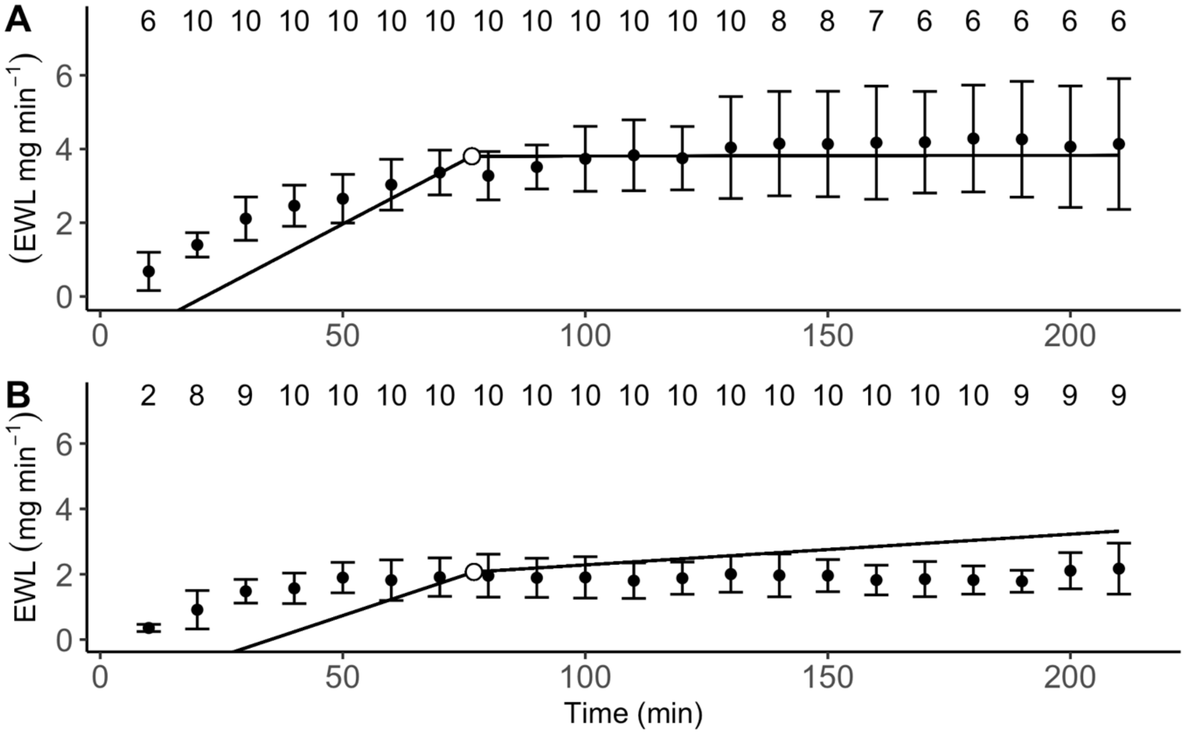

Figure 3.3. Rates of evaporative water loss (EWL) of wild-caught African Four-striped grass mice (*Rhabdomys pumilio* Sparrman 1784) exposed to a static wet-bulb temperatures of 31 °C generated using **A**. 70% and **B**. 90% RHs. The closed circles represent the mean EWL at each time interval, with the error bars indicating the ± SD over the mean. The total number of individuals included in the calculation of mean values are provided above each point. The open-circles indicate an inflection in the slope of the relationship of EWL over time identified using a breakpoint analysis.

Table 3.7. Linear mixed-effects models describing the significant relationships between prolonged exposure to identical wet-bulb temperatures with different heat and humidity components, and evaporative water loss (EWL) in the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784).

