© 2022. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2022) 225, jeb243860. doi:10.1242/jeb.243860

RESEARCH ARTICLE

Obligatory homeothermy of mesic habitat-adapted African striped mice, *Rhabdomys pumilio*, is governed by seasonal basal metabolism and year-round 'thermogenic readiness' of brown adipose tissue

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ABSTRACT

Small mammals undergo thermoregulatory adjustments in response to changing environmental conditions. Whereas small heterothermic mammals can employ torpor to save energy in the cold, homeothermic species must increase heat production to defend normothermia through the recruitment of brown adipose tissue (BAT). Here, we studied thermoregulatory adaptation in an obligate homeotherm, the African striped mouse (*Rhabdomys pumilio*), captured from a subpopulation living in a mesic, temperate climate with marked seasonal differences. Basal metabolic rate (BMR), non-shivering thermogenesis (NST) and summit metabolic rate (M_{sum}) increased from summer to winter, with NST and M_{sum} already reaching maximal rates in autumn, suggesting seasonal preparation for the cold. Typical of rodents, cold-induced metabolic rates were positively correlated with BAT mass. Analysis of cytochrome *c* oxidase (COX) activity and UCP1 content, however, demonstrated that thermogenic capacity declined with BAT mass. This resulted in seasonal differences in NST being driven by changes in BMR. The increase in BMR was supported by a comprehensive anatomical analysis of metabolically active organs, revealing increased mass proportions in the cold season. The thermoregulatory response of *R. pumilio* was associated with the maintenance of body mass throughout the year (48.3±1.4 g), contrasting large summer–winter mass reductions often observed in Holarctic rodents. Collectively, bioenergetic adaptation of this Afrotropical rodent involves seasonal organ adjustments influencing BMR, combined with a constant thermogenic capacity dictated by trade-offs in the thermogenic properties of BAT. Arguably, this high degree of plasticity was a response to unpredictable cold spells throughout the year. Consequently, the reliance on such a resource-intensive thermoregulatory strategy may expose more energetic vulnerability in changing environments of food scarcity and extreme weather conditions due to climate change, with major ramifications for survival of the species.

KEY WORDS: Seasonality, Basal metabolism, Non-shivering thermogenesis, Summit metabolism, Phenotypic plasticity

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Received 1 December 2021; Accepted 8 June 2022

INTRODUCTION

Defending a body temperature (T_b) higher than the ambient temperature (T_a) is energetically expensive, and the costs vary greatly between mammals. Size has a significant influence on the overall energy required to maintain basic bodily functions and also impacts the heat exchange dynamics in mammals (McNab, 1970; Schmidt-Nielsen, 1997). Smaller bodied mammals require less energy overall but gain or lose heat from their body faster than larger bodied mammals as a result of their higher surface-area-to-volume ratio. Because of this low thermal inertia, thermoregulatory costs are usually higher in smaller mammals, resulting in higher mass-specific metabolic rates (Schmidt-Nielsen, 1997; Tattersall et al., 2012). Nevertheless, all mammals must invest additional energy to increase endogenous heat production to defend their normothermic T_b when faced with cold conditions. We define cold in relation to a species' thermoneutral zone (TNZ) (Scholander et al., 1950), with the lower critical limit of thermoneutrality marking the onset of cold. To maintain normothermia, thermoregulatory costs increase linearly with lowering of T_a below TNZ. Often, to avoid large thermoregulatory costs, species that can may enter a state of facultative hypometabolism, i.e. torpor or hibernation, opting to defend a lower setpoint T_b (Geiser, 2004). In this case, the animal down-regulates metabolism, allowing its normothermic T_b to decrease until the new setpoint T_b is reached before actively thermoregulating, yielding large energetic savings. By contrast, species that are obligated to defend normothermia must pay the high energetic cost to sustain endogenous heat production, with the potential of exploiting exogenous sources of heat (e.g. sun basking), to compensate for heat lost from their body when confronted with cold conditions (Tattersall et al., 2012).

Given the low T_a in winter, thermoregulatory costs are high and small mammals undertake a suite of phenotypic changes to reduce energy expenditure and better cope with winter conditions. In general, they (1) shed body mass (M_b) to lower overall maintenance costs (i.e. basal metabolic rate, hereafter BMR), (2) increase heat production capacity by upregulating non-shivering thermogenesis (NST) and (3) show some increase in insulation (Heldmaier et al., 1986; Heldmaier, 1989; Lovegrove, 2005). For example, the bank vole *Myodes* (=*Clethrionomys*) *glareolus* shows an extreme reduction in mass of ∼46% accompanied by a reduction of ∼36% in BMR between summer and winter (Rosenmann et al., 1975; Boratyński and Koteja, 2009). In rare instances such as in Djungarian hamsters (*Phodopus sungorus*), the response is so attuned to season that individuals decrease M_b in winter even when food is available (Steinlechner et al., 1983). However, it is important to note that these generalizations are based largely on data from the Holarctic region where wintery conditions, although severe,

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are highly predictable. This is an important consideration as selection pressure differs between predictable and unpredictable environments. For example, species found in climatically unpredictable environments tend to have low BMRs compared with those found in predictable environments associated with high latitudes (Lovegrove, 2000, 2003).

Seasonal responses of species from less predictable environments unsurprisingly often deviate from the general trend mentioned above. Melano-bellied oriental voles (*Eothenomys melanogaster*) increase BMR and NST in winter relative to summer, but M_b remains the same (Xu et al., 2011), whereas African striped mice (*Rhabdomys pumilio*) increase NST (Haim and Fourie, 1980a,b,c), resting metabolic rate and M_b in winter relative to summer (Rimbach et al., 2018). Further, cold-acclimated rock elephant shrews (*Elephantulus myurus*) increased BMR relative to their warm-acclimated counterparts even though M_b was similar (Mzilikazi et al., 2007). The authors in this last study also found that there was no difference in NST between warm- and cold-acclimated individuals once the contribution of BMR was considered (i.e. the regulatory component of NST). The reason for such deviations is that seasonal responses also include physiological factors that are independent of, yet confounded by, T_a, including reproductive status, locomotion, diet and food availability, all of which influence the animal's energy budget (McNab, 1986; Bozinovic, 1992; Hammond and Diamond, 1992; Cruz-Neto et al., 2001; Lovegrove, 2001; Mzilikazi et al., 2007; Withers et al., 2016). For instance, Rimbach et al. (2018) studied seasonal responses in African striped mice in a karoo habitat, where it is known that they typically become food stressed in summer and lose about 12% of their M_b (Schradin and Pillay, 2004; Schradin, 2005). The summer–winter increase in metabolism thus accompanies an increase in M_b as a result of an increase in resources during winter. Would this seasonal response remain in a setting where resources were more abundant in summer?

Understanding the extent of a species' phenotypic plasticity also has a direct bearing on predicting how that species may respond to future conditions (Gienapp et al., 2008; Norin and Metcalfe, 2019). To this end, it has been shown that global climate change can have contrasting effects on seasonal survival in mammals (Reusch et al., 2019; Cordes et al., 2020), mediated by the capacity of individuals to deal with shifts in resource availability. This could be driven by changes in snow cover (Boratyński and Koteja, 2009; Cordes et al., 2020) and rainfall (Schradin and Pillay, 2005). Whereas the effects of climate change may have negative consequences for survival and population size in a particular season, say winter, they could enhance survival and promote an increase in population size in summer, for example (Cordes et al., 2020). To gain a better understanding of a species' risk of extinction as a result of climate change, it is necessary to progress past simplistic winter–summer comparisons as energetic demands and constraints vary seasonally. Similarly, placing more emphasis on intraspecific comparisons between individuals occupying different habitats, where they are likely to face different selection pressures, will afford better insight to the extent of phenotypic plasticity that the species is capable of. This would provide a more holistic context to gauge the species' resilience to climate change. It is unlikely that a species would exhibit the same response to climate change in all subpopulations across its entire range, especially for species with large distribution ranges.

The African striped mouse is an ideal study model to test for differences between subpopulations because the species is widely distributed throughout southern Africa, occupies starkly different habitats and much is already known about its behaviour, ecology and physiology (Haim and Fourie, 1980b; Korn, 1989; Haim et al., 1998; Schradin and Pillay, 2004; Schradin, 2005; Schradin and Pillay, 2005; Scantlebury et al., 2006; Schradin et al., 2007; Lovegrove, 2009; Scantlebury et al., 2010; Schradin et al., 2012; Rimbach et al., 2018, 2019; Schradin et al., 2019; Zduniak et al., 2019). African striped mice residing in arid succulent karoo habitats favour group living (Schradin and Pillay, 2004), whereas mice residing in more mesic habitats favour a solitary existence (Schradin, 2005). While living in a group, these mice benefit from communal breeding and parental care (Schradin, 2005), as well as from huddling (Scantlebury et al., 2006). Given the thermoregulatory benefits of huddling, it is possible that individuals living in a group may not need to invest as much energy in heat production during cold periods as those living alone, reducing their daily energy expenditure. Also, the African striped mouse is one of only a few diurnal rodent species (Roll et al., 2006) and individuals are known to delay activity during cold mornings to bask in the warm sunlight, further reducing their energy expenditure (Schradin et al., 2007; Scantlebury et al., 2010; Zduniak et al., 2019). This high degree of phenotypic plasticity may allow African striped mice to be adaptable to unpredictable environmental conditions, and observations based on individuals from only one or the other habitat are likely to bias our assessment of the species' vulnerability to future conditions.

