



Metabolic rate, evaporative water loss and thermoregulatory state in four species of bats in the Negev desert



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ARTICLE INFO

Article history:

Received 16 June 2015

Received in revised form 7 October 2015

Accepted 7 October 2015

Available online 13 October 2015

Keywords:

Bats

Respiratory water loss

Cutaneous evaporative water loss

Metabolic rate

Torpor

Desert

ABSTRACT

Life in deserts is challenging for bats because of their relatively high energy and water requirements; nevertheless bats thrive in desert environments. We postulated that bats from desert environments have lower metabolic rates (MR) and total evaporative water loss (TEWL) than their mesic counterparts. To test this idea, we measured MR and TEWL of four species of bats, which inhabit the Negev desert in Israel, one species mainly restricted to hyper-arid deserts (*Otonycteris hemprichii*), two species from semi-desert areas (*Eptesicus bottae* and *Plecotus christii*), and one widespread species (*Pipistrellus kuhlii*). We also measured separately, in the same individuals, the two components of TEWL, respiratory water loss (RWL) and cutaneous evaporative water loss (CEWL), using a mask. In all the species, MR and TEWL were significantly reduced during torpor, the latter being a consequence of reductions in both RWL and CEWL. Then, we evaluated whether MR and TEWL in bats differ according to their geographic distributions, and whether those rates change with T_a and the use of torpor. We did not find significant differences in MR among species, but we found that TEWL was lowest in the species restricted to desert habitats, intermediate in the semi-desert dwelling species, and highest in the widespread species, perhaps a consequence of adaptation to life in deserts. Our results were supported by a subsequent analysis of data collected from the literature on rates of TEWL for 35 bat species from desert and mesic habitats.

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1. Introduction

Mid-latitude deserts are characterized by high daytime ambient temperatures (T_a), intense solar radiation, low humidity, desiccating winds, scarce and unpredictable food supplies, and free-water availability (Noy-Meir, 1973), conditions that potentially exert strong selective pressures on biota. Some endothermic animals that live in deserts have morphological and behavioral adaptations, which include different degrees of fossoriality, nocturnality, or both, thus avoiding high heat loads and low air humidity during the day (Schmidt-Nielsen, 1964). Desert-dwelling birds and mammals are also physiologically adapted to cope with these environmental conditions; they have generally lower metabolic rates (MR) and lower rates of evaporative water loss (EWL) than their mesic counterparts (Schmidt-Nielsen and Schmidt-Nielsen, 1950; Lovegrove, 2000, 2003; Williams and Tieleman, 2005; Van Sant et al., 2012).

The only order of volant mammals, bats number over 1300 species, which occupy a variety of habitats on all continents, except Antarctica (Fenton and Simmons, 2014). Although bats expend some 15 times as much energy during flight than at rest (Speakman and Thomas, 2003), they have lower basal metabolic rates (BMR) than that of other mammals of the same body mass (m_b) (McNab, 1982; Speakman and Thomas, 2003; Marom et al., 2006). Bats have significantly larger surface to volume ratios than terrestrial mammals of similar m_b due to their membranous wings, which are vascularized and not insulated by fur (Hill and Smith, 1984), leading to the idea that cutaneous evaporative water loss should be significant in bats (Licht and Leitner, 1967; Thomas et al., 1991; Minnaar et al., 2014).

Total evaporative water loss (TEWL), the sum of respiratory water loss (RWL) and cutaneous evaporative water loss (CEWL), is the main avenue of water loss in small mammals, representing up to 80–85% of their total water loss (Studier, 1970). Some bats apparently lose twice as much water through evaporation than terrestrial mammals of the same body mass because of their relatively large surface areas (Studier, 1970; Webb, 1995), but these conclusions are based on studies

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with small samples sizes. Therefore, it is intriguing that bats live in many desert environments, since they harbor traits that potentially increase energy consumption and water loss.

Many species of bats can enter torpor, a state wherein bats allow their body temperature (T_b) to drop below normal by 5–15 °C, but as much as 20 °C, with a concomitant decrease in MR and possibly water loss (Herreid and Schmidt-Nielsen, 1966; Webb, 1995; Schmidt-Nielsen, 1997; Ruf and Geiser, 2014). Torpor may be a key feature that allows mammals to colonize and survive in a wide variety of habitats (Bozinovic and Marquet, 1991; Lovegrove and Raman, 1998; Rambaldini and Bringham, 2008). Bats may enter shallow torpor on a daily basis, reducing T_b by just a few degrees for several hours, or they may endure longer and deeper torpor bouts that can last from weeks to months. Prolonged bouts of deep torpor are used by species of bats from diverse environments and may occur at low T_a , hibernation in winter, for example, or when prey are scarce (Brack and Twente, 1985; Turbill et al., 2003; Geiser, 2004; Zubaid et al., 2006).

Of the 32 species of bats that occur in Israel, 12 species are found in the Negev Desert (Yom-Tov and Kadmon, 1998; Korine and Pinshow, 2004). Consequently it seems likely that these species have adaptations to conserve energy and prevent excessive rates of evaporative water loss (Kunz, 1982; Geiser, 2004; Marom et al., 2006; Muñoz-García et al., 2012a). Nonetheless, some studies report that desert-dwelling bat species do not have significantly lower MR or water loss than those of mesic species (Marom et al., 2006; Muñoz-García et al., 2012a,b), suggesting that desert bats might not show specific adaptations to live in these environments. However, few desert species have been examined. Also, measurements of TEWL available in the literature were mostly made on normothermic animals, i.e., animals defending high T_b , with few measurements on torpid bats, making it difficult to evaluate differences in energy expenditure and water fluxes between desert and mesic species.

Since metabolic rates and TEWL are reduced by 50–90% during torpor (Carpenter, 1969; Morris et al., 1994), the use of torpor by desert species of bats is of adaptive significance. Bats are not known to defecate or urinate during torpor (Webb, 1995), and therefore it is commonly assumed that all water losses during torpor are evaporative. Marom et al. (2006) measured the rates of TEWL in two species of bat that inhabit the Negev Desert: one that lives only in deserts, *Otonycteris hemprichii*, and another that is found throughout Israel, *Tadarida teniotis*. Bats entered torpor at low T_a s during the day, as determined by their significantly reduced T_b , compared to T_b in normothermic bats measured at night. The lower MR that ensues during torpor should lead to reduced RWL and, therefore, reduced TEWL. However, the authors did not find significant differences in TEWL between torpid and normothermic bats. These data suggest that during torpor, CEWL might be similar to or even increase over that during normothermy, a counterintuitive result. To test this idea, Muñoz-García et al. (2012a) measured CEWL and RWL in Kuhl's pipistrelle (*Pipistrellus kuhlii*) a widespread species but one that also inhabits the Negev Desert. The authors found that, when in deep torpor, CEWL of bats was similar or even higher than that of bats in shallow torpor, leading Muñoz-García et al. (2012a) to hypothesize that bats exert less physiological control over CEWL when torpid.

In the present study, we evaluated whether rates of metabolism and water loss in bats differ according to their geographic distributions, and whether those rates change with T_a and the use of torpor. We measured MR and TEWL of four sympatric species of bats in the Negev Desert: one species whose range is restricted mainly to hyper-arid deserts (*O. hemprichii*), two hyper-arid and semi-desert species (*Eptesicus bottae* and *Plecotus christii*), and one widespread species (*P. kuhlii*) (Yom-Tov and Kadmon, 1998; Nowak, 1999). We hypothesized that species of bat found only in deserts have adaptations to reduce energy expenditure and water loss, in contrast to bats that live in mesic environments. We predicted that, while euthermic, MR and TEWL of bats

at rest would be lowest in the desert species (*O. hemprichii*), intermediate in the semi-desert dwelling species (*E. bottae* and *P. christii*), and highest in the widespread species (*P. kuhlii*). To further test the hypothesis that there is a relationship between TEWL and habitat, we compared rates of TEWL for 35 bat species from desert and mesic habitats. We predicted that desert species have lower rates of TEWL than mesic species. Moreover, we predicted that bats in torpor have reduced MR and rates of TEWL, which result from reductions in both RWL and CEWL, and that these reductions are greater in desert species than in mesic species.

