

Metabolic, Ventilatory, and Hygric Physiology of the Gracile Mouse Opossum (*Gracilinanus agilis*)

C. E. Cooper^{1,2,*}

P. C. Withers^{1,2}

A. P. Cruz-Neto^{1,2,3}

¹Centre of Ecosystem Diversity and Dynamics, Department of Environmental Biology, Curtin University of Technology, P.O. Box U1987, Bentley Delivery Centre, Western Australia 6845, Australia; ²Department of Zoology, School of Animal Biology, Internal Mail M092, University of Western Australia, Crawley, Western Australia 6009, Australia; ³Departamento de Zoologia, Universidade Estadual Paulista, Avenida 24-A, Bela Vista, Rio Claro, 13506-960 São Paulo, Brazil

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ABSTRACT

We present the first complete study of basic laboratory-measured physiological variables (metabolism, thermoregulation, evaporative water loss, and ventilation) for a South American marsupial, the gracile mouse opossum (*Gracilinanus agilis*). Body temperature (T_b) was thermolabile below thermoneutrality ($T_b = 33.5^\circ\text{C}$), but a substantial gradient between T_b and ambient temperature (T_a) was sustained even at $T_a = 12^\circ\text{C}$ ($T_b = 30.6^\circ\text{C}$). Basal metabolic rate of $1.00 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ at $T_a = 30^\circ\text{C}$ conformed to the general allometric relationship for marsupials, as did wet thermal conductance ($5.7 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$). Respiratory rate, tidal volume, and minute volume at thermoneutrality matched metabolic demand such that O_2 extraction was 12.4%, and ventilation increased in proportion to metabolic rate at low T_a . Ventilatory accommodation of increased metabolic rate at low T_a was by an increase in respiratory rate rather than by tidal volume or O_2 extraction. Evaporative water loss at the lower limit of thermoneutrality conformed to that of other marsupials. Relative water economy was negative at thermoneutrality but positive below $T_a = 12^\circ\text{C}$. Interestingly, the Neotropical gracile mouse opossums have a more positive water economy at low T_a than an Australian arid-zone marsupial, perhaps reflecting seasonal variation in water availability for the mouse opossum. Torpor occurred at low T_a , with spontaneous arousal when $T_b > 20^\circ\text{C}$. Torpor resulted in absolute energy and water savings but lower relative water economy. We found no evidence that gracile

mouse opossums differ physiologically from other marsupials, despite their Neotropical distribution, sympatry with placental mammals, and long period of separation from Australian marsupials.

Introduction

Australia is widely perceived to be the major center of marsupial diversity (147 recent species in 55 genera of 16 families; Strahan 2002), and the majority of marsupial research involves Australian species. However, the marsupial fauna of South and Central America is also diverse, in terms of both species numbers (76 species representing 19 genera and four families) and occupied ecological niches (Emmons and Feer 1997; Eisenberg and Redford 1999). This American fauna represents a relict of a once more extensive marsupial radiation, and the four extant families that make up the Didelphimorphia are relatively distantly related, having been separate since the early Tertiary period (Tyndale-Biscoe 2005). The Didelphimorphia occupy a wide range of climates and habitats and include species with niches that have not been exploited by the Australian marsupial radiation (e.g., semiaquatic marsupials), but they are morphologically less diverse and show less dietary variation than Australian marsupials. This often-overlooked American fauna merits attention similar to that given to Australian marsupials, and it provides an important phylogenetic comparison with Australian species.

Our basic knowledge of the metabolic physiology of South American marsupials is limited to measurement of oxygen consumption ($\dot{V}\text{O}_2$) and body temperature (T_b) for 13 species (Morrison and McNab 1962; McNab 1978; Dawson and Olson 1988; Nespolo et al. 2002; Bozinovic et al. 2004, 2005, 2007; Ribeiro and Bicudo 2007). This data set includes a single individual of a species of mouse opossum thought to be *Marmosa microtarsus* (now *Gracilinanus microtarsus* Tate 1932; Morrison and McNab 1962; Gardner and Creighton 1989). We are not aware of any study of ventilatory or hygric physiology for a South American marsupial.

Here we examine a further species of South American marsupial, the gracile mouse opossum (*Gracilinanus agilis*; family Didelphidae), to present the first complete data set for thermal, metabolic, ventilatory, and hygric physiology for a didelphid marsupial. We compare its physiology with that of other marsupials to examine potential environmental and adaptive responses in a Neotropical habitat. Gracile mouse opossums are small (adult mass 12–45 g), sexually dimorphic, short-lived (1–1.5 yr), partially semelparous, solitary, nocturnal, and predom-

*Corresponding author; e-mail: c.cooper@curtin.edu.au.

inantly arboreal marsupials that inhabit the Cerrado biome in southeastern and southern Brazil (Eisenberg and Redford 1999; Costa et al. 2003; Martins et al. 2006a, 2006b; Rossi et al. 2006). Extensive studies of this species indicated a predominantly insectivorous diet, with some fruit. (Martins and Bonato 2004; Martins et al. 2006c, 2006d).

Material and Methods

Nine adult nonreproductive gracile mouse opossums (seven males and two females) were trapped at the Clube Náutico Araraquara (21°43'S, 48°01'W). The area is *cerradão*, a physiognomy of the cerrado biome characterized by a dense forest with tall trees and closed canopy, with marked seasonal variation in temperature and rainfall (see Martins et al. 2006a for a complete description of the study area). We consider that the species of mouse opossum studied here is *Gracilinanus agilis* based on the description, distribution, and habitat of this species (Tate 1933; Gardner and Creighton 1989; Costa et al. 2003; L. Costa, Universidade Federal do Espírito Santo, personal communication).