With the basic understanding of seasonal adjustments in whole-animal metabolism and M_b in captive African striped mice and wild-caught individuals from arid habitats, we sought to study the seasonal changes made by mesic habitat-adapted individuals using an integrated approach to correlate adjustments across multiple levels of organismal organisation. We included autumn and spring seasons to improve the context of seasonal adjustments throughout the year and investigated seasonal changes in M_b, BMR, NST and summit metabolism (M_{sum}) in wild-caught mice from the coastal city of Gqeberha (formerly Port Elizabeth), along the south eastern region of South Africa. As a secondary focus, we tested the assumption made by many studies that all members of a particular species will exhibit the same response to the effects of climate change, even though *in situ* conditions differ across space and time (Wiens et al., 2009). We did this by comparing our data with previously observed whole-animal seasonal responses for our study species. Given the contrast in habitat between our study site and that of the arid karoo, as well as between the unpredictability of the Afrotropical environment compared with the more predictable Holartic environment, we expected that the seasonal response of mice in our study would not conform to either that of African striped mice in the karoo or the general response by Holarctic small mammals. To test this, we developed two hypotheses. Firstly, we hypothesised that the thermoregulatory response of mice would involve decreases in M_b and BMR, and increases in NST and M_{sum} in winter compared with summer, aligned with the general response in Holartic species. Secondly, we hypothesised that the thermoregulatory response of mice would involve increases in M_b, BMR, NST and M_{sum} in winter compared with summer; aligned with the previous observations in arid-adapted African striped mice.

We also undertook a comprehensive anatomical analysis of metabolically active organs, including organ mass and metabolic activity to provide a more holistic view of seasonal adjustments and potential trade-offs, to better understand the physiological mechanisms governing whole-animal adjustments. Because of the physiological nexus between M_b, organ size and BMR (Schmidt-Nielsen, 1997), we expected correlated seasonal changes between these variables. Similarly, we also expected correlated seasonal changes in brown adipose tissue (BAT), uncoupling protein 1 content and oxidative capacity [measured as cytochrome *c* oxidase

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(COX) activity] related to NST (Cannon and Nedergaard, 2004). Lastly, we compared BMR and M_{sum} with published allometric predictions for similar sized mammals to add broader context to any observed seasonal adjustments and potential size-related trade-offs made by the mice in our study (Bozinovic, 1992; Lovegrove, 2000; 2003; Rezende et al., 2004; Sieg et al., 2009).

MATERIALS AND METHODS

Study site and animal capture

The study included 40 adult African striped mice, *Rhabdomys pumilio* (Sparrman 1784), caught at the Nelson Mandela University Nature Reserve (34°00′44″S; 25°39′21″E). The region experiences a temperate climate with relatively mild winter conditions, but the area is prone to sudden cold spells that occur sporadically throughout the year. The reserve spans 830 ha and the vegetation is a mosaic of St Francis dune fynbos and thicket. Local temperature and rainfall data were recorded at a weather station (0035209B1) less than 5 km from the study site and were obtained from the South African Weather Service. The average daily temperature and rainfall per season were 13.3±0.2°C and 4.6±1.3 mm during winter, 16.3±0.5°C and 1.1±0.4 mm during spring, 21.8±0.3°C and 2.5±1.5 mm during summer, and 17.4±0.4°C and 1.0±0.5 mm during autumn (Fig. 1). Mice were caught using Sherman live traps baited with a mixture of peanut butter and oats. Ten individuals were collected during four sampling periods between 16 June and 17 August 2011 (winter), 9 September and 12 October 2011 (spring), 11 January and 17 February 2012 (summer) and 29 March and 3 May 2012 (autumn), respectively (Fig. 1). Sampling effort was spaced to avoid the 2 weeks around the start and end of each season, which we deemed to be transitional periods. All captured individuals were carried in their traps to the nearby laboratory at Nelson Mandela University. Mice were sexed, weighed and females were inspected for signs of pregnancy including nipple swelling and vaginal perforation. We also conducted an abdominal palpation.

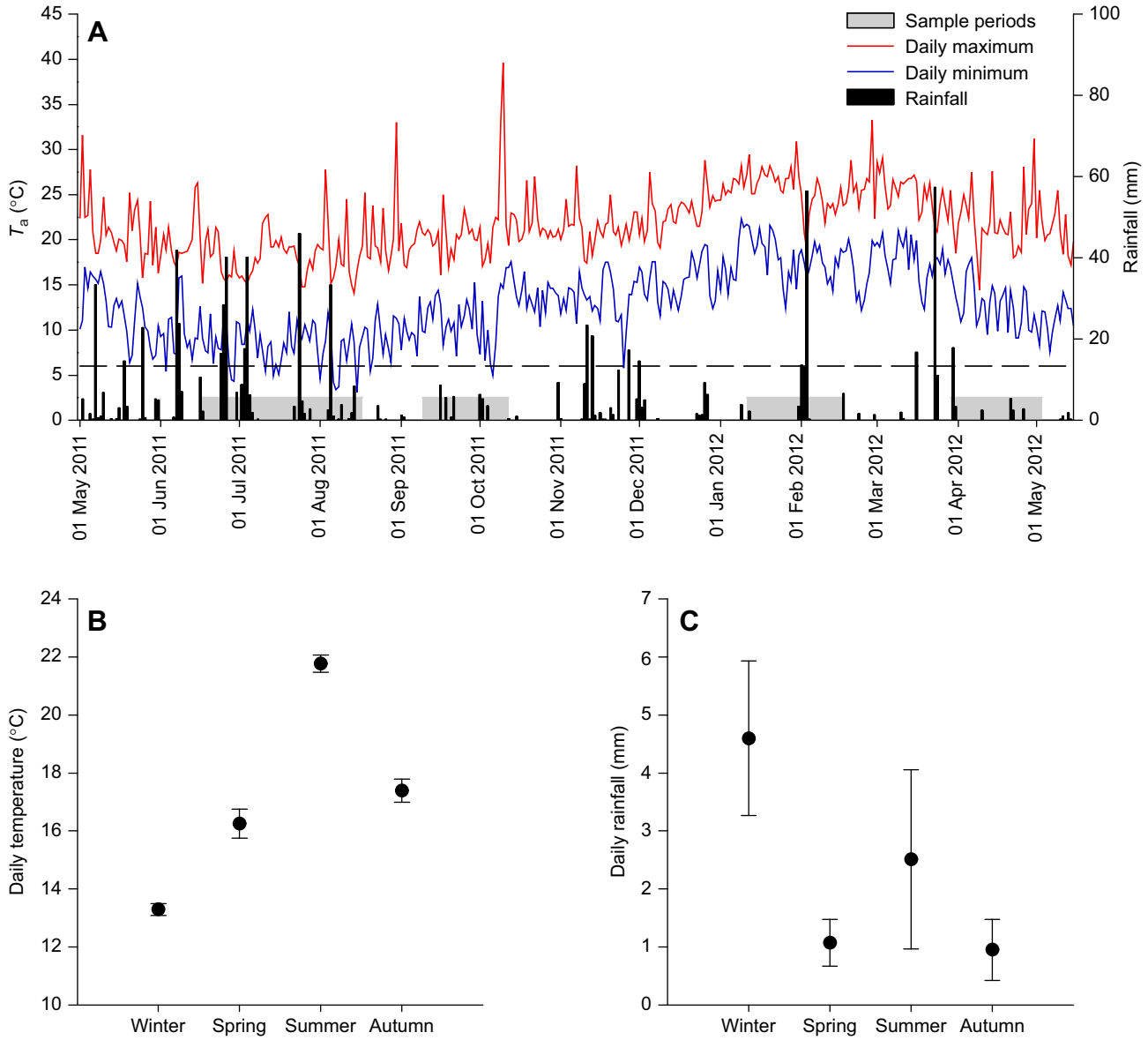

Fig. 1. Weather data for the study size between 1 May 2011 and 15 May 2012. (A) Rainfall, daily maximum and daily minimum temperatures. (B) Mean (±s.e.m.) daily temperature per season. (C) Mean (±s.e.m.) daily rainfall per season. The data were recorded at a national weather station within 5 km of the study site. The dashed line in A indicates 6°C, a temperature shown by Haim and Fourie (1980b) to illicit maximal cold-induced metabolic rates in African striped mice, *Rhabdomys pumilio*.

All sub-adult mice and females suspected to be pregnant were released. Captive mice were housed singly in glass terraria (60 cm×30 cm×30 cm L×B×H) and kept in an environmentally controlled room at 50% relative humidity, with the photoperiod and temperature set to resemble local conditions. While captive, animals had unrestricted access to water and food for up to 6 h before respirometry measurements. Mice were sustained on commercial rodent feed and all individuals spent a total of 3 days in captivity. Upon completion of metabolic measurements, the individuals were killed and their organs harvested.