2. Materials and methods

2.1. Experimental animals

We captured non-reproductive adult bats in the central Negev Highlands. Experiments were done on the Sede-Boqer Campus of Ben-Gurion University of the Negev. Bats were kept in separate, large, covered outdoor cages (2 × 2 × 4 m) and fed daily with sufficient *Tenebrio* larvae to enable them to maintain m_b . Water with vitamins (RIBOS, Biopet, Maabarot, Israel) was provided ad libitum. Bats were exposed to semi-natural weather and light conditions during captivity. The cage was shaded and provided with a bat box for roosting. Once measurements were completed, animals were released at their place of capture.

2.2. Measurements of resting metabolic rate and total evaporative water loss

We calculated MR, TEWL, CEWL and RWL in postabsorptive individuals using a multi-channel, open-flow respirometry system to measure O_2 consumption ($\dot{V} O_2$), CO_2 production ($\dot{V} CO_2$) and water loss. Measurements were made at four different ambient temperatures (T_a): 10 °C, 15 °C, 30 °C and 35 °C. Bats were weighed before and after measurements to ± 0.01 g on a digital balance (Scout SP202, Ohaus, NJ, USA). We recorded rectal temperature as a proxy for T_b before and after respirometry measurements using a sheathed, 36-gauge type-T thermocouple, read with a microprocessor thermometer (OMEGA, model HH23, Stanford, CT, USA). We also measured T_a continuously with a thermocouple (same type as above) located inside a controlled temperature cabinet (Thermo Scientific, model Precision 815, OH, USA) where metabolic chambers were placed.

To measure MR and TEWL, we placed each bat in a plastic metabolic chamber (Lock&Lock model HPL93, 0.35 L). For the smallest species, *P. kuhlii*, we built chambers using plastic containers (50 cm³) that were covered with dark tape to minimize stress to the animals during measurements. Each chamber was lined with 0.25 cm plastic-dipped hardware cloth from which the bats could hang upside down. Paraffin oil at the bottom of the chambers trapped feces and urine that could add water vapor to the air sample. Air was pumped into the metabolic chambers from outside the building through a purged gas generator (Purge Gas model #PCDA-1-12-m-32-C, Broomfield, CO, USA) providing dry, CO_2 -free air. Flow rates ranged from 160 to 180 mL/min for the medium-sized chambers and ~100 mL/min for the small chambers. From every chamber, a subsample of air was pumped through an eight-channel multiplexer (G244 gas switcher, Qubit Systems Inc.) that routed the sample of air from each chamber to the gas analyzers, enabling the sequential monitoring of up to six animals. The multiplexer was programmed to automatically select channels, alternating between a chamber containing an animal and the reference channel that had an empty chamber and served to generate a baseline for each cycle. Each cycle started with 300 s of reference chamber air, followed by 300 s from each of the chambers containing an animal. We separated the 300 s intervals with 60 s switches to the reference chamber. Air coming from the multiplexer was then routed through a dew point meter (RH-

300, Sable Systems International, Inc. Las Vegas, NV, USA), followed by a magnesium perchlorate column to dry the sample, and finally into the O₂ and CO₂ analyzers (FoxBox O₂ and CO₂ gas analyzer, Sable Systems International). Each trial lasted 2–4 h, enough time to ensure that bats were at rest when measurements were made. For calculations, we used the average values of $\dot{V} \text{CO}_2$ and $\dot{V} \text{O}_2$ from traces that remained stable for at least 5 min.

To measure CEWL and RWL, we fitted bats with a mask that covered the snout, following Muñoz-García et al. (2012a). To reduce stress in animals, only one bat was measured at a time at each T_a with this system for a maximum of 2 h (Muñoz-García et al., 2012a). If the bat removed the mask more than twice, we stopped the measurements and repeated the trial with the same animal another day. The chamber used for these measurements (Lock&Lock model HPL818, Hana Cobi, Korea, volume 1.56 L) had an inlet port and two outlet ports: one for air coming from the chamber and a second for air coming from the mask and the chamber. The flow rate of air entering the chamber was set at ~400 mL/min. An external pump (Qubit Systems, model P651, Kingston, ON, Canada) regulated the flow rate of the channel used to draw air from the mask, which was set at ~250 mL/min. This was always higher than the flow rate of the air stream pulled out of the chamber (~150 mL/min), to ensure that all respiratory gases were captured in the mask. Measurement cycles started with 300 s of reference air, followed by 300 s of air drawn from the outlet port of the chamber, followed by 60 s of reference air and finishing with 300 s of air drawn from the outlet port coming from the mask. The bat with its wings folded was immobilized in a horizontal position with small strips of Velcro that were attached to an aluminum mesh frame following Muñoz-García et al. (2012a). Paraffin oil was not used, given the complications of this method. In the event that feces and/or urine were found in the chamber, data were discarded. In each measurement session, we recorded the fractional concentration of O₂ (FO₂) or CO₂ (FCO₂), dew-point temperature, T_a at the dew-point meter, and barometric pressure.

Total evaporative water loss (g/h) was calculated using the following equation:

$$TEWL = (V_{ep_{out}} - V_{ip_{in}})$$

where V_{ip_{in}} and V_{ep_{out}} are the incurrent and excurrent flows (mL/min) times their respective water vapor densities corrected to standard temperature and pressure (g/m³, STP).

To calculate RWL, we followed Tieleman and Williams (2002):

$$RWL = (\rho_{mask} - \rho_{chamber}) \times V_{EM}$$

where ρ_{mask} is the water vapor density (g/m³, STP) in the sample of air exiting the mask, ρ_{chamber} is the water vapor density (g/m³, STP) of the air exiting the chamber, and V_{EM} is the corrected flow rate of air exiting the mask, calculated as:

$$\dot{V}_{EM} = \dot{V}_{IC} + \dot{V} \text{H}_2\text{O} + \dot{V} \text{CO}_2 - \dot{V} \text{O}_2$$

where \dot{V}_{IC} is the flow rate of incurrent air entering the chamber (mL/min), $\dot{V} \text{H}_2\text{O}$ is the volume of water vapor (mL/min) added by evaporation, $\dot{V} \text{CO}_2$ is the volume of CO₂ added (mL/min) by the animal and $\dot{V} \text{O}_2$ is the volume of O₂ it consumed (mL/min).

To estimate CEWL, we calculated the water vapor density of the air that left the chamber and the mask and multiplied it by the sum of the flow rates of the air streams coming from the mask and the chamber, as follows:

$$CWL = (\rho_{chamber} - \rho_{v-in}) \times (V_{EM} + V_{EC})$$

where ρ_{v-in} is the vapor density of the air entering the chamber (g/m³,

STP) and V_{EC} is the flow rate of the air leaving the chamber. $\dot{V} \text{O}_2$ was calculated using equation 4a of Withers (1977):

$$\dot{V} \text{O}_2 = [V_E(F_{I\text{O}_2} - F_{E\text{O}_2})] / (1 - F_{I\text{O}_2})$$

where F_IO₂ is the fraction of O₂ entering the mask, and F_EO₂ is the fractional concentration of O₂ exiting the mask; therefore, V_E(F_IO₂ - F_EO₂) is the difference in the concentration of O₂ between inlet and outlet air multiplied by the corrected flow rate (Withers, 1977).

