Evaporative water loss and dehydration during the night in hummingbirds

Pérdida de agua evaporativa y deshidratación nocturna en picaflores

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ABSTRACT

Nectar-feeding birds oscillate between avoiding overhydration when they are feeding and preventing dehydration during fasts. Here, we examined how resting rates of total evaporative water loss (TEWL) and metabolic water production (MWP) influence water balance in the green-backed firecrown (*Sephanoides sephanoides*), a Chilean hummingbird. We hypothesized that a circadian rhythm in TEWL would assuage the dehydration risk that hummingbirds face during the night. However, we did not find support for this idea. In resting hummingbirds, rates of TEWL during the day (54 ± 6 μL h^{-1}, n = 8) and night (65 ± 12 μL h^{-1}, n = 5) were similar. Rates of MWP were also similar between the day (22 ± 3 μL h^{-1}, n = 8) and night (23 ± 2 μL h^{-1}, n = 5). MWP rates were significantly lower than TEWL rates during both the day and night. Our findings both support the notion that hummingbirds dehydrate during extended fasts and illustrate that evaporative water loss is an important osmoregulatory consideration in hummingbirds. However, because the technique we used to estimate rates of TEWL and MWP at night was indirect, our findings should be interpreted cautiously until direct measurements are available.

Key words: circadian rhythm, evaporative water loss, hummingbird, osmoregulation, water balance.

RESUMEN

Las aves nectarívoras oscilan entre la sobrehidratación durante los períodos de alimentación y la prevención de la deshidratación durante el ayuno. Examinamos cómo las tasas de pérdida total de agua evaporativa (TEWL) y la producción de agua metabólica (MWP) durante el descanso influyen sobre el balance hídrico en el picaflor chileno (*Sephanoides sephanoides*). Hipotetizamos que el ritmo circadiano en la TEWL podría disminuir el riesgo de deshidratación que los picaflores enfrentan durante la noche. Sin embargo, no encontramos apoyo de esta hipótesis. En los picaflores en reposo, las tasas de TEWL durante el día (54 ± 6 μL h^{-1}, n = 8) y la noche (65 ± 12 μL h^{-1}, n = 5) fueron similares. Las tasas de MWP también fueron similares durante el día (22 ± 3 μL h^{-1}, n = 8) y la noche (23 ± 2 μL h^{-1}, n = 5). Independiente del período de medición las tasas de MWP fueron significativamente menores que las tasas de TEWL. Nuestros resultados apoyan la noción de que los picaflores se deshidratan durante períodos de ayuno prolongado y además ilustran que la pérdida de agua evaporativa es una consideración osmorregulatoria importante en picaflores. Sin embargo, debido a que la técnica que utilizamos para estimar las tasas de TEWL y MWP fue indirecta, nuestros hallazgos debieran ser interpretados con precaución hasta que medidas directas estén disponibles.

Palabras clave: balance hídrico, pérdida de agua evaporativa, picaflor, osmorregulación, ritmo circadiano.

INTRODUCTION

Subsisting on floral nectar poses an osmoregulatory dilemma for vertebrates. When feeding, nectarivorous birds ingest multiples of their body mass (M_B) per day in water (Beuchat et al. 1990, McWhorter & López-Calleja 2000, Martínez del Río et al. 2001) yet appear prone to rapid dehydration during fasts (Hartman Bakken et al. 2004, Lotz & Martínez del Río 2004). Although recent studies have illuminated how the endocrine (Fleming et al. 2004a, 2004b), intestinal (McWhorter et al. 2003), and renal systems respond to this

quandary (Goldstein & Bradshaw 1998, Hartman Bakken et al. 2004, McWhorter et al. 2004, Hartman Bakken & Sabat 2006), the osmoregulatory role of evaporative water loss remains relatively unstudied (Nicolson 2006). Here, we measure daytime and estimate nighttime rates of total evaporative water loss (TEWL) and metabolic water production (MWP) in resting green-backed firecrowns (*Sephanoides sephanoides* Lesson, 1827), a hummingbird (Trochilidae) widely distributed in Chile.