Fixed Factors	**Random Factor**	***df***	***logLik***	***AICc***	**∆*AICc***	w_i
Break Point						
Test	1|ID	5	-144.116	300.7	0.00	0.580
Test + M_b	1|ID	6	-142.889	301.4	0.70	0.409
Null	1|ID	3	-151.542	310	9.28	0.006
M_b	1|ID	4	-150.319	310.2	9.51	0.005
EWL At BP						
Test	1|ID	5	-38.617	89.7	0.00	0.970
Test + M_b	1|ID	6	-40.548	96.7	7.01	0.029
Null	1|ID	3	-48.231	103.4	13.65	0.001
Mean EWL After BP						
Test	1|ID	5	-38.084	88.7	0.00	0.96
Test + M_b	1|ID	6	-39.699	95.1	6.38	0.039
Null	1|ID	3	-47.907	102.7	14.07	0.001
β_{EWL} ***Before BP***						
Null	1|ID	3	46.875	-86.8	0.00	0.998
M_b	1|ID	4	41.807	-74	12.81	0.002
β_{EWL} ***After BP***						
Null	1|ID	3	66.32	-125.7	0.00	1.000

The final phase of the EWL response in the mice was characterised by the sustained use of the EWL at BP (Figure 3.7). Here the β_{EWL}following BP was best described by the null model (AICc = -125.7 w_i = 1.00; Table 3.7). However, the random variability akin to the null did not provide a significant predictor of the β_{EWL}following BP (t_{stat} = 0.567, df = 29, p = 0.58), nor did any other model generated. The mean β_{EWL} after the BP was 0.01 ± 0.007 mg min^{-2}. The test-specific values are provided in Table 3.4.

Table 3.8. Linear regression models describing the significant relationships between evaporative water loss (EWL) and prolonged exposure to identical wet-bulb temperatures with different heat and humidity components in the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784).

Parameter	***Regression Equation***
EWL At BP	$EWL = Test + 3.831$ $EWL = Test + 0.03304 \times M_b + 1.884$ $EWL = 1 + 3.187$
Mean EWL After BP	$\mu EWL = Test + 3.873$ $\mu EWL = Test + 0.03539 \times M_b + 1.791$ $\mu EWL = 1 + 3.159$
Rate of Change before BP	$\beta_{EWL} = 1 + 0.05582$

Discussion

This study sought to investigate the effect that different heat and humidity combinations would have on heat storage in *R. pumilio* when under identical T_w exposure. This was achieved by using open flow respirometry to expose 10 males to treatment bouts of three and a half hours in duration whereby they experienced constant T_w conditions of 31˚C under a RH of either 70% or 90%. The physiological indicators of heat stress were continuously measured such that data for shifts in T_b, MR and EWL were collected. It was found that although being exposed to identical T_w conditions, the heat storage effect typical of humid heat stress was not expressed equally between the two treatment types. Differences in the T_b defended between the two tests were detected, as well as the rate of EWL being used to maintain thermal homeostasis between the two treatment types. Metabolic rates, however, were not affected.

When under heat stress, it is well established that humidity will exacerbate the stress as a result of its capacity to reduce the efficiency of evaporative cooling and thereby hinder the capacity of endotherms to remove excess heat via EWL. When combining this with the physical hinderance that T_w measures, and therefore informs of conditions that impose thermal threats to endothermic organisms, it was hypothesised that when exposed equal T_ws, *R. pumilio* would exhibit greater stress responses when exposed to T_w conditions comprising of a greater humidity component. However, this is not what was found. When under the 90% RH treatments, all of the study animals were able to thermoregulate near that of their normothermic conditions (T_b = 36.8˚C; Lovegrove, 2009), whereby all were able to defend a constant T_b of 37.02˚C while relying significantly less on EWL to remain homeothermic (Table 3.3). In contrast, while under the 70% RH treatments, not only did the study animals rely on significantly greater rates of EWL while also defending a higher T_b of 38.1˚C (Table 3.3) than that expressed in the 90% treatments, but 40% of the study animals also had to be removed before the completion of their respective treatments as a result of becoming breaching the predetermined hyperthermic safety measures, as adopted from Chapter 2 (Figure 3.3).

