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Total Evaporative Water Loss in Birds at Different Ambient Temperatures: Allometric and Stoichiometric Approaches

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Valery M. Gavrilov (2017) Total evaporative water loss (*TEWL*) in Passeriformes and Non-Passeriformes was estimated by simultaneous measurements of energy expenditure and mass loss in resting birds. It was found that the percentage of heat dissipated by water evaporation depends on body size. Published data for 102 bird species were analyzed together with my own measurements for 157 bird species at thermally neutral temperatures (mostly 25°C) to establish the following relationship between *TEWL* and body mass: $TEWL25°C\ Aves = 0.28\ m^{0.701}$, $R^2 = 0.92$, where *TEWL* is in g H_2O/day and m is body mass (*g*). The scaling exponent 0.701 ± 0.007 is 0.05 greater than for the relationship of basal metabolic rate (*BMR*) to body mass. It was found that *TEWL* in passerines is higher than in non-passerines at all ambient temperatures by 50% at 25°C, 30% at 0°C, 39% at the lower critical temperature, and 59% at the upper critical temperature. The dependence of water loss on body mass at different ambient temperatures (T_A) was found to vary in the same manner as evaporative heat loss. *TEWL* in Passeriformes is approximately 25-60% higher than in Non-Passeriformes (particularly at high T_A), which is consistent with the ratio of their *BMR* levels. Within the thermoneutral zone, the proportion of heat dissipated by evaporation increases by approximately 2.6-fold in small passerines and by almost 4.1-fold in large passerines with the transition from the lower to upper critical temperature. In non-passerines, the proportion of evaporative heat losses increases by approximately 2.7 times within the thermoneutral zone in both large and small birds. The high basal metabolic rate in Passeriformes involves benefits like a higher maximum metabolic power and the ability to breed at lower ambient temperatures, but it comes with a cost: a significant expenditure of evaporative water. This cost is important because it is found to increase with body size in Passeriformes due to the forced evaporative heat loss, but it shows virtually no increase with body size in Non-Passeriformes. Thus, despite a high *BMR* significantly increasing ecological opportunities, this way of expanding the ecological niches is possible for the small size class only. These findings suggest that the high level of basal metabolic rate in Passeriformes in comparison to Non-Passeriformes determines the necessity for the former to utilize considerably larger amounts of water for evaporation to maintain the needed heat balance, especially at higher ambient temperatures and at larger body sizes.

Key words: Water loss, Heat dissipation, Thermoregulation, Metabolic heat production, Energetic equivalent of the loss body mass, Birds.

BACKGROUND

Evaporation is a key component of an organism's heat balance. It depends on metabolic rate, ambient temperature and relative humidity. Total evaporative water loss (*TEWL*) is a part of the animal's heat balance that is especially important under warm conditions. Birds display a broad spectrum of morphological and functional adaptations aimed at improving the water balance. *TEWL* studies provide insight into those ecological and morphological properties that determine the geographic distribution of avian species. In individual animals of a given species, *TEWL* is

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influenced by water availability, feeding patterns and protein content of the diet (Bernstein 1971; Lasiewski et al. 1971; Marder and Ben-Asher 1983; Marder and Gavrieli-Levin 1987; Webster and Bernstein 1987; Wolf and Walsberg 1996; Arieli et al. 2002; Ophir et al. 2002; McKechnie and Wolf 2004; DeNardo et al. 2004; Hoffman et al. 2007). Several studies have shown that rates of evaporative heat loss are correlated with metabolic rates (Hoffman and Walsberg 1999; Marder et al. 1989; McKechnie and Wolf 2004; Webster and King 1987; Withers and Williams 1990; Clark and Dudley 2009, 2010). The rate of heat dissipation is proportional to *TEWL* and strongly influenced by acclimatization and, presumably, by natural selection; thus, it varies among species (Williams and Tieleman 2002).

The need to maintain water balance restricts certain species to areas with sufficient rainfall, as demonstrated by studies of bird population density in Taiwan's subtropical mountains (Walther et al. 2017). Rodríguez and Barba (2016) found that various physiological and behavioral adaptations related to water balance allow nestlings of small altricial birds to maintain a stable body temperature at high air temperatures during the breeding season.

Basal metabolic rate (*BMR*) and *TEWL* are considered to be fundamental characteristics of the animal life history (Tieleman et al. 2006; Versteegh et al. 2008). It is known that Passeriformes and Non-Passeriformes differ in their *BMR*: Passeriformes' *BMR* is about 1.5 times higher (McNab 2009, 2016). An important question is how this difference in *BMR* values relates to evaporative heat losses at rest and, especially, during flight. Does a high *BMR* imply that closely related species or individuals are characterized by a high peak power output and therefore high energy expenditures for ordinary existence and additional works (flight in particular)? Does *BMR* level determine the ratio of evaporative and non-evaporative heat loss? If *BMR* determines the other components of the energy budget, the energy expenditures for flight and the level of evaporative cooling in passerine birds should be higher than in non-passerines. In other words, if *BMR* determines the upper metabolic limit, then the ratio of *BMR* to the upper metabolic limit should be relatively constant. It could then be expected that the ratio of *TEWL* to oxygen consumption in passerines is be equal to those in non-passerines. Therefore, at the same ambient temperature, Passeriformes would have a higher *TEWL* than Non-Passeriformes. In this paper, heat dissipation in resting birds is analyzed at various ambient temperatures using the terms described below (the stoichiometric approach).