A suite of physiological processes in nectar-feeding birds exhibit diel variation, and the apparent rhythms in evaporative water loss (Collins et al. 1980, Collins 1981), metabolism (Collins et al. 1980, Powers 1991), hormone secretion (Fleming et al. 2004a), and renal function (Hartman Bakken et al. 2004, Hartman Bakken & Sabat 2006) putatively reflect these birds' need for water elimination and conservation during feeding and fasting, respectively. Understanding if and how evaporative water losses are regulated is of interest because modulations could lessen the risk of dehydration these birds confront during nonfeeding periods. Because TEWL appears to reflect hydration status (MacMillen & Hinds 1998), time of day (Withers 1992, MacMillen & Hinds 1998), and environmental conditions (Williams 1996, Tieleman et al. 2002, Sabat et al. 2006), we predicted that resting TEWL rates would be greater during the day compared to the night. If supported, this hypothesis suggests the need to conserve body water at night is facilitated by a reduced rate of TEWL. Additionally, we used our nighttime estimate of TEWL to gauge the extent of overnight dehydration in hummingbirds.

MATERIAL AND METHODS

The protocols we followed for this work conformed to the bioethical guidelines for animal care and experimentation established by the Universidad de Chile. Using mist-nets, we captured male green-backed firecrowns in San Carlos de Apoquindo, Región Metropolitana de Santiago, Chile (33°23' S, 70°31' W). Maintenance conditions for captive hummingbirds were as previously described (Hartman Bakken & Sabat 2006). During

experiments, the scotophase ranged from 11.48 to 11.53 h per 24 h. All the measurements we describe below were made at 25 ± 1 °C.

Daytime measurements

To measure TEWL ($\mu L \ h^{-1}$), oxygen consumption (VO_2; mL h^{-1}), and MWP ($\mu L \ h^{-1}$) in resting green-backed firecrowns ($M_B = 5.90 \pm 0.86$ g, n = 8), we followed standard flow-through respirometry and hygrometry methods (Tieleman et al. 2002, Sabat et al. 2006). We calibrated our respirometry system with chromatographically certified 20 % O_2 in N_2 (INDURA, Santiago, Región Metropolitana de Santiago, Chile). H_2O-free air was pumped to the stainless steel metabolic chamber (~1 L) through Bev-A-Line tubing (Thermoplastic Processes, Stirling, New Jersey, USA) at 500 mL min^{-1} from a mass flow controller (MFC-2, Sable Systems, Las Vegas, Nevada, USA). This regime is unlikely to produce hypoxic conditions inside the metabolic chamber (McNab 2006). Air leaving the metabolic chamber was sent at a rate of 290 mL min^{-1} through a relative humidity/dewpoint analyzer (RH-200, Sable Systems, Las Vegas, Nevada, USA) then scrubbed of both H_2O and CO_2 before passing through our O_2 analyzer (FC-10a, Sable Systems, Las Vegas, Nevada, USA). We digitized analogue output from both the relative humidity/dewpoint and O_2 analyzers with a Universal Interface II (Sable Systems, Las Vegas, Nevada, USA). Data were recorded using the ExpeData software package (Sable Systems, Las Vegas, Nevada, USA).

To minimize the influence that activity has on rates of TEWL (Collins et al. 1980, Collins 1981, Withers 1992, Lotz & Nicolson 2002, Lotz et al. 2003) and to enable comparisons between day and night, we obtained measurements from fasting, postabsorptive birds at rest inside of dark metabolic chambers during the photophase. To trap excretory water, birds perched atop a wire-mesh grid above a pool of mineral oil. After steady state conditions were visually evident (Tieleman et al. 2002, Sabat et al. 2006), hummingbirds remained in the metabolic chamber for 20 min. H_2O vapour pressure and O_2 concentration in the air leaving the chamber were averaged over this 20 min period to determine both TEWL and VO_2 (Tieleman et al. 2002). Steady state

was achieved in 2.20 ± 0.51 h (n = 8) and trial length was 2.53 ± 0.51 h (n = 8). To check that these measurements were obtained from normothermic birds, we measured cloacal temperature using a digital thermometer (± 0.1 ºC) and a Cu-Cn thermocouple (± 0.1 ºC) immediately after each trial. Our criterion for normothermia was any cloacal temperature reading ≥ 39.0 ºC. We calculated TEWL and absolute humidity after Withers (1977), Williams & Tieleman (2000), and Lide (2001), respectively. To estimate MWP at rest, we assumed that hummingbirds were oxidizing endogenous lipid (Suarez et al. 1990, Carleton et al. 2006, Welch et al. 2006), 0.57 mg lipid are oxidized per mL O_2 consumed (Schmidt-Nielsen 1997), and that the catabolism of 1 mg lipid produces 1.07 µL H_2O. With these assumptions, resting MWP is:

