

Partitioning of Evaporative Water Loss into Respiratory and Cutaneous Pathways in Wahlberg's Epauletted Fruit Bats (*Epomophorus wahlbergi*)

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ABSTRACT

The relative contributions of respiratory and cutaneous evaporation to total evaporative water loss (TEWL) and how the partitioning of these two avenues varies with environmental temperature has received little attention in bats. We trained Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) captured in Pretoria, South Africa, to wear latex masks while hanging in respirometry chambers, and we measured respiratory evaporative water loss (REWL) and cutaneous evaporative water loss (CEWL) over air temperatures (T_a) from 10° to 40°C. The bats' normothermic body temperature (T_b) was approximately 36°C, which increased at higher T_a to 40.5° ± 1.0°C at $T_a \approx 40^\circ\text{C}$. Both TEWL and resting metabolic rate (RMR) increased sharply at $T_a > 35^\circ\text{C}$, with a mean TEWL at 40°C equivalent to 411% of that at 30°C. The increase in TEWL was driven by large increases in both CEWL and REWL. CEWL comprised more than 50% of TEWL over the entire T_a range, with the exception of $T_a \approx 40^\circ\text{C}$, where REWL accounted for 58% of evaporative water loss. Surface area-specific CEWL increased approximately sixfold with increasing T_a . Thermoregulation at T_a approaching or exceeding T_b involved a considerable energetic cost, with RMR at $T_a \approx 40^\circ\text{C}$ exceeding by 24% that measured at $T_a \approx 10^\circ\text{C}$. Our data do not support recent arguments that respiratory gas exchange across the wing membranes represents 5%–10% of the total in *E. wahlbergi*.

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Introduction

Evaporative heat loss is a significant component of the energy and water fluxes that occur between terrestrial endotherms and their environments and has far-reaching implications for many aspects of their ecology and evolution (Gordon and Olson 1995). Evaporative heat dissipation is of particular significance to species that routinely encounter environmental temperatures approaching or exceeding their body temperature (T_b), with birds and mammals typically responding to hot conditions with large increases in their rates of evaporative water loss (EWL) to prevent potentially lethal increases in T_b (King and Farner 1961; Herreid and Schmidt-Nielsen 1966; Licht and Leitner 1967). Total evaporative water loss (TEWL) is the sum of cutaneous evaporative water loss (CEWL) and respiratory evaporative water loss (REWL), although there is empirical evidence that other avenues of evaporation, including the ocular surfaces and the avian cloaca, may also contribute significantly to total rates of evaporation (Pinshow et al. 1982; Hoffman et al. 2007).

In bats, evaporation may constitute 80%–85% of overall water flux (Studier 1970; Arad and Korine 1993; Bassett et al. 2009) and has particular thermoregulatory significance for insectivorous species roosting in hot microsites (Maloney et al. 1999; Marom et al. 2006; Cory Toussaint and McKechnie 2012). Relatively little is known about rates of respiratory evaporation versus cutaneous evaporation in bats (Bassett et al. 2009), particularly compared with birds, where it is well established that the relative importance of REWL and CEWL for thermoregulation at air temperatures (T_a) approaching and exceeding T_b varies widely among orders (Wolf and Walsberg 1996; Tieleman and Williams 2002; McKechnie and Wolf 2004).

Chew and White (1960) were the first to partition TEWL into the respiratory and cutaneous components in bats. These authors estimated from the temperature and humidity of inspired air that REWL is 22%–29% of TEWL at $T_a \approx 26^\circ\text{C}$ in pallid bats (*Antrozous pallidus*), although Herreid and Schmidt-Nielsen (1966) subsequently asserted that there were errors in these calculations. Vogel (1969) separated TEWL in bats by simultaneously measuring REWL and CEWL, using a partitioned chamber similar to ones more recently used for birds (e.g., Wolf and Walsberg 1996; McKechnie and Wolf 2004), and found that *Rhinopoma hardwickei* dissipated heat predominantly by REWL, whereas in *Myotis* sp. and *Rhinolophus* sp.,

CEWL was the predominant route. Laburn and Mitchell (1975) partitioned TEWL in Egyptian fruit bats (*Rousettus aegyptiacus*) by first measuring TEWL and then measuring REWL by placing plastic bags over the bats' bodies in such a way as to leave just their heads exposed. These authors found that REWL comprised most of TEWL in *R. aegyptiacus* at $T_a > 37^\circ\text{C}$. Most recently, Muñoz-García et al. (2012) partitioned TEWL in the insectivorous Kuhl's pipistrelle (*Pipistrellus kuhlii*) using a mask and found that REWL decreased with resting metabolic rate (RMR) and that CEWL was less precisely regulated when *P. kuhlii* was in deep torpor compared with normothermy or shallow torpor. In that study, bats were restrained during measurements of REWL and CEWL, and the significantly higher TEWL of restrained bats (i.e., those wearing a mask) compared with unrestrained individuals raised the possibility that observed REWL/CEWL ratios were influenced by stress arising from the experimental setup (Muñoz-García et al. 2012).

Notwithstanding the above studies, the relative roles of REWL and CEWL in evaporative heat and water fluxes need additional investigation. A clearer understanding of the relative roles of these avenues of evaporative heat dissipation is important for elucidating the selective pressures driving the evolution of evaporative cooling capacity in bats, particularly in those taxa that roost in very hot environments. Notably, most of the above studies involved small insectivorous species, and water balance in pteropodid fruit bats may well differ on account of the high water content of their diets, larger body mass, and use of arboreal roosts sometimes exposed to high solar heat loads (e.g., Strahan 1991; Welbergen et al. 2008). Information on evaporative cooling capacity and upper thermoregulatory limits is also important in the light of the threat posed to bats by higher maximum T_a and more frequent and intense heat waves under climate change scenarios (IPCC 2011). Understanding the links between EWL partitioning and the upper limits of bats' evaporative cooling capacity is likely to be vital for predicting their ability to avoid hyperthermia during extremely hot weather. The vulnerability of some bats to extreme weather is exemplified by recent large-scale, heat-associated mortality events among Australasian flying foxes (Welbergen et al. 2008).

