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## Respiratory evaporative water loss during hovering and forward flight in hummingbirds

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## ABSTRACT

Hummingbirds represent an end point for small body size and water flux in vertebrates. We explored the role evaporative water loss (EWL) plays in management of their large water pool and its use in dissipating metabolic heat. We measured respiratory evaporative water loss (REWL) in hovering hummingbirds in the field (6 species) and over a range of speeds in a wind tunnel (1 species) using an open-circuit mask respirometry system. Hovering REWL during the active period was positively correlated with operative temperature ( $T_e$ ) likely due to some combination of an increase in the vapor-pressure deficit, increase in lung ventilation rate, and reduced importance of dry heat transfer at higher  $T_e$ . In rufous hummingbirds (*Selasphorus rufus*; 3.3 g) REWL during forward flight at 6 and 10 m/s was less than half the value for hovering. The proportion of total dissipated heat (TDH) accounted for by REWL during hovering at  $T_e > 40^\circ\text{C}$  was  $<40\%$  in most species. During forward flight in *S. rufus* the proportion of TDH accounted for by REWL was  $\sim 35\%$  less than for hovering. REWL in hummingbirds is a relatively small component of the water budget compared with other bird species ( $<20\%$ ) so cutaneous evaporative water loss and dry heat transfer must contribute significantly to thermal balance in hummingbirds.

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## 1. Introduction

A key mechanism for dissipating excess metabolic heat is evaporative water loss (EWL). In birds evaporative heat loss occurs across the skin (cutaneous evaporative water loss; CEWL) and through the respiratory tract (respiratory evaporative water loss; REWL). The rate of heat dissipation and the proportional contribution of CEWL and REWL to total evaporative water loss (TEWL) is strongly influenced by both acclimatization and, presumably, natural selection, and thus varies among species (Williams and Tieleman, 2002). Much of the recent work on EWL has focused on the physiological regulation of CEWL in birds adapted to different environmental regimes (e.g. Haugen et al., 2003). REWL has been more difficult to measure since it requires that birds be masked or otherwise restrained and their

heads physically isolated from the rest of their body (e.g. Webster and King, 1987; Wolf and Walsberg, 1996). Such protocols impose an unavoidable lack of realism in measurement conditions.

Many studies have been done on EWL in nonflying birds resulting in the description of adaptations that help birds balance the need to dissipate heat and protect against dehydration (Williams and Tieleman, 2002). However only a handful of studies using either direct measurement or indirect estimations have explored the role EWL plays in both thermoregulation and water budget management during flight (e.g. Berger et al., 1971; Berger and Hart, 1972; Tucker, 1968; Engel et al., 2006a, 2006b). No activity in vertebrate animals has a higher energetic demand than flight (Schmidt-Nielsen, 1972), and because the muscles that power flight have a low mechanical efficiency ( $\sim 10\%$ ; Wells, 1993), they generate large amounts of metabolic heat that must be dissipated. Recently Engel et al. (2006a, 2006b) in studies of rose-colored starlings (*Sturnus roseus*) and European starlings (*S. vulgaris*) showed that EWL plays a meaningful role in dissipating heat during flight but can also result in significant water loss if flights are of extended duration (e.g. migration). Further, they estimated that REWL accounts for up to 88% of TEWL making REWL a major avenue for water loss during flight.

To date most studies of EWL in birds have focused on the effects of high temperature and/or water restriction. This is understandable

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since the competing needs of heat dissipation and water retention present a physiological challenge in animal design. Hummingbirds are unique among birds in that their small size makes thermoregulation difficult, and flight, particularly sustained hovering in still air, which is unique to this family, has an energetic cost that can exceed  $10\times$  basal metabolism (Lasiewski, 1963; Bartholomew and Lighton, 1986; Clark and Dudley, 2010). The dilute nectars consumed by hummingbirds result in daily water turnover rates that can equate to  $>2\times$  their body mass (Powers and Nagy, 1988; Weathers and Stiles, 1989; Beuchat et al., 1990; Powers and Conley, 1994) and an osmoregulatory physiology that is strikingly similar in many ways to fish and amphibians (Calder and Hiebert, 1983; Hartman Bakken and Sabat, 2007). When hummingbirds are feeding about 80% of dietary water is absorbed (McWhorter and Martínez del Rio, 1999) creating a large water pool that could facilitate high rates of evaporative cooling. However, total EWL in resting hummingbirds is  $\sim 1.5\text{--}2.0$  mL/day (Lasiewski, 1964; Lasiewski and Lasiewski, 1967; Powers, 1992) and accounts for less than 25% of total daily water efflux. EWL during flight in hummingbirds is largely unstudied. The only available data suggest REWL during hovering at moderate and low temperatures and in dry air accounted for no more than 11% of hourly water efflux (Berger and Hart, 1972). These previous data suggest EWL is a relatively small proportion of total water efflux in foraging hummingbirds.