Gracile mouse opossums were captured in Sherman cage traps placed on tree branches 150–180 cm above ground level and baited with peanut butter and rolled oats, cat biscuits, and cod liver oil. Mouse opossums were returned to Universidade Estadual Paulista (UNESP), Rio Claro, and housed in individual wooden cages in an animal room maintained at approximately 23°C with a 12L : 12D cycle. Opossums were provided with ad lib. food (tinned cat food, dry cat biscuits, mango, pawpaw, and banana) and water. Experiments commenced within 2 wk of capture, over the period of December–February 2006/2007.

Standard flow-through respirometry was used to measure rates of $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), and evaporative water loss (EWL) at ambient temperatures (T_a 's) of 12°, 20°, 28°, 30°, and 32°C. We used three separate metabolic systems. Two systems consisted of an Aalborg mass flow controller (GFC17) that regulated compressed air at flow rates of 500 to 1,000 mL min⁻¹ (depending on T_a). Air then passed through a 360-mL metabolic chamber that was in an Electrolab 122FC temperature control cabinet before passing through a Vaisala HMP 45A temperature and humidity probe. Calculated 99% equilibrium times were 1.7–3.3 min (calculated after Lasiewski et al. 1966). A manifold was placed after the humidity probe, and 100 mL min⁻¹ of excurrent air was subsampled to measure CO₂ and O₂ levels (after air was dried with Drierite), using a Sable Systems Foxbox. The Vaisala temperature and humidity outputs were connected to the analog inputs of the Foxbox. The third system consisted of a Sable Systems two-channel mass flow controller (V 1.1), relative humidity (RH) meter (100 RH/Dewpoint meter), TC-1000 thermometer, CA-2A CO₂ analyzer, FC-1B O₂ analyzer, and PP-2 dual subsampling pump (V 1.0). Flowmeters were calibrated using a bubble flowmeter, O₂ analyzers were calibrated to room air (20.95% O₂), and CO₂ analyzers were calibrated using a Cameron Instruments mass-flow gas mixer (model GF3/MP) at concentrations of 0.5%, 1.0%, and 1.5% CO₂ in a N₂/O₂ mix. The calibration of the RH probes

was confirmed using two points, 1% RH (dried with Drierite to 0.005 mg L⁻¹; http://www.drierite.com/default.cfm?goto_page=page4a.cfm) and 100% RH (saturated; generated by breathing on the probe).

The two Foxboxes were each connected via an RS232 interface to a laptop PC, and the voltage outputs for O₂, CO₂, RH, and T_a were recorded every 10 s with a custom-written Visual Basic (ver. 6) data acquisition program (P. Withers). The analyzers for the third system were interfaced to a PC using a Sable Systems UI2 analog-to-digital converter, and the output was recorded every 10 s with the same data acquisition program.

Ventilatory data were measured using the metabolic chamber as a whole-body plethysmograph (Malan 1973; Withers 1977; Dawson et al. 2000; Larcombe 2002; Cooper and Withers 2004b). Pressure changes due to the warming and humidifying of inspired air were detected with a custom-made pressure transducer (Motorola MPX2010 sensor; Physiology Department, University of Western Australia; systems 1 and 2) or a Sable Systems PT-100B pressure transducer (system 3). Analog voltage outputs from the pressure transducers were converted to digital signals using a Pico Technology ADC 11 data logger and were recorded on a PC every 15 ms for approximately 30 s using PicoScope. Between two and six sets of ventilatory data were obtained for an individual opossum at each T_a , so a single mean was calculated for each ventilatory variable for each opossum at each T_a . The plethysmography system was calibrated after Szewczak and Powell (2003).

Gracile mouse opossums were fasted for 24 h before the commencement of experiments. They were measured in the metabolic systems for no less than 8 h at each T_a , during their inactive phase (day), until $\dot{V}O_2$, $\dot{V}CO_2$, and EWL had become stable and minimal. We calculated $\dot{V}O_2$, $\dot{V}CO_2$, and EWL at each T_a from the average of each variable over the 20-min period where they were stable and minimal. Calculations were after Withers (2000) and were accomplished using a custom written Visual Basic data analysis program (P. Withers). Respiratory exchange ratio (RER) and evaporative quotient (EQ) were calculated as $\dot{V}CO_2/\dot{V}O_2$ and EWL/ $\dot{V}O_2$, respectively. Wet (C_{wet}) and dry (C_{dry}) thermal conductance (J g⁻¹ h⁻¹ °C⁻¹) were calculated as $MR/(T_b - T_a)$ and $(MHP - EHL)/(T_b - T_a)$, respectively, where MR is metabolic rate, MHP is metabolic heat production, and EHL is evaporative heat loss, and where EWL was converted to EHL using 2.4 J mg⁻¹ H₂O (Withers 1992) and MR was converted to MHP using the oxycaloric coefficient at the measured RER for that experiment (interpolated from table 4.2 in Withers 1992; e.g., 19.5 J mL⁻¹ O₂ at RER = 0.7 to 21.4 J mL⁻¹ O₂ at RER = 1.0). Metabolic water production (MWP; mL g⁻¹ h⁻¹) was also calculated using the measured RER for that experiment after Withers (1992). Relative water economy (RWE) was calculated as MWP/EWL. The CO₂ analyzer of one Foxbox (system 2) was not functional; therefore, for nine (of 32) experiments, the $\dot{V}O_2$ and EWL were calculated after Koteja (1996), assuming an RER of 0.85. An RER of 0.85 was also assumed when converting MR to joules and calculating MWP for these nine experiments.