To gain a clearer understanding of the partitioning of evaporative water losses and upper limits to thermoregulation in large-bodied frugivorous bats, we investigated the partitioning of TEWL into CEWL and REWL in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*). Like other fruit bats (e.g., Ochoa-Acuña and Kunz 1999), free-ranging *E. wahlbergi* pant and wing-fan during hot weather (I. A. Minnaar, personal observation). We hence predicted that both REWL and CEWL in *E. wahlbergi* should increase sharply at high T_a , contrary to the pattern typical of many birds, where either REWL or CEWL, but generally not both, increase rapidly as T_a approaches normothermic T_b (reviewed by Wolf and Walsberg 1996). We tested this prediction by training individuals to wear masks, so that we could obtain CEWL and REWL measurements while they were hanging in a natural posture within respirometry chambers, presumably reducing restraint-associated stress (Muñoz-

García et al. 2012). Although the focus of our study was evaporative water and heat fluxes, our experimental design also permitted an evaluation of recent evidence that a significant fraction (5%–10%) of respiratory gas exchange occurs across the wings in *E. wahlbergi* (Makanya and Mortola 2007).

Material and Methods

Study Animals

We captured 10 adult Wahlberg's epauletted fruit bats *Epomophorus wahlbergi* (Sundevall, 1846; nine nonreproductive females, one male; mean \pm standard deviation [SD] body mass [M_b] = 84.1 ± 7.9 g) at the Pretoria National Botanical Gardens, Pretoria, in Gauteng Province, South Africa ($25^\circ44'S$, $28^\circ16'E$), during March 2012 using mist nets (Ecotone ultrathin mist nets, Gdynia, Poland). Breeding occurs throughout the year in this species (Monadjem et al. 2010), but we used only individuals that showed no signs of reproductive activity. The male and female fruit bats were housed separately in outdoor aviaries (each 5 m long \times 2.5 m wide \times 2.5 m high) at the University of Pretoria's experimental farm (3 km from the capture site) between March and December 2012. Bats were maintained on a diet recommended by Barnard (2009), composed of a mixture of seasonal fruits, such as banana, apple, pear, and papaya, and supplemented with vitamins and minerals. Food, placed in hanging baskets, and drinking water were provided ad lib. The EWL measurements were conducted between September and November 2012 (austral spring).

Measurements of Gas Exchange and Body Temperature

Rates of EWL, oxygen consumption (\dot{V}_{O_2}), and carbon dioxide production (\dot{V}_{CO_2}) were measured using a flow-through respirometry system. Bats were placed individually in 4-L airtight plastic chambers (Lock and Lock, Hana Cobi, Korea) with a 1-cm layer of mineral oil at the bottom of each to prevent evaporation from urine and feces from affecting estimates of EWL. A plastic mesh platform was placed 10 cm above the mineral oil to prevent the bat from coming into contact with the oil. A three-sided plastic mesh frame was placed inside the container to provide adequate opportunity for the bat to hang in a natural posture.

We separated CEWL and REWL using a mask (Tieleman and Williams 2002; Muñoz-García et al. 2012). Masks were constructed using liquid latex painted onto a mold made of modeling clay, the dimensions of which were determined by measuring male and female *E. wahlbergi* skulls. Velcro strips secured the mask in place over the bat's muzzle (fig. 1). Openings for the eyes and ears were cut into the mask, so that evaporation from these areas was included in estimates of CEWL. The mask fitted the bat's head snugly but permitted air to be drawn into the mask from the chamber, with a piece of flexible Tygon tubing connected to the apex of the mask used to pull air through it (fig. 1).

Masked bats, hanging individually in chambers, were placed inside a darkened, temperature-controlled cabinet (model KMF



Figure 1. A Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) wearing a mask used for separating respiratory and cutaneous evaporative water losses. The mask was constructed from latex and was held in position by Velcro strips. A color version of this figure is available online.

720, Binder, Tuttlingen, Germany). Air temperature within each chamber was measured using a thermistor probe (Sable Systems, Las Vegas, NV) inserted through the lid via a small hole with a rubber grommet sealed by the probe. Signals from the thermistor were received by an analog to digital converter (UI-2, Sable Systems). Atmospheric air provided by a compressor was scrubbed of water vapor (to a dewpoint $\approx -50^{\circ}\text{C}$) and CO_2 by an adsorption dryer (Ecodyr K-MT 3, Parker Zander, Charlotte, NC) and supplied to the chamber at a constant flow rate between 3 and 10 L min^{-1} (higher flow rates were used at higher T_a to keep chamber humidities low) by a mass flow controller (model FMA5520, Omega Engineering, Bridgeport, NJ) regularly calibrated against a soap bubble flow meter (Baker and Pouchot 1983). Some of the air entering the chamber was then drawn through the mask at a constant flow rate between 1.5 and 2.1 L min^{-1} using a custom-built air pump and rotameter (FL-2012, Omega Engineering) calibrated as above, with the remainder of the chamber incurrent air exiting through an outlet fitting. These flow rates maintained water vapor partial pressures below 0.4 and 1.7 kPa (equivalent to dewpoints of -5° and 15°C) in the chamber and mask, respectively, intended to ensure adequate water vapor pressure gradients for unimpeded evaporative heat dissipation. The $[\text{O}_2]$ in the mask remained within 0.5% of baseline values at all times. The time required for air to reach 99% equilibrium within the chamber ranged from 1.8 to 6.1 min (Lasiewski et al. 1966).

Inlet and outlet fittings were placed at the bottom and top of the chamber, respectively, to maximize mixing of air. During measurements, we could verify from CO_2 and O_2 concentrations in the chamber that air did not escape from the mask,

because these remained near baseline levels, whereas water vapor pressure increased through CEWL. On rare occasions that bats removed masks during measurements, data were discarded, and the measurements were repeated. Air was subsampled sequentially from a baseline channel, the chamber, and the mask using a TR-RM8 respirometry multiplexer (Sable Systems) and SS-3 subsampler (Sable Systems).