All experimental procedures were reported to and approved by the Nelson Mandela Metropolitan University Animal Ethics Committee (A10-SCI-ZOO-005) and permits to capture study animals were issued by the Eastern Cape Department of Economic Development and Environmental Affairs (CRO 56/10CR & CRO 78/11CR).

Gaseous exchange measurements

We used a flow-through respirometry system (Lighton, 2008) to measure the rate of oxygen consumption ($\dot{V}_{O_2}$) in African striped mice. Animals were weighed immediately before and after measurements, and were held in respirometers constructed from modified airtight clear plastic containers. During measurements of BMR and NST, atmospheric air was flowed through 1.25 l respirometers at a constant rate between 600 and 750 ml min^{-1} using a pump, needle value and mass flow meter system (MFS-2, Sable Systems, Las Vegas, NV, USA) plumbed downstream of the respirometer to draw air past the animal and to measure the excurrent flow rate. For measurement of M_{sum}, a mass flow controller (FMA 5520, Omega, Bridgeport, NJ, USA) was used to push compressed helox gas (79.7% He, 20.3% O_2) through 1.7 l respirometers at a constant rate of 2 l min^{-1}. Using helox gas as a respiratory medium elicits a cold response without exposing the individual to extreme temperatures (Swanson et al., 1996; Minnaar et al., 2014). All flow rates were sufficient to maintain O_2 concentration within the respirometer between 20.0% and 20.8%.

The excurrent airstream from the animal respirometer and a control airstream from an empty respirometer were sequentially subsampled using a pump and flow meter (SS-4 sub-sampler, Sable Systems) and flowed through a series of gas analysers to measure the water vapour content and fractional concentration of O_2. A RM-8 Flow Multiplexer (Sable Systems) was used to switch between the airstreams. The sub-sampled air was first flowed through a water vapour analyser (RH-300, Sable Systems) and then passed through a column of silica gel to dry the air, then a column of soda lime to remove CO_2 and another column of silica gel to remove any remaining water vapour, before entering an O_2 analyser (FC-10a; Sable Systems). The digital outputs of the instruments were recorded on a personal computer, at 1 s intervals using Expedata data acquisition software (Sable Systems).

All gaseous exchange traces were synchronized using Expedata before calculating $\dot{V}_{O_2}$. For all measurements other than M_{sum}, where compressed gas was used, the flow rate was corrected for the presence of water vapour using the equation:

$$\text{Flow rate}_{\text{corrected}} = \text{Flow rate}_{\text{measured}} \times \frac{\text{BP} - \text{WVP}}{\text{BP}}, \quad (1)$$

where BP is the barometric pressure and WVP is the water vapour pressure (Lighton, 2008). Thereafter, we calculated $\dot{V}_{O_2}$ as:

$$\dot{V}_{O_2} = \text{Flow rate}_{\text{corrected}} \times \frac{F_{I_{O_2}} - F_{E_{O_2}}}{1 - F_{E_{O_2}}}, \quad (2)$$

where $F_{I_{O_2}}$ represents the fractional concentration of oxygen in the airstream entering the respirometer (assumed to be 0.2095) and $F_{E_{O_2}}$ represents the fractional concentration of oxygen in the airstream leaving the respirometer to the O_2 analyser, with water vapour and CO_2 scrubbed from the excurrent airstream before the analyser (Lighton, 2008). When using an excurrent flow measurement flow-through respirometry design, it is ideal to remove CO_2 from the airstream before the flow meter. Our design may thus have led to a slight, though probably negligible, alteration to the absolute values of $\dot{V}_{O_2}$ recorded during BMR and NST determination. However, this would have no impact on the overall seasonal comparison. The O_2 analyser was spanned before every measurement and the water vapour analyser was calibrated at the start of and approximately mid-way through each sampling period, following the same procedure described by Welman et al. (2017). The mass flow controller was calibrated using a 1 litre soap bubble flow meter (Baker and Pouchot, 1983).

Gaseous exchange experimental protocol

The experimental temperatures were regulated using a temperature-controlled cabinet (Humidity Chamber, Labcon, Krugersdorp, South Africa) and the air temperature within the respirometers was recorded using iButtons (DS1922L; Thermochron, Dallas, TX, USA; resolution: 0.0625°C). All experiments were performed on solitary mice. NST measurements immediately followed BMR measurements, and these were conducted during the night-time rest phase between 21:00 h and 04:00 h. BMR was determined from the most level continuous 10 min section of the $\dot{V}_{O_2}$ trace observed over a 6 h measurement period, measured in post-absorptive mice kept at 32±1°C. The appropriate section of the trace was identified using built-in functions of the Expedata software package, and BMR was generally reached 2.5–4 h into the trial. NST was determined from the peak 5 min section of the $\dot{V}_{O_2}$ trace following a noradrenaline injection (NA mg kg^{-1}=2.53$M_b^{-0.4}$; Wunder and Gettinger, 1996). In retrieving mice to inject NA, opening the temperature-controlled cabinet caused the experimental temperature to decrease to room temperature (±23°C). We then kept the door of the cabinet slightly ajar to allow us to view the mice and to maintain experimental temperatures at room temperature to accommodate dissipation of excess body heat. Further, observing the mice allowed us to distinguish between periods of activity and rest, and we only considered NST from sections of the trace when mice were lying flat on their bellies, presumably to offload heat through maximum exposure of thermal windows. Control measurements were performed using NaCl. M_{sum} was measured during the day-time active phase between 06:00 h and 18:00 h. We left mice to recover for 1 day between NST and M_{sum} measurements. M_{sum} was determined as the peak 5 min section of the $\dot{V}_{O_2}$ trace using a sliding cold exposure protocol in a helox atmosphere (Swanson et al., 1996). Mice typically reached their peak metabolic rate at helox temperatures between 15 and 5°C. To determine baseline values, respirometers were flushed with helox for 10 min before and after mice were placed into the chambers. Mice were exposed to helox for no longer than 40 min and the experiment was immediately terminated when mice became hypothermic, indicated by a steady decrease in $\dot{V}_{O_2}$ over time (Swanson et al., 1996). We used a K-type thermocouple (Digital thermometer VC9808$^+$, ACDC Dynamics, Edenvale, Gauteng, South Africa) to measure the rectal temperature of mice upon completion of the experiment to confirm hypothermia (Fig. S1). Subsequently, mice were transferred to a warm room to recover. The M_b corresponding to each of the physiological variables was determined from a regression of the animals' mass at the start and end of each experiment.

Organ harvest, tissue homogenization and western blot protocol

All visible BAT deposits, liver, heart, kidneys, lungs, pancreas, stomach and intestines were harvested and weighed individually. Tissues were flash frozen in liquid nitrogen and stored at −80°C. Tissues were cut on dry ice and separated into 2 ml Eppendorf tubes. A 7 mm stainless steel bead was added to each tube and tissues were homogenized using a Tissue Lyser 2 (Qiagen) at 30 shakes per second for 30 s in 10 µl of tissue buffer (33.9 mmol l^{-1} KH_2PO_4, 66.1 mmol l^{-1} $K_2HPO_4{\cdot}3H_2O$, 2 mmol l^{-1} EDTA) per mg frozen tissue. If necessary, tissues were homogenized again for a further 30 s to achieve an even consistency and cooled on ice. We determined the total protein concentration in BAT and liver samples using a Pierce BCA Protein Assay Kit (Thermo Scientific) and a PHERAstar FS (BMG-Labtech) plate reader, following the steps described in the kit's manual.

With known but variable starting protein concentrations, samples were aliquoted and diluted with tissue buffer to a standard protein concentration of 0.8 µg $µl^{-1}$; 25 µl of this dilution was combined with 8 µl of a pre-mixed 100 mmol l^{-1} DTT and 1× NuPAGE LDS sample buffer (Invitrogen) solution and incubated at 95°C for 5 min, then centrifuged at 14,000 rpm for 2 min. We added 10 µg of protein per sample to wells of gel cassettes (15 wells, 15 µl, Mini-Protean, TGX any KD stain-free; Bio-Rad) for protein electrophoresis at 200 V in a medium of 25 mmol l^{-1} Tris, 192 mmol l^{-1} glycine and 0.1% w/v SDS, pH 8.3. A Trans-Blot Turbo transfer system (Bio-Rad) was used to transfer the proteins to a blotting membrane (trans-blot turbo transfer pack, mini format 0.2 µm nitrocellulose; Bio-Rad), and the membrane was rinsed for 1 h in Odyssey blocking buffer (LI-COR Bioscience). The primary antibody (anti-UCP1 rabbit; Philipps-University Marburg) was added to the solution at a concentration of 1:10,000 and incubated on a shaker at 4°C overnight. The membrane was washed twice for 20 min with PBS-T (PBS and 0.1% Tween 20 solution), twice for 20 min with only PBS and then incubated in the dark for 1 h in Odyssey blocking buffer containing the secondary antibody [anti-rabbit IgG (H&L) (goat) Antibody IRDye® 800 conjugated; Rockland Immunochemicals, Inc.] at a concentration of 1:10,000. After incubation, the membrane was washed again with PBS-T and PBS as previously described. The relative concentration of UCP1 in BAT was determined from the UCP1 signal strength detected by fluorescence at a wavelength of 800 nm (Odyssey FC Imager; LI-COR Bioscience). Protein containing mouse UCP1 was used as a positive control, while liver samples were used as a negative control. A representative western blot is provided in Fig. S2.