For the calculation of MR of torpid bats, we used $\dot{V} \text{CO}_2$, rather than $\dot{V} \text{O}_2$ because the signal to noise ratio of the O₂ analyzer was too low to give accurate readings (Ben-Hamo et al., 2013); $\dot{V} \text{CO}_2$ was calculated as follows:

$$\dot{V} \text{CO}_2 = V_I [1 - \Delta\text{CO}_2 / (1 - (1/0.71))]$$

where V_I is the flow of air incurrent to the chamber and ΔCO₂ is the increase of CO₂ produced by the animal in the air sample. We assumed a respiratory exchange ratio of 0.71 for a post-absorptive bat catabolizing only fat (Schmidt-Nielsen, 1997; Muñoz-García et al., 2012a; Ben-Hamo et al., 2013). We used this value to convert $\dot{V} \text{CO}_2$ into $\dot{V} \text{O}_2$ and then to mW, assuming 19.61 J is produced for each mL of O₂ consumed (Schmidt-Nielsen, 1997).

2.3. Data analysis

All the physiological variables measured in this study are presumably affected by torpor. Many researchers have treated torpor as a categorical variable, either in torpor or not, and determined an arbitrary cutoff of drop in T_b for designating the point at which animals become torpid; others have used the difference between T_a and T_b to classify animals as torpid or normothermic. However, T_b in heterothermic animals is a continuous variable, and individuals experience different depths of torpor depending on environmental conditions, body mass and taxonomic affiliation (see Muñoz-García et al., 2014, and references therein). Therefore, we calculated the thermoregulatory index (TRi), an indicator of the thermoregulatory state of heterothermic endotherms, described by Muñoz-García et al. (2014), as a proxy for depth of torpor. TRi is calculated as:

$$\text{TRi} = [m_b(T_b - T_a)C_p(\text{MR})^{-1}] e^{E_i[\text{Abs}\Delta T]/(k \cdot T_b \cdot T_a)}$$

where C_p is the specific heat capacity of tissues (3.5 J/g °C) (Hart, 1951), E_i is the energy of activation for biochemical reactions (with an average of 62.72 kJ/mol), AbsΔT is the absolute value of the difference between T_b and T_a, and k is the Boltzmann constant (1.381 × 10⁻²³ J/K). TRi can be interpreted as the length of time it takes an animal to generate or dissipate the amount of heat that it will gain or dissipate by physical means (convection, conduction, radiation) to maintain a given temperature gradient with the environment. We used TRi to compare the physiological variables of interest among individuals of different species enduring different depths of torpor.

To test for differences in body temperature (T_b), we used a mixed-effects ANOVA with bat ID as a random factor nested in species, and with species and ambient temperature (T_a) as categorical predictors. We used a two-way ANOVA, with T_a and species as fixed factors, to test for significant differences in TRi. To evaluate differences in MR, we used ANCOVA, with m_b as a covariate, and T_a and species as fixed factors. To compare RWL among species, we divided it by m_b^{0.75}, since RWL scales allometrically with MR (Schmidt-Nielsen, 1970).

We estimated the surface area of bats using the following morphometric measurements from 4 to 7 individuals of each species: length of the body, length of the tail, width of the body, length of the forearm,

length of the third digit, and width and length of the ear. With these measurements we calculated total surface area as the sum of the surface area of the wing, the ears, the uropatagium and the body. We correlated total surface area with body mass of individuals, for each species, and we used the allometric coefficients generated from this correlation to estimate total surface area for each bat used in our study.

We used ANCOVA to test for significant differences in surface specific (ss) TEWL, metabolic rate corrected (ms) RWL and ssCEWL, using TRi as a covariate, and T_a and species as fixed factors. Finally, to evaluate if there were differences at which CEWL and RWL decreased with depth of torpor, we regressed CEWL vs RWL, using TRi as a covariate, separately for each bat species.

All statistical analyses were done with R, version 3.1.2., using the 'nlme' package, and SPSS 22. Data are reported as means \pm SD and we chose α of 0.05 to reject our null hypotheses. We log-transformed data when they were heteroscedastic.

To compare values of TEWL in this study with those of other species, we collected TEWL data from the literature and calculated allometric equations for 35 species of bat (see Table 1). Species were designated as mesic ($n = 21$) or desert ($n = 14$), based on habitat description by the authors. We collected TEWL data for bats within their thermal-neutral zones (TNZ), which ranged from ~ 20 to 39.5°C , and did a phylogenetic signal k-test (Blomberg et al., 2003) on the values of TEWL and m_b for all species. For these analyses, we used a macro for Microsoft Office Excel 2007 (by P. C. Withers, School of Animal Biology, University of Western Australia) to calculate the expected values and predicted 95% confidence intervals for each variable. Since the phylogenetic signal was not significant ($k_{mb} = 0.18$, $P = 0.17$, $k_{TEWL} = 0.06$, $P = 0.41$), we did not make phylogenetic corrections. We used ANCOVA to compare TEWL between mesic and desert species, with m_b as a covariate and "habitat" (mesic vs. desert) as a factor. First, we evaluated a full model with the interaction " m_b " \times "habitat" to test for significant differences in the slopes between m_b and TEWL. Since the slopes were not significantly different, we removed the interaction term from the analysis and tested for significant differences in the intercepts.

3. Results

The average values of all the variables measured in each bat species in this study are presented in Table 1.

Table 1

Physiological variables measured in the individuals of four species of bats at four different ambient temperatures (T_a), measured with mask or without it ($n = 6$ for *Eptesicus bottae* and *Otonycteris hemprichii*, $n = 7$ for *Plecotus christii* and $n = 9$ for *Pipistrellus kuhlii*). Values are means \pm SD. T_b = Body temperature; MR = metabolic rate; TEWL = total evaporative water loss; RWL = respiratory water loss; CEWL = cutaneous evaporative water loss.