Subsampled air was pulled through a Sable Systems RH-300 water vapor analyzer, a CO_2 analyzer (CA-10a, Sable Systems), and an O_2 analyzer (FC-10B, Sable Systems). The CO_2 and water vapor analyzers were zeroed using nitrogen (Afrox, Johannesburg, South Africa) and spanned using a certified span gas containing 2,000 ppm CO_2 (Afrox, Johannesburg, South Africa) and the oxygen dilution method of Lighton (2008), respectively. The O_2 analyzer was spanned to 20.95% using atmospheric air scrubbed of CO_2 and water vapor using soda lime and magnesium perchlorate (Merck, Modderfontein, South Africa), respectively. Data from the gas analyzers and temperature sensor were acquired and digitized using a Universal Interface II analog-digital convertor (Sable Systems) and recorded using ExpeData (Sable Systems) software on a desktop personal computer.

We measured T_b with temperature-sensitive passive integrated transponder (PIT) tags (Bio-Thermo 12-mm microchip, Destron Fearing, St. Paul, MN; resolution: 0.1°C) injected subcutaneously in each bat's interscapular region. Cory Toussaint and McKechnie (2012) calibrated a subsample of the same batch of PIT tags, and we used the same calibration curves here. Subcutaneous temperature is representative of core T_b in bats (Gorman et al. 1991). Body temperature data were con-

tinuously received via a loop antenna (Racket Antenna, Biomark, Boise, ID) that was placed adjacent to each chamber and attached to a receiver and data logger (model FS2001F-ISO, Biomark).

Experimental Protocol

Over a 2-wk period in September 2012, we habituated the bats to wearing the masks. Initially, they wore the masks for 30 min at a time inside the metabolic chambers, but following the habituation protocol, they wore masks for up to 5 h during measurements. We monitored the bats while they were in the chambers using a surveillance camera with an infrared light source (CCD 420TV, Sony, Tokyo, Japan) connected to an LCD monitor, and we could thus verify that the bats appeared calm during measurements. Experiments were conducted during the day (i.e., the rest phase of the bats). By removing all uneaten food by 2200 hours the previous night, we ensured that bats were postabsorptive during experiments, because they had been without food for at least 8 h before experiments started.

After fitting each bat with a mask, we induced the bat to hang from the three-sided mesh frame before lowering it into the chamber. The chamber was placed inside the darkened temperature-controlled cabinet at least 30 min before data collection to allow the bat time to habituate to the experimental setup. We subsampled baseline air for 5–10 min at the start of each run until stable $[O_2]$, $[CO_2]$, and water vapor pressure readings were obtained. We then sequentially subsampled from the chamber followed by the mask, switching between the two channels during periods of stable readings (typically 7–15 min for each source). These sampling periods were thus longer than the 99% equilibrium times for the chamber. Ideally, gas concentrations from chamber and mask air should have been measured simultaneously using dual analyzers; we were unable to do so because of limits to equipment availability. However, because (1) the gas traces were always stable during measurements and (2) we verified that the bats were calm via the surveillance camera, we are confident that these sequential measures represent valid resting values. Air from the chamber was again subsampled after mask air to confirm that the mask was still in place and had not become dislodged (a dislodged mask was easily detected by large deviations in chamber $[O_2]$ and $[CO_2]$ from baseline values). Each run ended with a 5-min baseline reading. This cycle was repeated for between 2 and 5 h, except at 38° and 40°C, where runs did not last longer than an hour, and bats were offered water at the end of experiments. Bats were weighed before and after measurements, with the average M_b used for calculations.

We measured EWL and MR at $T_a = 11.4^\circ \pm 0.4^\circ\text{C}$, $20.7^\circ \pm 0.1^\circ\text{C}$, $30.4^\circ \pm 0.1^\circ\text{C}$, $34.9^\circ \pm 0.7^\circ\text{C}$, $37.7^\circ \pm 0.3^\circ\text{C}$, and $40.0^\circ \pm 0.5^\circ\text{C}$ (hereafter referred to as 10°, 20°, 30°, 35°, 38°, and 40°C, respectively). One bat was measured at a time, with the sequence of T_a values randomized. However, measurements at 38° and 40°C were conducted only after all other T_a , because these are above the T_a range experienced by wild bats in Pre-

toria. Measurements were obtained from all 10 bats at each T_a , with each bat resting for at least 2 d between successive runs.

Several weeks before training the bats to wear the masks, we measured TEWL, T_b , and RMR in the same individuals at T_a between 10° and 30°C in increments of approximately 5°C, with a sample size of 5–6 individuals per T_a . We used these measurements to test whether the masks had any effect on TEWL, T_b , or RMR, in light of the observation by Muñoz-García et al. (2012) that TEWL was significantly higher in *Pipistrellus kuhlii* when bats were restrained and wearing a mask than when they were not. They interpreted the increased TEWL as an effect of stress associated with restraint (Muñoz-García et al. 2012).

Data Analyses

Rates of CEWL and REWL were estimated using the same approach as Tieleman and Williams (2002) and Muñoz-García et al. (2012). CEWL was estimated from the water vapor pressure in the chamber and the incurrent flow rate (corrected for the addition of water vapor). REWL was estimated from the water vapor pressure in the mask, using the chamber water vapor pressure as the baseline and with mask excurrent flow rate corrected for changes in water vapor pressure and O_2 and CO_2 fractional concentrations. We estimated oxygen consumption (\dot{V}_{O_2}) using equation (9.4), carbon dioxide production (\dot{V}_{CO_2}) using equation (9.5), the rates of EWL using equation (9.6) (in the case of REWL, modified so as to estimate EWL from the difference in water vapor pressure between the mask and the chamber), and the excurrent flow rate using equation (9.3) from Lighton (2008). To quantify respiratory gas exchange across the wings (Makanya and Mortola 2007), we calculated respiratory \dot{V}_{CO_2} from the difference in $[CO_2]$ between chamber and mask excurrent air as well as nonrespiratory \dot{V}_{CO_2} from the difference between incurrent and chamber excurrent air. A similar evaluation of nonrespiratory \dot{V}_{O_2} was precluded by the high flow rates we used to maintain low chamber humidity levels and the lower sensitivity of the O_2 analyzer compared with the CO_2 analyzer. RMR and EWL values were calculated as the 1-min averages of the lowest stable values. We estimated T_b as the mean value corresponding to the time over which RMR and EWL were estimated. We determined body surface area (A_b) as did Marom et al. (2006) and estimated the area of the head enclosed in the mask by assuming it to be a truncated cone. Body and head dimensions were measured with digital callipers. To correct CEWL estimates for the skin area covered by the mask, we used the estimated rate of surface area-specific CEWL.