COX activity measurements

The COX activity of BAT homogenate was measured polarographically in a temperature-controlled chamber at 34±1°C. The tissue homogenate was diluted 1:4 with a solution of tissue buffer, 10 mmol l^{-1} reduced glutathione and 0.1% w/v detergent (*n*-dodecyl β-D-maltoside); the detergent was used to release the protein from the membrane fractions. This dilution was incubated in 350 µl of air-saturated measurement medium (79 mmol l^{-1} $K_2HPO_4{\cdot}3H_2O$, 21 mmol l^{-1} KH_2PO_4, 5 mmol l^{-1} EDTA, 0.125 mol l^{-1} ascorbate; pH 7.4) to which 1.5 mmol l^{-1} horse cytochrome *c* (Sigma-Aldrich) was added. We assumed that the final solution contained 421 nmol O_2 ml^{-1} (Reynafarje et al., 1985). Additionally, we also estimated COX activity using plate-based respirometry of the XF96 analyser (Seahorse, Agilent). While the machine is usually used for biological material adherent to the multi-well bottom, and cannot be used for quantitative determination of soluble COX, as oxygen concentration will also vary in the well outside the 2.28 µl microchamber, we found a strong correlation between BAT COX activity in our chamber-based versus plate-based setup ($F_{1,38}$=4.40, P=0.043, r=0.72), which could resolve seasonal effects. Following this observation, we used the high-throughput setup of plate-based respirometry to estimate the COX activity of several visceral organs purely qualitatively, with the benefit that one can process many samples simultaneously, reducing the overall time. In short, we measured oxygen uptake of COX dissolved in 150 µl of measurement medium before and after the addition of 0.75 mmol l^{-1} horse cytochrome *c* into the well.

Statistical protocol

All statistical procedures were performed using Minitab® Statistical Software and tests were conducted using α=0.05. We confirmed that the data were normally distributed using a Ryan–Joiner test (similar to a Shapiro–Wilk test) and had homogeneous variances using a Levene's test. We tested for seasonal and sex-dependent differences in M_b at capture using one-way analysis of variance (ANOVA) tests. Where applicable, we used a simple regression analysis to describe the relationship between metabolic variables as well as between M_b and whole-animal metabolism. However, we used a forward selection stepwise regression analysis to describe the relationship between organ mass and BMR. The forward selection approach determines the best model using a subset of all variables in the dataset through an iterative process of adding explanatory variables into the model until the inclusion of additional explanatory variables no longer improves model performance. In this case, various organs were included in the order of their explanatory power of BMR. Of all possible permutations, the Minitab® Statistical Software automatically returns the model with the fewest explanatory variables based on the lowest Akaike information criterion (AIC) score.

For all seasonal comparisons, we used mixed-effects general linear models (GLMs), with sex included as a random effect to account for potential sex-dependent differences. Because the determination of total protein concentration and UCP1 signal strength in BAT, as well as the COX activity of organs were based on equal amounts of tissues, comparisons were straightforward. By contrast, for seasonal comparison of BMR, NST, M_{sum} and organ mass, we included M_b as a covariate in the model. Further, following Gębczyński's (2008) argument that BMR should be considered as an obligatory component of non-shivering heat production, we differentiate between the maximum metabolic response observed following a NA injection (MMR_{NA}), which would include the contribution of BMR to the individual's NST capacity, and the individual's regulatory NST response (MMR_{NA}−BMR). All significant main effects were followed by a Tukey HSD test to identify specific inter-seasonal differences.

Following McClune et al. (2015), we compared the metabolism of mice in our study with allometric predictions based on published allometric equations. Data were generated for each mouse by substituting its M_b into the respective allometric equations and these were compared with observed values using mixed-effects GLMs. Given the seasonal variability in metabolism (see Results), separate comparisons were made for each season. To account for the repeated nature of the comparison, i.e. the metabolism of each mouse would be compared with multiple predictions based on its mass, we included the individual ID as a random effect in the model. This was followed by a Dunnett's many-to-one contrast *post hoc* test to identify specific differences. Lastly, to determine whether mice responded to short-term temperature variability, we conducted pairwise Pearson correlation tests between metabolism and the

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minimum and maximum temperatures experienced by mice in the week leading up to capture.

Unless stated otherwise, the data are presented as means±s.e.m. and all comparisons included n=10 per season, except for comparisons involving M_{sum}, where n=8 for winter.

RESULTS

Body mass and metabolism

There was no seasonal effect on M_b ($F_{3,36}$=0.32, P=0.812) and mean M_b at capture was 48.3±1.4 g (winter 47.1±3.0 g, spring 48.9±2.7 g, summer 46.8±3.4 g, autumn 50.3±2.4 g). Similarly, there was no difference in M_b between males (47.7±2.6 g, n=16) and females (48.6±1.7 g, n=24; $F_{1,38}$=0.10, P=0.757). BMR scaled positively with M_b during winter ($F_{1,8}$=6.90, P=0.030), summer ($F_{1,8}$=7.60, P=0.025) and autumn ($F_{1,8}$=21.64, P=0.002), but not within spring ($F_{1,8}$=4.87, P=0.058; Fig. 2A). MMR_{NA} scaled positively with M_b during summer ($F_{1,8}$=10.76, P=0.011) but there was no relationship between MMR_{NA} and M_b during other seasons (Fig. 2B). M_{sum} scaled positively with M_b during autumn ($F_{1,8}$=5.62, P=0.045) but there was no relationship between M_{sum} and M_b during other seasons (Fig. 2C).

Seasonal comparison of metabolism

There was a significant seasonal difference in BMR ($F_{3,34}$=18.89, P<0.001), regulatory NST ($F_{3,34}$=4.288, P=0.011), MMR_{NA}