A commonly cited allometric analysis of *TEWL* at thermally neutral temperatures is that of Crawford and Lasiewski (1968). They tabulated data for 42 species ranging in size from a 3-g hummingbird to a 100-kg ostrich (*Struthio camelus*). Williams (1996) noted that the allometric equation in Crawford and Lasiewski relating *TEWL* to body mass in birds was based on a relatively small sample size and constructed using procedures that might have biased the parameter estimation. In his report, Williams (1996) analyzed *TEWL* for 102 bird species ranging in size from hummingbirds to ostriches using both least-squares regression and phylogenetically independent contrasts. Using this approach, Williams (1996) established that (1) the slope of the allometric relationship between *TEWL* and body mass is higher than in the equation of Crawford and Lasiewski (1968), (2) birds from arid environments have lower *TEWL* than birds from more mesic environments, and (3) small and large birds have similar ratios of *TEWL* to oxygen consumption. The latter finding negates the idea that small desert birds replenish proportionately less of their TEWL with metabolic water than larger species do. Williams (1996) selected data taken at 25°C because this temperature is (1), at or near the lower critical temperature for many birds (2), not thermally stressful for most birds, and (3), the same temperature chosen by Crawford and Lasiewski (1968) for their analysis.

In this paper we address the following questions: (1) How do *TEWL* estimates based on the energy equivalent of the body mass loss (*q*) correspond to *TEWL* estimates obtained by conventional methods reviewed by Williams (1996)? Since McNab (2009, 2016) convincingly demonstrated that Passeriformes and Non-Passeriformes differ in their basal metabolic rate, we also address these questions: (2) What is the relationship between *TEWL* and body mass in Passeriformes and Non-Passeriformes birds at different ambient temperatures? And (3) How does the share of evaporative heat loss in Passeriformes and Non-Passeriformes vary from within the thermoneutral zone, *i.e.* from the lower critical temperature to the upper critical temperature?

MATERIALS AND METHODS

Bird sampling

We analyzed more than 60 species of passerine birds representing the order's full body size range-from the goldcrest (*Regulus regulus*, 5.5 g) to the Raven (*Corvus corax* 1,208 g) - and 30 species of non-passerine birds of a similar size range (25-4,000 g). All birds were kept in big aviaries at natural day lengths and temperatures. For migratory birds and subtropical species, the aviaries were heated in winter to maintain temperatures of 5-10°C. These conditions allow for an accurate analysis of seasonal acclimatization (Proser 1991). Measurements of energy values were made in winter (November-January, February) and summer (late May-June, late August-September) on non-molting birds. Studies of seasonal variation in energy expenditure, both at rest and at ordinary existence levels, were done at experimentally controlled temperatures, where T_A varied from -28°C to + 40°C to construct a thermal energy profile for each species.

Measurements of body mass variations in birds

The following experiments were performed for a more precise determination of the rate at which food passes through the alimentary canal and the magnitude of mass loss at night. Groups of five chaffinches (*Fringilla coelebs*) and three wood or Carolina ducks (*Aix sponsa*) were placed immediately after evening feeding into a small light cage with a mesh floor. The excrements dropped sank in a cuvette with liquid mineral oil which prevented evaporation of water. The cage and the cuvette were connected to scale-levers to register body mass changes during the night.

The rate of body mass loss after evening feeding stabilizes at different times in different-sized birds: on average, it occurs 2-4 h after feeding in small birds, and 6-8 h in large birds. Further loss proceeded at constant rates determined by water evaporation. Figure 1 exemplifies this trend, showing that the alimentary canal became empty five hours after feeding in Carolina ducks (body mass 470 g) and three hours after feeding in chaffinches (body mass 21 g). Based on our experiments, we assumed that 3 hours after the last feeding in small birds and 10-12 hours in large birds is sufficient for the alimentary canal to empty.

All birds fasted for at least 3-12 h and were in the postabsorptive state during measurements. We thus assume that during measurements the body mass loss occurred at a constant rate.

Metabolic rate measurements

All oxygen consumption values were corrected to standard pressure and temperature according to the equations of Depocas and Hart (1957). Respirometry in our modification is based on measurements of air pressure and is therefore very sensitive to temperature changes. We carefully controlled the temperature in the laboratory and employed sealed chambers during metabolic rate measurements (Gavrilov 1997 2012a b 2014 2015). If temperature fluctuations in the sealed chamber within one hour of measurements exceeded 0.3°C, the corresponding data were not used. The average volume of consumed oxygen from the entire period of metabolic rate measurements was transformed into the volume at standard temperature and pressure and converted to kJ day^{-1} according to the equation 1 L of O_2 = 15.97 + 5.16RQ (kJ) (Schmidt-Nielsen 1997). Oxygen consumption was measured at rest at different ambient temperatures in all species studied.

Measurement of RQ

Respiratory quotient (RQ) was determined by Haldane gas analyzer (Dolnik and Gavrilov 1973 1979; Gavrilov 1997 2015; Gavrilov et al. 2013). We measured energy expenditures at rest and the respiratory quotient in 26 species of passerine birds and 16 species of non-passerine birds in winter and summer. Measurements were made at different temperatures ranging from + 5°C to + 35°C.