Bats had an average respiratory exchange ratio ($RER = \dot{V}_{CO_2}/\dot{V}_{O_2}$) of 0.74 during experiments, indicating a predominance of lipid metabolism. A few values fell below the 0.71–1.00 range, in which case we assumed an RER value of 0.71 when converting oxygen consumption to MR. RMR was estimated from \dot{V}_{O_2} and RER using the thermal equivalence data in table 4.2 in Withers (1992).

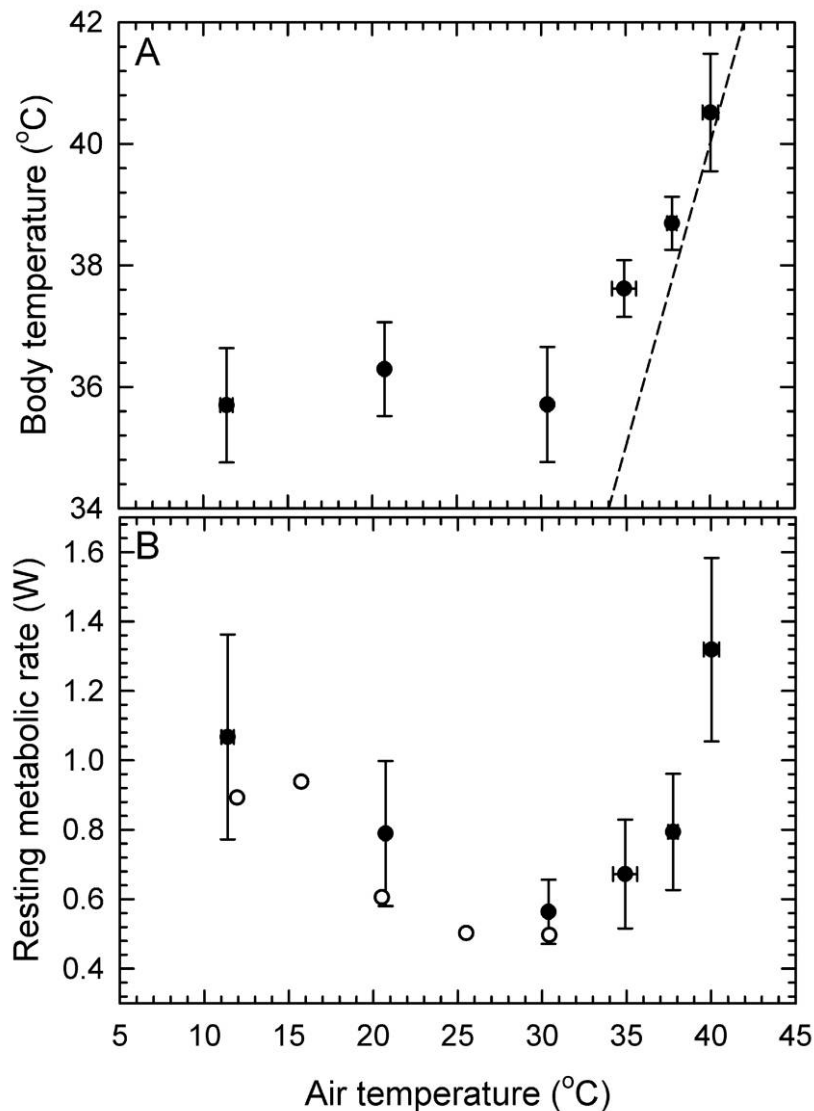


Figure 2. The relationships between air temperature (T_a) and body temperature (T_b ; A) and resting metabolic rate (B) in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) from Pretoria, South Africa. Sample size was 10 fruit bats (same individuals) per T_a value, and error bars indicate standard deviations. The dashed line shows $T_a = T_b$. The open circles in B indicate the mean resting metabolic rate values for bats that were not wearing masks.

The dry heat transfer coefficient (C ; conductance) was calculated following Dawson and Schmidt-Nielsen (1966):

$$C = \frac{\text{RMR} - \text{EHL}}{(T_b - T_a)A_b},$$

where EHL is evaporative heat loss (W) and A_b is surface area in centimeters squared, estimated as described above.

Data were analyzed using SigmaPlot 12.5 and Statistica statistical software, version 10 (<http://www.statsoft.com>). Because the same individuals were used for each experimental T_a , we used Spearman rank order correlation coefficient (ρ) to test the statistical significance of correlations (Zar 1999). We compared TEWL between bats wearing and not wearing a mask

using a repeated-measures analysis of covariance (RM-ANCOVA) with mask (yes/no) as a categorical predictor and T_a as a covariate. We compared T_b and RMR using the same approach. Values are presented as means \pm SD.