Ventilatory measurements were made at the end of each

experiment, after which the mouse opossum was immediately removed from the chamber, and its T_b was measured using a plastic-tipped thermocouple (connected to a Radio Spares 611–234 thermocouple meter) inserted ~ 1 cm into the cloaca. Ventilatory variables (respiratory frequency, f_R ; tidal volume, V_T ; minute volume, V_I , and oxygen extraction, EO_2) were calculated after Malan (1973) and Cooper and Withers (2004b). We used the technique of Szewczak and Powell (2003) to mathematically convert our open plethysmograph system to a closed system to account for variation in the time course of calibration injections and breathing pressure pulses. A custom-written Visual Basic data analysis program (P. Withers and C. Cooper) was used for ventilatory calculations. Values of EO_2 were calculated using the $\dot{V}O_2$ at the time of ventilatory measurements. Values of T_b were obtained for some torpid animals (for which it was certain that they had attained a steady and minimal torpor $\dot{V}O_2$ and EWL; $n = 4$ of 6 at $T_a = 11.5^\circ\text{C}$, and $n = 3$ of 5 at $T_a = 20^\circ\text{C}$) by removing the animals from the chamber, measuring their T_b , and then returning them to the chamber to allow them to arouse.

All values are presented as mean \pm SE, where n = number of individuals. Ventilatory values are presented at body temperature and pressure saturated conditions, although standard temperature and pressure dry conditions were used to calculate EO_2 . Effects of T_a and torpor on physiological variables were examined with ANOVA with linear a priori contrasts and Student-Newman-Keuls (SNK) post hoc tests. Statistical analyses were conducted using SPSS (student ver. 11.0) and statistiXL (ver. 1.6). The experimental procedures were approved by the UNESCO ethics committee (056/06-CEA).

Results

Body mass for the seven male gracile mouse opossums was 29.1 g (range 12–43.6 g) and for the two females was 19.6 g (range 12–26 g). Mean T_b of normothermic mouse opossums ranged from $30.6^\circ \pm 1.50^\circ\text{C}$ at $T_a = 12.1^\circ\text{C}$ ($n = 8$) to $34.4^\circ \pm 0.44^\circ\text{C}$ at $T_a = 32.1^\circ\text{C}$ ($n = 9$; Fig. 1A). There was a significant effect of T_a on T_b (linear contrast $P = 0.001$), with T_b at 11.5°C significantly different from that at 32.1°C (SNK $P = 0.021$). Gracile mouse opossums entered torpor at 12° ($n = 6$) and 20°C ($n = 5$), with T_b significantly lower than for normothermic opossums at these values of T_a ($F_{1,17} = 49.6$, $P < 0.001$). The lowest T_b we measured was 14.6°C at $T_a = 12^\circ\text{C}$. Two mouse opossums with $T_b < 20^\circ\text{C}$ did not spontaneously arouse from torpor, even after disturbance, although they did return to a normothermic T_b when allowed to rewarm at room temperature. Mouse opossums did, however, always spontaneously arouse at $T_b > 20^\circ\text{C}$.

There was a significant effect of T_a on $\dot{V}O_2$ (linear contrast $P < 0.001$; Fig. 1B) for normothermic gracile mouse opossums, with MR at 12°C ($2.28 \pm 0.214 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$) being higher (SNK $P < 0.001$) than that at 20°C ($2.17 \pm 0.108 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$). Values of MR at 12° and 20°C were also higher (SNK $P < 0.001$) than at T_a 's of 28° ($1.12 \pm 0.098 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$),

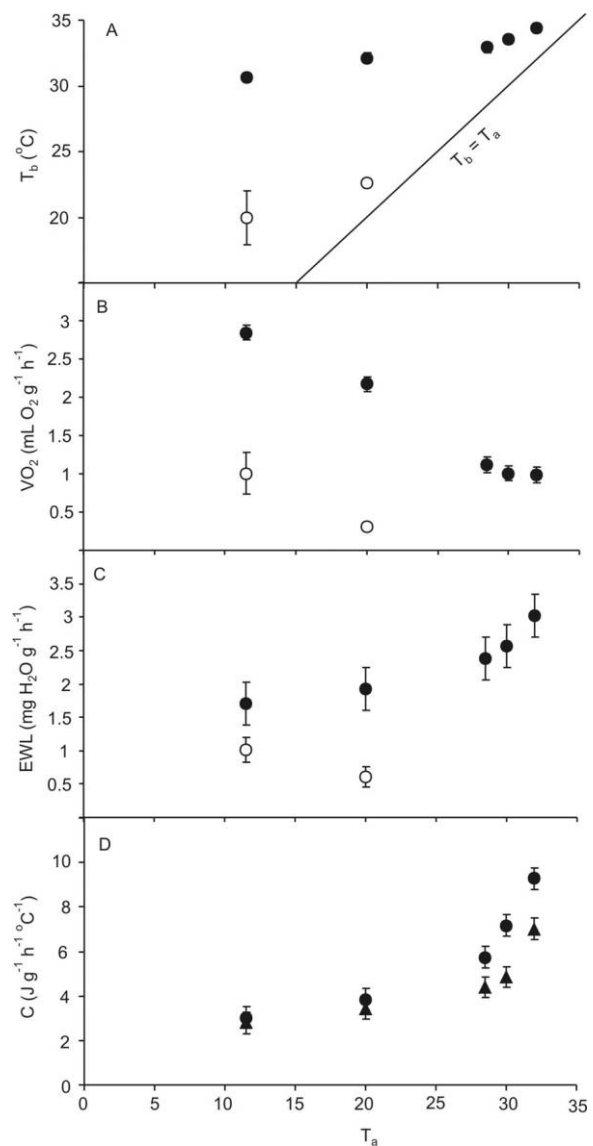


Figure 1. Body temperature (A), metabolic rate (B), evaporative water loss (C), and wet (circles) and dry (triangles) thermal conductance (D) of gracile mouse opossums at various ambient temperatures. Values are mean \pm SE. For normothermic animals (solid symbols), $n = 6$ at each T_a , except 20°C , where $n = 8$. For torpid animals (open symbols), $n = 6$ at $T_a = 11.5^\circ\text{C}$, and $n = 5$ at $T_a = 20^\circ\text{C}$.