The stoichiometric approach for estimating total evaporative water loss

This paper applied the stoichiometric approach for the determination of *TEWL* developed and described by Gavrilov (2014 2015). This method includes direct simultaneous measurements of the energetic equivalent of body weight loss (*q*) as the ratio between heat production (metabolic rate, MR, determined by the rate of oxygen consumption) and body weight loss (d*m*) at various T_A:

$$q = \mathrm{MR}/\mathrm{d}m$$

Body weight loss of an animal in the postabsorptive state at a constant relative humidity is primarily determined by water evaporation. Therefore, the caloric equivalent is directly proportional to the specific caloric value of metabolically oxidized compounds and inversely proportional to the body weight loss d*m*. The latter is the sum of evaporative and cloacal water losses and is equal to the difference between the weights of consumed oxygen and liberated CO_2, excreted products of nitrogen metabolism (protein oxidation), and excreted gastroliths. The complete algorithm used to calculate evaporative heat loss (H_e) from the energetic equivalent of body weight loss at rest for any combination of oxidized compounds was published elsewhere (Gavrilov 2015). In this work, we shall only give exponential equations for the relationships between evaporative heat loss as

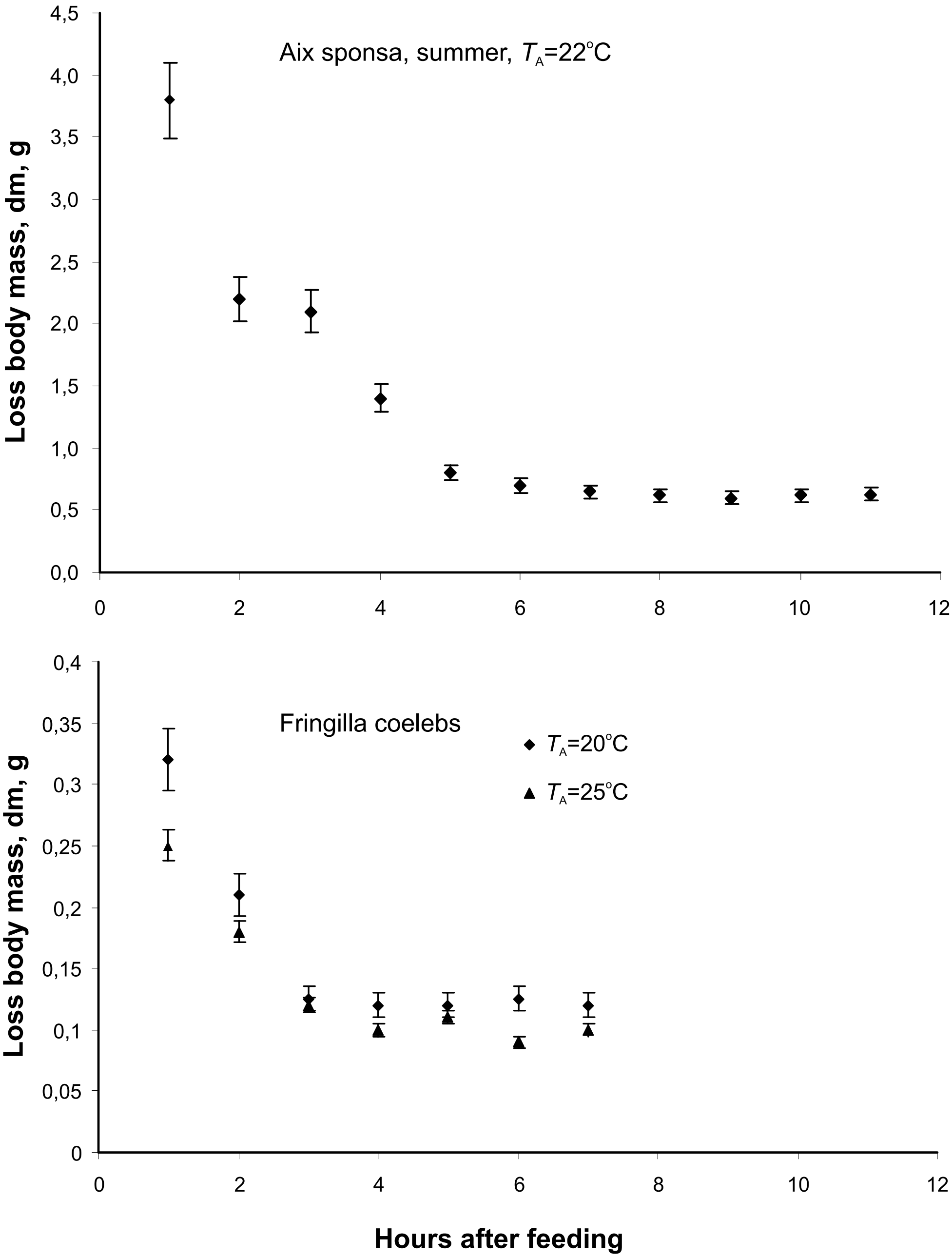

Fig. 1. Body mass loss per hour as a function of time after feeding for small (upper panel) and large (bottom panel) birds. Data for upper panel were slightly displaced horizontally to prevent overlapping. Body mass was measured every hour and thus body mass loss per hour is a mass difference between two successive measurements.

a percentage of total heat loss (H_e, %) and the energetic equivalent of the body weight loss at rest. For purely lipid metabolism (lipid oxidation is the main source of energy for birds in winter) we have

$$\% H_e = 238.3q^{-0.98}, \text{ where } q \text{ is in kJ/g.}$$

The oxidized substrate ratio is different in summer and the following coefficients were assumed: 0.7 for lipids, 0.1 for carbohydrates, and 0.2 for proteins (Dolnik and Gavrilov 1979; Gavrilov 2014 2015). Therefore, the energetic equivalents of body weight loss at rest were 0.7*q* for lipids, 0.l*q* for carbohydrates, and 0.2*q* for proteins. The exponential equation for summer is

$$\% H_e = 239.3q^{-1.05}, \text{ where } q \text{ is in kJ/g.}$$

The evaporative heat loss (as a percentage of total heat loss) can be derived from the energetic equivalent of body weight loss at rest. From this relationship, we can determine the ratio between evaporative and non-evaporative heat losses at any ambient temperature.