Although seeming counter intuitive, when inspecting the respective conditions that the grass mice experienced when under the 70% and 90% treatments, the reason for these responses is evident. While under the 70% RH treatments, the grass mice were exposed to T_a conditions of 35.8˚C, merely 0.9 ˚C below their normothermic T_b, but when under the 90% RH treatments they were exposed to T_as of 32.2˚C, 4.5˚C below their normothermic T_b (Figure 3.1). Considering that the upper critical limit (T_{crit}) for this species is ~ 32˚C (Haim and Fourie, 1980), this would imply that while under the 70% RH treatments, the animals were exposed to T_a conditions 3.6˚C above their T_{crit}, while the 90% RH treatments were only 0.2˚C above their T_{crit}. Therefore, although being exposed to a T_w that is known to illicit a hyperthermic response in this species (Chapter 2), the degree of thermal stress imposed from the T_a conditions was not sufficient to stress the grass mice under in the 90% RH treatments. Under these conditions, although exploiting latent heat loss (Figure 3.4), the thermal conditions would have been low enough to permit the grass mice to predominantly rely on sensible heat loss to thermoregulate. As such, these findings support the general consensus from previous literature proposing that high ambient humidity will only become a limiting factor when ambient temperatures are sufficiently high to force endotherms to rely solely on latent heat loss through evaporative cooling to offload excess body heat (Bohmanova et al., 2007; Eichna et al., 1945; Haldane, 1905; Sherwood, 2018). These findings in turn also highlight several key insights how T_w stress will operate across the heterogenous shifts to be experienced in the coming century (Coffel et al., 2018; Sherwood and Huber, 2010).

The Effect of Different Heat and Humidity Conditions Under Identical Wet-bulb Temperatures on the Physiological Responses of Rhabdomys pumilio

When heat stressed, endotherms exhibit several key metrics that can be used to study the degree of stress and the capacity for the endotherm to counter these perturbations in thermoneutrality. Of these, the most easily measured is that of T_b as it indicates the degree of success by the endotherm in maintaining homeothermy. Building on the findings of Chapter 2, it was hypothesised that when under T_w conditions of 31˚C, that the grass mice would become mildly hyperthermic and attempt to defend a T_b of 38.2˚C, as was found to occur in all T_w stresses ranging from T_ws of 30 – 33˚C. In this study, it was found that this only occurred under the 70% RH treatment, but not in the 90% RH treatment. This finding, considering the above argument, is not surprising for the conditions for the 70% RH treatment mirrored those required for the 31 ˚C T_w treatment in Chapter 2 and therefore should induce an equitable hyperthermic stress response, while the 90% RH conditions were not sufficient to be able to do so.

What is interesting, however, is that the duration until the animals defended a stable T_b did not differ. Considering that there was a significant difference in the degree of heat stress experienced between the two test conditions, one would expect the duration of thermal storage to be greater in 70% RH treatments as the mice were defending T_bs of 1.1˚C higher than those being defended in the 90% RH treatments and therefore would require more energy to reach this T_b. However, under both treatment conditions the duration of time until defending a constant body temperature was 67 minutes (Table 3.3), mirroring the findings for the 31˚C T_w treatment in Chapter 2. Although seeming odd, a possible reason for this finding may be as a result of the differences in T_bs expressed by the grass mice at the initiation of each test. In the 70% RH treatment, the mean starting T_b was 34.53˚C, whereas in the 90% RH treatment was 33.70˚C, nearly a full degree lower and of similar proportions to the difference between the defended T_bs. As such, although more thermal energy would have been required to reach the elevated T_b of the 70% RH treatment, the lower T_bs expressed at the start of the 90% RH treatments would have biased this finding. By calculating the duration of time that it would have taken for the mice to reach their respective defended T_bs between the two treatments from thermoneutrality using the rates of increase from Table 3.3, the thermal loading periods would have reduced to 35.25 minutes and 5.33 minutes for the 70% and 90% treatments respectively, supporting the notion that the greater stress was experienced under the 70% RH treatments.