Results

Body Temperature and Metabolic Rate

Body temperature of the fruit bats remained stable at $\sim 36^\circ\text{C}$ and was independent of T_a between 10° and 30°C , but it was significantly and positively correlated with T_a above 30°C (fig. 2A; Spearman rank order $\rho = 0.859$, $P < 0.001$, $n = 30$). Mean T_b at $T_a \approx 40^\circ\text{C}$ was $40.5^\circ \pm 1.0^\circ\text{C}$ (fig. 2A), with one individual

reaching $T_b = 42.9^\circ\text{C}$ at a T_a of 40.7°C . At $T_a \approx 40^\circ\text{C}$, bats panted and sometimes licked their snouts and muzzles. Values of T_b at T_a between 10° and 30°C did not differ significantly between when the bats wore a mask and when they did not (RM-ANCOVA: $F_{1,4.03} = 4.651$, $P = 0.097$). RMR followed the typical endothermic pattern of decreasing with increasing T_a between 10° and 30°C (fig. 2B). Minimum RMR was 0.564 ± 0.092 W at $T_a \approx 30^\circ\text{C}$. RMR increased rapidly at $T_a > 30^\circ\text{C}$, and at $T_a \approx 40^\circ\text{C}$, RMR reached 234% of that at $T_a \approx 30^\circ\text{C}$ and 124% of that at $T_a \approx 10^\circ\text{C}$ (fig. 2B). In contrast to T_b and TEWL (see below), RMR at T_a between 10° and 30°C was significantly higher when bats wore a mask compared to when they did not (RM-ANCOVA: $F_{1,9.82} = 5.337$, $P = 0.044$). At $T_a \approx 10^\circ$, 20° , and 30°C , when the bats wore masks, mean RMR was 19.5%, 35.6%, and 9.8% higher, respectively, compared to when they did not.

Evaporative Water Loss

TEWL increased with increasing T_a , with a much steeper slope at $T_a > 35^\circ\text{C}$ (fig. 3). At $T_a \approx 40^\circ\text{C}$, TEWL was 28.66 ± 7.80 mg min^{-1} , equivalent to more than twice the corresponding value at $T_a \approx 38^\circ\text{C}$ and 411% of that at $T_a \approx 30^\circ\text{C}$ (fig. 3). The mean rate of TEWL at $T_a \approx 40^\circ\text{C}$ was equivalent to $2.05\% \pm 0.52\%$ of M_b per hour. The model that best described the relationship between TEWL and T_a , identified following Song et al. (1997), was a two-segment linear regression with an inflection at $T_a = 37.3^\circ\text{C}$ (fig. 3), which provided a better fit than either an exponential or power model. TEWL at T_a between

10° and 30°C did not differ significantly between when the bats wore a mask and when they did not (RM-ANCOVA: $F_{1,10.58} = 3.074$, $P = 0.108$).

Both REWL and CEWL were low and approximately stable at $T_a < 30^\circ\text{C}$ and increased at higher T_a , with a more gradual increase in CEWL and a rapid increase in REWL (fig. 4). Between $T_a \approx 30^\circ\text{C}$ and $T_a \approx 40^\circ\text{C}$, for instance, CEWL and REWL increased by factors of 2.1 and 13.7, respectively. Rates of CEWL were greater than those of REWL at all T_a values below 40°C , with the fractional contribution of REWL to TEWL ranging from $18.1\% \pm 6.5\%$ ($T_a \approx 30^\circ\text{C}$) to $37.9\% \pm 18.5\%$ ($T_a \approx 38^\circ\text{C}$). At $T_a \approx 40^\circ\text{C}$, however, REWL accounted for the majority of evaporative heat loss, namely, $57.7\% \pm 12.3\%$ of TEWL (fig. 4). Estimated surface area-specific CEWL (CEWL/ A_b) increased approximately sixfold from 0.025 ± 0.013 $\text{mg min}^{-1} \text{cm}^{-2}$ at $T_a \approx 10^\circ\text{C}$ to 0.139 ± 0.065 $\text{mg min}^{-1} \text{cm}^{-2}$ at $T_a \approx 40^\circ\text{C}$.

Dry Heat Transfer Coefficient

The C of the bats was not significantly correlated with T_a between 10° and 20°C (Spearman rank order $\rho = 0.030$, $P = 0.896$), with a mean of 0.454 ± 0.154 $\text{mW } ^\circ\text{C}^{-1} \text{cm}^{-2}$ over this range (fig. 5). At higher T_a , C increased rapidly, with a mean of 5.462 ± 3.915 $\text{mW } ^\circ\text{C}^{-1} \text{cm}^{-2}$ ($n = 9$) at $T_a \approx 38^\circ\text{C}$ (fig. 5).

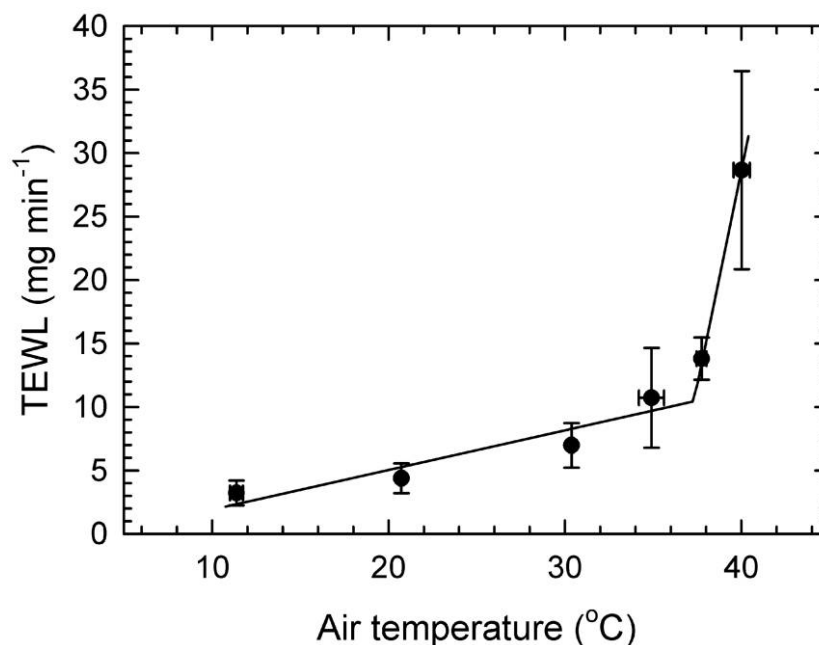


Figure 3. The relationship between air temperature (T_a) and total evaporative water loss (TEWL) in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) from Pretoria, South Africa. Sample size was 10 fruit bats (same individuals) per T_a value, and error bars indicate standard deviations. The solid line indicates the two-piece linear regression model that provided the best fit to the data: $T_a < 36.96^\circ\text{C}$: $\text{TEWL} = 0.312 \cdot T_a - 1.205$; $T_a > 37.31^\circ\text{C}$: $\text{TEWL} = 6.755 \cdot T_a - 241.570$.