30° ($1.00 \pm 0.051 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$), and 32°C ($0.98 \pm 0.068 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$). We consider the mean $\dot{V}O_2$ at 30°C of $1.00 \pm 0.051 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ to be basal MR (BMR; mean mass = $31.4 \pm 2.64 \text{ g}$; range = 26.1–43.6 g; all adult individuals). Data for $\dot{V}CO_2$ essentially mirrored those for $\dot{V}O_2$ and are not presented separately here. RER was not influenced by T_a (linear contrast $P = 0.181$), with the mean RER for all animals over all experiments being 0.87 ± 0.081 . During torpor, $\dot{V}O_2$ was significantly lower ($F_{1,21} = 103$, $P < 0.001$; Fig. 1B) than in normothermic opossums, being 1.00 ± 0.272 and $0.30 \pm 0.035 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ at $T_a = 12^\circ$ and 20°C , respectively, or 35% and 14% of the respective normothermic $\dot{V}O_2$ values.

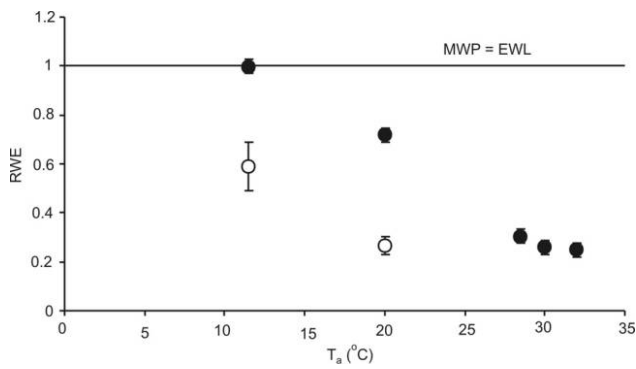


Figure 2. Relative water economy (RWE) of gracile mouse opossums calculated as metabolic water production (MWP)/evaporative water loss (EWL) at various ambient temperatures (T_a 's). Values are mean \pm SE. For normothermic animals (solid symbols), $n = 6$ at each T_a , except 20°C, where $n = 8$. For torpid animals (open symbols), $n = 6$ at $T_a = 11.5^\circ\text{C}$, and $n = 5$ at $T_a = 20^\circ\text{C}$.

There was a significant influence of T_a on both C_{wet} and C_{dry} for gracile mouse opossums (Fig. 1D). Both measures of conductance increased with T_a from 3.00 ± 0.178 (C_{wet}) and $2.78 \pm 0.161 \text{ J g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ (C_{dry}) at 12°C to 9.86 ± 1.492 (C_{wet}) and $6.17 \pm 0.934 \text{ J g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ (C_{dry}) at 32°C (linear contrast $P < 0.001$ for both C_{wet} and C_{dry}). We found that C_{wet} was significantly higher than C_{dry} ($F_{1,54} = 12.2$, $P = 0.001$), and this difference increased as T_a (and thus EWL) increased.

EWL increased with T_a from 12° to 32°C (linear contrast $P < 0.001$; Fig. 1C). EWL at $T_a = 12^\circ\text{C}$ ($1.70 \pm 0.083 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) was lower than values at $T_a = 30^\circ$ ($2.56 \pm 0.218 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$; SNK $P = 0.030$) and 32°C ($3.03 \pm 0.190 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$; SNK $P = 0.001$). EWL at $T_a = 20^\circ\text{C}$ ($1.93 \pm 0.142 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) was lower than that at 32°C (SNK $P = 0.002$). There was a significant effect of T_a on EQ, with EQ increasing with T_a (linear contrast $P < 0.001$) from 0.61 to $3.13 \text{ mg H}_2\text{O mL}^{-1} \text{ O}_2$ at 11° and 32°C , respectively. EQ at $T_a = 12^\circ$ and 20°C was significantly lower (SNK $P < 0.001$) than at $T_a = 28^\circ$, 30° , and 32°C . EQ at $T_a = 28^\circ\text{C}$ was lower than at $T_a = 32^\circ\text{C}$ (SNK $P = 0.009$). During torpor, EWL was significantly lower than for normothermic mouse opossums ($F_{1,19} = 39.7$, $P < 0.001$), being 68% ($1.15 \pm 0.202 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) and 31% ($1.70 \pm 0.083 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) of respective normothermic EWL, at $T_a = 12^\circ$ and 20°C , respectively.

MWP decreased with T_a from 12° to 32°C (linear contrast $P < 0.001$), mirroring the effects of T_a on $\dot{V}\text{O}_2$. RWE was strongly influenced by T_a (linear contrast $P < 0.001$; Fig. 2), increasing as T_a decreased, from 0.20 ± 0.034 at $T_a = 32^\circ\text{C}$ to 1.06 ± 0.085 at $T_a = 12^\circ\text{C}$. RWE at $T_a = 12^\circ\text{C}$ was significantly higher than that at all other T_a 's (SNK $P < 0.001$), and RWE at $T_a = 20^\circ\text{C}$ (0.74 ± 0.152) was higher than at 28° , 30° , and 32°C ($P < 0.001$). RWE of torpid mouse opossums (0.26 ± 0.035 at $T_a = 20^\circ\text{C}$ and 0.65 ± 0.143 at $T_a = 12^\circ\text{C}$) was lower than that of normothermic mouse opossums at equivalent T_a ($F_{1,19} = 31$, $P < 0.001$).