The cause for these lower T_bs likely as a result of excessive cooling that may have occurred during the pre-trial isolation period. It is also possible that the low T_bs are as a result of the animals having pressed themselves against the wall of the respirometer, allowing for contact between their PIT tags and the colder respirometer wall to skew the reading. However, this would be unlikely as *R. pumilio* is known to huddle when cold, which would move the PIT tags away from the wall. Also, from personal observations of where the PIT tags would be located following autopsies on deceased individuals whereby the PIT tags were found around the animal’s testes, structures known to be highly regulated for reproductive purposes, makes the influence of the respirometer on the measured T_b even more unlikely. This does imply that the animals were out of thermal comfort zones at the start of the treatments, however owing to the lack of MR response (Table 3.4) whereby at temperatures lower than 32˚C, *R. pumilio* is known to elevate its MR to maintain thermoregulatory homeostasis (Haim and Fourie, 1980; Scantlebury et al., 2006), the degree to which these lowered initial T_bs have impacted the responses of the animals is likely negligible.

When under humid heat stress, small terrestrial endotherms have been found to experienced reduced rates of evaporative cooling and concomitant increases in MR as a result (Baudinette, 1972; Christian, 1978; Edwards and Haines, 1978; Lasiewski et al., 1966). These are caused by the combination of the reduced capacity for EWL to be effective in thermoregulatory processes by virtue of decreased WVP gradients between the animal’s periphery and their environment, and the concomitant Arrhenius upcycling being experienced as a result of the increase in thermal energy available to biochemical processes stimulating the rates of biochemical conversions within the animal’s system. Regarding the findings in this study however, these relationships were both not found to have occurred. Under both treatments, there was no effect of the thermal conditions on MRs, and no apparent inhibitive effect of the thermal conditions on the capacities for the grass mice to rely on EWL to prevent severe hyperthermia from ensuing.

Regarding the effect of the treatments on the grass mice’s reliance on EWL, although a significantly different use of EWL was found to have occurred between the 70% and the 90% RH treatments such that a lower rate of EWL was found to occur in the 90% RH treatment, this difference is not likely to be as a result of humid inhibition. Considering that that 90% RH treatment did not see the grass mice to be significantly thermally stress, thus relied mainly on sensible heat loss to thermoregulate, the difference in EWL is not reflective of the inhibitive nature of the elevated humidity exposure, but rather is reflective of the differing degrees of thermal stress experienced between the two treatment types.

With respect to the lack of effect of either treatment on the measured MRs of the grass mice, this remains odd. Considering that even though thermal inhibition as a result of the reduced efficiency in EWL was not found, there were still significantly different thermal environments being experienced between the 70% and 90% RH treatments. Previous studies on *R. pumilio* have shown that once exposed to T_a above 32˚C, this species does experienced Arrhenius upcycling (Haim and Fourie, 1980). As such, there should be a difference in MR found between the treatments such that the 70% RH treatment would show a higher MR than that found in the 90% treatment. However, this does not appear to be a unique response to this species. In the case of the Californian Ground Squirrel (*Otospermophilus beecheyi*), when exposed to differing humid heat stresses, there showed to be an independence of T_b and MR from ambient humidity conditions (Baudinette, 1972). In White mice (*Mus musculus*), Ewing and Studier (1973) further echoed this finding, illustrating that at lower WVDs the predicted linear increase in MR as a result of T_a were found to occur until the mice were exposed to water vapour pressures (WVPs) of 30 mm Hg and temperatures ranging from 34 - 40˚C. For context, this study used WVPs of 43.97 mm Hg and 35.98 mm Hg for the 70% and 90% RH treatments respectively, falling above the threshold established by Ewing and Studier (1973). Withers and Cooper (2014) also showed similar to occur in the Red Kaluta (*Dasykaluta rosamondae*), and Walters et al. (2004) even found this to occur in Rhesus monkeys (*Macaca mulatta*). However, to date the reason behind the phenomenon is not yet known.