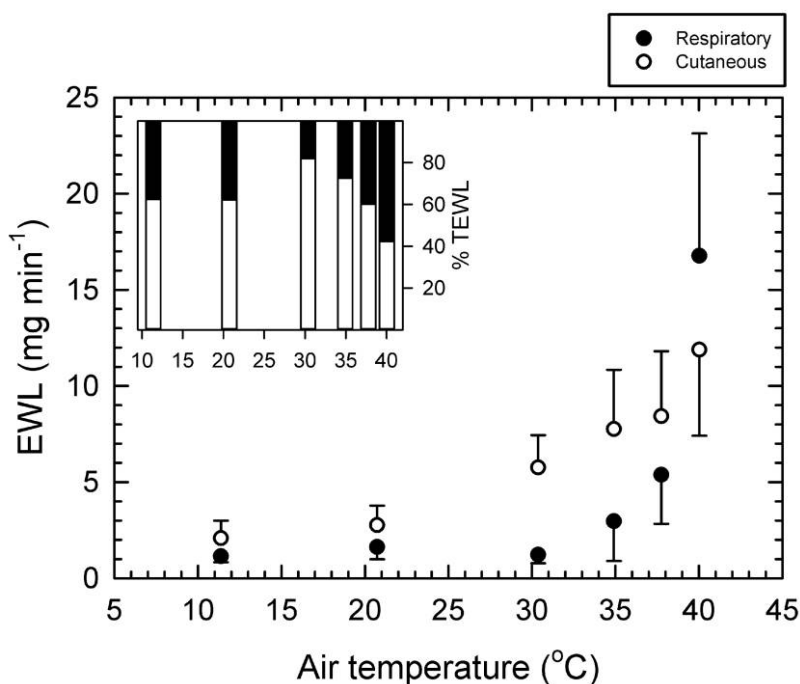


Figure 4. The relationships between air temperature (T_a) and cutaneous and respiratory evaporative water loss (EWL) in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) from Pretoria, South Africa. Sample size was 10 fruit bats (same individuals) per T_a value, and error bars indicate standard deviations. The inset graph shows the partitioning of total EWL (TEWL) into cutaneous (open bars) and respiratory (filled bars) evaporation as percentages of TEWL.

Nonrespiratory CO_2 Production

Nonrespiratory \dot{V}_{CO_2} measured from chamber air represented <3% of total \dot{V}_{CO_2} at all T_a values. At both $T_a \approx 10$ and $20^\circ C$, estimated nonrespiratory \dot{V}_{CO_2} averaged $<0.01 \text{ mL min}^{-1}$, with the 95% confidence intervals for the means including zero. At higher T_a , the 95% confidence intervals did not include zero, with estimated contributions of nonrespiratory \dot{V}_{CO_2} to total values ranging from $1.9\% \pm 0.4\%$ ($T_a \approx 40^\circ C$) to $2.9\% \pm 1.4\%$ ($T_a \approx 35^\circ C$).

Discussion

Cutaneous evaporation made up most of TEWL in *E. wahlbergi* at T_a of $38^\circ C$ and below. At higher temperatures, however, the fruit bats were strongly reliant on respiratory heat dissipation, with REWL comprising $\sim 58\%$ of evaporative heat dissipation at $T_a \approx 40^\circ C$. Our data also reveal that thermoregulation at T_a above normothermic T_b is energetically costly in *E. wahlbergi*; RMR at $T_a \approx 40^\circ C$ was 24% higher than RMR at $T_a \approx 10^\circ C$.

The rates of TEWL we observed in *E. wahlbergi* over the T_a range of $10^\circ\text{--}35^\circ C$ are considerably higher than those reported previously for this species by Downs et al. (2012). For instance, in our study, mean TEWL at $T_a \approx 35^\circ C$ was $7.73 \text{ mg g}^{-1} \text{ h}^{-1}$, whereas Downs et al. (2012) reported values around $4 \text{ mg g}^{-1} \text{ h}^{-1}$. These authors estimated TEWL gravimetrically by passing excurrent air through a condenser at an unspecified temperature. However, the fact that they subsequently passed excurrent

air through a silica gel column to remove residual water vapor implies that the condenser temperature may not have been low enough to trap all evaporated water, meaning that TEWL could have been underestimated.

The relationship between TEWL and T_a at values that approach or exceed normothermic T_b in *E. wahlbergi* follows the same pattern of rapid increases of evaporative heat loss observed in other endotherms. In small bats, TEWL typically increases two- to 12-fold between $T_a < 30^\circ C$ and $T_a \approx 40^\circ\text{--}43^\circ C$ (Herreid and Schmidt-Nielsen 1966; Licht and Leitner 1967; Maloney et al. 1999; Cory Toussaint and McKechnie 2012). As is typical in endotherms, increases in heat dissipation in *E. wahlbergi* at T_a approaching T_b also included a nonevaporative component, with the changes in dry heat transfer coefficient being quantitatively similar to those in other species (e.g., Marom et al. 2006).

The fractional increases in TEWL we observed in *E. wahlbergi* are quantitatively similar to those observed in other yinpterchiropterans with comparable M_b , such as *Dobsonia minor* (Bartholomew et al. 1970) and male *Rousettus aegyptiacus* (Laburn and Mitchell 1975). Moreover, the increases in T_b we observed at $T_a > 30^\circ C$ in *E. wahlbergi* are qualitatively similar to those documented in Australasian pteropodids, including *Pteropus hypomelanus*, *Paranyctimene raptor*, *Nyctimene cyclotis*, *Rousettus amplexicaudatus*, *D. minor*, *Dobsonia praedatrix*, and *Dobsonia moluccensis* (Ochoa-Acuña and Kunz 1999; McNab and Bonaccorso 2001).