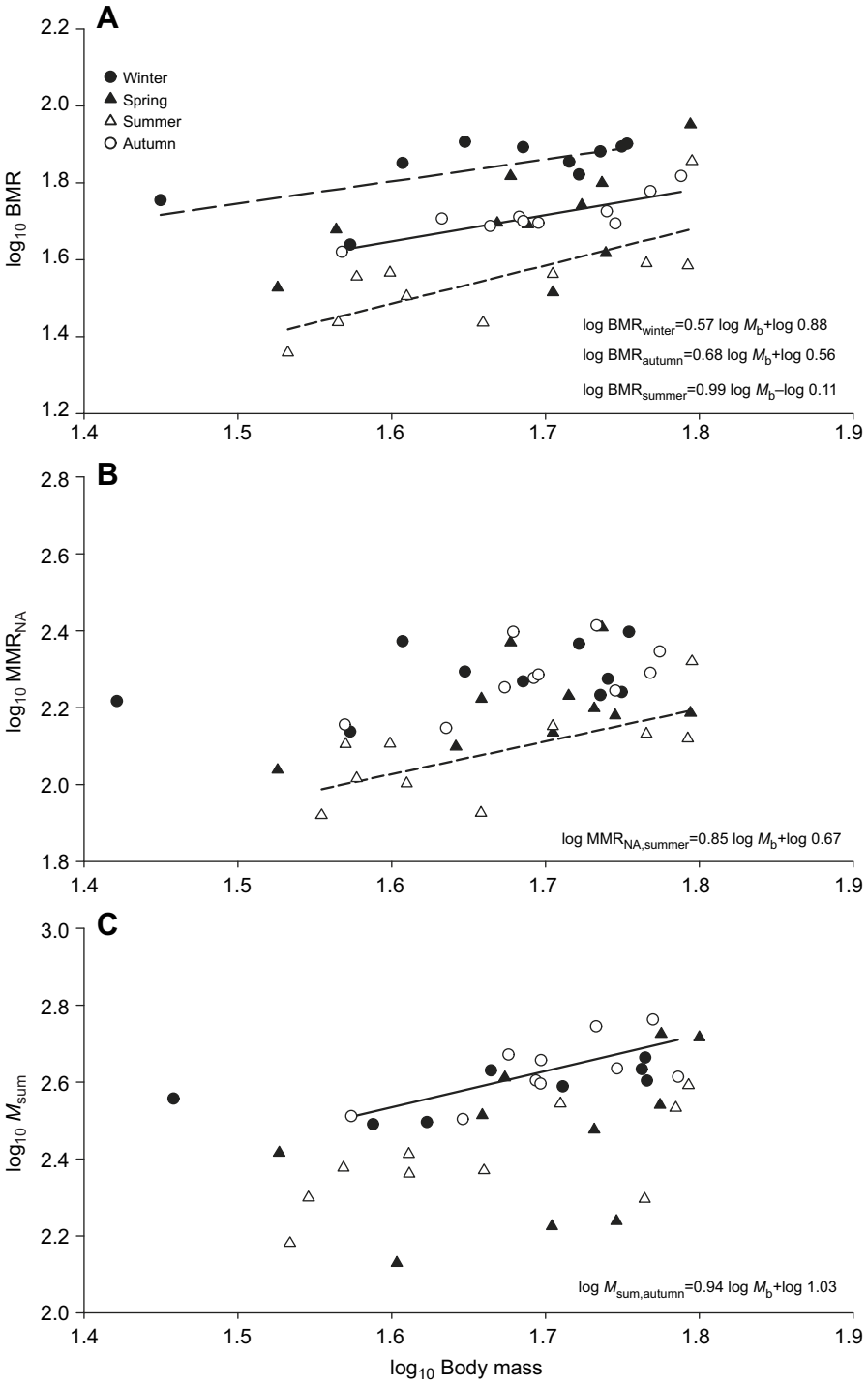

Fig. 2. Scatterplots of body mass and metabolism in wild-caught African striped mice, *Rhabdomys pumilio*. (A) Scatter plot of body mass (g) and basal metabolic rate (BMR; ml O_2 h^{-1}). (B) Scatter plot of body mass (g) and overall non-shivering thermogenesis (measured as the maximum metabolic rate following noradrenaline injection, MMR_{NA}; ml O_2 h^{-1}). (C) Scatterplot of body mass (g) and summit metabolic rate (M_{sum}; ml O_2 h^{-1}). Long-dashed lines represent significant relationships between body mass and metabolism in winter, solid lines represent significant relationships between body mass and metabolism in autumn, and short-dashed lines represent significant relationships between body mass and metabolism in summer. The equations describing the respective lines are also presented. All significant relationships are at P<0.05.

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($F_{3,34}$=8.17, P<0.001) and M_{sum} ($F_{3,29}$=10.57, P<0.001). BMR in winter (1.17±0.06 ml O_2 min^{-1}) was almost twice as high as that in summer (0.61±0.07 ml O_2 min^{-1}), while spring (0.88±0.09 ml O_2 min^{-1}) and autumn (0.87±0.03 ml O_2 min^{-1}) values did not differ from each other but both differed from winter and summer levels (Fig. 3A). Regulatory NST was higher in autumn (2.38±0.19 ml O_2 min^{-1}) than in summer (1.46±0.13 ml O_2 min^{-1}), but no other seasonal differences were detected (winter: 2.06±0.16 ml O_2 min^{-1}, spring: 1.89±0.22 ml O_2 min^{-1}) (Fig. 3C). MMR_{NA} was similar between winter (3.23±0.19 ml O_2 min^{-1}) and autumn (3.25±0.21 ml O_2 min^{-1}), and the *post hoc* test determined that MMR_{NA} in both of these seasons was higher than in summer (2.08±0.19 ml O_2 min^{-1}). However, the *post hoc* test failed to detect a difference between MMR_{NA} in spring (2.77±0.24 ml O_2 min^{-1}) and other seasons (Fig. 3B). Notably, three mice caught in spring experienced an anomalous heatwave during the week before capture and all three individuals achieved M_{sum} levels (range 2.24–2.80 ml O_2 min^{-1}) that were only slightly higher than their respective MMR_{NA} levels (range 1.82–2.63 ml O_2 min^{-1}). The inclusion of these data points did not alter the finding of an overall seasonal effect in the model (low spring values included: $F_{3,32}$=7.95, P<0.001 versus low spring values excluded: $F_{3,29}$=10.57, P<0.001), nor did it influence the finding that M_{sum} in summer (4.32±0.40 ml O_2 min^{-1}) was significantly lower compared with that in autumn (7.23±0.45 ml O_2 min^{-1}) and winter (6.44±0.33 ml O_2 min^{-1}). But, the inclusion of the low spring values did change the outcome of the *post hoc* comparison between spring and both summer and autumn. When excluding these low values, M_{sum} in spring (6.41±0.67 ml O_2 min^{-1}) was statistically similar to winter and autumn levels, and higher than the summer level (Fig. 3D), whereas their inclusion resulted in a decrease in mean M_{sum} in spring (5.28±0.74 ml O_2 min^{-1}) to a level that was similar to that in summer, and significantly lower than that in autumn (Fig. 3D).

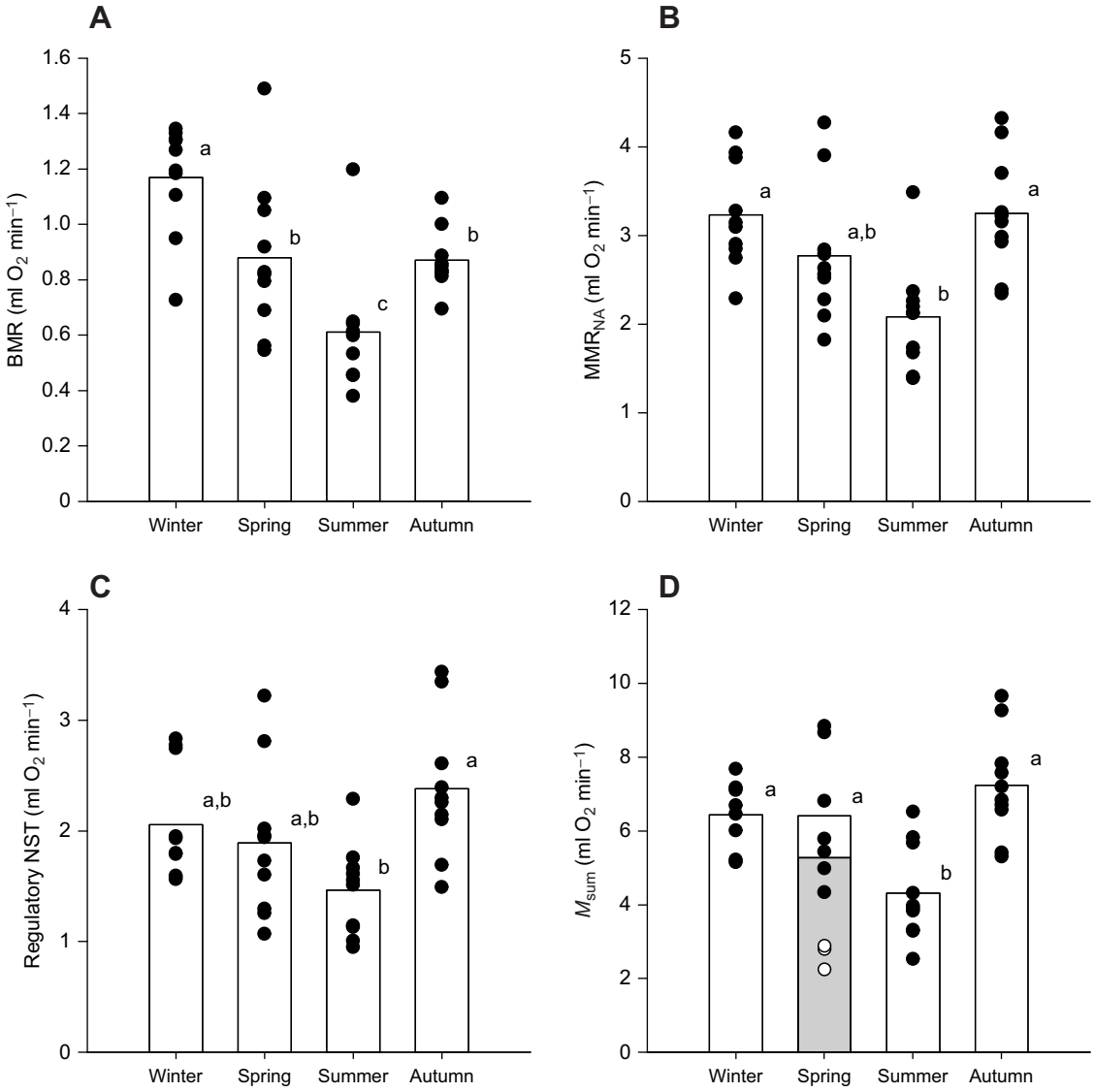

Fig. 3. Mean seasonal metabolic rate in wild-caught African striped mice, *R. pumilio*. (A) BMR, (B) MMR_{NA}, (C) regulatory non-shivering thermogenesis (NST=MMR_{NA}−BMR) and (D) M_{sum}. For each variable, means that do not share a letter differ significantly (P<0.05). Individual data points are presented as circles with the potential outlier values for M_{sum} in spring indicated as open circles. The grey bar indicates the mean M_{sum} in spring when low values are included, which is statistically similar to M_{sum} in winter and summer, and lower than M_{sum} in autumn.