The Importance of Humidity in Heat Stress

The effect of humidity on thermoregulation has long been appreciated in literature (Candas et al., 1982; Christian, 1978; Haldane, 1905; Walters et al., 2004; Withers and Cooper, 2014), with authors noting the negative relationship between humidity and evaporative cooling in response to the reduced water vapour pressure gradients between thermoregulating organisms and their respective environment (Edwards and Haines, 1978; Haldane, 1905; Lasiewski et al., 1966; Powers, 1992; Withers and Cooper, 2014). However, owing to the complex nature in which water vapour interacts with both pressure and temperature (Lighton, 2008), the methodological challenges involved in ensuring controlled ambient conditions has led to a paucity in the understanding of humid heat stress on thermoregulatory processes (Kenney and Zeman, 2002; van Dyk et al., 2019; Walters et al., 2004; Withers and Cooper, 2014). One echoed proponent among all of these studies however is that humidity is a secondary driver of heat stress in thermoregulating organisms, where T_a remains the dominant factor (Bohmanova et al., 2007; Eichna et al., 1945; Haldane, 1905; Raymond et al., 2017; Sherwood, 2018). In the above investigation, this was echoed, indicating that the capacity for heat stress between heat and humidity combinations under identical T_ws is not equal.

A significant amount of literature highlights the danger of T_w once reaching conditions of 2˚C below an endotherms T_b (e.g. Im et al., 2017; Pal and Eltahir, 2016; Raymond et al., 2017; Raymond et al., 2020; Sherwood and Huber, 2010) with the previous chapter indicating that this threat is an underestimate and that T_w will more likely pose a threat once reaching at least 4˚C below an endotherms T_b. However, this study found that the importance of T_w may be limited to conditions whereby heat and humidity collectively inhibit thermoregulatory processes, otherwise the key concern between climates will be that of T_a extremes irrespective of T_w. This implies that environments where T_w would be a thermal threat by virtue of T_w conditions alone would be limited, and that the future threats imposed by climate change would be heterogenous in nature depending on the surrounding climates.

Regarding conditions whereby T_w would be of key concern, Raymond et al. (2020) inform of two key environmental types that would promote dangerous T_w conditions. The most common instance one where an environment experiences severe T_a exposure near a large, slow-moving water body with an above average surface temperature. Collectively these conditions operate in unison such that the elevated surface temperature of the water body does not require significant thermal investment to reach an oppressive vapour pressure and therefore does not limit the capacity of the T_a to enforce thermally stressful conditions. This also exasperates the conditions further for the T_w condition combination would provide a stable yet stressful climate while the ambient water vapour gradually loses heat over a prolonged period as a result of the high heat carrying capacity of water. For this reason, areas such as the horn of Africa, coastal Eastern India and the coastal South West United States of America will become areas dangerous to endothermic organism, with T_w maxima of 35˚C having already been recorded regionally in these areas (Pal and Eltahir, 2016; Raymond et al., 2020). The second, less frequent environmental condition combination that will cause oppressive T_w conditions will be areas where there is an efficient inland transport of humid air as a result of summer monsoons and large-scale irrigation, characteristics found to occur in Western South Asia (Im et al., 2017; Matthews, 2018).