| T_a ($^\circ\text{C}$) | Species | T_b ($^\circ\text{C}$) | | MR (mW) | | TEWL (g/h) | | RWL (g/h) | CEWL (g/h) |
|----------------------------|----------------------|----------------------------|-----------------|--------------------|---------------------|-----------------|-----------------|-----------------|-----------------|
| | | No mask | Mask | No mask | Mask | No mask | Mask | | |
| 10 | <i>E. bottae</i> | 19.03 \pm 6.5 | 17.25 \pm 5.1 | 27.96 \pm 35.89 | 63.37 \pm 88.48 | 0.24 \pm 0.12 | 0.53 \pm 0.15 | 0.21 \pm 0.08 | 0.32 \pm 0.12 |
| | <i>P. christii</i> | 19.65 \pm 6.6 | 16.1 \pm 1.4 | 32.41 \pm 57.07 | 35.45 \pm 54.54 | 0.29 \pm 0.15 | 0.58 \pm 0.22 | 0.19 \pm 0.1 | 0.39 \pm 0.2 |
| | <i>O. hemprichii</i> | 13.3 \pm 1.1 | 26.78 \pm 1.3 | 29.27 \pm 19.18 | 776.23 \pm 106.05 | 0.56 \pm 0.16 | 1.58 \pm 0.35 | 0.6 \pm 0.2 | 0.98 \pm 0.22 |
| | <i>P. kuhlii</i> | 15.24 \pm 3.6 | 15.94 \pm 2.1 | 22.85 \pm 31.04 | 52.33 \pm 53.23 | 0.26 \pm 0.19 | 0.59 \pm 0.17 | 0.23 \pm 0.11 | 0.36 \pm 0.08 |
| 15 | <i>E. bottae</i> | 20.38 \pm 3.5 | 25.7 \pm 5.1 | 19.45 \pm 14.79 | 309.65 \pm 260.21 | 0.45 \pm 0.27 | 0.81 \pm 0.18 | 0.39 \pm 0.25 | 0.41 \pm 0.22 |
| | <i>P. christii</i> | 24.01 \pm 7.2 | 23.3 \pm 4.3 | 61.02 \pm 71.67 | 138.06 \pm 137.78 | 0.35 \pm 0.11 | 0.73 \pm 0.17 | 0.31 \pm 0.06 | 0.42 \pm 0.12 |
| | <i>O. hemprichii</i> | 18.38 \pm 1.7 | 28.55 \pm 2.4 | 24.13 \pm 23.93 | 648.21 \pm 84.13 | 0.42 \pm 0.09 | 1.69 \pm 0.36 | 0.72 \pm 0.21 | 0.97 \pm 0.24 |
| | <i>P. kuhlii</i> | 18.9 \pm 4.7 | 19.2 \pm 3.5 | 25.3 \pm 37.09 | 68.6 \pm 95.98 | 0.28 \pm 0.2 | 0.81 \pm 0.39 | 0.38 \pm 0.22 | 0.43 \pm 0.19 |
| 30 | <i>E. bottae</i> | 32.93 \pm 1.8 | 32.15 \pm 1.3 | 91.18 \pm 48.36 | 113.69 \pm 96.58 | 1.26 \pm 0.7 | 1.22 \pm 0.53 | 0.5 \pm 0.38 | 0.71 \pm 0.49 |
| | <i>P. christii</i> | 33.26 \pm 2.1 | 32.9 \pm 2.1 | 46.26 \pm 27.54 | 63.11 \pm 34.99 | 0.5 \pm 0.1 | 0.96 \pm 0.19 | 0.44 \pm 0.18 | 0.52 \pm 0.19 |
| | <i>O. hemprichii</i> | 32.21 \pm 1.1 | 33 \pm 0.6 | 117.64 \pm 39.8 | 151.43 \pm 32.56 | 0.86 \pm 0.25 | 2.36 \pm 0.29 | 1.16 \pm 0.09 | 1.19 \pm 0.23 |
| | <i>P. kuhlii</i> | 32.36 \pm 1.6 | 31.7 \pm 1 | 34.49 \pm 15.24 | 55.64 \pm 35.31 | 0.41 \pm 0.12 | 1 \pm 0.22 | 0.47 \pm 0.13 | 0.53 \pm 0.16 |
| 35 | <i>E. bottae</i> | 35.03 \pm 0.6 | 35.2 \pm 1 | 60.19 \pm 28.17 | 85.36 \pm 44.91 | 1.06 \pm 0.25 | 1.95 \pm 0.73 | 0.99 \pm 0.6 | 0.96 \pm 0.17 |
| | <i>P. christii</i> | 36.06 \pm 0.5 | 35.65 \pm 1.1 | 68.58 \pm 31.59 | 70.14 \pm 36.05 | 1.53 \pm 0.68 | 1.44 \pm 0.65 | 0.68 \pm 0.37 | 0.77 \pm 0.28 |
| | <i>O. hemprichii</i> | 35.88 \pm 0.7 | 36.16 \pm 0.7 | 115.41 \pm 51.28 | 135.76 \pm 19.56 | 1.67 \pm 0.43 | 2.57 \pm 0.33 | 1.32 \pm 0.32 | 1.25 \pm 0.27 |
| | <i>P. kuhlii</i> | 35.62 \pm 0.6 | 34.77 \pm 1.2 | 41.88 \pm 5.42 | 48.94 \pm 17.08 | 0.54 \pm 0.16 | 1.28 \pm 0.17 | 0.73 \pm 0.11 | 0.55 \pm 0.12 |

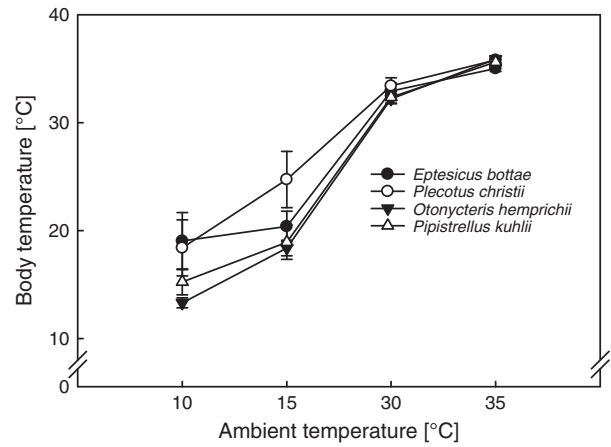


Fig. 1. The relationship between body temperature (T_b) and ambient temperature (T_a) in *Eptesicus bottae*, *Otonycteris hemprichii*, *Plecotus christii* and *Pipistrellus kuhlii*. Values are means \pm SD. Body temperature decreased with T_a , but there were no significant differences among species.

3.1. Body temperature

Body temperature increased significantly with T_a in all species (T_a : $F_{1,75} = 368.97$, $P < 0.001$, Fig. 1). *P. christii* had higher T_b than *O. hemprichii* ($P = 0.04$) and *P. kuhlii* ($P = 0.005$) at all values of T_a (Fig. 1; Species: $F_{3,75} = 4.28$, $P < 0.01$), but the T_b of *P. christii* did not differ significantly from that of *E. bottae* ($P = 0.2$).

3.2. Metabolic rate and thermoregulatory index

We found a significant interaction of " T_a " by "species" for MR ($F_{9,95} = 2.90$, $P = 0.004$, Fig. 2). We suggest that this result can be explained by the fact that there was large variability in MR in all species of bats, probably because individuals experienced different levels of torpor during measurements. Therefore, to control for differences in thermoregulatory states, we calculated TRi for all bats and incorporated this variable in our analyses. We found that TRi decreased significantly with T_a in all species, indicating that torpor was deeper at low T_a (Fig. 3; T_a : $F_{3,105} = 91.9$, $P < 0.001$). We also found differences among

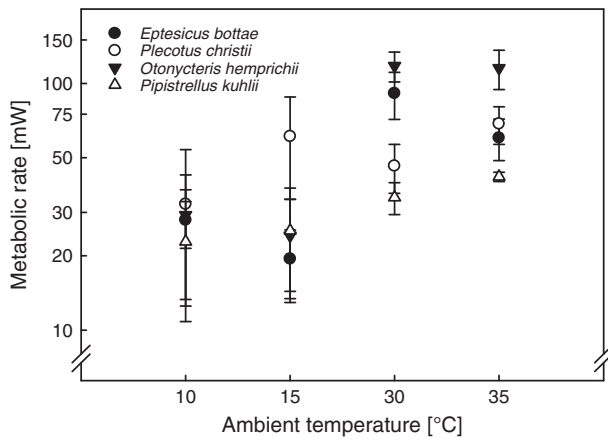


Fig. 2. The relationship between metabolic rate (MR) and ambient temperature (T_a) in *Eptesicus bottae*, *Otonycteris hemprichii*, *Plecotus christii* and *Pipistrellus kuhlii*. Values are means \pm SD. There was high variability in MR at all T_a s, probably because bats experienced different levels of torpor.

species, *O. hemprichii* having significantly lower TRI than the other species ($F_{3,105} = 3.87$, $P < 0.02$). Thus, individual *O. hemprichii* entered shallower torpor than the other bat species.