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Organ mass and organ metabolic activity

Total organ mass differed seasonally ($F_{3,34}$=45.37, P<0.001) (Fig. 4), with the largest store of brown adipose tissue being located within the interscapular region (Fig. 5A). The collective organ mass was heaviest in winter (8.92±0.52 g), followed by spring (7.22±0.63 g), but was similarly low in summer (5.36±0.45 g) and autumn (5.89±0.28 g). Of all organs studied, the pancreas was the only one that did not differ in mass between seasons ($F_{3,34}$=2.10, P=0.162; pooled mean 0.39±0.03 g). The organs whose mass best explained the intra-seasonal variability in BMR differed between seasons (Table 1). The forward selection stepwise regression analysis determined that BMR during winter was best described using the mass of the heart, pancreas and kidneys ($F_{3,6}$=14.18, P<0.05, r^2=0.88), BMR in spring was best described using the mass of the intestines, pancreas, stomach and BAT ($F_{4,5}$=9.99, P<0.05, r^2=0.89), BMR in summer was best described using the mass of the heart only ($F_{1,8}$=13.13, P<0.05, r^2=0.62), whereas BMR in autumn was best described using the mass of the liver, pancreas, heart and stomach ($F_{5,4}$=28.55, P<0.05, r^2=0.97).

There was a significant seasonal difference in the mass of BAT ($F_{3,34}$=8.72, P<0.001), with mice recruiting approximately twice as much BAT in winter (0.63±0.05 g) compared with summer (0.37±0.05 g) and approximately 1.5-fold more compared with autumn (0.41±0.03 g). The mass of BAT in spring (0.55±0.8 g) did not differ from that in other seasons. Further, MMR_{NA} had no relationship with the mass of BAT within seasons but scaled positively with the mass of BAT across seasons ($F_{1,38}$=6.38, P=0.016, r^2=0.14; Fig. 5B). By contrast, regulatory NST had no relationship with the mass of BAT. Seasonal comparisons of the mass of other organs are presented in Table S1.

Total protein concentration in BAT ($F_{3,35}$=6.56, P<0.001) was approximately 25% lower in winter (21.85±0.74 µg µl^{-1}) compared with the rest of the year (autumn: 28.29±1.08 µg µl^{-1} versus summer: 29.12±1.08 µg µl^{-1} versus spring: 27.66±1.81 µg µl^{-1}), yet UCP1 signal strength did not differ seasonally ($F_{3,35}$=1.33, P=0.282), even when considering total UCP1 signal strength, measured as UCP1 signal strength multiplied by BAT mass ($F_{3,35}$=2.59, P=0.068). Similarly, there was no seasonal difference in BAT COX activity ($F_{3,32}$=2.69, P=0.063; pooled mean 362.82±23.72 nmol O_2 min^{-1} mg^{-1}), but there was a seasonal difference in total BAT COX activity measured as BAT COX activity multiplied by BAT mass ($F_{3,32}$=5.177, P=0.005; Fig. 5C). Total BAT COX activity in spring (225,891.5±26,907.7 nmol O_2 min^{-1}) was approximately twice that of summer (110,800.1±14,692.7 nmol O_2 min^{-1}), with no other differences being detected (winter: 196,110.8±26,907.7 nmol O_2 min^{-1}; autumn: 177,645.9±16,190.4 nmol O_2 min^{-1}). Regulatory NST scaled positively with total COX activity ($F_{1,38}$=7.45, P=0.009, r^2=0.16; Fig. 5D). BAT COX activity scaled negatively with the mass of BAT across seasons ($F_{1,38}$=6.72, P=0.013, r^2=−0.15; Fig. 5E) but positively with UCP1 signal strength across seasons ($F_{1,38}$=41.22, P=0.001, r^2=0.52; Fig. 5F).

There was no seasonal difference in the COX activity of liver tissue ($F_{3,35}$=1.72, P=0.181; pooled mean 2781±278 pmol O_2 min^{-1} mg^{-1}), lung tissue ($F_{3,35}$=0.55, P=0.653; pooled mean 963±221 pmol O_2 min^{-1} mg^{-1}) or kidney tissue ($F_{3,35}$=1.08,

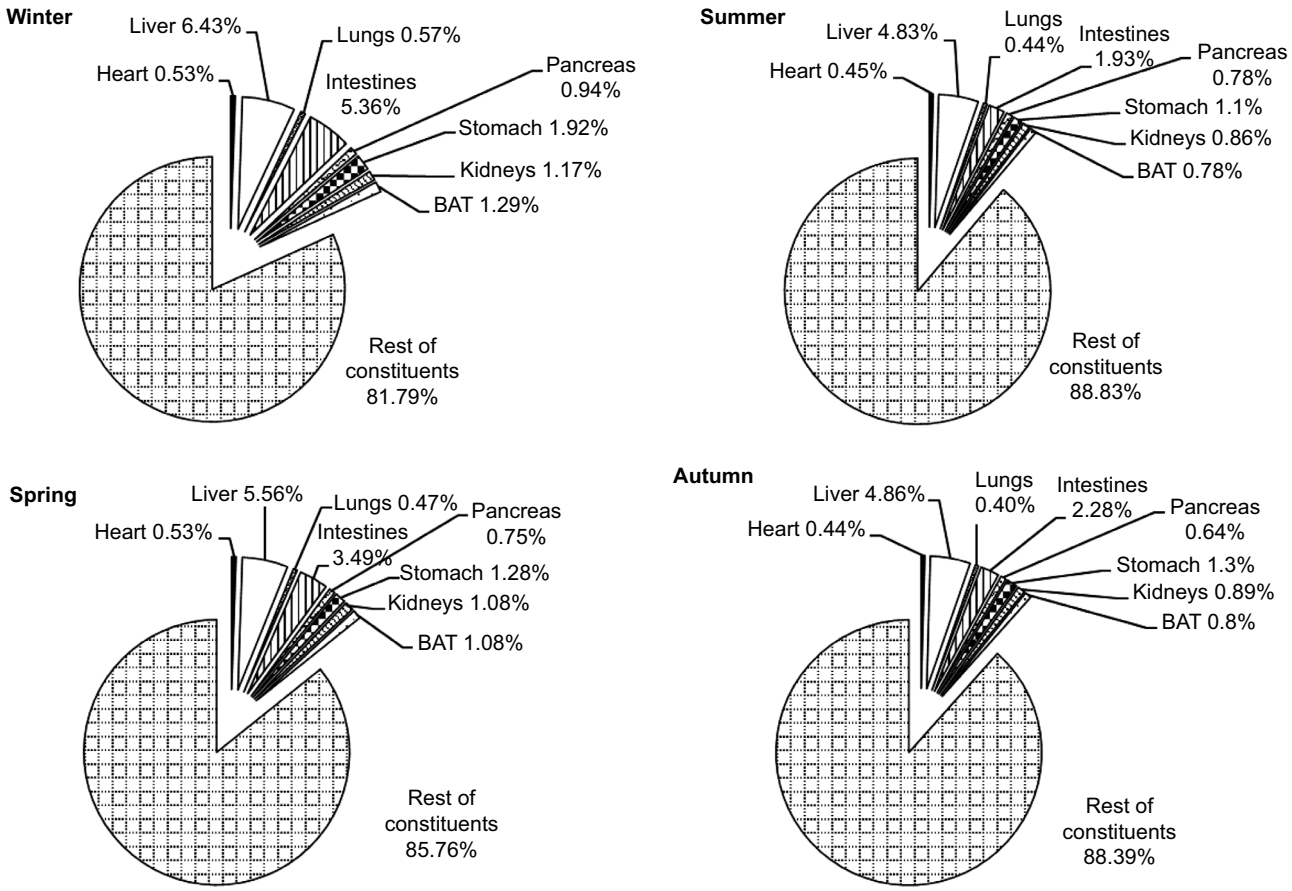

Fig. 4. The relative contribution of individual organs to body mass in wild-caught African striped mice, *R. pumilio*, in each season. BAT, brown adipose tissue.