In this study, we measured REWL in six species of free-living hovering hummingbirds and captive rufous hummingbirds (*Selasphorus rufus*) during hovering and forward flight in a wind tunnel. Because hummingbirds have high water turnover rates due to their nectar diet and generate large amounts of heat due to their small size, high metabolic rate, and muscle activity during flight we predicted that REWL in hovering hummingbirds would be the primary avenue of heat dissipation at high temperatures (prediction 1) yet be a relatively small component of total water efflux compared to other birds due to their high urinary output (prediction 2). Further we predicted for forward flight that REWL would be lower than during hovering due to lower metabolic rates for forward flight (prediction 3) (Clark and Dudley, 2009, 2010).

## 2. Material and methods

### 2.1. Study sites and animals

#### 2.1.1. Field studies

Field data were collected during July 2006 along Harshaw Creek (Patagonia Mts.) and July 2007 at the Southwestern Research Station (Chiricahua Mts.) in southeastern Arizona. The Harshaw Creek site (HC) is 1370 m in elevation and characterized by riparian vegetation that included large sycamores (*Platanus wrightii*) surrounded by oak-mesquite (*Quercus* sp., *Prosopis* sp.) habitat. During this study measurements were made (see System design and Operative temperature below) at operative temperatures ( $T_e$ ) between 15 and 45 °C and measurements were acquired for all species between 20 and 40 °C.  $T_e$  integrates radiative and convective factors with ambient temperature ( $T_a$ ) to more accurately model an organism's thermal environment (Bakken, 1976; Robinson et al., 1976; Campbell, 1977). Ambient water vapor pressure (WVP) decreased with increasing  $T_e$ . For comparison between study sites WVP at 25 °C was 1.4 kPa and at 40 °C 1.3 kPa. Hummingbird species studied at HC were black-chinned hummingbirds (*Archilochus alexandri*; males 3.0 g, females 3.6 g), broad-billed hummingbirds (*Cyananthus latirostris*; males 3.3 g, females 3.4 g), rufous hummingbirds (*S. rufus*; males 3.6 g) and violet-crowned hummingbirds (*Amazilia violiceps*; 5.5 g). The Southwestern Research Station site (SWRS) is 1650 m in elevation and characterized by riparian vegetation that included large sycamores surrounded by oak-pine (*Pinus* sp.) habitat. Measurements were made over a  $T_e$  range of 22–42 °C including measurements for both study species between 25 and 40 °C. Ambient WVP at 25 °C was 1.1 kPa and at 40 °C 0.7 kPa. At SWRS we

studied magnificent hummingbirds (*Eugenes fulgens*; males 7.7 g, females 7.4 g) and blue-throated hummingbirds (*Lampornis clemenciae*; males 8.2 g). All mass values are population means measured at nearby banding sites within 1 week of data collection (Hummingbird Monitoring Network, S. Wethington, personal communication).

Because our field data were collected on free-living hummingbirds the total number of individuals sampled for each species is unknown. We estimated the total number of individuals for each species present at our study sites from banding data from the Hummingbird Monitoring Network (S. Wethington, personal communication) and our video analysis. The estimated total number of individual birds present at our study sites (IP), number of individual birds known to have visited our feeders (KI), and total number of measurements (n) are: *C. latirostris* (male, IP=15, KI=4, n=71; female, IP=10, KI=4, n=10), *A. alexandri* (male, IP=20, KI=3, n=8; female, IP=21, KI=5, n=25), *S. rufus* (male, IP=3, KI=3, n=15), *A. violiceps* (unknown, IP=3, KI=3, n=7), *E. fulgens* (male, IP=20, KI=5, n=51; female, IP=18, KI=3, n=14), and *L. clemenciae* (male, IP=4, KI=4, n=83). Values for KI represent minimum individual birds sampled at least once since all individuals sampled could not be positively identified from video recordings. We only analyzed measurements in which the sampling duration was at least 3 s to insure that experimental values were well above baseline.

#### 2.1.2. Laboratory studies

Laboratory measurements during hovering and forward flight were made at the University of Portland (UP), Portland, OR during summer 2006. All measurements were made on *S. rufus* females (3.2–3.4 g, n=4) collected at the Tilikum Retreat Center located in Yamhill Co., OR under permits from the US Fish and Wildlife Service and Oregon Department of Fish and Wildlife. Following capture, birds were transported to the UP where they were housed in 1.0 m<sup>3</sup> cages lined with fine mesh nylon screen to minimize damage to their plumage. They were fed ad libitum a 50:50 mixture of 20% sucrose solution (weight/volume) and Nektar Plus™ on which the birds were able to maintain mass for the duration of the study. All laboratory measurements were made at room temperature (21 °C; WVP=1.2 kPa). All protocols involving captive birds were approved by the University of Portland Institutional Animal Care and Use Committee.