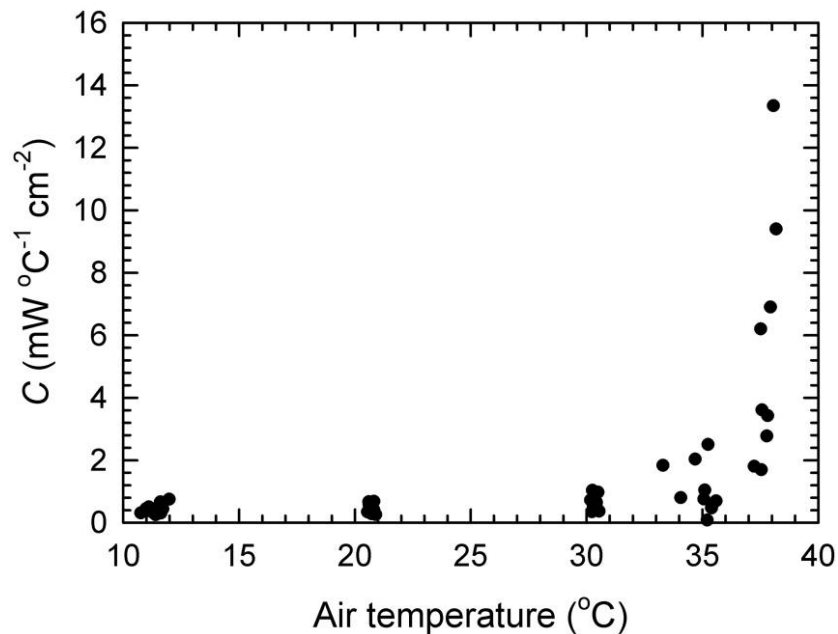


Figure 5. Dry heat transfer coefficient (C ; conductance) as a function of air temperature (T_a) in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) from Pretoria, South Africa. The same individuals were used at each of the T_a values (i.e., $\sim 10^\circ$, 20° , 30° , 35° , and 38°C).

The predominantly respiratory evaporative cooling that we observed at high T_a is qualitatively consistent with Laburn and Mitchell's (1975) findings for *R. aegyptiacus*. However, their use of a plastic bag to prevent cutaneous evaporation more than likely caused an overestimation of the importance of REWL, because the prevention of water vapor dissipation must have reduced CEWL through the humidification of air around the bats' bodies. Vogel's (1969) data for three species, collected using a partitioned chamber, suggest that the relative roles of REWL and CEWL in bats vary with phylogeny. Vogel (1969) reported REWL to predominate in *Rhinopoma hardwicki*, but CEWL was the primary avenue of heat dissipation at higher T_a in *Myotis* sp. and *Rhinolophus* sp. In *P. kuhlii*, CEWL was the major avenue of evaporative heat loss between $T_a = 5^\circ$ and 40°C , representing 60%–80% of TEWL (Muñoz-García et al. 2012). CEWL/ A_b in *P. kuhlii* doubled between $T_a \approx 25^\circ\text{C}$ and $T_a \approx 40^\circ\text{C}$ (Muñoz-García et al. 2012). In their study, bats wearing masks were restrained in a horizontal position and had significantly higher rates of TEWL than individuals that were not wearing masks and that were unrestrained in respirometry chambers. Muñoz-García et al. (2012) interpreted these higher TEWL values in mask-wearing bats as a consequence of stress associated with restraint, but they noted that the likely effect of stress on CEWL/REWL ratios was unclear. In our study, *E. wahlbergi* appeared completely calm during measurements, and TEWL and T_b between $T_a = 10^\circ$ and 30°C were statistically indistinguishable between mask-wearing and unrestrained bats. However, the significantly higher RMR values associated with the masks suggest that our experimental setup nevertheless caused a degree of stress to the bats. The most likely conse-

quence would be an increase in REWL, with the lack of a corresponding increase in TEWL suggesting that the fractional contribution of CEWL may be reduced under these conditions. This observation raises the possibility that, under natural conditions and in the absence of any artificial stressors, CEWL/TEWL may be higher than under the artificial conditions of this study, reinforcing the conclusion that CEWL is the predominant avenue of evaporation at moderate T_a . Although we cannot rule out the possibility that percentage REWL was overestimated at higher T_a , the relatively small magnitude of the mask-associated increases in RMR suggests that any such effect would have been small compared to the large increases associated with the onset of panting. Overall, however, the fact that TEWL and T_b at moderate T_a were not significantly affected by the masks suggests that our experimental setup and training protocol obviated a large component of the stress-related issues potentially encountered by Muñoz-García et al. (2012).

Another source of error in our estimates of REWL and CEWL concerns the observation that fruit bats sometimes licked their snouts and muzzles at high T_a , a pattern of heat dissipation behavior commonly observed in pteropodids roosting under natural conditions (e.g., Nelson 1965; Ochoa-Acuña and Kunz 1999). Although we corrected REWL and CEWL estimates for CEWL from the area of the skin enclosed by the mask, this approach assumes that CEWL/ A_b in this region is the same as that of the rest of the skin. Thus, behavioral augmentation of evaporation from the skin surface by licking may mean that we have overestimated the fractional contribution of REWL. However, given the relatively small skin area that a bat was able to lick while wearing a mask, we suggest that this source of

error did not appreciably affect our conclusions regarding the relative importance of respiratory heat loss at high T_a .

Our data do not support Makanya and Mortola's (2007) findings that 5%–10% of respiratory gas exchange occurs across the wing membranes in *E. wahlbergi*. Even if there was zero air mixing between the mask and chamber in our experimental setup and our estimated cutaneous \dot{V}_{CO_2} values exclusively reflect cutaneous gas exchange, this avenue represented <3% of total CO_2 production. However, the small concentrations of CO_2 present in the chamber (i.e., before the air was drawn into the mask) could also represent an experimental artifact on account of the mixing of air between the mask and the chamber, in which case the actual contribution of cutaneous \dot{V}_{CO_2} would be even lower. Conversely, the small magnitude of estimated \dot{V}_{CO_2} in the chamber verifies that even if mixing occurred between the mask and the chamber, the extent of this mixing was very small and likely a negligible source of error in our estimates of REWL and CEWL.