There was a significant negative relationship between T_a and f_R for normothermic mouse opossums (linear contrast $P <$

0.001 ; Fig. 3A), with f_R ranging from $57 \pm 11.6 \text{ breaths min}^{-1}$ at $T_a = 32^\circ\text{C}$ to $155 \pm 11.6 \text{ breaths min}^{-1}$ at $T_a = 12^\circ\text{C}$. At $T_a = 12^\circ\text{C}$, f_R was significantly higher than at all other T_a 's (SNK $P < 0.001$), while f_R at $T_a = 20^\circ\text{C}$ ($101 \pm 11.2 \text{ breaths min}^{-1}$) was higher than that at $T_a = 30.3^\circ$ ($59 \pm 10.8 \text{ breaths min}^{-1}$) and 32.1°C . There was no effect of T_a on V_T (linear contrast $P = 0.818$; Fig. 3B), with the mean V_T of all animals at all temperatures being $0.59 \pm 0.042 \text{ mL}$. However, T_a did have a significant effect on V_I (linear contrast $P < 0.001$; Fig. 3C), with V_I at $T_a = 12^\circ$ ($83 \pm 12.8 \text{ mL min}^{-1}$) and 20°C (79 mL min^{-1}) significantly higher than at 28° ($33 \pm 12.8 \text{ mL min}^{-1}$), 30° ($38 \pm 9.4 \text{ mL min}^{-1}$), and 32°C ($34 \pm 8.6 \text{ mL min}^{-1}$). There was no effect of T_a on EO_2 (linear contrast $P = 0.966$; Fig. 3D), with the mean for all animals and temperatures being $12.4\% \pm 0.94\%$. Ventilation could not be measured reliably for torpid gracile mouse opossums because of their infrequent breaths with very small pressure changes, reflecting their small $T_b - T_a$ difference.

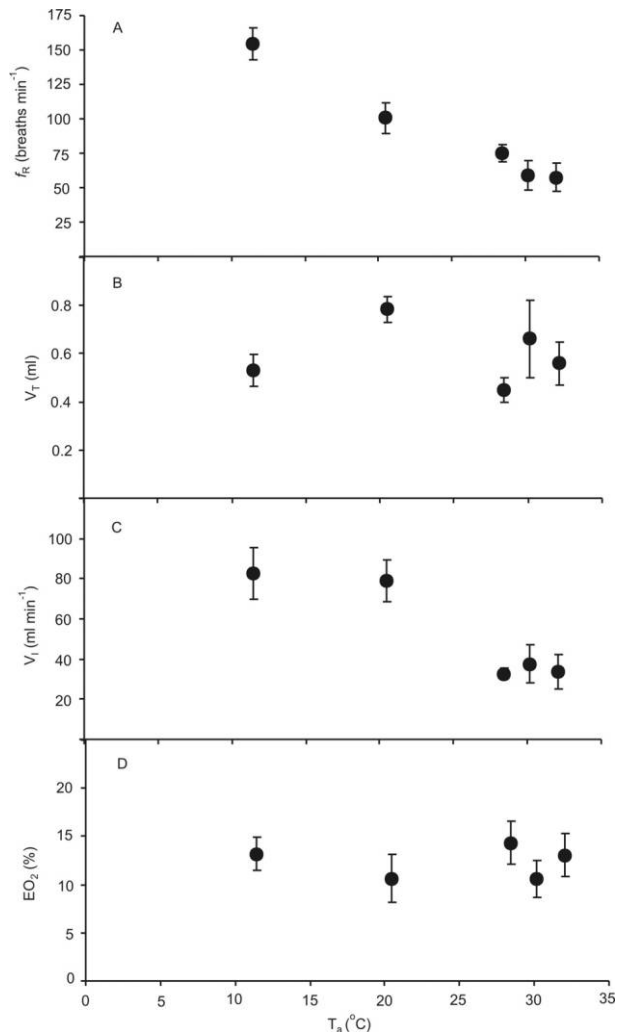


Figure 3. Ventilatory frequency (A), tidal volume (B), minute volume (C), and oxygen extraction (D) of gracile mouse opossums at various ambient temperatures. Values are mean \pm SE. For all animals, $n = 6$ at each T_a , except 30°C , where $n = 5$.

Discussion

The metabolic, hygric, and ventilatory physiology of the gracile mouse opossum is consistent with that of other marsupials. We find no evidence that this Neotropical marsupial species differs physiologically from other marsupials, despite its geographical distribution and sympatric long-term survival with placental mammals. Significant adaptation of physiological variables of marsupials is therefore not essential for survival of marsupials in the Americas.

Thermal and Metabolic Physiology

The thermoneutral rest phase T_b ($T_a = 30^\circ\text{C}$) of $33.5 \pm 0.68^\circ\text{C}$ for normothermic gracile mouse opossums is slightly lower than the T_b of approximately 34.9°C for the mouse opossum (presumably *Gracilinanus microtarsus*) measured by Morrison and McNab (1962) at 30°C . Our T_b value is 96% of that predicted for a marsupial of equivalent mass (Withers et al. 2006). This T_b conforms to the general marsupial allometric relationship, both before and after correction for phylogenetic history, falling within the 95% prediction limits for a further datum (data from and calculations after Voss et al. 2005; Cooper and Withers 2006; Withers et al. 2006). Normothermic mouse opossums are quite thermolabile, with T_b decreasing significantly with decreasing T_a . However, they are able to maintain a substantial $T_b - T_a$ differential during normothermia, even at T_a 's as low as 12°C ($\Delta T = 19.3^\circ \pm 1.0^\circ\text{C}$).