3.3. Total evaporative water loss

We found high variability in surface-specific TEWL (ssTEWL) among species and at different T_a , probably because bats were at different torpor depths (Fig. 4B). To account for these differences, we included TRI as a covariate in our analyses, and found that TRI covaried significantly with ssTEWL (Fig. 4A; $F_{1,103} = 110.3$, $P < 0.001$), namely it is apparent that as torpor depth increased, ssTEWL decreased (Fig. 4A). We found that, for bats, at the same depth of torpor, ssTEWL was significantly lower at low T_a (10 and 15 °C) than at higher T_a (30 and 35 °C) (Fig. 4A; T_a : $F_{3,103} = 10.73$, $P < 0.01$). Comparing among species, and after correction for torpor depth, ssTEWL of *O. hemprichii* was lower than that of the other species of bats (Fig. 4A; Species: $F_{3,103} = 40.74$, $P < 0.001$).

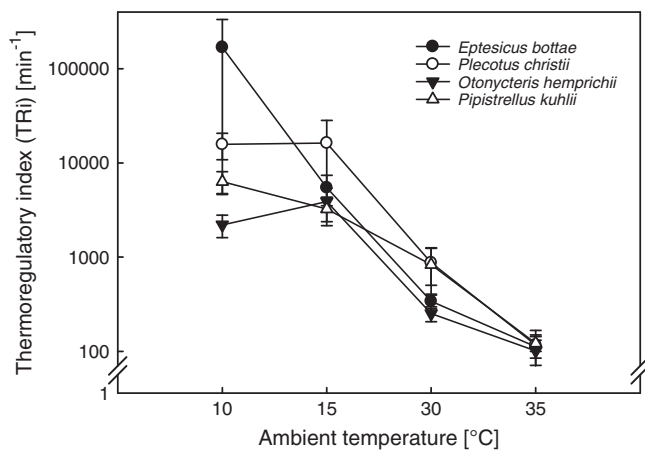


Fig. 3. The relationship between the thermoregulatory index (TRI) and ambient temperature (T_a) in *Eptesicus bottae*, *Otonycteris hemprichii*, *Plecotus christii* and *Pipistrellus kuhlii*. Values are means \pm SD. The TRI decreased with T_a , indicating that bats entered deeper torpor at low T_a . *O. hemprichii* had a significantly lower TRI than that of the other species of bats.

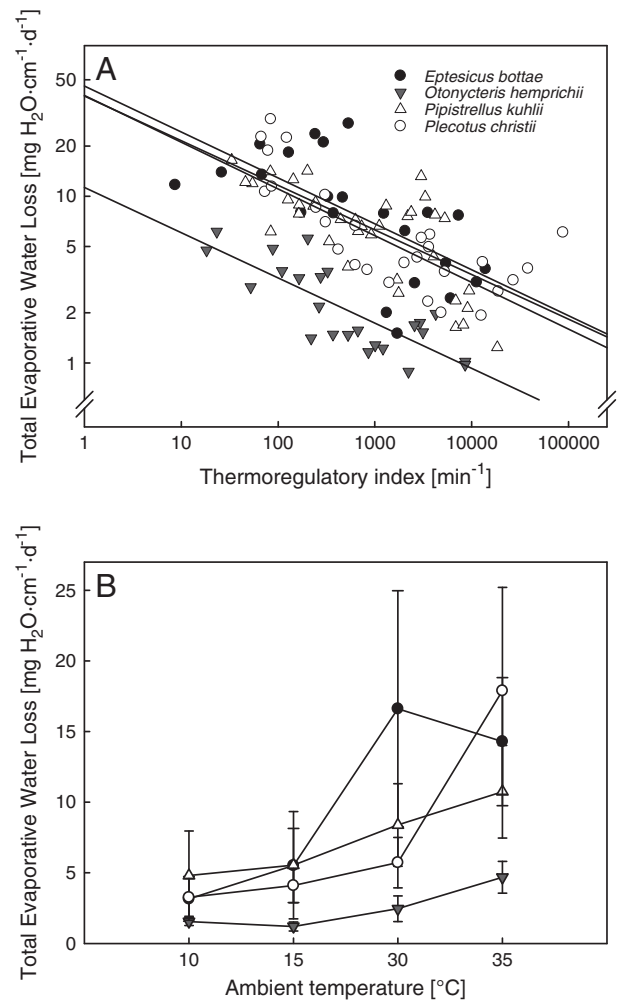


Fig. 4. (A) The relationship between surface-specific total evaporative water loss (ssTEWL) and the thermoregulatory index (TRI) in *Eptesicus bottae*, *Otonycteris hemprichii*, *Plecotus christii* and *Pipistrellus kuhlii*. As torpor depth increased, ssTEWL decreased in all species. *O. hemprichii* had significantly lower ssTEWL than the other species, after accounting for differences in the thermoregulatory state of individuals. (B) The relationship between ssTEWL and ambient temperature (T_a) in *E. bottae*, *O. hemprichii*, *P. christii* and *P. kuhlii*. Values are means \pm SD.

To compare TEWL of the four species we studied with TEWL of other species of bats from different environments, we collected data for TEWL from the literature, and divided the species into those that live in the desert ($n = 14$) and those that live in mesic environments ($n = 21$, Table 2, Fig. 5). We used only data from normothermic bats, in their TNZs. ANCOVA of TEWL for all the species, with m_b as a covariate, did not indicate a significant interaction term between habitat and m_b ($P = 0.68$); therefore, the slope of the relationship between TEWL and m_b was the same for species living in different habitats. After removing the interaction term, we found that habitat (desert or mesic) was significant ($F = 22.7$, $P < 0.001$), indicating that the y-axis intercept of the regression line relating TEWL and m_b was significantly different between desert and mesic species. The estimated rate of TEWL for a desert species with a body mass of 10 g was 40.86 mg water/h, whereas for mesic species, the calculated TEWL for a 10-g bat was 61.88 mg water/h, indicating that TEWL in desert bats is lower than that of mesic bats. The four species used in the present study had the lowest TEWL among all of the species of bats for which we have data (Fig. 5).

Table 2
Body mass (m_b), total evaporative water loss (TEWL) during normothermy, taxonomic affiliation and habitat of 35 species of bats.