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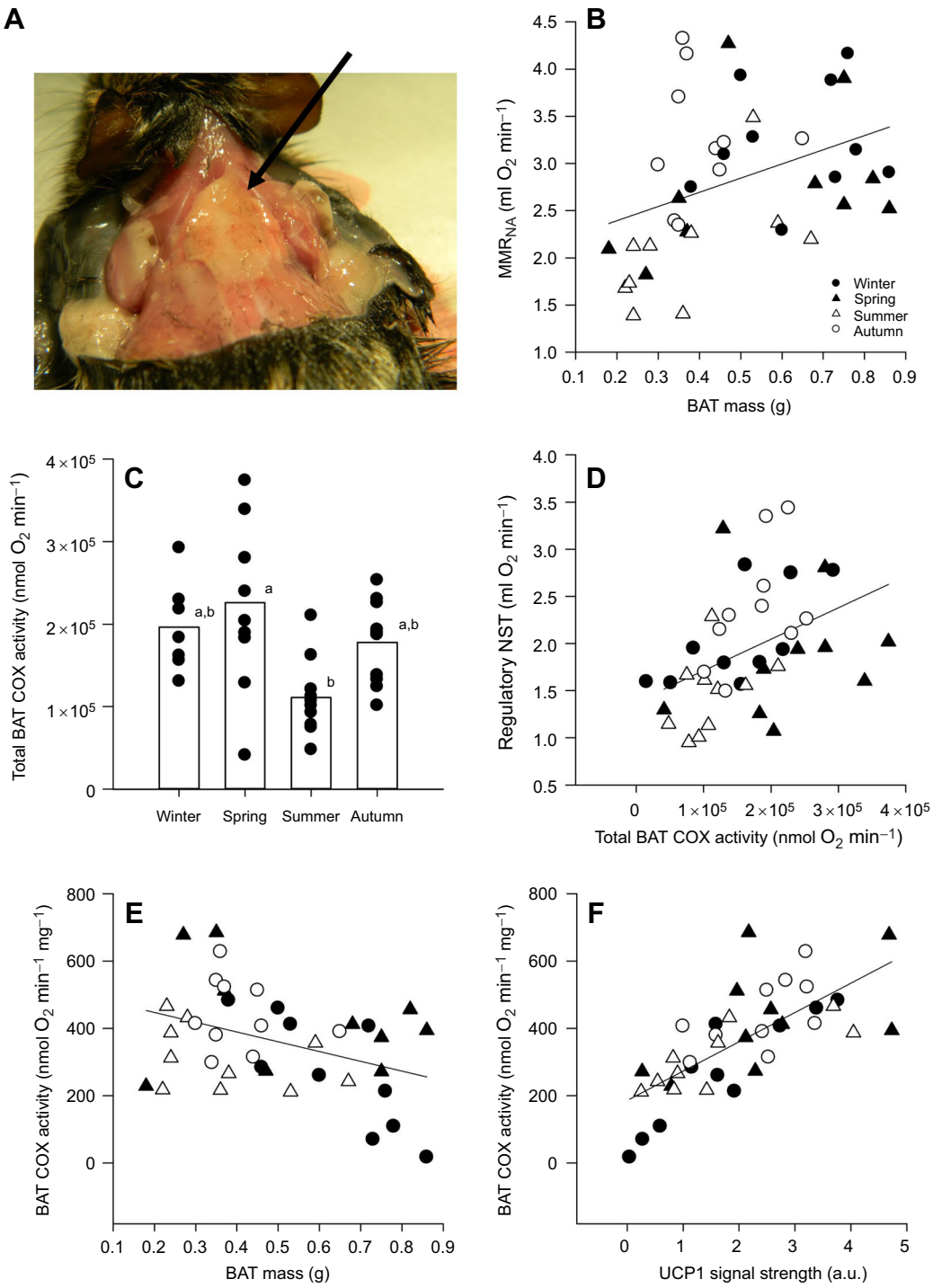

Fig. 5. Relationship between BAT and NST of wild-caught African striped mice, *R. pumilio*. (A) Appearance of interscapular adipose deposit (arrow) of a winter-caught individual, illustrating the primary BAT store. (B) Scatterplot of BAT mass and overall NST (MMR_{NA}). (C) Mean seasonal total BAT cytochrome *c* oxidase (COX) activity in BAT homogenates. Individual data points are also provided. (D) Scatterplot of regulatory NST and total BAT COX activity. (E) Scatterplot of BAT COX activity and bat mass. (F) Scatterplot of BAT COX activity and UCP1 signal strength (a.u., arbitrary units). The solid lines in scatterplots indicate significant relationships (P<0.05).

P=0.372; pooled mean 6170±525 pmol O_2 min^{-1} mg^{-1}). But, there was a seasonal difference in the COX activity of heart tissue ($F_{3,35}$=10.55, P<0.001), with COX activity being high in spring (5492±494 pmol O_2 min^{-1} mg^{-1}) and low in other seasons (autumn: 3508±314 pmol O_2 min^{-1} mg^{-1} versus summer: 2974±301 pmol O_2 min^{-1} mg^{-1} versus winter: 2992±303 pmol O_2 min^{-1} mg^{-1}).

Allometric predictions of metabolism versus metabolic rate in wild-caught mice

The BMR observed in wild-caught mice differed significantly from allometrically predicted values in all seasons (winter: $F_{7,63}$=73.36, P<0.001; spring: $F_{7,63}$=34.67, P<0.001; summer: $F_{7,63}$=117.30, P<0.001; autumn: $F_{7,63}$=173.59, P<0.001). There were only two instances when BMR of wild-caught mice matched predicted values

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Table 1. Best-fit forward selection linear regression models describing the relationship between organ mass and basal metabolic rate (BMR) in wild-caught African striped mice, *Rhabdomys pumilio*

Regression model	Model statistics
BMR_{winter}=3.93 (Heart)−0.57 (Pancreas)+0.76 (Kidneys)−0.024	$F_{3,6}$=14.18, P=0.004, r^2=0.88
BMR_{spring}=0.52 (Intestines)−0.52 (Pancreas)+0.63 (Stomach)−0.36 (BAT)−0.054	$F_{4,5}$=9.99, P=0.013, r^2=0.89
BMR_{summer}=4.94 (Heart)−0.46	$F_{1,8}$=13.13, P=0.007, r^2=0.62
BMR_{autumn}=0.27 (Liver)+1.21 (Pancreas)+0.66 (BAT)−3.03 (Heart)−0.107 (Stomach)+0.28	$F_{5,4}$=28.55, P=0.003, r^2=0.97

(Table 2); otherwise, relative to allometric predictions, BMR was lower in spring, summer and autumn (46% to 88% of allometric predictions). By contrast, BMR in winter was higher than all but one of the allometrically predicted values (Table 2). Conversely, the M_{sum} observed in wild-caught mice only differed from predicted values in summer ($F_{1,9}$=41.74, P<0.001), when it was 30% lower than predicted. There was no change in the outcome of the comparison between allometrically predicted values and the M_{sum} observed in wild-caught mice in spring, irrespective of whether the three low values were included ($F_{1,9}$=4.45, P=0.064) or excluded ($F_{1,6}$=0.33, P=0.586).

Correlation between T_a and metabolism in wild-caught mice

BMR only correlated with T_a in spring, displaying a negative correlation with the maximum (r=−0.78, P=0.008) and minimum (r=−0.76, P=0.012) temperatures experienced before capture (Table S2). MMR_{NA} displayed a negative correlation with the maximum (r=−0.78, P=0.008) temperatures experienced before capture in summer, as well as the maximum (r=−0.65, P=0.042) and minimum (r=−0.77, P=0.009) temperatures experienced before capture in autumn (Table S2). M_{sum} displayed a negative correlation with the maximum and minimum temperatures experienced before capture in both spring (T_{max}: r=−0.85, P=0.002; T_{min}: r=−0.83, P=0.003) and autumn (T_{max}: r=−0.77, P=0.009; T_{min}: r=−0.85, P=0.002) (Table S2).

DISCUSSION

The seasonal regulation of M_b is an important strategy for many small mammals as it dictates their total energy requirements (Li and Wang, 2005a,b; Wang et al., 2006; Zhang et al., 2012). Small mammals from the climatically predictable Holartic region decrease M_b and BMR to conserve energy during winter while thermoregulatory costs are high and food availability is low (Lovegrove, 2005), whereas African striped mice in a less predictable karoo environment increase M_b and BMR during winter, facilitated by an increase in food availability (Rimbach et al., 2018). The summer–winter increase in BMR in mesic habitat-adapted African striped mice thus aligned more closely with the seasonal change displayed by conspecifics in the karoo. Unfortunately, methodological differences between studies precluded a direct comparison of metabolism between mice in our study and those in Rimbach et al. (2018) but BMR of mice in summer was similar to that reported in Haim and Fourie (1980b), while BMR of mice in winter was higher than that reported in Haim and Fourie (1980a). However, unlike their arid habitat-adapted counterparts or Holarctic small mammals, mesic habitat-adapted African striped mice maintained a constant M_b throughout the year while still managing to achieve pronounced seasonal regulation of BMR. This was done through nuanced regulation of their organs, which probably benefited mice by increasing minimum heat production capacity in winter while reducing some of the increase in total energetic requirements that would otherwise have been incurred as the result of a higher M_b. This high level of phenotypic plasticity reaffirms the idea that responses of individuals may not be applicable across individuals inhabiting contrasting environments, especially those in areas with high climate unpredictability. The results also show that commonly used allometric calculations do not accurately predict the energetic requirements of this species, illustrating that the use of such an approach to study species' responses to climate change may not be appropriate (Buckley et al., 2018; Norin and Metcalfe, 2019).

It has long been known that organs such as the brain, heart, liver, kidneys and gastrointestinal tract have the greatest contribution to BMR (Krebs, 1950; Holliday et al., 1967) and metabolism usually scales with M_b because changes to the overall mass encompass all changes to organ mass. However, the relationship between BMR and individual organs may differ between species as well as between individuals of the same species depending on factors such as diet and aerobic performance, all of which vary seasonally (Konarzewski and Diamond, 1995; Ksiazek et al., 2004; Song and Wang, 2006; Xu et al., 2011). For example, small mammals that remain active at low temperatures tend to increase the mass and length of their digestive tract as an adaptive response to enhance digestive efficacy and assimilation rate to accommodate higher thermoregulatory costs and improve maintenance of M_b (Bozinovic et al., 1990; Bozinovic and Nespolo, 1997; Chi and Wang, 2011).