Regarding conditions whereby T_w stress would be less relevant in comparison to T_a stress, this would be subject to areas that would experience anomalously high T_as that dwarf an endotherms evaporative cooling capacity. Such conditions are already well associated with mass mortality events that have increased in frequency as a result of climate change in areas such as Australia (Welbergen et al., 2008) and South Africa (McKechnie et al., 2021). For example, on 7 January 2014, following T_as of 43-44.6˚C being recorded in eastern Queensland Australia, a minimum loss of ~45

000 flying-foxes (*Pteropus* sp.) succumbed to hyperthermic mortality. This dwarfs the number of flying foxes having suffered the same fate over 19 extreme heat wave events between 1994 – 2007 across Australia (~ 30 000; Welbergen et al., 2008). Considering that extreme heat waves are expected to continue to increase in frequency, intensity and sporadicity (Coumou and Robinson, 2013; Diffenbaugh et al., 2017; Dosio et al., 2018; Herold et al., 2017; Meehl and Tebaldi, 2004; Schär, 2016; Sippel and Otto, 2014; Stocker et al., 2013), although T_w may pose a threat in certain regions, the threat of merely heat stress alone will still be a predominate driver in hyperthermic related mortalities.

Conclusion

This research sought to understand how thermal stress imposed by identical T_w conditions comprised of different heat and humidity levels would impact thermoregulatory processes. It was found that although T_w may be a highly stable metric globally, and that a threat upon thermoregulation may be imposed once T_w = 35˚C, owing to the multifaceted nature in which T_w is attained, the thermal stress imposed by T_w conditions will not be equal between environments. While humidity may lower the efficiency of endotherms to exploit latent heat loss at elevated humidity conditions, the domineering threat imposed by T_w stress arises from its heating component with the inhibitory nature upon EWL being a secondary factor in imposing thermal stress. From this research it is evident that although concerns regarding the effects of humid heat stress are to be appreciated, the greater threat to thermoregulatory processes is that of the ever-increasing T_as and the associated extremes to which they will force endotherms to defend against. As such although T_w shifts will pose a thermal threat to endothermic organisms, the degree to which future conditions will be experienced and defended against will be heterogenous in nature with greater stress being experienced in conditions with lower humidity and elevated temperatures, rather than conditions in which humidity is elevated and thermal conditions are comparatively lowered.

Chapter Four: Synthesis and Conclusions

Wet-bulb temperature (T_w) measures the lowest temperature that an object can reach when experiencing peak evaporative cooling (Rogers and Yau, 1989; Sherwood and Huber, 2010). As evaporative cooling is also the final physiological countermeasure against heat stress in homeothermic endotherms (Romanovsky, 2018; Sherwood et al., 2013; Withers and Cooper, 2014) T_w has also been proposed to represent the lower limit on thermoregulation (Sherwood and Huber, 2010). However, empirical studies of this limit have been limited. Considering that future T_w conditions are set to exceed the tolerance limits of many homeothermic endotherms (Lovegrove, 2012; Raymond et al., 2020; Sherwood and Huber, 2010), this lack of study has also left a critical gap in the understanding of how T_w conditions may affect these animals. Therefore, this thesis sought to investigate the effect of extreme T_w exposure, concomitant with future climate change conditions, to provide amongst the first empirical insights into how thermoregulatory processes may be affected in homeothermic endotherms. Using an obligate homeothermic small mammal, the Four-striped grass mouse (*Rhabdomys pumilio* Sparrman 1784), as a study model the aims of this thesis were two-fold. Firstly, it sought to investigate the limiting capacity of T_w conditions to provide an empirical measure of the T_w limit. Secondly, as identical T_w conditions can be generated using different heat and humidity conditions, this thesis also sought to understand how heat and humidity contribute to T_w heat stress. In doing so, this thesis therefore would be able to assist in understanding the severity of future T_w heat stress and add to this limited body of knowledge.