$$MWP = VO_2 \times 0.57 \times 1.07, \qquad \text{equation 1}$$

Nighttime estimates

To estimate the resting rate of TEWL during the night, we monitored the nighttime rate of M_B change (ΔM_B, mg h^{-1}) in green-backed firecrowns (M_B = 5.33 ± 0.51 g, n = 5) by hanging the only available cage perch from an electronic balance (± 0.01 g). In a fasting hummingbird, ΔM_B summarizes three separate rates: TEWL, MWP, and lipid oxidation rate (L, mg h^{-1}). Therefore, we estimated resting TEWL indirectly during the night (µL h^{-1}) as:

$$TEWL = \Delta M_B + MWP - L, \qquad \text{equation 2}$$

where L is the product of VO_2 measured during the day as described above and the oxygen consumption to lipid catabolism ratio of 0.5. This indirect approach assumes that there is no diel variation in VO_2 and that birds are normothermic throughout the night phase. To check the latter assumption, we affixed a Cu-Cn thermocouple (± 0.1 ºC) to the cage perch and used a digital thermometer (± 0.1 ºC) to make body temperature estimates as previously described (Hartman Bakken et al. 2004). Briefly, in order to use perching temperature as a proxy for body temperature during the night, we measured the perching temperature and its corresponding cloacal temperature for each bird in a separate experiment. Based on these measurements, a perching temperature of 31.1 ± 1.5 ºC (range: 28.5–32.2 ºC, n = 5) corresponded to our minimum threshold of 39.0 ºC for normothermia. However, due to differences in birds' perching styles, we made normothermia determinations on bird-by-bird basis. M_B and perching temperature measurements were taken every 0.5 h throughout the night phase. A drop of 0.1 ºC below each bird's minimum normothermic value was considered hypothermia.

Statistical analyses

We compared means to hypothesized values with one-sample t-tests; to compare daytime measurements and nighttime estimates, we used paired t-tests. In all other cases, we analyzed data using standard least squares linear regression. We report data as means ± SD.

RESULTS

Total evaporative water loss rates

We found no evidence to support our hypothesis of a TEWL rhythm in resting green-backed firecrowns: TEWL rates during the day and night were 54 ± 6 (n = 8) and 65 ± 12 µL h^{-1} (n = 5), respectively (Fig. 1A). These rates were similar (paired t-test, t_4 = 1.33, P = 0.2539, Fig. 1A). Daytime TEWL was not different from Williams' (1996) allometric expectation of 49 µL h^{-1} for mesic-adapted birds (one-sample t-test, t_7 = 2.11, P = 0.0726); however, nighttime TEWL was significantly greater than Williams' (1996) prediction of 46 µL h^{-1} for mesic-adapted birds (one-sample t-test, t_4 = 3.52, P = 0.0244). Neither day- nor nighttime TEWL were related to either VO_2 (day: r^2 = 0.08, P = 0.4978, n = 8; night: r^2 = 0.00, P = 0.9405, n = 5) or M_B (day: r^2 = 0.01, P = 0.8745, n = 8; night: r^2 = 0.02, P = 0.8393, n = 5).

Oxygen consumption and lipid oxidation rates

VO_2 during the day was 36.5 ± 4.1 mL O_2 h^{-1} (n = 8); hummingbirds used in our nighttime experiment had an VO_2 equal to 37.7 ± 4.0 mL O_2 h^{-1} (n = 5). Mass-specific rates of were 6.2 ± 0.8 (n = 8) and 6.6 ± 0.5 mL O_2 g^{-1} h^{-1} (n = 5)

during the day and night, respectively. These values are greater than the mass-specific basal metabolic rate of 3.17 mL O_2 g^{-1} h^{-1} previously reported in green-backed firecrowns; however, we obtained our VO_2 measurements at 25 ± 1 ºC, which is below this species' lower critical temperature of 28 ºC (López-Calleja & Bozinovic 1995). Based on our daytime VO_2 measurements, nighttime L in green-backed firecrowns was 19 ± 2 mg h^{-1} (n = 5).