Table 2. Mean deviation in metabolism of wild-caught African striped mice, *R. pumilio*, relative to published allometric equations for small mammals (100%)

Variable	Method	Relationship	Reference	Winter	Spring	Summer	Autumn
BMR	OLS	$4.29M_b^{0.71}$	Bozinovic (1992)	109%*	76%***	56%***	75%***
BMR	OLS	$3.13M_b^{0.718}$	Lovegrove (2000)	144%***	101%	75%***	99%
BMR	IC	$4.20M_b^{0.679\pm0.027}$	Lovegrove (2003)	125%***	88%*	65%***	86%***
BMR	OLS	$5.21M_b^{0.642}$	Lovegrove (2003)	115%***	81%***	60%***	80%***
BMR	IC	$4.36M_b^{0.689\ (0.619–0.759)}$	Rezende et al. (2004)	116%***	81%***	60%***	80%***
BMR	OLS	$4.98M_b^{0.660\ (0.602–0.719)}$	Rezende et al. (2004)	113%***	80%***	59%***	78%***
RMR	PGLS	$9.34M_b^{0.553\pm0.024}$	Sieg et al. (2009)	91%***	65%***	47%***	64%***
M_{sum}	OLS	$27.14M_b^{0.68}$	Bozinovic (1992)	106%	99%	68%***	111%

BMR, basal metabolic rate; RMR, resting metabolic rate; M_{sum}, summit metabolic rate; OLS, ordinary least squares linear regression analysis; IC, independent contrasts; PGLS, phylogenetic generalised least square regression analysis. Table contents were adapted from White (2011). ***P<0.001; **P<0.01; *P<0.05.

Similarly, there is also an increase in the size and/or metabolic activity of the liver in response to low temperatures. Unlike the digestive tract, the liver has a direct association with thermoregulation, playing a crucial role in heat production through the conversion of glycogen to glucose to fuel metabolism (Villarin et al., 2003; Xu et al., 2011; Zhang et al., 2019). Yet, neither of these organs appeared to explain the variability of winter BMR in African striped mice, even though both were significantly heavier in winter compared with other seasons. Instead, the results highlight the pancreas as an important predictor of BMR.

We found that the pancreas was the only organ studied that did not show seasonal changes in mass. Yet, the results show that intraspecific variation in the pancreas was related to the variation in BMR in all seasons but summer. The pancreas regulates blood glucose levels through the production of insulin and glucagon hormones (Chang and Leung, 2014). It also contributes to digestion through the production of the hormone gastrin and various enzymes that assist the breakdown of fats, carbohydrates and proteins (Chang and Leung, 2014). Therefore, although the underlying mechanisms of pancreatic function or its seasonal regulation are not fully understood (Chang and Leung, 2014), as argued for the liver and digestive tract, the pancreas' role in energy production and digestive efficacy justifies its seasonal importance.

Seasonal changes in BAT mass did not accurately reflect the seasonal changes in NST capacity. African striped mice are known to increase NST following acclimation to cold or short photoperiods (Haim and Fourie, 1980a,b). Thus, a summer–winter increase in BAT mass and NST capacity was not surprising. However, the inclusion of autumn and spring seasons revealed an unexpected yearly pattern. Curiously, although we measured signs of adaptivity of BAT, the important parameters UCP1 and COX (for oxidative capacity) did not exhibit the pronounced seasonal increases as seen in most Holarctic rodent species, where changes in photoperiod alone quadruple the oxidative power of BAT (Heldmaier et al., 1981). Further, while rodents generally increase NST in winter through increased BAT stores, brown adipocyte phenotype or BAT mitochondrial activity (Klingenspor et al., 1996; Van Sant and Hammond, 2008; Beaudry and McClelland, 2010), we found that NST capacity in African striped mice already reached maximal rates in autumn and mice then remained in a constant state of 'thermogenic readiness' until summer. To do so, mice relied on trade-offs between BAT mass and COX activity. While overall NST capacity (MMR_{NA}) correlated with BAT mass, regulatory NST did not. Regulatory NST was however related to BAT COX activity but the inverse relationship between BAT mass and COX activity illustrates that the oxidative power of BAT was not maintained at a high level throughout the season. Thus, to maintain NST during winter, spring and autumn, mice regulated BAT COX activity in relation to BAT mass to achieve a similar overall oxidative power (i.e. total BAT COX activity).

So far, the results illustrate that African striped mice upregulate BMR and NST immediately after summer but their respective overall seasonal patterns differ throughout the rest of the year. Because BMR is dictated by changes to organs, its thermogenic contribution is fixed over a long period of time. Mice would benefit from seasonally higher BMR because it would allow them to defend normothermia at relatively cooler temperatures before needing to activate the BAT-mediated component of NST (Heldmaier et al., 1990). Thus, the change in BMR can be viewed as a slower paced adjustment in minimum heat production capacity responding to the general change in temperature over a relatively longer period of time, whereas the change in NST represents a more immediate response to changes in minimum and maximum temperature on a daily basis. Both BMR (Rosenmann et al., 1975; Bozinovic et al., 1990; Bozinovic, 1992; Rezende et al., 2004) and NST (Heldmaier and Buchberger, 1985; Cannon and Nedergaard, 2004) correlate with M_{sum} in placental mammals. However, we found that the seasonal change in M_{sum} was identical to that of NST. This confirms the overall strategy adopted by these mice as increasing heat production almost all year round so that they are able to defend body temperature against low winter-like temperatures. Notably, for their size, African striped mice showed M_{sum} comparable to that of Neotropical species, many of which inhabit much colder climates (Bozinovic, 1992).

Presumably, the year-round availability of food explains how these mice could accrue enough energy reserves to allow them to maintain a high heat production capacity. But, why did mice adopt such an energetically expensive strategy to maintain this high level throughout the year? We propose the high climatic unpredictability of their habitat as the reason. Climatic unpredictability is known to cause aseasonal use of torpor in many species (Mzilikazi et al., 2002; Mzilikazi and Lovegrove, 2004; Nowack et al., 2015; Doty et al., 2016; Nowack et al., 2016). It is also proposed as the explanation why rock elephant shrews seem to recruit sufficient BAT reserves to maintain 'thermogenic readiness' throughout the year, not just in winter (Mzilikazi et al., 2007). Certainly, a high NST capacity would allow these elephant shrews to deal with low ambient temperatures and resource unpredictability faced in their habitat, facilitating a return to normothermia from a bout of torpor. By contrast, African striped mice are obligatory homeothermic animals and must rely on endogenous heat production to defend normothermia at all times, in spite of any climatic unpredictability. We argue that sporadic cold spells at the study site pressurized African striped mice to maintain a high level of heat production throughout the year. These mice have exceptionally poor insulation and lose heat readily (Haim and Fourie, 1980b), which poses a challenge at night when temperatures are lowest and passive exogenous heat gain is not possible. For mice in our study, the difficulty of defending normothermia at low temperature would also have been compounded by their ecology, with mesic habitat-adapted mice opting for a solitary living. Thus, they would not have benefited from huddling to keep warm and to save energy during cold periods (Schradin, 2005).

To illustrate the point we raise above, we selected 6°C as a threshold T_a for severe cold, where mice are likely to be challenged by temperatures close to their tolerable limits. This decision was based on the observation that exposure to 6°C elicited a response in African striped mice that was close to their maximum heat production capacity (Haim and Fourie, 1980b). Although minimum temperatures at the study site most often dropped below 6°C in winter, we noted that temperatures also approached and occasionally dropped below the threshold in spring and autumn as well. Arguably, the need to deal with these sporadic low temperatures, while faced with other seasonally variable selective pressures such as reproduction or changes in diet, resulted in trade-offs between M_b, organ mass and BAT thermogenic capacity. This is of concern as the thermoregulatory strategy used by African striped mice commits them to securing enough food to meet the associated higher energetic costs. These mice are known to have poor survival and slow growth when faced with limited resources (Rimbach et al., 2018, 2021). Given the likelihood of decreased food security due to changing weather patterns as climate change continues, the collective evidence suggests that African striped mice are vulnerable to large mesic population declines in the future.

Acknowledgements
We thank Rebecca Oelkrug, Saskia Muller and Maria Kutschke for technical assistance and discussions related to mitochondrial respiration and western blotting. We also thank the two anonymous reviewers for their valuable contribution to improving the manuscript.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: S.W., M.J., N.M.; Methodology: S.W., M.J.; Formal analysis: S.W.; Investigation: S.W.; Writing - original draft: S.W.; Writing - review & editing: M.J., N.M.; Supervision: N.M.; Funding acquisition: N.M.

Funding
This study was partially funded by the Research Capacity Development stream of the Nelson Mandela University and the National Research Foundation of South Africa. Opinions expressed and conclusions arrived at are those of the authors and are not necessarily to be attributed to any of the above-mentioned donors.

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