Wet-bulb temperature as thermal threat to endotherms

Classically, T_w has been thought to enforce a thermoregulatory limit once reaching 2˚C below an endotherm's T_b (Sherwood and Huber, 2010). Following this proposal in 2010, this theoretical limit has been widely adopted in literature (Im et al., 2017; Pal and Eltahir, 2016; Raymond et al., 2017; Raymond et al., 2020; Sherwood and Huber, 2010), and is predicted to be exceeded given current climate change predictions (Coffel et al., 2018; Raymond et al., 2018; Sherwood and Huber, 2010). Furthermore, Raymond et al. (2020) have already found that there are several areas that have exceeded this limitation for humans, and by extension over 80% of endothermic species (Lovegrove, 2012). Regarding the capacities of small homeothermic endotherms to counter this type of stress, T_w not only limited thermoregulatory efficiency, but that this limitation occured significantly lower than predicted. Despite being able to rely on facultative hyperthermia to increase their heat stress tolerance to T_w conditions of 30˚C – 32˚C, once under T_w exposures of T_w 33˚C, 4˚C below their reported T_bs, the grass mice experienced significantly greater rates of heat storage both prior to and after relying on their elevated homeothermic T_bs of 38.2˚C (Chapter 2) despite the increased use of evaporative cooling. Furthermore, under the 4˚C T_b – T_w differential, despite initially relying on facultative hyperthermia, the majority of the mice were unable to prevent becoming pathologically hyperthermic, with only 50% of the mice persisting past 110 minutes of exposure and only 2 out of the 11 individuals completing a full 220-minute trial (Chapter 2). As such, these finding support Vecellio et al. (2022) by advocating for a widening of the perceived safety margins to prevent exposure pathological T_w heat stress. This widening however does imply that over 90% of at least mammals will be at risk of suffering T_w induced hyperthermia under future climate change scenarios (Figure 2.7).

Globally, T_w conditions are known to be highly stable between significantly different environmental conditions (Sherwood and Huber, 2010). Currently the mean T_w, irrespective of habitat, is 27˚C (Coffel et al., 2018; Sherwood and Huber, 2010). However, despite this relative homogeneity, the conditions under which these T_ws are generated are highly heterogenous. As such, this brought the question as to whether the stress experienced between different environmental conditions would be equitable, and therefore by extension whether the T_w limit would be a fixed measure or climate dependent. The data presented in this thesis indicate that T_w heat stress will be highly heterogenous in nature and will be driven by the T_a conditions of respective environments. Although elevated humidity conditions are known to limit evaporative cooling (Baudinette, 1972; Christian, 1978; Edwards and Haines, 1978; Lasiewski et al., 1966), in all of the tests conducted on the grass mice there were no limitations on their capacities to rely on EWL to thermoregulate. Rather, the rates of heat storage in relation to T_a showed to be the key driver towards hyperthermic stress, agreeing with previous studies (Bohmanova et al., 2007; Eichna et al., 1945; Haldane, 1905; Raymond et al., 2017; Sherwood, 2018). When under T_ws comprised of T_a near their upper critical limits, the grass mice were able to thermoregulate efficiently compared to conditions of equitable T_ws but presenting T_a conditions well above that of their upper critical limits. Again, these findings echo Vecellio et al. (2022) where although a lowered T_w limit may have been found, this will be even lower in areas with greater T_as rather than humidity levels, making the T_w limit flexible based on environmental conditions. As such, these findings suggest that the key climatic driver towards hyperthermia will be climate dependant, dividing areas of concern into T_w dominated and T_a regions.

Drawing off the conclusions from Raymond et al. (2020), T_w dominated systems will be limited to areas where there are large, slow moving bodies of water with an above average surface temperature, such as gulf systems. In these conditions the replacement of heated water will be limited and lead to greater rates of evapotranspiration under heat wave conditions. Collectively these areas will then provide a significantly elevated humidity level in combination with elevated heat components. For this reason, in the immediate future, T_w heat stress studies would be advised to conduct *in situ* measures of endothermic tolerances and thermoregulatory methods in areas such as the South West United States, the Horn of Africa and along coastal India where T_w conditions have already breached 35˚C (Pal and Eltahir, 2016; Raymond et al., 2017; Raymond et al., 2020). However, considering the comparative rarity at which extreme T_w conditions are recorded in comparison to conventional heat wave events (Sherwood and Huber, 2010), a significantly greater number of endotherms are likely to be affected by T_a dominated events.