Metabolic water production rates

The rates of MWP in resting hummingbirds were 22 ± 3 (n = 8) and 23 ± 2 μL h^{-1} (n = 5) during the day and night, respectively (Fig. 1B). TEWL was significantly greater than MWP during both the day (paired t-test, t_7 = −14.52, P < 0.0001) and night (paired t-test, t_4 =

−7.53, P = 0.0017). The day- and nighttime ratios of MWP to TEWL were 0.42 ± 0.06 (n = 8) and 0.36 ± 0.07 (n = 5), respectively. These ratios were not different (paired t-test, t_4 = −1.39, P = 0.2360). Please note, however, that our nighttime estimates of TEWL and MWP are not independent (equation 2).

Body temperature

Cloacal temperature immediately after our daytime trials was 40.6 ± 0.7 ºC (range: 39.4–41.2 ºC, n = 8), indicating that birds were normothermic during the measurement period. The assumption of normothermia we made for our nighttime estimates, however, was not satisfied: green-backed firecrowns were hypothermic for 11 ± 5 % of the night phase (1.30 ± 0.57 h, n = 5). Consequently, the
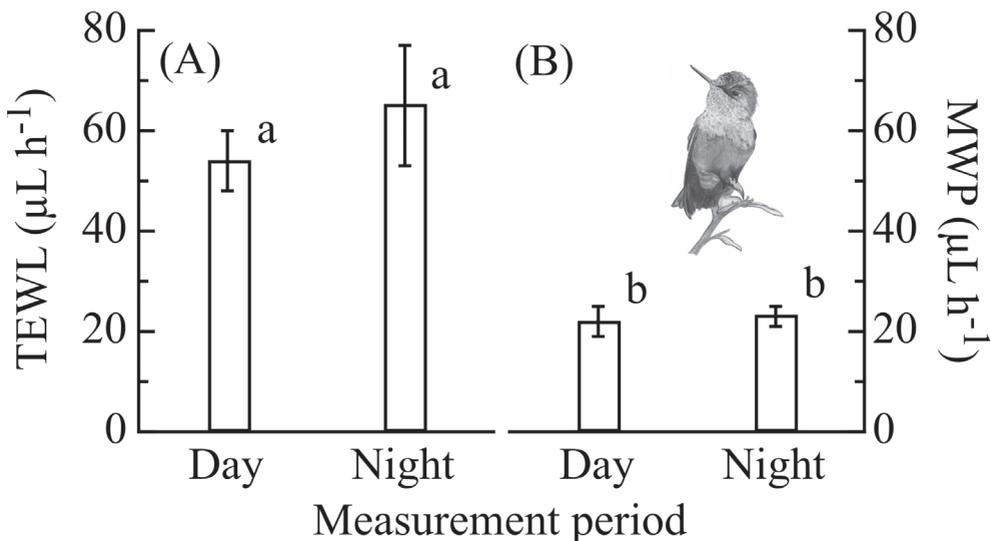
Fig. 1: Total evaporative water loss (TEWL) and metabolic water production (MWP) in resting green-backed firecrowns (*Sephanoides sephanoides*) during both the day and night. (A) We hypothesized a circadian rhythm in TEWL would facilitate body water conservation at night; however, we did not find any support for this idea. Rates of TEWL were similar during the day (54 ± 6 μL h^{-1}, n = 8) and night (65 ± 12 μL h^{-1}, n = 5). (B) Although the rates of MWP were significantly lower than rates of TEWL, there was no diel variation in MWP (day: 22 ± 3 μL h^{-1}, n = 8; night: 23 ± 2 μL h^{-1}, n = 5). Note that the nighttime rates of TEWL and MWP are not independent (equation 2). See Discussion for caveats regarding these findings. Lowercase letters denote significant differences from paired t-tests. Data are means ± SD.