| Species | m_b (g) | TEWL (g/h) | Family | Habitat | Reference (original) |
|----------------------------------|-----------|------------|------------------|---------|-----------------------------------|
| <i>Macroderma gigas</i> | 107.20 | 0.227 | Megadermatidae | Mesic | Baudinette et al., 2000 |
| <i>Rhinonycteris aurantius</i> | 8.27 | 0.092 | Hipposideridae | Mesic | Baudinette et al., 2000 |
| <i>Natalus stramineus</i> | 5.59 | 0.071 | Natalidae | Mesic | Studier, 1970 |
| <i>Noctilio albiventris</i> | 39.90 | 0.110 | Noctilionidae | Mesic | Roverud and Chappell, 1991 |
| <i>Taphozous mauritanicus</i> | 26.2 | 0.031 | Emballonuridae | Desert | Toussaint and McKechnie, 2012 |
| <i>Nycteris thebaica</i> | 11.7 | 0.055 | Nycteridae | Desert | Toussaint and McKechnie, 2012 |
| <i>Macrotus californicus</i> | 11.70 | 0.086 | Phyllostomidae | Desert | Bell et al., 1986 |
| <i>Leptonycteris curasoae</i> | 22.00 | 0.175 | Phyllostomidae | Desert | Carpenter and Graham, 1967 |
| <i>Leptonycteris sanborni</i> | 22.00 | 0.090 | Phyllostomidae | Mesic | Carpenter and Graham, 1967 |
| <i>Glossophaga soricina</i> | 11.31 | 0.112 | Phyllostomidae | Desert | Studier, 1970 |
| <i>Artibeus hirsutus</i> | 48.00 | 0.178 | Phyllostomidae | Mesic | Carpenter and Graham, 1967 |
| <i>Tadarida brasiliensis</i> | 16.90 | 0.038 | Molossidae | Desert | Herreid and Schmidt-Nielsen, 1966 |
| <i>Tadarida teniotis</i> | 31.97 | 0.028 | Molossidae | Desert | Marom et al., 2006 |
| <i>Molossus coibensis</i> | 14.71 | 0.080 | Molossidae | Mesic | Studier, 1970 |
| <i>Mops condylurus</i> | 23.24 | 0.037 | Molossidae | Desert | Maloney et al., 1999 |
| <i>Sauromys petrophilus</i> | 11.0 | 0.012 | Molossidae | Desert | Toussaint and McKechnie, 2012 |
| <i>Miniopterus schreibersii</i> | 10.91 | 0.063 | Vespertilionidae | Mesic | Baudinette et al., 2000 |
| <i>Chalinobolus gouldii</i> | 17.50 | 0.032 | Vespertilionidae | Mesic | Hosken and Withers, 1997 |
| <i>Lasiurus cinereus</i> | 32.50 | 0.183 | Vespertilionidae | Desert | Cryan and Wolf, 2003 |
| <i>Nyctophilus geoffroyi</i> | 8.00 | 0.020 | Vespertilionidae | Mesic | Hosken and Withers., 1999 |
| <i>Nyctophilus gouldi</i> | 8.00 | 0.011 | Vespertilionidae | Mesic | Morris et al., 1994 |
| <i>Nyctophilus major</i> | 13.60 | 0.036 | Vespertilionidae | Mesic | Hosken, 1997 |
| <i>Plecotus auritus</i> | 9.12 | 0.113 | Vespertilionidae | Mesic | Webb, 1995 |
| <i>Pipistrellus pipistrellus</i> | 6.20 | 0.084 | Vespertilionidae | Mesic | Webb, 1995 |
| <i>Myotis lucifugus</i> | 9.00 | 0.073 | Vespertilionidae | Desert | Procter and Studier, 1970 |
| <i>Myotis daubentonii</i> | 10.00 | 0.066 | Vespertilionidae | Mesic | Webb, 1995 |
| <i>Myotis nigricans</i> | 4.02 | 0.047 | Vespertilionidae | Mesic | Studier, 1970 |
| <i>Myotis nattereri</i> | 8.70 | 0.036 | Vespertilionidae | Mesic | Willis et al., 2011 |
| <i>Myotis velifer</i> | 9.70 | 0.033 | Vespertilionidae | Mesic | Studier et al., 1970 |
| <i>Myotis yumanensis</i> | 5.00 | 0.044 | Vespertilionidae | Mesic | Licht and Leitner, 1967 |
| <i>Myotis thysanoides</i> | 8.00 | 0.066 | Vespertilionidae | Mesic | Studier et al., 1970 |
| <i>Plecotus christii</i> | 6.8 | 0.003 | Vespertilionidae | Desert | This study |
| <i>Pipistrellus kuhlii</i> | 6.8 | 0.003 | Vespertilionidae | Desert | This study |
| <i>Eptesicus bottae</i> | 8.95 | 0.006 | Vespertilionidae | Desert | This study |
| <i>Otonycteris hemprichii</i> | 29.71 | 0.001 | Vespertilionidae | Desert | This study |

3.4. Respiratory and cutaneous water loss

Total evaporative water loss, the sum of CEWL and RWL, in bats wearing a mask to separate CEWL from RWL, was significantly higher than TEWL of the same individuals measured mask-free for all species

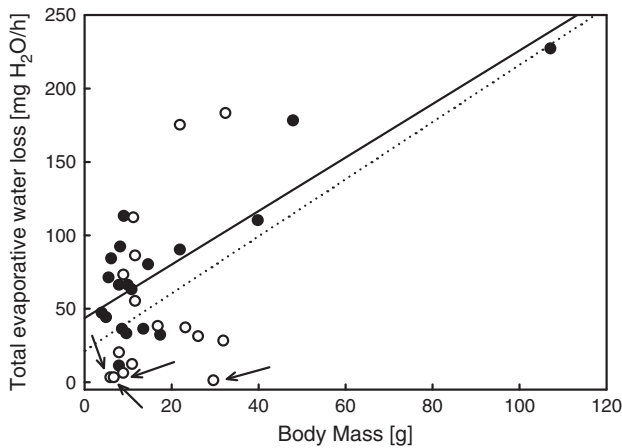


Fig. 5. Relationship between total evaporative water loss (TEWL) and body mass (m_b) in mesic species (filled symbols and solid line; $TEWL = 43.66 + 1.82 m_b$, $R^2 = 0.71$; $P < 0.001$) and desert species (unfilled symbols and dashed line; $TEWL = 21.46 + 1.94 m_b$, $R^2 = 0.10$; $P = 0.23$). TEWL was significantly lower in desert species than in mesic species after accounting for m_b . Arrows indicate the species that were measured in this study.

(paired t -test, $t_{109} = 13.6$, $P < 0.001$), a difference that we ascribe to stress (see Muñoz-García et al., 2012a). The purpose of fitting the bats with a mask was to calculate separately the respiratory and cutaneous components of TEWL. For bats wearing a mask, and using TR_i as a covariate, we found the same patterns between TEWL and T_a and species as

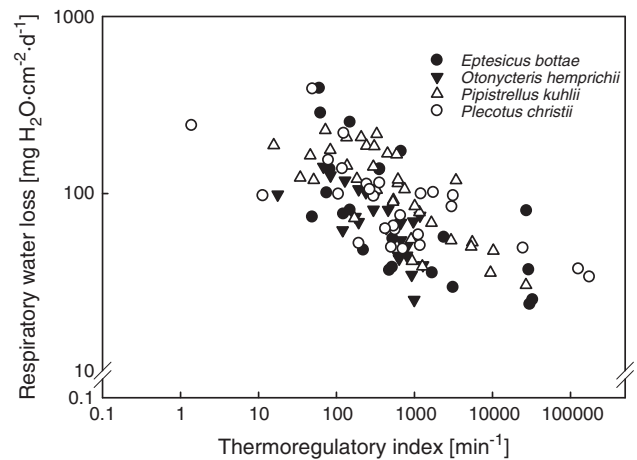


Fig. 6. The relationship between surface-specific respiratory water loss (ssRWL) and the thermoregulatory index (TR_i) in *Eptesicus bottae*, *Otonycteris hemprichii*, *Plecotus christii* and *Pipistrellus kuhlii*. As torpor depth increased, ssRWL decreased in all species. *O. hemprichii* had significantly lower ssRWL than the other species, *P. christii* and *E. bottae* had intermediate values for ssRWL, and *P. kuhlii* had a significantly higher ssRWL than the other three species, after accounting for differences in the thermoregulatory state of individuals.

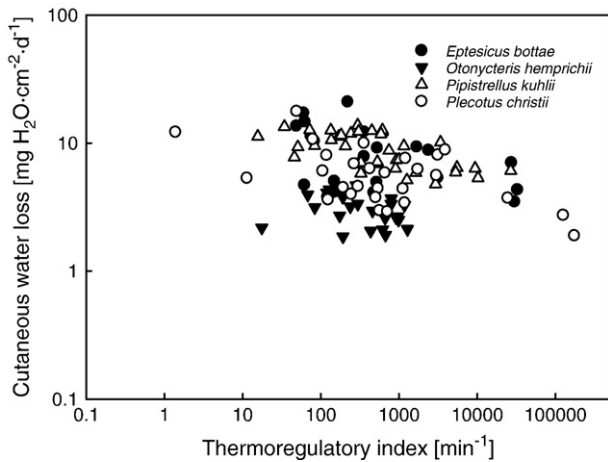


Fig. 7. The relationship between surface-specific cutaneous evaporative water loss (ssCEWL) and the thermoregulatory index (TRI) in *Eptesicus bottae*, *Otonycteris hemprichii*, *Plecotus christii* and *Pipistrellus kuhlii*. As torpor depth increased, CEWL decreased in all species, except in *O. hemprichii*, where it remained constant. *O. hemprichii* had a significantly lower ssCEWL than the other species, after accounting for differences in the thermoregulatory state of individuals.

for bats measured without a mask, i.e., TEWL of bats measured with the mask decreased significantly with T_a ($F_{3,102} = 9.96$, $P < 0.001$), and TEWL differed significantly among species ($F_{3,102} = 55.7$, $P < 0.001$), with *O. hemprichii* having the lowest TEWL and *P. kuhlii* having the largest TEWL. Taking this into account, and since all individuals were subjected to the same experimental conditions, we consider our results of RWL and CEWL comparable among species.