Regarding areas that would be predominantly driven by T_a stress, these would be areas that experience anomalously elevated T_as that exceed the capacities of evaporative cooling to assist endotherms from thermoregulating. Considering the growing aridity of the world climate in response to global warming (Arias et al., 2021) these T_a dominated areas will not only increase in their regularity regarding the stress imposed by T_a conditions but will also increase in size as the world continues to warm. Furthermore, considering that extreme heat wave events are to increase in frequency, intensity and sporadicity (Coumou and Robinson, 2013; Diffenbaugh et al., 2017;

Dosio et al., 2018; Herold et al., 2017; Meehl and Tebaldi, 2004; Schär, 2016; Sippel and Otto, 2014; Stocker et al., 2013) this implies that the threat posed by T_a stress alone is likely to be of more immediate concern compared to that of extreme T_w. Therefore, in conclusion, although T_w may pose a threat to future endothermic populations and that this limit is currently significantly underestimated, the growing regularity of conventional extreme heat wave events and rising T_a conditions concomitant with global warming need to remain the key focus of mitigation methods. Although T_w events may be of a significant threat in some areas in future, the rising regularity of extreme T_as are already driving mass mortality events globally without T_w limitation (McKechnie et al., 2021; Ratnayake et al., 2019; Welbergen et al., 2008; Welbergen et al., 2014).

Limitations and recommendations

Body temperature distributions within the mammalian clade are constrained to a narrow range, with 50% of mammal species defending T_bs between 35 – 37.9˚C (Lovegrove, 2012). However, the distribution of sizes within this is significantly greater, ranging from 1.8 g in the Etruscan shrew (*Suncus etruscus*; Fons, 1970) to 6000 kg in Africa Bush Elephants (*Loxodonta africana*; Larramendi, 2015) for terrestrial mammals alone. It is well established that size plays a significant role in an animal's thermoregulatory capacity by effecting key attributes that determine how heat stress is both experienced and responded to. For example, by virtue of size differences not only are water economies available to evaporative cooling affected (McKechnie and Wolf, 2010; Scholander et al., 1950), but so are capacities for thermal inertia and behavioural countermeasures available to animals of different size classes (Calder, 1996; Tattersall et al., 2012). Therefore, further testing into how different size clades within endotherms may respond to T_w heat stress, is needed. Granted, the responses by the grass mice to high T_w exposure show to be similar to those seen in humans (*sensu* Vecellio et al., 2022), these responses may differ in other clades in both clinical and natural settings.

Furthermore, although *Rhabdomys pumilio* may present a robust model species for T_w heat stress responses owing to being one of the few diurnal semi-arid rodents and being known to tolerate a wide range of habitat conditions (Coetzee and van der Straeten, 2008; Du Toit et al., 2012; Ganem et al., 2020; Kingdon, 1974; Schradin and Pillay, 2004; Skinner and Chimimba, 2005), this study can only use this model to infer how small obligate homeotherms may respond. Considering that there are also different thermoregulatory modes within endothermic, such of heterothermy, these phylogenetic differences may also present different thermoregulatory tolerances and limitations compared to those found in this thesis. Heterothermy, for example, has been shown to provide endotherms a broader thermal tolerance when under heat stress, extending their upper critical limit beyond that of their homeothermic counterparts (Boyles et al., 2011; Hetem et al., 2014; Welman et al., 2017). In freetail bats (*Mormopterus sp.*) for example, by becoming heterothermic and thermoconforming, they have shown to allow their T_b to increase from to 33.2˚C as high as 45.8°C, tolerating T_as up to 47.9˚C for a period of 4.2 hours (Bondarenco et al., 2014). Considering that T_a showed to be the key driver towards pathological hyperthermia in this study, such an elevation in tolerance may provide heterotherms with a key competitive edge compared to their homeothermic counterparts, despite T_w limitations. As such, an investigation into how heterotherms may respond to high T_w exposure will also be critical to fully understanding how T_w conditions may affect future endothermic populations.

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