### 2.2. Respiratory evaporative water loss

#### 2.2.1. System design

For both field and laboratory studies we measured respiratory evaporative water loss (REWL) using a negative-pressure open-circuit system. Hummingbirds were trained to feed from a respiratory mask constructed from the barrel of a 25 mL syringe (21.5 mm ID) into which was inserted either a 1 or 10 mL feeder syringe to attract the birds. Use of a mask-respirometry system to collect respiratory gases from hovering hummingbirds has been thoroughly reviewed by Welch (2011). Air was pulled from the mask and through a relative humidity sensor (Sable Systems RH-100; resolution 0.001% RH, accuracy 1% of recorded value) at a flow rate of 2–3 L/min using a Sable Systems Mass Flow System (MFS-2; resolution 1 mL/min, accuracy <2% of full scale). Bev-A-Line IV tubing, which is impermeable to water, was used throughout the system. Output from the relative-humidity sensor was recorded using Warthog LabAnalyst (Mark Chappell, UC Riverside) running on a MacBook Pro laptop computer. The water content of expired air was estimated as the difference between the water vapor pressure (WVP; kPa; measured by the RH-100) of ambient air (baseline) and the WVP of expired air from the hummingbird. Air expired by a hummingbird during a typical measurement results in a WVP increase of  $\sim 200\text{--}300$  Pa which is well within the sensitivity range of the RH-100 (0.035–0.050% RH at 36 °C). WVP measurements made while holding a hummingbird's bill near the mask without its head inserted did not differ from ambient background WVP validating that our REWL

estimates result from exhaled water vapor and likely some evaporation from the head and did not include water from cutaneous evaporation from the body.

In the field all measurements were made on free-living hovering hummingbirds using the respiratory-mask system described above. Laboratory measurements were made by placing the respiratory mask in a wind tunnel designed for studies of avian flight (Tobalske et al., 2005). The working section of the tunnel in which the bird flies is square in cross-section, 60×60×85 cm inner diameter at the inlet, with clear acrylic (Lexan™) walls to provide a view of the bird. Birds were acclimated to the working section of the wind tunnel for an interval of 2–3 h, during which the birds were in still air with free access to a feeder and a perch.

### 2.2.2. Calculation of REWL

The water vapor pressure (WVP; kPa) of the airstream pulled from the respiratory mask was measured by the RH-100 and converted to water vapor density (WVD; g/m<sup>3</sup>) using the unit conversion option in LabAnalyst which is based on the following equation as described in Tracy et al. (1980):

$$WVD = WVP / (T * R_w)$$

where T is temperature in Kelvin (K) and R<sub>w</sub> is the gas constant for water vapor (461.5 J kg<sup>-1</sup> K<sup>-1</sup>). Flow rate of water in the air stream (VH<sub>2</sub>O; mg/min) was calculated as:

$$VH_2O = FR * WVD * 0.001$$

where FR is the excurrent flow rate (mL/min) from the mask and 0.001 a multiplier used to convert the VH<sub>2</sub>O to mg/min. Because VH<sub>2</sub>O measurements were rarely long enough to reach steady-state actual REWL (mg/min) was calculated as:

$$REWL = TEW / MT$$

where TEW is total evaporated water (mg) calculated by integrating the area under the measurement peak and MT the measurement time (min) defined as the total time the hummingbird's head was completely inserted into the respiratory mask. We tested the accuracy of our TEW measurement by injecting 10 mL boluses of air of known water content (4 mg H<sub>2</sub>O/L air; similar in magnitude to our REWL measurements) into the airstream flowing through the RH-100 with a calibrated syringe. Then the water content of each bolus was calculated by integration as described above. The known water content of the injected air was within the range of injection error for our calculated values (mean ± SD = 4.2 ± 0.4 mg/L) suggesting that our measurements of TEW are reasonably accurate. MT was measured using video recordings. Video recordings were made in the field using a Canon HV20 digital camcorder (30 frames/s) and in the lab using a Redlake PCI-2000 (500 frames/s).

### 2.3. Operative temperature

We recorded operative temperature (T<sub>e</sub>) during all measurements of REWL in free-living birds. T<sub>e</sub> was measured using a hollow-copper sphere thermometer (40 mm diameter) similar to that described by Walsberg and Weathers (1986). The sphere was painted flat gray to achieve mid-range reflectivity. A cu-cn thermocouple was inserted into the sphere to measure temperature. Temperature recordings from thermocouples were made using a Sable Systems TC-1000 thermocouple reader (resolution 0.01 °C, accuracy 0.2 °C). The sphere was located within 30 cm of the respiratory mask. A second identical sphere thermometer was placed in a constantly shaded environment sheltered from wind to measure ambient temperature (T<sub>a</sub>) for comparison.

### 2.4. Heat dissipation

Respiratory evaporative heat loss (REHL; W/kg) was calculated as:

$$REHL = (REWL * 2260 \text{ J/g}) / 60$$

where REWL is mg g<sup>-1</sup> min<sup>-1</sup> and 2260 J/g is the latent heat of vaporization of water.