Since bats measured wearing a mask were at different torpor depths, we corrected for this variable using TRI as a covariate. We found that TRI was a significant covariate for metabolism-specific RWL [$\text{msRWL} = \text{RWL}/(m_b^{0.75})$] (Fig. 6A; $F_{1,102} = 45.2$, $P < 0.001$), indicating that msRWL decreased as depth of torpor increased. After correcting for depth of torpor, msRWL increased significantly with T_a ($F_{3,102} = 8.70$, $P < 0.001$). We also found significant differences in msRWL among species; for bats at the same torpor depth, *O. hemprichii* had the lowest msRWL, *P. christii* and *E. bottae* had intermediate rates of msRWL, and *P. kuhlii* had the highest msRWL (Fig. 6; $F_{3,102} = 2.91$, $P < 0.04$).

We found that TRI covaried significantly with ssCEWL ($F_{1,102} = 24.2$, $P < 0.001$), suggesting that ssCEWL decreased as depth of torpor increased. For bats at the same depth of torpor, ssCEWL increased significantly with T_a ($F_{3,102} = 4.63$, $P < 0.005$). We also found significant differences in ssCEWL among species; for bats at the same torpor depth, *O. hemprichii* had the lowest ssCEWL, *P. christii* had intermediate ssCEWL, and *E. bottae* and *P. kuhlii* had the highest ssCEWL (Fig. 7; $F_{3,102} = 48.1$, $P < 0.001$).

Using ANCOVA with TRI as a covariate, we regressed CEWL on RWL and found significant interactions for *O. hemprichii* and *P. kuhlii*, indicating that the slopes of these relationships were significantly different ($P < 0.04$), i.e., CEWL and RWL did not decrease at the same rate with TRI in these species (Fig. 8). In *P. christii* and *E. bottae*, CEWL was significantly higher than RWL at the same torpor depth ($P < 0.04$), but the slopes of these relationships were not significantly different ($P > 0.21$; Fig. 8).

4. Discussion

Contrary to our predictions, but in agreement with Marom et al. (2006), *P. kuhlii* the only widespread species that we studied, did not have significantly higher MR than the semi-desert species. However,

there was high individual variation in MR in all four species, particularly *E. bottae* and *P. christii*. This high variability in MR was due to the fact that some bats entered torpor during measurements, whereas others remained normothermic. Therefore, to compare the physiological variables of interest, we chose to calculate TRI, a continuous variable that estimates the thermoregulatory state of a heterothermic endotherm (Muñoz-García et al., 2014). We found that TRI was correlated with T_a , indicating that, as expected, depth of torpor increased as T_a decreased. When we compared TRI among species, we found that TRI of *O. hemprichii* was significantly higher than that of the other species, suggesting that *O. hemprichii* used shallower torpor at low temperatures than the others.

The minimum TRI of *O. hemprichii* was calculated for $T_a = 15^\circ\text{C}$, whereas for the other species minimum TRI was calculated for $T_a = 10^\circ\text{C}$. Moreover, TRI for *O. hemprichii* at 15°C was similar to TRI of the other species at 10°C , indicating that *O. hemprichii* reached the same depth of torpor at 15°C as the other species did at 10°C . It is possible that the minimum preferred temperature (T_{pm}), the value of T_a at which torpor metabolic rate is lowest, is higher in desert species than in mesic species. As a “true” desert species, *O. hemprichii* probably does not experience low T_a s most of the year and, from an evolutionary perspective, we think that it is reasonable to assume that 10°C is below the T_{pm} for this species. Taken together, these results suggest that, at low T_a , bats use torpor as an energy and water loss saving mechanism (Speakman and Thomas, 2003; Geiser, 2004). It seems, however, that *O. hemprichii* has a relatively high T_{pm} , perhaps an adaptation to hot, arid environments.

Surface-specific TEWL (ssTEWL) decreased with depth of torpor in all species, as we predicted. After correction for torpor depth (as indicated by TRI), rates of ssTEWL in *O. hemprichii* were significantly lower, but ssTEWL of *P. kuhlii* was not significantly higher, than those of the other species, a result that partially supports our predictions. Desert populations of the widespread *P. kuhlii* have similar rates of ssTEWL as those of other desert-dwelling species, perhaps because of genetic differences among populations that live in different environments, or because of acclimatization to desert environments, ideas that need to be tested. In house sparrows (*Passer domesticus*), Muñoz-García et al. (2008) found that acclimatization accounted for most of the variation observed in CEWL between desert and mesic populations. Similar results were obtained for Merriam’s kangaroo rat, *Dipodomys merriami* (Tracy and Walsberg, 2001). However, it seems that TEWL in laboratory rats, *Rattus norvegicus*, shows a major genetic component (Furuyama and Ohara, 1993).

To further explore the association of TEWL with habitat, we collected data for other species of bats from the literature. We found evidence that rates of TEWL might be lower in desert than in mesic species (see also Maloney et al., 1999; Toussaint and McKechnie, 2012). All the species we examined in this study had significantly lower rates of TEWL than predicted by the allometric equation that we generated. This might be due to methodological differences between studies; for example, in some studies TEWL was measured gravimetrically, whereas in others rates of TEWL were estimated using open-flow respirometry. There might also be unaccounted-for differences in the torpor depth of bats. We chose species measured within at thermoneutrality, but slight differences in the depth of torpor might alter rates of TEWL.

In all species in this study, except *O. hemprichii*, both ssCEWL and msRWL decreased with depth of torpor, as we predicted. Interestingly, in *O. hemprichii* ssCEWL did not change with TRI. *P. kuhlii* had the highest rates of msRWL and ssCEWL of all the four species, after accounting for torpor depth. *O. hemprichii* had significantly lower ssRWL and ssCEWL than the other species, at the same TRI. Both *P. christii* and *E. bottae* showed intermediate values for ssRWL and ssCEWL. These results support our hypothesis that ssCEWL and msRWL are negatively correlated with aridity of the habitat of the studied species.