The BMR of adult gracile mouse opossums ($1.00 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ at $T_a = 30^\circ\text{C}$) is 96% of that predicted for a marsupial of equivalent mass, calculated by least squares regression from \log_{10} -transformed data of Withers et al. (2006), using Hayes and Shonkwiler's (2006, 2007) correction for an unbiased antilog transformation for prediction from a log-transformed allometric equation. This correction is necessary because the estimated y value from a double log regression is an arithmetic mean of logarithms, which is equivalent to a geometric mean when untransformed to the original scale and thus underestimates the actual predicted y value (Hayes and Shonkwiler 2006). The BMR of mouse opossums conforms to this allometric relationship both before and after phylogenetic correction (calculated after Withers et al. 2006), falling well inside the 95% prediction limits for a further datum. We find no evidence for an elevated BMR for this South American marsupial compared with other marsupials, despite McNab's (1978) suggestion that Neotropical South American marsupials have an elevated BMR. The BMR of $1.8 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ for a 13-g *G. microtarsus*, as reported by Morrison and McNab (1962), is appreciably higher than the BMR of 31-g *Gracilinanus agilis*, even after accounting for the mass difference (a 13-g *G. agilis* would have a BMR of $1.24 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$; MR is proportional to $\text{mass}^{0.75}$). However, they measured two MRs for their animal in thermoneutrality; $1.8 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ was reported as BMR and $1.00 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ was suggested as reflecting a state of hypothermia. Our results for *G. agilis* suggest that their value of $1.00 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ was BMR, and the higher

value of $1.8 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ might have been a hypermetabolic state related to activity and elevated T_b during experiments. This interpretation is supported by consideration of C_{wet} (we can calculate a similar C_{wet} for animals from both studies, indicating that the higher MR of *G. microtarsus* is consistent with their similarly elevated T_b).

Gracile mouse opossums are significantly thermolabile in their response to varying T_a . Nevertheless, they have a substantial thermoregulatory response to decreasing T_a , although the variation in T_b with T_a reduces their metabolic response below that expected from their C_{wet} , with the slope of the line relating MR and T_a being only $0.10 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$, compared with a calculated thermoneutral C_{wet} of $0.36 \pm 0.152 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$. By attenuating their metabolic response to low T_a , mouse opossums reduce their $T_b - T_a$ differential, reducing heat loss and lowering O_2 consumption.

Thermoneutral C_{wet} of the gracile mouse opossum is 111% of that predicted from the allometric relationship for marsupials, calculated by least squares regression from \log_{10} -transformed data of Withers et al. (2006), using Hayes and Shonkwiler's (2006, 2007) unbiased antilog transformation. This C_{wet} conforms to the general marsupial regression, falling within the 95% prediction limits both before and after phylogenetic correction (analysis after Cooper and Withers 2006; Withers et al. 2006). We find no evidence that gracile mouse opossums have a significantly elevated C_{wet} compared with other marsupials, despite McNab's (1978) suggestion that Neotropical South American marsupials have a higher C_{wet} than other marsupials. The C_{wet} of $0.36 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ measured in this study at 30°C is appreciably higher than the $0.25 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ value reported for *G. microtarsus* by Morrison and McNab (1962; calculated from the slope of $\dot{V}\text{O}_2$ against $T_b - T_a$). This discrepancy presumably results from differences in calculation techniques and physiological state of the animals. Indeed, if we calculate C_{wet} as $\text{MR}/(T_b - T_a)$ for Morrison and McNab's mouse opossum at $T_a = 30^\circ\text{C}$, it is $0.36 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ ($= 1.80/[34.9 - 30]$), the same as we found for *G. agilis*. This indicates that the higher MR of *G. microtarsus* at thermoneutrality is consistent with their similarly elevated T_b .

Ventilation

Variation of ventilatory parameters of gracile mouse opossums from allometrically predicted values (calculated by least squares regression from \log_{10} -transformed data) range from 116% of predicted for V_T to 142% of predicted for V_I (Nicol and Maskrey 1980; Hallam and Dawson 1993; Chappell and Dawson 1994; Dawson et al. 2000; Larcombe 2002; Cooper and Withers 2004b; Larcombe and Withers 2006; Larcombe et al. 2006), using Hayes and Shonkwiler's (2006, 2007) correction. The value of EO_2 is 76% of that predicted for a 30.7-g marsupial. All ventilatory parameters conform to the allometric relationships for other marsupials both before and after correction for phylogenetic history (analysis after Cooper and Withers 2006; Withers et al. 2006), falling well within the 95% prediction limits for each relationship.

Endothermic mammals accommodate their increased demand for O_2 at low T_a by increasing their V_I , or their EO_2 , or both. Like most other marsupials, gracile mouse opossums increase V_I rather than EO_2 when their MR is high at low T_a . An increase in V_I is accommodated by an increase in f_R , V_T , or both. The degree by which f_R and V_T increase to accommodate a greater V_I in marsupials is dependent on body mass, with larger marsupials having a proportionally larger change in V_T and smaller marsupials a proportionally larger change in f_R at low T_a compared to thermoneutral conditions (Cooper and Withers 2004b). The ventilatory response of gracile mouse opossums to low T_a is typical of small marsupials, with a substantial and statistically significant increase in f_R at low T_a and no significant change in V_T .

These ventilatory data for a South American marsupial suggest that these animals are similar to Australian marsupials in terms of their ventilatory physiology. Most ventilatory variables in mammals are constrained by the same physical and chemical parameters (e.g., diffusion, convection, thoracic compliance, blood gases), so any differences between taxa are likely to reflect differing metabolic demands rather than intrinsic differences in the functioning of the respiratory system. Therefore, we would expect ventilatory variables of gracile mouse opossums to conform to those of other marsupials, since their metabolic physiology also conforms to that of other marsupial species.

Evaporative Water Loss

The EWL of $2.56 \pm 0.218 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$ for the gracile mouse opossum at the lower limit of thermoneutrality ($T_a = 30^\circ\text{C}$) is 79% of that predicted from the allometric equation for marsupials, calculated by least squares regression of \log_{10} -transformed data from Withers et al. (2006), using Hayes and Shonkwiler's (2006, 2007) correction. This EWL conforms to the general marsupial regression, falling within the 95% prediction limits both before and after phylogenetic correction (analysis after Cooper and Withers 2006; Withers et al. 2006). Despite the Neotropical distribution of gracile mouse opossums and their diet high in preformed water, we find no indication that they have a high water loss compared with Australian marsupials. If anything, EWL of mouse opossums tends to be low rather than high for marsupials. This is surprising, considering that environmental variables do influence the EWL of marsupials (Withers et al. 2006). The low EWL of gracile mouse opossums may reflect a need to conserve water during the dry season, when free water and food (insects; Martins and Bonato 2004; Martins et al. 2006b, 2006c) are less available. Therefore, this Neotropical marsupial may be under similar selection pressure for water conservation as a generalized Australian marsupial, for at least the dry part of the year, and therefore an intrinsically lower EWL would be an adaptive advantage.