Total metabolic power (MP; W/kg) during hovering and forward flight were calculated from published or previously measured values of oxygen consumption. Oxygen consumption was converted to equivalent heat units assuming 1 L O<sub>2</sub> = 20.1 kJ. For calculations involving free-living birds, values for *A. alexandri*, *S. rufus*, and *L. clemenciae* are from Lasiewski (1963) and Lasiewski and Lasiewski (1967). Values for *C. latirostris*, *A. violiceps*, *E. fulgens*, *L. clemenciae*, and wind tunnel measurements for *S. rufus* are from the current study. Total dissipated heat (TDH; W/kg) was calculated using the following equation:

$$TDH = (MP * 0.9) - BMR$$

where BMR is basal metabolism (W/kg). The 0.9 multiplier reduces MP by the amount of metabolic power that is converted to mechanical energy in the flight muscles of hummingbirds (10%; Wells, 1993). BMR values for *A. alexandri*, *S. rufus*, *E. fulgens*, and *L. clemenciae* are from Lasiewski (1963) and Lasiewski and Lasiewski (1967). No BMR values have been measured for *C. latirostris* or *A. violiceps* so values for *S. rufus* and Anna's hummingbird (*Calypte anna*) (Lasiewski, 1963), similarly sized species, were used. Since measured BMR values for hummingbirds show little variation it is unlikely that substituting these values results in meaningful error. Because we could not track changes in body temperature during measurements our calculation of TDH assumes no heat is stored during flight. If heat is stored during flight actual TDH would be lower than that estimated by the above equation.

The percent TDH accounted for by REWL was calculated as:

$$\% \text{Heat dissipated} = (REHL / TDH) * 100.$$

### 2.5. Statistical analysis

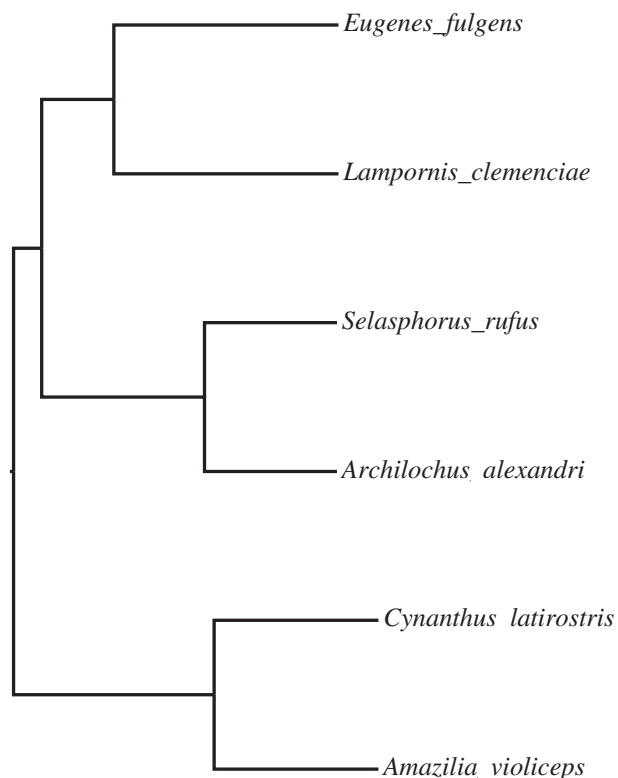
Standard statistical analysis was done using SPSS version 18.0 (IBM, Somers, New York). The effects of M<sub>b</sub> and T<sub>e</sub> on REWL were tested using standard least-squares regression (Zar, 1974). Mean values measured in our wind tunnel studies were tested for statistical difference using a student t-test and analysis of variance (ANOVA) (Zar, 1974). Post hoc testing (Tukey) was used to evaluate differences between specific groups when ANOVA results were significant (Zar, 1974). We calculated the relationship between log REWL and log body mass (M<sub>b</sub>; g) using phylogenetically independent contrasts (PIC; Felsenstein, 1985; Garland et al., 1992) and the PDAP module (v. 1.14) of Mesquite v. 2.73 (Maddison and Maddison, 2010; Midford et al., 2008). The phylogeny and relative branch lengths used in our PIC analysis were based on McGuire et al. (2009) and Garcia-Deras et al. (2008) (Fig. 1). Standardized contrasts were positivized according to Garland et al. (1992). We performed least-squares regression through the origin on positivized contrasts of log REWL versus log M<sub>b</sub>.

## 3. Results

### 3.1. REWL during hovering and forward flight

REWL values ranged from 0.7 to 1.9 mg g<sup>-1</sup> min<sup>-1</sup> and differed significantly among species (F<sub>5,277</sub>, P < 0.001; Table 1). Male and female REWL are reported separately for three species but were only significantly different for *E. fulgens* (t = 3.38, df = 63, P = 0.001). Male and female REWL data were combined for *A. alexandri* and *C.*





**Fig. 1.** Presumed phylogeny of hummingbirds used in our phylogenetic independent contrasts. Phylogeny is based on McGuire et al. (2009) with placement of *C. latirostris* based on Garcia-Deras et al. (2008).

*latirostris* in subsequent analyses. Because REWL in at least some free-living hummingbirds appears to vary with  $T_e$  (see below) the means reported in Table 1 represent the average REWL during the measurement period. Both the standard and PIC regressions of log REWL and log  $M_b$  had similar slopes showing a negative correlation between REWL and  $M_b$  (Fig. 2). The PIC regression just missed significance with  $P = 0.054$ .