Pérdida total de agua evaporativa (TEWL) y producción de agua metabólica (MWP) de reposo en picaflores (*Sephanoides sephanoides*) durante el día y la noche. (A) Hipotetizamos que el ritmo circadiano en la TEWL podría facilitar la conservación del agua corporal durante la noche; sin embargo, no encontramos apoyo para esta idea. Las tasas de TEWL fueron similares durante el día (54 ± 6 μL h^{-1}, n = 8) y la noche (65 ± 12 μL h^{-1}, n = 5). (B) Aun cuando las tasas de MWP fueron significativamente menores que las tasas de TEWL, no hubo variación diaria en la MWP (día: 22 ± 3 μL h^{-1}, n = 8; noche: 23 ± 2 μL h^{-1}, n = 5). Notar que las tasas de TEWL y MWP nocturnas no son independientes (ecuación 2). Véase Discusión para una advertencia respecto de estos hallazgos. Letras minúsculas denotan diferencias significativas de una prueba pareada de t. Los datos se muestran como promedio ± DE.

nighttime rates of TEWL and MWP are over- and underestimated, respectively (Withers 1992, Fig. 1). In our discussion, we approximate the magnitude of these inaccuracies. There was no relationship between night length and percent of night spent hypothermic ($r^2 = 0.00$, $P = 0.9275$, $n = 5$).

Nighttime changes in body mass

During the night phase, ΔM_B in green-backed firecrowns was -62 ± 12 mg h^{-1} ($n = 5$). Figure 2A shows a typical overnight M_B profile for the hummingbirds in this experiment. On average, birds lost 714 ± 141 mg (14 ± 3 % of initial M_B; $n = 5$) overnight; however, time spent hypothermic exerted a significant effect on overnight M_B loss ($y = -965 + 193x$, $r^2 = 0.61$, $P = 0.0045$, $n = 5$, Fig. 2B).

DISCUSSION

We suspected that a circadian rhythm in resting TEWL would lessen the risk of nighttime dehydration in hummingbirds; however, we found no evidence to support this hypothesis (Fig. 1A). In our discussion, we first consider the significance that an invariable resting TEWL rate has on the processes hummingbirds use to maintain water balance. We then discuss how nocturnal hypothermia and pre-night hyperphagia in hummingbirds may have affected our nighttime measurements.

Osmoregulatory implications

An invariable rate of TEWL in resting hummingbirds suggests that, after expected increases in water loss with activity (Collins 1981, Withers 1992, Lotz & Nicolson 2002, Lotz et al. 2003), the need for water elimination during the day is not assuaged by a higher rate of TEWL. However, with no daily oscillations in resting TEWL (Fig. 1A), the notion that hummingbirds must cope with an extreme risk of dehydration at night is supported. In this study, green-backed firecrowns lost 750 ± 141 μL ($n = 5$) of body water during the night due to evaporation. In terms of total body water (Hartman Bakken & Sabat 2006), this volume corresponds to a total loss of 25 ± 5 % ($n = 5$)
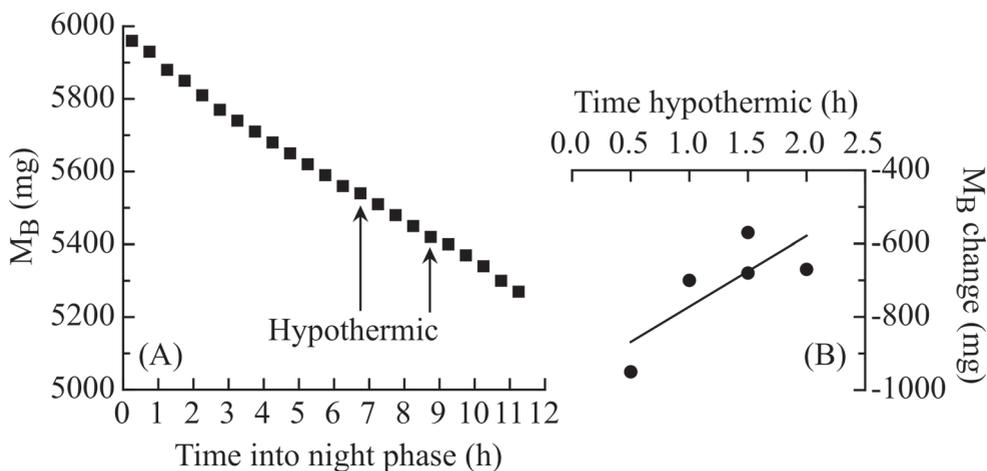
Fig. 2: (A) A nighttime body mass (M_B) profile for one representative green-backed firecrown (*Sephanoides sephanoides*). On average, green-backed firecrowns lost M_B at a rate of -62 ± 12 mg h^{-1} ($n = 5$) and were hypothermic for 1.30 ± 0.57 h during the night (11 ± 5 % of the night phase; $n = 5$). (B) However, this rate of loss was significantly affected by the time hummingbirds spent hypothermic ($y = -965 + 193x$, $r^2 = 0.61$, $P = 0.0045$, $n = 5$). The green-backed firecrown in panel (A) was hypothermic during two 0.5 h measurement periods (denoted by arrows).