Far more is known about the partitioning of TEWL into respiratory and cutaneous avenues in birds than in bats or other mammals. For instance, it is well established that, at T_a approaching and exceeding normothermic T_b , passerine birds rely predominantly on increased REWL to defend T_b , whereas columbiformes (pigeons and doves) rely predominantly on increased CEWL (Marder and Gavrieli-Levin 1986; Webster and Bernstein 1987; Withers and Williams 1990; Wolf and Walsberg 1996; Tieleman and Williams 2002; McKechnie and Wolf 2004). The general avian pattern of EWL at $T_a > T_b$ seems to be that either REWL or CEWL strongly predominates evaporative heat dissipation. Based on the limited available data, evaporative cooling in bats appears to be characterized by simultaneous increases in both REWL and CEWL to a greater degree than is the case in birds. In our study, both CEWL and REWL increased substantially with T_a ; CEWL was 4.3-fold higher and REWL was 10.3-fold higher at $T_a \approx 40^\circ C$ than at $20^\circ C$ (fig. 4). Relative fractional increases in CEWL and REWL with increasing T_a were similar in *P. kuhlii* (Muñoz-García et al. 2012). This pattern contrasts with that in passerine birds. For example, in verdins (*Auriparus flaviceps*), the contribution of CEWL to TEWL decreased sharply from ~60% at $T_a \approx 30^\circ C$ to less than 20% at $T_a > 40^\circ C$ (Wolf and Walsberg 1996), while qualitatively similar changes occurred in four species of larks (Tieleman and Williams 2002). Conversely, in heat-acclimated white-winged doves (*Zenaida asiatica*), CEWL represented nearly 80% of TEWL at $T_a \approx 45^\circ C$ (McKechnie and Wolf 2004). The limited available data hence suggest that, at T_a above normothermic T_b , both REWL and CEWL increase in bats in general, whereas the birds that have been examined so far tend to increase one, not both, of these avenues of evaporative heat dissipation.

The partitioning of TEWL into cutaneous and respiratory components may have important consequences for maximum evaporative cooling capacity and therefore tolerance of extreme heat. Cutaneous heat dissipation may be a more efficient means of thermoregulating during hot weather, because it does not require the muscle action associated with panting and/or gular flutter: in white-winged doves and rock pigeons, heat-accli-

ated individuals with higher CEWL had significantly lower metabolic rates at $T_a > 40^\circ C$ compared to cool-acclimated individuals in which a greater proportion of TEWL occurred via REWL (Marder and Arieli 1988; McKechnie and Wolf 2004). The sharp increase in RMR that occurred in *E. wahlbergi* at $T_a > 35^\circ C$ reveals that avoidance of hyperthermia incurs a significant metabolic cost and suggests that these fruit bats may not be able to tolerate the T_a maxima 2° – $5^\circ C$ higher than at present predicted for the end of the twenty-first century (IPCC 2011). Indeed, large-scale mortality among *Pteropus* flying foxes has been noted during extreme heat waves along the east coast of Australia (Welbergen et al. 2008), and recent years have seen media reports of similar mortality among fruit bats in India (e.g., Priyadarshi 2012).

Bats that spend much of their time in hot roosts, especially during summer, likely lose substantial fractions of their total body water daily (Studier 1970; Maloney et al. 1999). To estimate how much of the total daily water turnover of Wahlberg's epauletted fruit bats is evaporative in hot weather at our study site, we obtained hourly weather records for a week of unusually high T_a in Pretoria during November 2012 (data from weather station Pretoria UNISA, South African Weather Service). The long-term average daily maximum for November is $27^\circ C$ (South African Weather Service); the maximum T_a value on November 20, 2012, was $35.8^\circ C$. Wahlberg's epauletted fruit bats roost in deep shade within the canopies of leafy trees (Monadjem et al. 2010), and in the absence of strong wind, weather station T_a is probably a reasonable estimate of the fruit bats' operative temperature (Bakken 1976). Using the hourly T_a data for November 20, 2012, and our TEWL data for the fruit bats, we estimate that an 84-g roosting individual would lose 6.8 g H_2O by evaporation between sunrise and sunset, equivalent to 8.1% of its M_b . At $T_a = 35.8^\circ C$, the maximum chamber and mask water vapor pressures experienced by bats during our study were 0.4 and 1.7 kPa, respectively, whereas average values during November in Pretoria are typically in the 2.9–3.5-kPa range (South African Weather Service). However, bats roosting in tree canopies probably experience higher humidity levels, and hence the efficiency of evaporative cooling on hot days may well be much lower in individuals roosting in the wild than in the conditions of our study. Thus, we consider the above estimate of daily EWL a minimum; higher humidities and/or any radiative heat load will likely elevate it considerably.

Wahlberg's epauletted fruit bats also occur in regions considerably hotter than Pretoria, such as low-lying areas along the east coast of southern Africa (Skinner and Chimimba 2005), where, by the same calculation, their daily evaporative water losses would be considerably higher. Increasing all the temperatures of the hourly T_a curve we used by $5^\circ C$, for instance, would lead to a sunrise-to-sunset TEWL of 12.7 g H_2O , or 15.1% of a bat's M_b , which suggests that, in areas where T_a exceeds $40^\circ C$, the fruit bats would be at the very edge of their ability to maintain T_b by evaporative cooling. Although previous studies suggest that dehydration equivalent to 23%–32% of M_b is lethal for bats (Studier 1970), these values were as-

sociated with chronic dehydration at mild T_a and are probably not applicable to conditions of rapid dehydration during acute heat stress.

In conclusion, our data reveal that both respiratory and cutaneous rates of evaporation increase substantially at T_a values above normothermic T_b in an 84-g yinpterochiropteran bat. The predominance of REWL at high environmental temperatures contrasts with the situation at lower T_a and is associated with high metabolic costs of thermoregulation. Taken together with recent reports of large-scale mortality among flying foxes during heat waves, our data highlight the need for additional studies that explore the capacity of fruit bats to adjust the partitioning of TEWL into respiratory and cutaneous avenues in response to acclimatization or acclimation to hot conditions as well as their dehydration tolerance limits under conditions of acute heat stress.

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