We used the above exponential equations to calculate the percentage of evaporative heat loss (H_e) from experimental measurements of *q*. Using the obtained percentage of evaporative heat loss and total heat production (*SMR* or *BMR*), we calculated the evaporative heat loss at various ambient temperatures: H_e0°C, H_eT_{lc}-the lower critical temperature, H_e25°C, and H_eTuc-the upper critical temperature. Taking into account that the evaporative heat of 1 gram of water is equal to 2.4 kJ (Garai 2009), total evaporative water loss can be represented as

$$TEWL = (BMR *\% H_e /100)/2.4,$$

where *BMR* is the total heat produced at any thermoneutral temperature T_A and $\%H_e$ is the percentage of total heat loss through evaporation at this T_A.

Scaling analysis and statistic

We performed a scaling analysis of evaporative water loss in Passeriformes and Non-Passeriformes birds at different ambient temperatures and seasons. First, we studied the relationship between *TEWL* and body mass by collating *TEWL* data at various ambient temperatures.

We tested the ANOVA difference between the model in R statistic (R Development Core Team 2014) for log-transformed values of *TEWL* (*g* H_2O/day) and body mass (*g*).

The study was performed according to the laws of the Russian Federation and Moscow State University regarding the capture and maintenance of wild animals. All birds were released after the experiment.

RESULTS

RQ measurements

RQ values estimated in 26 species of passerine birds and 16 species of non-passerine birds at night in winter at ambient temperatures 5-35°C fell within the range 0.69-0.75 and averaged 0.72 ± 0.03 (*n* = 1024). These data confirmed that lipids were the primary source of energy expenditure in winter at night.

Summer RQ values for the same species at night with ambient temperatures 5-35°C fell within the range 0.74-0.82 and averaged 0.77 ± 0.08 (*n* = 1024). Such RQs can be measured at the following ratios of oxidizable substrates: 0.7 for fat, 0.2 for carbohydrates and 0.1 for proteins, such that RQ = 0.7*0.7 + 0.2*1 + 0.1*0.82 = 0.77.

Measurements of metabolic rate, energetic equivalent of body mass loss, and total evaporative water loss

All the data obtained on heat loss at rest (*BMR*, *SMR*) and the associated values measured (T_{lc}, T_{uc},*q*, and *TEWL*) for all bird species for two seasons are summarized in appendix 1. As an example, the energy metabolism values obtained in summer for one passerine species, the goal tit (*Parus ater*), are presented graphically in figure 2.

The dependence on T_A of the resting metabolic indices at night (Fig. 2 top) corresponds exactly to Scholander's model: *SMR* decreases with rising T_A and theoretically attains zero at a certain T_A equal to body temperature (T_B) under moderately cold stress, which corresponds to the beginning of the thermoneutral zone. Quantitatively, the metabolism or heat loss (*SMR*) is related to T_A by a linear equation

$$SMR = h_l(T_B\text{-}T_A),$$

where h_l is the heat transfer coefficient or thermal conductance; it includes losses of heat by radiation, conduction and convection at low ambient temperatures where h_l is minimal. The decrease in *SMR* discontinues at T_A = T_{lc}, (T_{lc}

is the lower critical temperature). At this point, *SMR* becomes equal to basal metabolic rate (*BMR*). With a further increase in T_A, the energy expenditure remains unchanged, whereas the bird passes from the minimal wet thermal conductance (h_l) to the maximal (h_u) attained at $T_A = T_{uc}$ (T_{uc} is the upper critical temperature): $h_u = (BMR)/(T_B - T_{uc})$.

Evaporative heat loss (H_e) dissipates 9.3% of heat at 0°C, 16.2-16.7% at T_{lc} and 35.1-60.2% at T_{uc}. At low T_A, the role of heat loss via evaporation is minor: virtually all of the energy used in thermoregulation (*SMR-BMR*) is expended by conduction, convection, and radiation. Evaporative heat loss increases significantly in the thermoneutral zone, even though the birds increase thermal conductance.