Unfortunately, methodological differences between studies make comparisons of EWL for marsupials (and other endotherms) difficult (Cooper et al. 2005). Varying RH in the metabolic chamber, long- versus short-term experimental durations, gravimetric versus instantaneous measurement

techniques, and a relatively small number of species measured render the EWL data set for marsupials more variable than that for other physiological parameters (e.g., BMR; Withers et al. 2006). Therefore, it is difficult to evaluate unequivocally the relative EWL of the gracile mouse opossum with respect to other marsupial species. Our measurement protocol is also likely to measure lower rates of EWL than the protocols of many of the studies contributing to the current marsupial EWL data set. We use a flow-through thin-film capacitance hygrometer to measure near-instantaneous evaporative water loss, with long measurement durations, ensuring that minimal water loss values, equivalent to BMR for energy use (i.e., the minimum 20-min mean), are obtained. Gravimetric methods must result in higher values than our near-instantaneous technique and potentially include increased EWL due to bouts of activity, grooming, and even urination/defecation. Short measurement durations (i.e., <4 h) in studies that contribute to the marsupial EWL data set probably also overestimate EWL of small marsupials, by ~45% (Cooper and Withers 2009).

Relative Water Economy

The EQ of opossums at the lower limit of thermoneutrality is very high ($2.6 \pm 0.27 \text{ mg H}_2\text{O mL}^{-1} \text{ O}_2$), indicating poor water economy at these high T_a 's. By comparison, an Australian dasyurid, the stripe-faced dunnart, has a thermoneutral EQ of 1.28 and the North American cactus mouse (*Peromyscus eremicus*) has an EQ of 1.2. Some mammals have thermoneutral EQs <1; laboratory mice and rats have EQs of only 0.85 and 0.94, and desert rodents may have EQs as low as 0.5–0.59 (Hudson 1962).

The RWE of gracile mouse opossums at low T_a indicates that they may not always have an abundance of water but are adapted to conserve water. The point of relative water economy (PRWE), where MWP balances EWL, occurs at approximately 11.5°C , and the mouse opossums achieve positive water balance at $T_a < 11.5^\circ\text{C}$. The T_a at which the PRWE occurs is an index of a species' adaptation to limited water availability and is useful for interspecific comparison (MacMillen and Hinds 1983; MacMillen 1990). Hinds and MacMillen (1986) calculated the PRWE for dasyurid marsupials to be -3.3°C and that of granivorous heteromyid rodents to be 14.1°C . Granivorous birds have PRWEs between -18.3° and 18°C , with a predicted PRWE for a 30-g granivorous bird about 12.5°C (MacMillen 1990; MacMillen and Baudinette 1993). Thus, the tropical, insectivorous/omnivorous gracile mouse opossum has a PRWE comparable with that of a granivorous desert species. This is somewhat surprising because we may expect a granivorous desert species to have evolved a more efficient water economy. Interestingly, the desert-dwelling stripe-faced dunnart has a substantially lower basal EWL ($1.81 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$) and EQ than the gracile mouse opossum but does not attain a positive water economy at $T_a > 11^\circ\text{C}$ (Cooper et al. 2005).

For normothermic endotherms, the potential to attain a positive water economy at low T_a appears to depend mainly on the pattern of change in EWL with decreasing T_a . For gracile mouse opossums, EWL decreased with decreasing T_a . Thus an

increase in MWP (resulting from increased heat production) at low T_a could balance, and then exceed, EWL. The low EQ for mouse opossums at low T_a compared with the higher EQ at higher T_a (Fig. 2) clearly demonstrates this difference. However, EWL remains constant below the lower critical temperature for many dasyurid marsupials (Hinds and MacMillen 1986) and numbats (Cooper and Withers 2002) and even increases for stripe-faced dunnarts (Cooper et al. 2005) and bandicoots (Larcombe and Withers 2006; Larcombe et al. 2006).

Variation in EWL below the thermoneutral zone presumably results from respiratory water loss increasing as a result of increased ventilation (required to accommodate a higher MR at low T_a). In some species, this increased ventilatory water loss balances or even exceeds the reduction in cutaneous water loss at lower T_a , resulting in a counterintuitive increase in (or constancy of) total EWL. Presumably, differences in the relationship of V_i and T_a between species and the efficiency of countercurrent heat and water exchange by the respiratory system determine the pattern of EWL below thermoneutrality and, therefore, the potential for a species to achieve positive water economy.

Gracile mouse opossums experience high humidity during periods of higher T_a in the wet season (Martins et al. 2006b, 2006d), which will reduce EWL in their subtropical environment. They may have a greater need to achieve a more favorable EQ during the cooler but less humid dry season, requiring a large T_a effect on RWE. Unfortunately, the current paucity of detailed and comparable data on EWL, respiration, and RWE makes it difficult to elucidate ecological correlates and infer the adaptive significance of water economy for marsupials. This is an area that merits further investigation.

Torpor

During torpor in *G. microtarsus*, T_b drops to as low as 14.6°C, and spontaneous arousal is observed when $T_b > 20^\circ\text{C}$ (at $T_a = 12^\circ$ and 20°C), thus confirming the occurrence of true torpor in another South American marsupial species. However, these mouse opossums may lack the thermogenetic capacity to rewarm from low T_b at low T_a . Morrison and McNab (1962) measured T_b values of 27.5°C and calculated T_b values of approximately 16°C for *G. microtarsus* when torpid. These minimum defended T_b values for mouse opossums are typical of those for Australian dasyurids and most other didelphid marsupials that undergo daily torpor (McNab 1978; Geiser 1994, 2004b), although the Chilean mouse opossum (*Thylamys elegans*) has a lower minimum defended T_b of $<5^\circ\text{C}$ (Bozinovic et al. 2005).