Hovering REWL for free-living *S. rufus* was significantly higher (31%) than our wind tunnel measurement ( $t = 2.396$ ,  $df = 33$ ,  $P = 0.011$ ; Fig. 3). REWL during forward flight at 6 and 10 m/s did not differ but both were significantly lower than wind tunnel hovering REWL, which was more than 2× the values for forward flight ( $F_{2,35} = 39.96$ ,  $P < 0.001$ ).

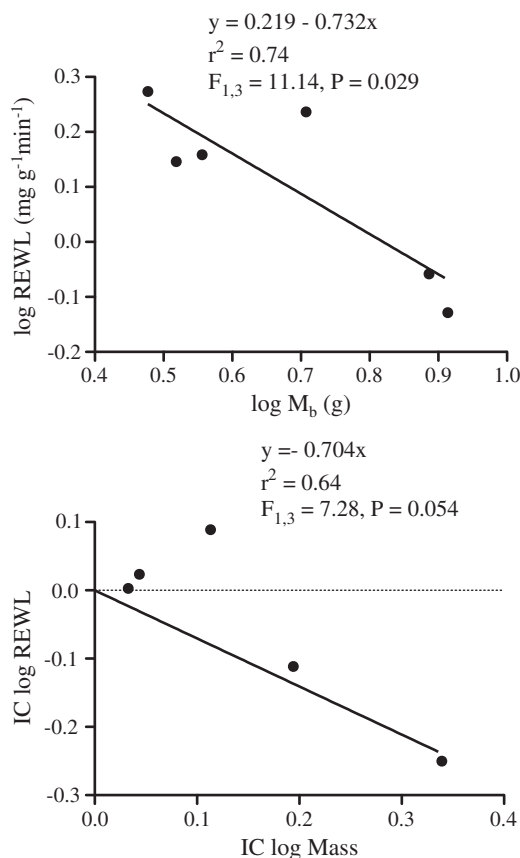
3.2. Temperature effects on REWL

We measured REWL in free-living hummingbirds at  $T_e$  values ranging from 15 to 44 °C. Over this temperature range log REWL was positively correlated with log  $T_e$  except for in *S. rufus* and *A. violiceps* (Fig. 4). For species in which REWL was correlated to  $T_e$  REWL approximately doubled over the  $T_e$  range measured with slopes

**Table 1**  
Mass-specific REWL for free-living hummingbirds in southeastern Arizona. Data are mean + SD.

Species	REWL ( $\text{mg g}^{-1} \text{min}^{-1}$ ) Male	Female
<i>Archilochus alexandri</i>	1.88 + 0.94	1.63 + 1.07
<i>Cynanthus latirostris</i>	1.40 + 0.63	1.31 + 0.52
<i>Selasphorus rufus</i>	1.44 + 0.75	-
<i>Amazilia violiceps</i>	1.72 + 0.89 <sup>a</sup>	-
<i>Eugenes fulgens</i>	0.88 + 0.36	1.29 + 0.55
<i>Lampornis clemenciae</i>	0.74 + 0.27	-

<sup>a</sup> Not sexually dimorphic. Sex unknown.

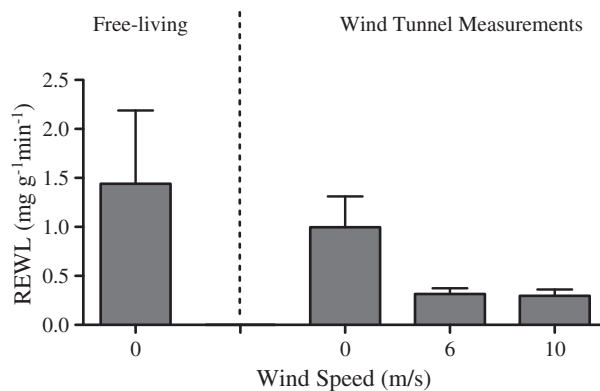


**Fig. 2.** The relationship between REWL and  $M_b$  for hummingbird species in this study ranging from ~3 to 8 g. Standard linear regressions are on top and linear regression of independent contrasts are on the bottom.