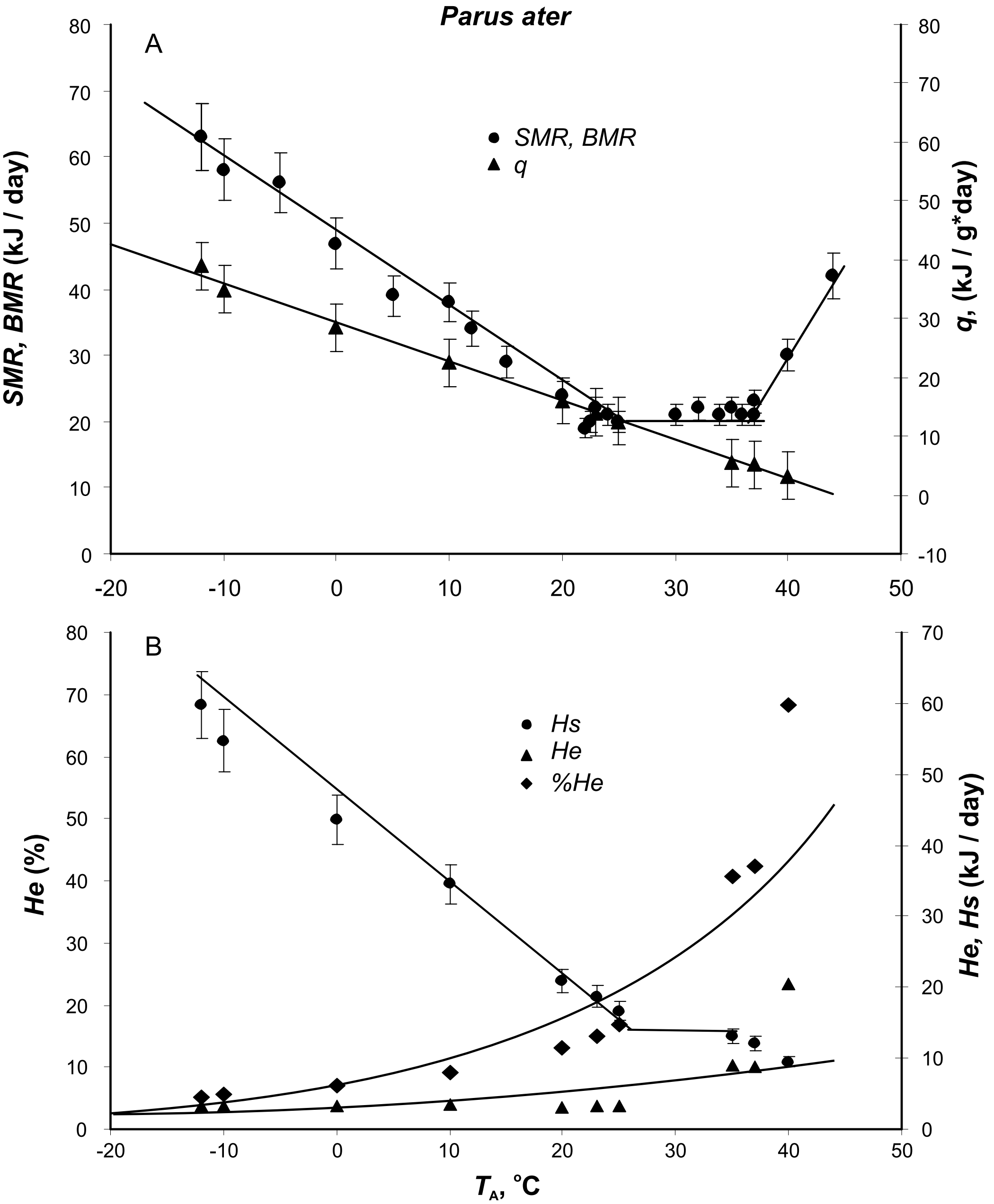

Fig. 2. (A) energy expenditure at rest (*SMR*, *BMR*, kJ per day, left scales) and the energy equivalent of lost body mass (*q*, kJ per g, right scales) as the functions of ambient temperature (T_A, °C). Each symbol is a mean for several measurements in several birds at a given T_A, vertical bars are SD. (B) Evaporative heat loss (H_e, kJ per day, right scale), non-evaporative heat loss (H_s, kJ per day, right scales) as the functions of ambient temperature (T_A, °C) and percentage of heat loss through evaporation (H_e, %, left scales) as the functions of ambient temperature (T_A, °C) in the Goal Tits (Parus ater) in winter.

Allometric analysis

Our data for 157 bird species (Appendix 1) with a thermally neutral temperature (25°C) indicate the following relationship between *TEWL* and body weight: *TEWL* at 25°C Aves = 0.27$m^{0.71}$, r^2 = 0.93, where the *TEWL* is expressed as g H_2O *g*/day, and body weight is expressed as *g* (Fig. 3A).

Evaporative water loss in Aves (based on combined data from this and Williams' (1996) review)

Comparison of our results for *TEWL*-obtained using measurements of the energy equivalent to body mass loss (*q*) and calculated for the same temperature (25°C)-with the results obtained by conventional methods (Williams 1996) revealed a good agreement between the two approaches, as shown in figure 3A: *TEWL*25°C (Willams), all = 0.29$m^{0.68}$, r^2 = 0.90; *TEWL*25°C (This study),