(A) Perfil representativo de la masa corporal (M_B) en un picaflor (*Sephanoides sephanoides*). En promedio, los picaflores pierden M_B a una tasa de -62 ± 12 mg h^{-1} ($n = 5$) y se mantuvieron hipotérmicos por 1.30 ± 0.57 h durante la noche (11 ± 5 % de la fase nocturna; $n = 5$). (B) Sin embargo, esta tasa de pérdida fue significativamente afectada por el tiempo en que los picaflores se mantuvieron hipotérmicos ($y = -965 + 193x$, $r^2 = 0.61$, $P = 0.0045$, $n = 5$). El picaflor en el panel (A) se mantuvo hipotérmico durante dos períodos de 0.5 h (indicado por flechas).

or a loss of ~2 % each hour. If accurate, these values constitute extreme levels of dehydration; however, we explain below why both our nighttime measurements and dehydration estimates are likely exaggerated.

Hypothermia and hyperphagia: direction and magnitude of their effects on our nighttime measurements

The indirect method we used to estimate both TEWL (equation 2) and MWP (equation 1) at night makes two critical assumptions: 1) there is no diel variation in VO_2 and 2) hummingbirds are normothermic throughout the night phase. Even though we did not measure nighttime VO_2, our observation that hummingbirds were hypothermic for ~10 % of the night phase indicates both assumptions of our indirect technique were violated (Lasiewski 1963, Hainsworth & Wolf 1970, Withers 1992, López-Calleja & Bovinovic 1995). What impact did this have on our nighttime measurements? Although we cannot gauge the effect precisely, we can assign maxima to our inaccuracies. If we accept the extreme (and improbable) scenario that VO_2 during hypothermic bouts is zero, the rate of TEWL at night will be overestimated by no more than the nighttime percentage of hypothermia (11 ± 5 %, n = 5). By this reasoning, the range of means for the rate of TEWL at night is 58–65 μL h^{-1} (Fig. 1A). Using the same rationale, we underestimated nighttime MWP rate by no more than 11 ± 5 % (n = 5). The range of means for the MWP rate at night, therefore, is 23 – 26 μL h^{-1} (Fig. 1B). Taking into account these ranges, the range of means for our nighttime MWP to TEWL ratio is 0.35–0.45. Although this sensitivity analysis suggests our conclusion that hummingbirds do not show a circadian rhythm in TEWL is robust (Fig. 1), our analysis does not consider the effect a nighttime dip in VO_2 would have on our estimates (Powers 1991, Withers 1992). Consequently, our findings should be treated cautiously until direct nighttime measurements are available.

Concerning our estimates of nighttime dehydration, the hyperphagic (and thus polydipsic) behaviour of nectar-feeding birds before lights-off in the evening (Collins 1981, López-Calleja et al. 1997) may have caused us to overstate these. That is, there will be a positive relationship between dietary water in nonabsorptive regions of the intestinal tract (i.e., crop) and our estimation of the proportion of total body water lost to evaporation during the night.

Despite the problems discussed above, it is clear that nightly evaporative water loss in hummingbirds is substantial. When considered together with their inability to form concentrated urine (Lotz & Martínez del Río 2004), it appears that hummingbirds arrest renal filtration at night (Hartman Bakken et al. 2004, Hartman Bakken & Sabat 2006) in order to prevent a lethal loss of body water. Direct measurements of nighttime TEWL, however, are needed, and coupling them to a study of how body water volumes and osmolarities change over the course of fasting periods would be an informative approach.

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