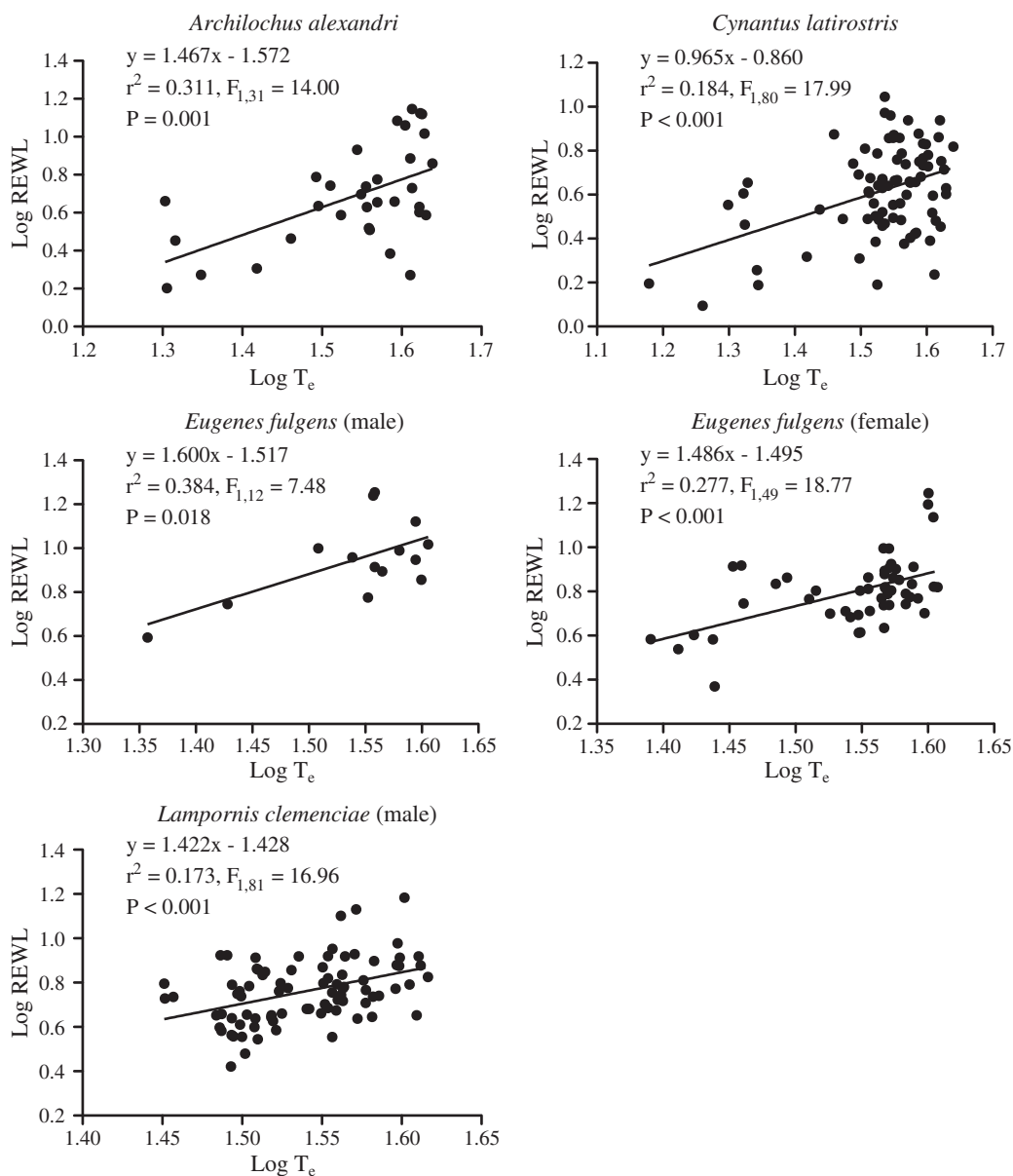
ranging from 0.97 to 1.60. The largest increase in REWL was 4.5× in *L. clemenciae*.

3.3. Heat dissipation by REWL

With the exception of *A. violiceps* less than 50% of TDH was dissipated by REWL even at high temperatures (Fig. 5). In *A. violiceps* REWL accounted for 50.6% of TDH. For the remaining species our low value was 7% for *A. alexandri*, *E. fulgens* females, and *L. clemenciae* males at ~20 °C. Our high value was 37% for *C. latirostris* at 44 °C. The



**Fig. 3.** Wind tunnel measurements of REWL during hovering and forward flight in *S. rufus*. Hovering REWL measured in free-living *S. rufus* is included for comparison. Wind speed = 0 is hovering while wind speed > 0 is forward flight speed. Hovering REWL in free-living individuals was significantly higher than in the wind tunnel. REWL during forward flight was significantly lower than during hovering. Values reported are mean ± SD.



**Fig. 4.** Standard linear regressions of log REWL (mg/min) and log  $T_e$  ( $^{\circ}$ C) for free-living hummingbird species in this study showing a significant temperature effect. Male and female were combined except for *E. fulgens* where the difference between genders was significant. No females were measured for *L. clemenciae* so only male data is shown.

mean REWL percentage of TDH for the four species whose rates of REWL were temperature dependent was 10.2% at 20  $^{\circ}$ C. Values for *A. alexandri*, *E. fulgens* females, and *L. clemenciae* males were all similar (range 6.9–7.5%) whereas *C. latirostris* was 19.2%. If *C. latirostris* is removed the average is 7.2%. At 40  $^{\circ}$ C all four species were similar ranging from 24 to 34% with an average of 28.5%. Again the high value of 34% was from *C. latirostris*. TDH for *S. rufus* (54.3 W/kg; 19.4%), *A. violiceps* (64.9 W/kg; 50.6%), and *E. fulgens* males (48.6 W/kg; 33.7%) was not related to temperature.