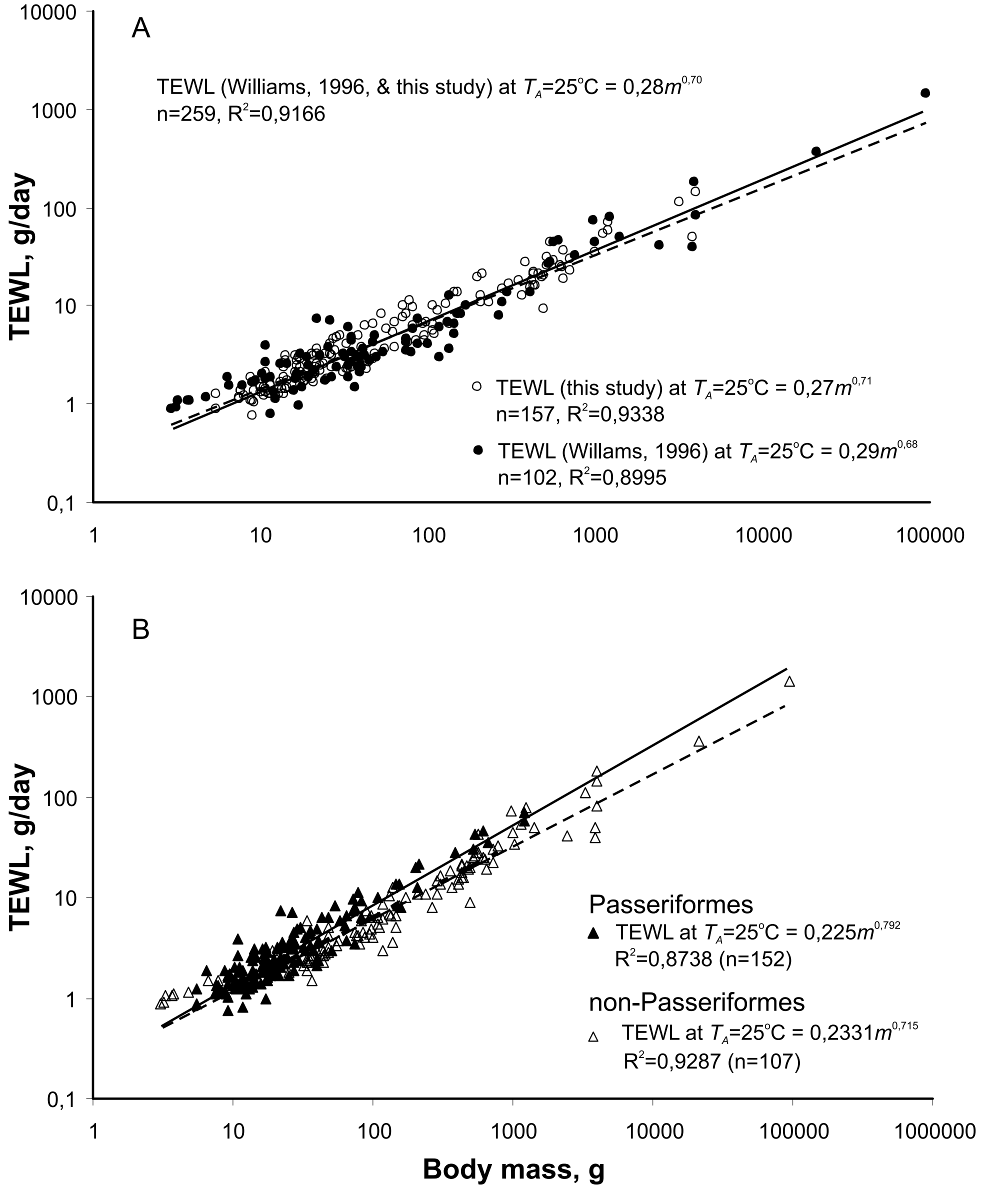

Fig. 3. (A) total evaporative water loss (*TEWL*) at T_A = 25°C as a function of body mass in all birds (this study and Willams, 1996). (B) total evaporative water loss (*TEWL*) at T_A = 25°C as s function of body mass in Passeriformes and Non-Passeriformes (this study and Willams 1996).

all = $0.27m^{0.71}$, $r^2 = 0.93$. The differences are not significant (the difference in slope is ns $F = 1.4578$, $p = 0.2284$; the difference in the y-intercepts is ns $F = 0.8987$, $p = 0.344$).

The data comparison demonstrates that determinations by the stoichiometric approach of total evaporative water loss yielded estimates that fit into the confidence intervals of all equations from the literature.

After combining the data from table 1 of Williams (1996) for 102 species of birds with my data for 157 species of birds (Appendix 1) at a thermally neutral temperature (25°C), the resulting relationship between *TEWL* and body weight is: *TEWL* at 25°C Aves = $0.28m^{0.70}$, $r^2 = 0.92$, where the *TEWL* is expressed as g H_2O g/day, and m is body mass (g) (Fig. 3A).

If the dichotomy between passerines and non-passerines is taken into account, we have *TEWL* at 25°C = $0.225m^{0.792}$ ($n = 152$ $r^2 = 0.874$) SD = 0.025 for Passeriformes and *TEWL* at 25°C = $0.233m^{0.715}$ ($n = 107$ $r^2 = 0.929$) SD = 0.019 for Non-Passeriformes. The differences are statistically significant for both the slope and y-intercept. On average, at T_A = 25°C passerines expend 50% more water than non-passerines. The difference grows with increasing body size (Fig. 3B).

Note that the highest slope of the regression line of the *TEWL* on body mass is in passerines at 25°C, because 25°C in some species is outside the thermoneutral zone.

Total evaporative water loss in Passeriformes and non-Passeriformes at different ambient temperatures

The established allometric relationships for three different ambient temperatures for passerines and non-passerine birds are shown in figures 4A, B, C. Table 1 shows the statistical significance of each pair of equations as the slope and y-intercept

Table 1. Fitted curves (*TEWL* = am^b, *TEWL g/day*, *a - intersept*, *b - slope*, *m - body mass*, *g*) for total evaporative water loss in various avian groups of species and comparison of *TEWL* in Passeriformes and non-Passeriformes birds at different ambient temperatures

Groups of species	Ambient temperatures T_A	N	Lim m, g	*a - intersept*	*b - slope*	r^2	Differences between Passeriformes and non-Passeriformes		
							Differences in slope	Differences in intersepts	Excess of TEWL in Passeriformes by
Aves, N + D, S + W	T_A = 25°C	259	2.7-100000	0.28	0.70	0.917			
Non-Passeriformes, N + D, S + W	T_A = 25°C	107	2.7-100000	0.23	0.747	0.874	$t = 2.458$ DF = 255 $p = 0.01464$	$t = -2.846$ DF = 255 $p = 0.00479$	50%
Passeriformes, N + D, S + W	T_A = 25°C	152	5.5-1208	0.225	0.792	0.929	The slopes are DIFFERENT at $p < 0.05$	The intercepts are DIFFERENT at $p < 0.05$	
Non-Passeriformes, N, S + W	T_A = 0°C	53	25.2-4010	0.21	0.710	0.970	$t = 1.549$ DF = 153	$t = -38.336$ DF = 153	30%
Passeriformes, N, S + W	T_A = 0°C	103	5.5-1208	0.32	0.68	0.982	$p = 0.12343$ The slopes are NOT different at $p < 0.05$	$p = 0.00000$ The intercepts are DIFFERENT at i < 0.05	
Non-Passeriformes, N, S + W	$T_A = T_{lc}$	53	25.2-4010	0.25	0.71	0.930	$t = 1.264$	$t = -42.675$	39%
Passeriformes, N, S + W	$T_A = T_{lc}$	103	5.5-1208	0.16	0.74	0.910	DF = 153 $p = 0.20804$ The slopes are NOT different at $p < 0.05$	DF = 153 $p = 0.00000$ The intercepts are DIFFERENT at $p < 0.05$	
Non-Passeriformes, N, S + W = T_{uc}	$T_A = T_{uc}$	53	25.2-4010	0.45	0.73	0.985	$t = -1.178$	$t = -9.823$	59%
Passeriformes, N, S + W	$T_A = T_{uc}$	103	5.5-1208	0.56	0.78	0.969	DF = 153 $p = 0.24052$ The slopes are NOT different at $p < 0.05$	DF = 153 $p = 0.00000$ The intercepts are DIFFERENT at $p < 0.05$	