Torpor can substantially reduce energy requirements (e.g., Song and Geiser 1997; Cooper and Withers 2004a; Geiser 2004a, 2004b) and is presumably important for mouse opossums to balance their daily energy budget, even in a tropical environment with relatively high primary productivity. A 14%–35% reduction in MR at low T_a is similar to that measured for *G. microtarsus* (Morrison and McNab 1962) and dasyurid marsupials during daily torpor (Geiser 1994, 2004b; Cooper et al.

2005). A greater MR reduction, to about 1% of normothermic, is observed for the Chilean mouse opossum at a T_a of 5°C , since their minimum defended T_b during torpor is lower (Bozinovic et al. 2005).

During torpor, absolute EWL is reduced to significantly below normothermic values, with gracile mouse opossums conserving 7–17 mg $\text{H}_2\text{O h}^{-1}$ compared with normothermic animals, representing decreases of 31%–68% of normothermic EWL. There are data on EWL during torpor for only one other marsupial, the stripe-faced dunnart (Cooper et al. 2005). This small dasyurid marsupial also achieves significant absolute water savings during torpor (50–55 mg $\text{H}_2\text{O h}^{-1}$). These reductions are greater than those of the mouse opossum, which may reflect a more important role for torpor in water conservation by the stripe-faced dunnart in its arid habitat.

Despite the absolute water savings, torpor does not facilitate relative water savings for either gracile mouse opossums or stripe-faced dunnarts. The RWE of torpid gracile mouse opossums is significantly lower than that of normothermic individuals (Fig. 2), while for stripe-faced dunnarts, torpor did not change their already negative RWE (Cooper et al. 2005). RWE remains the same for normothermic and torpid gerbils (*Gerbillus pusillus*; Buffenstein 1985) and is less favorable for torpid cactus mice (MacMillen 1965). This unfavorable effect of torpor on RWE presumably results from a smaller reduction in EWL with torpor than the corresponding reduction in MR (and thus MWP). The loss of a thermoregulatory metabolic increment, and the consequent decrease in T_b and MR by a Q_{10} effect (Withers 1992; Guppy and Withers 1999; Geiser 2004a), would substantially reduce MR during torpor even at a moderate T_a of 20°C , whereas the lesser reduction in EWL presumably reflects a lesser decrease in either or both of the cutaneous and respiratory components of EWL.

Ecological and Adaptive Significance

Despite 110–130 million yr of separate evolution (Wroe and Archer 2006), the physiological bauplan of the gracile mouse opossum provides no evidence that this South American marsupial differs from other marsupials. Marsupials are physiologically conservative, and there is little adaptive variation apparent in their physiology (McNab 2005; Withers et al. 2006).

Body mass is by far the greatest determinant of physiological variables such as BMR, C , T_b , EWL, and ventilatory parameters (McNab 2005; Withers et al. 2006), and there is less residual variation in the allometric relationships for these variables in marsupials than in placental mammals and birds. McNab (1978) suggested that Neotropical marsupials have a higher BMR and C than Australian marsupials, reflecting climatic and dietary adaptation. However, our standard physiological data set for the Brazilian gracile mouse opossum provides no evidence that this species differs significantly in terms of any of the physiological variables from other marsupials. There are correlates of physiological variables with environmental factors such as climate and food habits for marsupials, but although highly significant, they explain very little of the overall variation

in physiological traits (Withers et al. 2006). The various significant environmental correlates for marsupials are too subtle to result in a sufficient difference for a single species to be statistically different from other species (with the exception of the honey possum and marsupial mole; Withers et al. 2006). Thus, the general physiology of marsupials is sufficient to allow them to exploit a range of environmental and biological niches with only minimal adaptive change.

Marsupial physiology appears preadapted to a low-energy niche; on average, marsupials have low rates of standard energy metabolism compared with rates of placental mammals (Dawson and Hulbert 1969), and associated with these values of low MR are comparatively low T_b , EWL, and V_i (e.g., Dawson and Hulbert 1969; Cooper and Withers 2004b). McNab (1986, 2005) observed that many groups of placental mammals have BMR and T_b as low as, or even lower than, those of marsupials, but there are no hypermetabolic marsupials, presumably a constraint of the phylogenetic history of this group. This apparent absence of hypermetabolic marsupials may explain the poor competitive record of marsupials with placentals. In Australia, the recent (within the last 4,000 yr) human-facilitated introduction of placental mammals (other than rodents and bats) has been implicated in the competitive and predatory extinction of many Australian marsupial species (Lee and Cockburn 1985; Strahan 2002; Corbett 2006). However, South American marsupials have persisted despite a much longer association with placental mammals (about 60 million yr; Wroe and Archer 2006), but it has not been necessary for South American marsupials like the gracile mouse opossum to differ physiologically from Australian marsupials to persist in the face of competition/predation from placentals. This may reflect marsupials' low-energy insectivorous/omnivorous niche. It is interesting to note that those marsupials that have persisted in the Americas are generalist insectivores/omnivores that presumably are at no physiological disadvantage and may have MRs similar to those of placental species filling the same niche (McNab 1986, 2005).

Comparison of South American and Australian marsupials enables us to better understand the conservative pattern of ecological adaptation within this mammalian group. However, more complete and reliable physiological data, especially EWL and respiratory variables, are needed for South American marsupials in particular, to more comprehensively compare the physiology of Australian and South American marsupials and to elucidate any adaptive environmental factors.

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