The percent of TDH accounted for by REWL during hovering in the wind tunnel for *S. rufus* was 37.6 W/kg. Values for forward flight did not differ between flight speeds but were significantly lower (30% less) than hovering (11.4 W/kg;  $F_{2,35} = 39.96$ ;  $P < 0.001$ ).

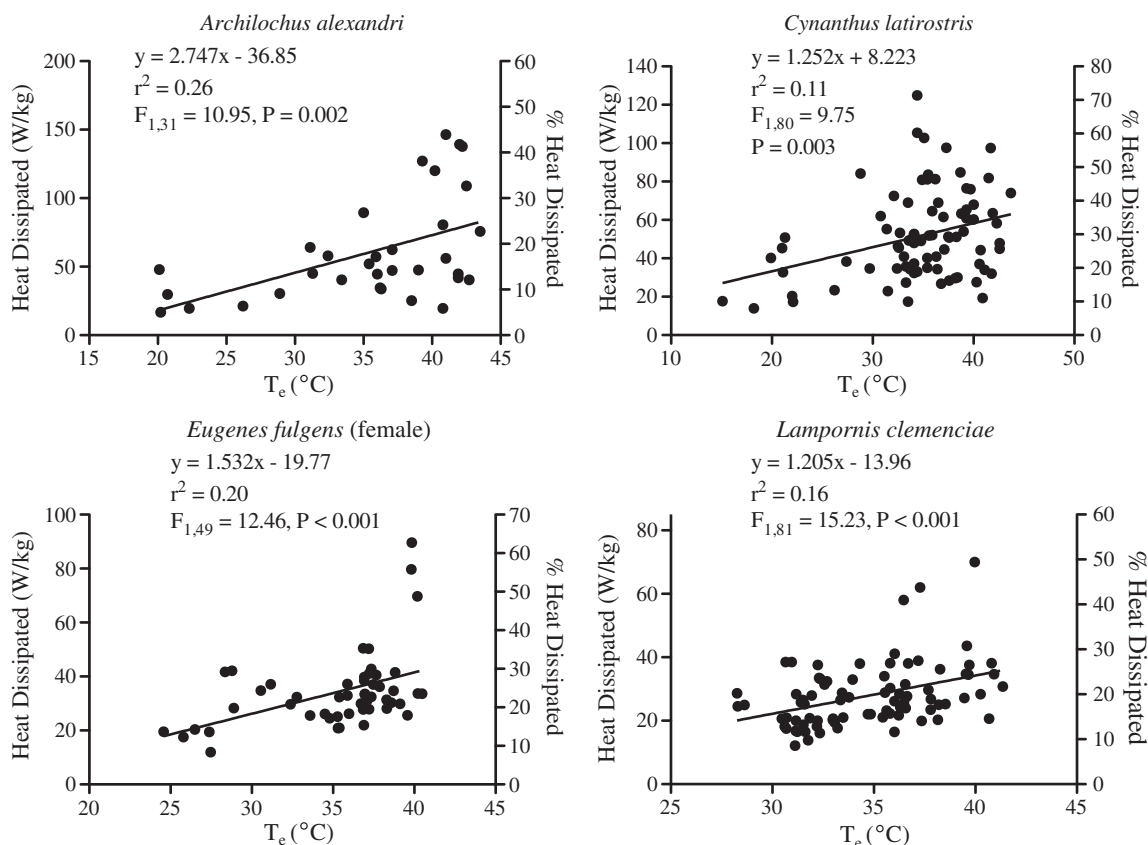
### 3.3.1. Contribution of REWL to daily water turnover

Powers and Conley (1994) measured daily water turnover rate in *A. alexandri* and *L. clemenciae* at SWRS using doubly-labeled water. Water turnover rate in both species was  $1.7 \text{ mL g}^{-1} \text{ d}^{-1}$ . If we assume that hummingbirds fly approximately 20% of their active period

(e.g. Wolf and Hainsworth, 1971; Powers and Conley, 1994) then REWL accounts for about 16% of total water turnover in *A. alexandri* and 7% in *L. clemenciae*. These values should be considered approximations as total flight time is highly variable (Wolf and Hainsworth, 1971) and this estimate was based solely on REWL during hovering which is higher than REWL during forward flight based on our wind tunnel measurements.

## 4. Discussion

Our results show that REWL during flight in hummingbirds is a relatively small component ( $\leq 20\%$ ) of total daily water flux (prediction #2) and is lower during forward flight than during hovering ( $< 50\%$ ) corresponding with the lower metabolic rates associated with forward flight (prediction #3). Contrary to our prediction (1), REWL accounted for a lower than expected proportion of TDH (typically  $< 40\%$ ) even at high temperatures (requiring that some combination of CEWL and dry heat transfer contribute significantly to thermal balance).



**Fig. 5.** Standard linear regressions of total metabolic heat dissipated by REWL and the proportion of TDH accounted for by REWL as a function of  $T_e$  in hovering free-living hummingbirds in this study. Regressions are shown only for four species/genders where the relationship is significant.