D - Measurements were made during the active (daytime) phase of the avian circadian cycle; N - Measurements were made during the resting (nighttime) phase of the avian circadian cycle; W - Measurements were made during the nonproductive "winter" phase of the avian annual cycle; S - Measurements were made during the nonreproductive "summer" phase of the avian annual cycle.

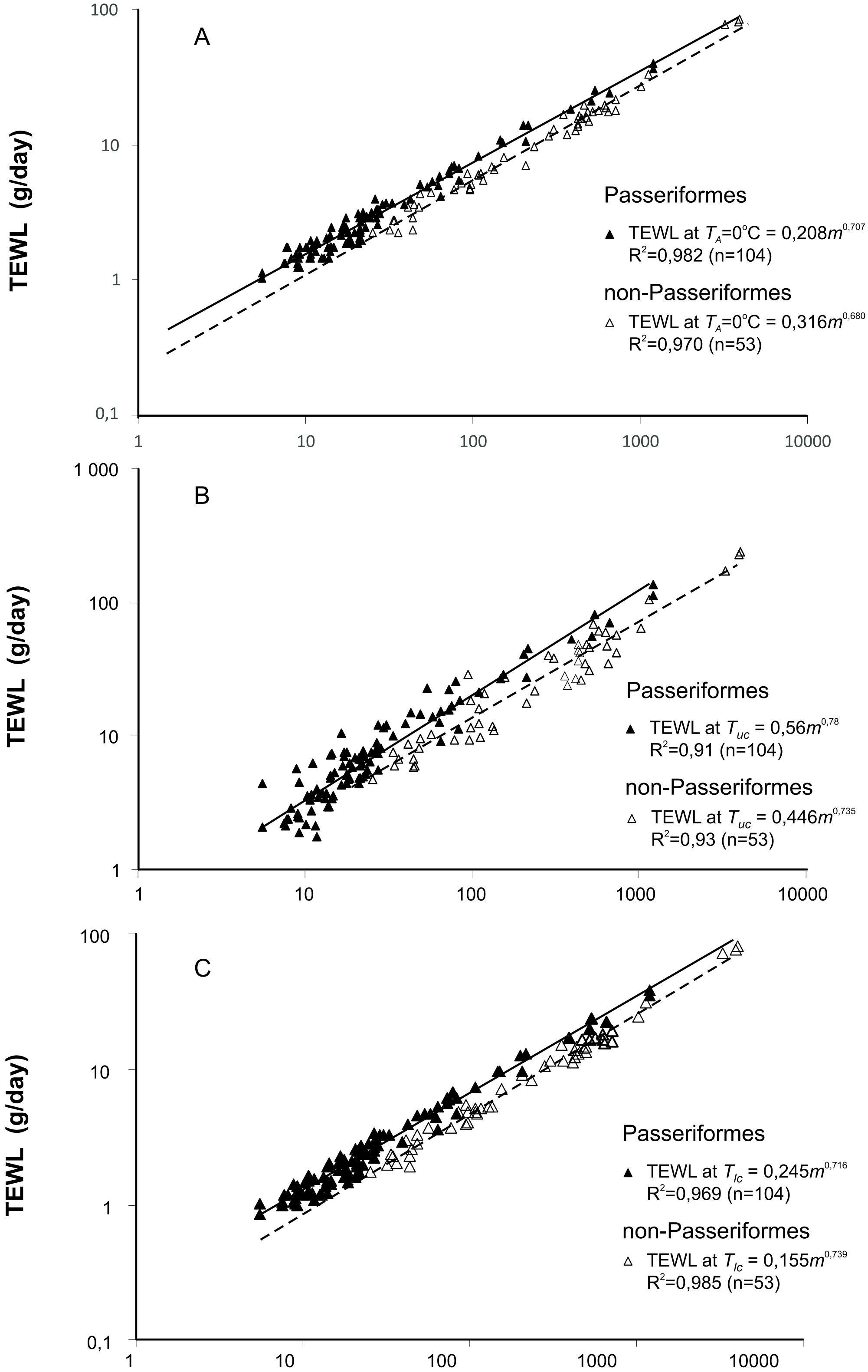

Fig. 4. (A) total evaporative water loss (*TEWL*) at T_A = 0°C as function of body mass in Passeriformes and Non-Passeriformes (this study). (B) total evaporative water loss (*TEWL*) at T_A $T_A = T_{lc}$ as function of body mass in Passeriformes and Non-Passeriformes (this study). (C) total evaporative water loss (*TEWL*) at T_A $T_A = T_{uc}$ as function of body mass Passeriformes and Non-Passeriformes (this study).

for Passeriformes and Non-Passeriformes.

The smallest amount of water evaporates at $T_A = T_{lc}$ in Passeriformes as well as in non-Passerifomes. The corresponding equations are:
for Non-Passeriformes *TEWL* at $T_A = T_{lc}$, $TEWL = 0.155m^{0.739}$ ($n = 53$ $r^2 = 0.985$) SD = 0.013,
for Passeriformes *TEWL* at $T_A = T_{lc}$, $TEWL = 0.245m^{0.716}$ ($n = 104$ $r^2 = 0.969$) SD = 0.013.
The slopes are not different at $p < 0.05$, the intercepts are different at $p < 0.05$. Passerine birds on average expend 39% more water than non-passerines.