In considering the relationship between RWL and CEWL with TR_i for each species, interesting patterns emerged. Respiratory water loss decreased with TR_i at the same rate in all species, i.e., the slopes of the relationships between RWL and TR_i were the same for all species ($P > 0.05$, ANCOVA with TR_i as a covariate). This suggests that the decrease in RWL is a by-product of the decrease in MR concomitant with torpor. In *P. christii* and *E. bottae*, RWL and CEWL decreased at the same rate with increasing TR_i . However, we found that CEWL decreased with TR_i at a slower rate than RWL in *P. kuhlii*. Supporting these results, Muñoz-García et al. (2012a) studied the relationship between CEWL and torpor depth in *P. kuhlii* and, based on their results, hypothesized that during deep torpor, animals do not efficiently regulate CEWL. The authors concluded that CEWL in *P. kuhlii* increased with torpor depth, even though they used the gradient between T_b and T_a ($T_b - T_a$) as a proxy for depth of torpor. The use of $T_b - T_a$ as a measure of the thermoregulatory state of a bat is problematic, since thermoneutral bats might have the same score as bats in deep torpor (Muñoz-García et al., 2014). We did not find CEWL to increase with depth of torpor in *P. kuhlii*, rather it decreased. However, CEWL decreased at a slower rate than RWL during deep torpor, reinforcing the hypothesis that during deep torpor, regulation of CEWL is somehow impaired. We also found a significant interaction term between CEWL and RWL in *O. hemprichii*. In this case, RWL decreased with TR_i , but CEWL remained constant with TR_i , suggesting that CEWL in

O. hemprichii is mainly determined by a structural component, that is probably independent of body temperature.

One of the main determinants of the rates of CEWL in endotherms is the lipid composition of the stratum corneum (SC), the outer layer of the epidermis (Williams and Tieleman, 2005; Lillywhite, 2006; Muñoz-García et al., 2008). Lipids in the SC are arranged in layers and organized in an ordered fashion which prevents excessive passage of water vapor across the skin (Muñoz-García et al., 2008). The function of the skin as a barrier to evaporation depends on the composition and organization of the lipids of the SC, a structural component, and a regulatory component, including enzyme activity, changes in the pH within layers of the SC, vasoconstriction and vasodilation (see Elias, 2004), which together determine the permeability of the barrier under different environmental conditions. During deep torpor, the structural components of the permeability barrier are unlikely to change substantially, but the regulatory, active mechanisms that increase resistance to water vapor diffusion across the skin are probably less effective, since they require energy expenditure for proper functioning. The fact that CEWL did not change significantly with depth of torpor in *O. hemprichii*, suggests that the structural component of the permeability barrier is more important than the regulatory components in this species. It is possible that the composition and organization of the lipids of the SC in *O. hemprichii* are such that results in low rates of CEWL at high values of T_a , at the cost of low flexibility when

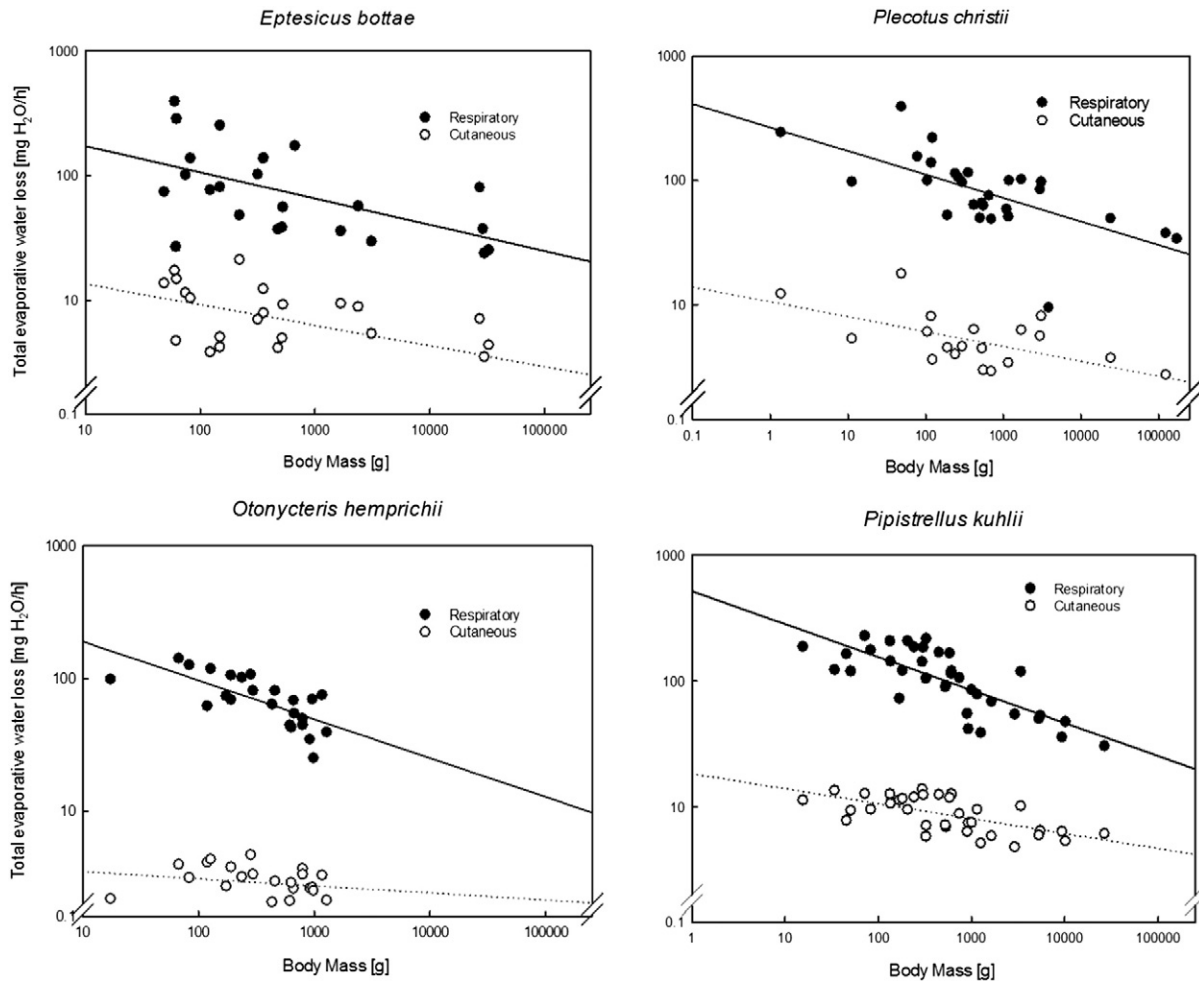


Fig. 8. The relationship between surface-specific respiratory water loss (ssRWL) and surface-specific cutaneous evaporative water loss (ssCEWL), using the thermoregulatory index (TR_i) as a covariate, in *Eptesicus bottae*, *Otonycteris hemprichii*, *Plecotus christii* and *Pipistrellus kuhlii*. At the same depth of torpor, ssCEWL was higher than ssRWL in *E. bottae* and *P. christii*. For *O. hemprichii* and *P. kuhlii* we found a significant interaction term, indicating that ssRWL and ssCEWL did not decrease with depth of torpor at the same rate.

T_{as} drop and individuals are torpid. Further studies are needed to explore this idea.

To conclude, we hypothesized that bats living in desert environments would be physiologically adapted to minimize energy expenditure and water loss. We predicted that MR and evaporative water loss would therefore be lower in desert species than in mesic species. Our results are not sufficient to make an unequivocal statement that desert-dwelling bats do have lower MR, but they indicate that they have lower rates of TEWL, RWL and CEWL than those of mesic species. We also hypothesized that evaporative water loss would decrease with torpor depth in bats. As predicted, we observed a significant reduction in RWL and CEWL with torpor depth.

Acknowledgments

This research was supported by grant number 2008469 of the US-Israel Binational National Science Foundation to CK and JBW, and a Blaustein Postdoctoral Fellowship and a VATAT fellowship from the Council of Higher Education, awarded to AM-G. APCN was supported by a travel grant from The Graduate Program of the State University of São Paulo (PROPG – UNESP) and by a research grant from FAPESP (#08/57687-0). This research was done under permit 37066 to Carmi Korine from the Israel Nature and Parks Authority and under permit IL-71-12-2010 from the Ben-Gurion University committee for the ethical care and use of animals in experiments. We thank two anonymous reviewers for useful comments in the preparation of the last version of this manuscript. This is paper number 884 of the Mitrani Department of Desert Ecology.

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