Mean REWL in free-living hummingbirds during hover flight is negatively correlated with body mass ranging from  $0.9 \text{ mg H}_2\text{O g}^{-1} \text{ min}^{-1}$  in *L. clemenciae* (8.2 g) to  $1.7 \text{ mg H}_2\text{O g}^{-1} \text{ min}^{-1}$  in *A. alexandri* (3.2 g) (Fig. 2). The slopes of the normal regression of hovering REWL and  $M_b$  and the PIC regression are similar although the PIC regression just misses statistical significance (Fig. 2) so we cannot rule out phylogenetic effects. Predicted hovering REWL values are 3 to 5 times higher than total evaporative water loss (TEWL) in resting Anna's hummingbirds (*Calypste anna*; 4.5 g) at 37 °C and an ambient humidity comparable to those measured in this study (Powers, 1992), which highlights the importance of understanding REWL within the context of flight.

REWL measurements were made at  $T_e$  ranging from 15 to 45 °C and were significantly positively correlated with  $T_e$  for most species (Fig. 4). The increase in REWL with  $T_e$  is likely influenced by increased vapour-pressure deficits (VPD) between inhaled air in the lungs and ambient air and possibly ventilation rate of the lungs (Engel et al., 2006b), and the increasing importance of evaporative heat dissipation as  $T_e$  approaches and exceeds body temperature (Evangelista et al., 2010). Evangelista et al. (2010) estimated that in Anna's hummingbird (*Calypste anna*) heat dissipation during hovering by means including EWL at 40 °C would need to be about 0.5 W (half hovering MR). Heat dissipated at 40 °C in this study ranged from 0.14 to 0.36 W (Fig. 5) suggesting that REWL can account for a meaningful portion of the required heat dissipation during hovering when dry heat transfer (especially convection) is less effective.

Mean hovering REWL in free-living *S. rufus* is 31% higher than hovering REWL measured in a wind tunnel. We identify four factors that could contribute to this difference. First, hovering metabolic rate increases with altitude in response to decreased air density (Welch and Suarez, 2008). At ~1100 m higher elevation our free-living measurements would be predicted to increase about 8% (Welch and Suarez, 2008). Second, temperature difference between our free-living and

wind tunnel measurements would also contribute to lower wind tunnel measurements of REWL. Although a temperature effect was not apparent in our free-living *S. rufus* measurements most measurements were made at  $T_e > 30$  °C whereas all wind tunnel measurements were made at 21 °C. Third, it is possible that free-living birds experienced higher body temperature due to stored heat resulting from muscle activity during an extended flight to the feeder. However current evidence suggests that at least some of the heat produced by flight activity can be used to reduce thermoregulatory cost (Chai et al., 1998; Welch and Suarez, 2008) which would perhaps limit body temperature elevation except when  $T_e$  is near or above body temperature. Finally, there may have been a reduction in aerodynamic power required for flight within a flight chamber versus free flight (Rayner, 1994). Tobalske et al. (1999) estimated a mechanical power saving in zebra finches (*Taenopygia guttata*; ~13 g) of up to 6%; and, based upon their size and flight position in the tunnel, we estimate values for hovering hummingbirds could be at this level.

REWL during forward flight in the wind tunnel was less than half that measured during hovering. The reduced REWL during forward flight is consistent with the lower metabolic rate in hummingbirds during flight at these speeds compared to hovering (Clark and Dudley, 2009; 2010) which would require lower lung ventilation rates. Proportional heat dissipation by REWL during forward flight is significantly less than during hovering but is likely compensated for by increased convective heat loss to air flowing across body surfaces. Lower REWL during forward flight might be advantageous for hummingbirds that make long migratory flights since dehydration can be a factor that limits the duration of a migratory flight (Carmi et al., 1992).

Free-living species for which we had measurements across a broad  $T_e$  range showed a significant relationship between the amount of heat dissipated and  $T_e$  during hovering (Fig. 5). When  $T_e$  was

>40 °C REWL accounts for the dissipation of 0.2–0.9 W of heat, which equates to ~30–40% of metabolic heat production depending on species (Fig. 5). These data reveal that the contribution of REWL to metabolic heat dissipation during hovering is higher than at rest (Powers, 1992). Even so, the contribution of REWL to dissipation of metabolic heat is less than we predicted, particularly at high temperature where REWL can be the dominant component of TEWL (e.g. Wolf and Walsberg, 1996; Engel et al., 2006b). During forward flight in *S. rufus* at 21 °C the proportion of metabolic heat dissipated is ~10–12%, which is 35–40% lower than during hovering. For contrast at temperatures <14 °C REWL in *S. roseus* accounted for 14% of heat production (Engel et al., 2006a).

The contribution of REWL to heat dissipation and its impact on total body water is relatively small. At  $T_e \geq 40$  °C REWL typically accounted for <40% of TDH in and did not appear influenced by body size. If hummingbirds store heat during flight then our predictions of total heat dissipated by REWL would be underestimates. At present the heat-storage capacity of hummingbirds during flight is unstudied.

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