At $T_A = 0°C$, the amount of evaporated water somewhat increases, while the exponents of the equations decrease (more in passerines than in non-passerines):
for Non-Passeriformes *TEWL* at 0°C = $0.210m^{0.707}$ ($n = 53$ $r^2 = 0.983$) SD = 0.013,
for Passeriformes *TEWL* at 0°C = $0.316m^{0.680}$ ($n = 104$ $r^2 = 0.970$) SD = 0.012.
The slopes are not different at $p < 0.05$, the intercepts are different at $p < 0.05$.
Passerine birds on average expend 30% more water than non-passerines.
At $T_A = T_{uc}$, the amount of evaporated water rises sharply, especially in passerine birds:
for Non-Passeriformes *TEWL* at $T_A = T_{uc}$, $TEWL = 0.445m^{0.735}$ ($n = 53$ $r^2 = 0.928$) SD = 0.029,
for Passeriformes *TEWL* at $T_A = T_{uc}$, $TEWL = 0.560m^{0.780}$ ($n = 104$ $r^2 = 0.908$) SD = 0.025.
The slopes are not different at $p < 0.05$, the intercepts are different at $p < 0.05$. Passerine birds on average spend 59% more water than non-passerines.

With temperature increasing from T_{lc} to T_{uc}, the amount of evaporated water increases in the smallest passerines by 2.5 times compared to 4 times in the largest passerines. Meanwhile in non-passerines, the amount of evaporated water rises by approximately 3 times in both large and small birds. The established relationship between *TEWL* and body mass indicates that *TEWL* in passerine birds is higher than in non-passerine birds at all ambient temperatures (0°C, T_{lc}, 25°C, and T_{uc}). Passerines spend more water for evaporation than non-passerines: by 50% at 25°C, by 30% at 0°C, by 39% at the lower critical temperature, and by 59% at the upper critical temperature.

Evaporative heat loss in Passeriformes and Non-Passeriformes at different ambient temperatures

The share of heat dissipated by evaporation ($\%H_e$) is the smallest at $T_A = 0°C$:
for Non-Passeriformes $\%H_e$ at 0°C, $\%H_e = 4.379m^{0.154}$ ($n = 53$ $r^2 = 0.738$) SD = 0.013,
for Passeriformes $\%H_e$ at 0°C, $\%H_e = 4.679m^{0.183}$ ($n = 104$ $r^2 = 0.655$) SD = 0.013.
Passerines average a 1.2-fold higher $\%H_e$ than non-passerines.
At $T_A = T_{lc}$ the share of heat dissipated by evaporation is almost independent of body mass; it is about 14-18% and slightly higher in non-passerine birds:
for Non-Passeriformes $\%H_e$ at $T_A = T_{lc}$, $\%H_e = 13.919m^{0.033}$ ($n = 53$ $r^2 = 0.131$) SD = 0.012,
for Passeriformes $\%H_e$ at $T_A = T_{lc}$, $\%H_e = 13.467\ m^{-0.019}$ ($n = 104$ $r^2 = 0.004$) SD = 0.030.
At $T_A = T_{uc}$ the share of heat dissipated by evaporation increases by 38-60%, especially in passerines:
for Non-Passeriformes $\%H_e$ at $T_A = T_{uc}$, $\%H_e = 38.001m^{0.039}$ ($n = 53$ $r^2 = 0.062$) SD = 0.021,
for Passeriformes $\%H_e$ at $T_A = T_{uc}$, $\%H_e = 35.950m^{0.083}$ ($n = 104$ $r^2 = 0.177$) SD = 0.018.
The share of heat dissipated by evaporation increases within the thermoneutral zone (*i.e.* with temperature rising from T_{lc} to T_{uc}) by approximately 2.6-fold in small passerines and by almost 4.1-fold in large passerines. In non-passerines, both large and small birds increase the share of evaporative heat losses within the thermoneutral zone by approximately 2.7 times. At the lower critical temperature T_{lc}, the share of heat dissipated by evaporation is approximately similar in passerine and non-passerine birds (14-18%) and is the same for large and small birds (the regression slopes practically do not differ from 0). At the upper critical temperature T_{uc} in non-passerine birds, the regression slopes only slightly differ from 0, while in passerines it is much higher. It is much more difficult for large passerine birds to maintain their heat balance at high ambient temperatures (Figs. 5, 6).

DISCUSSION

This paper presents a stoichiometric approach to the calculation of total evaporative water loss in Passeriformes and Non-Passeriformes. Body mass loss of a bird in the post-absorptive state at a constant relative humidity is primarily determined by water evaporation (Lasiewski et al. 1966a b; Dawson 1982). Comparison of the data obtained in this study with previously published data in the literature is shown in figure 3. A major advantage of the present method for determining *TEWL* at low ambient temperatures is that condensation or freezing of water vapor does not affect the accuracy of the resulting estimates in contrast to estimates based on measurements of humidity, dew point, or water vapor pressure. This method eliminates error caused by different gradients of water absorption at various temperatures and the difference between the density of saturating vapor and the actual density of vapor fed into the system from the surfaces of respiratory organs and skin. The experiments were performed at a constant relative humidity; we did not use dry air because it would stimulate evaporation. In traditional methods, as a rule, dry air is used. Also, there was no conflict between the theoretical oxidation constants of the substrates when *q* was measured over a long period because the birds were in the post absorptive state throughout the experiment. The measurements were performed in seasons in which the bird did not reproduce; therefore, birds did not transform the energy-rich compounds within their bodies and did not absorb water from the environment. The birds only expended

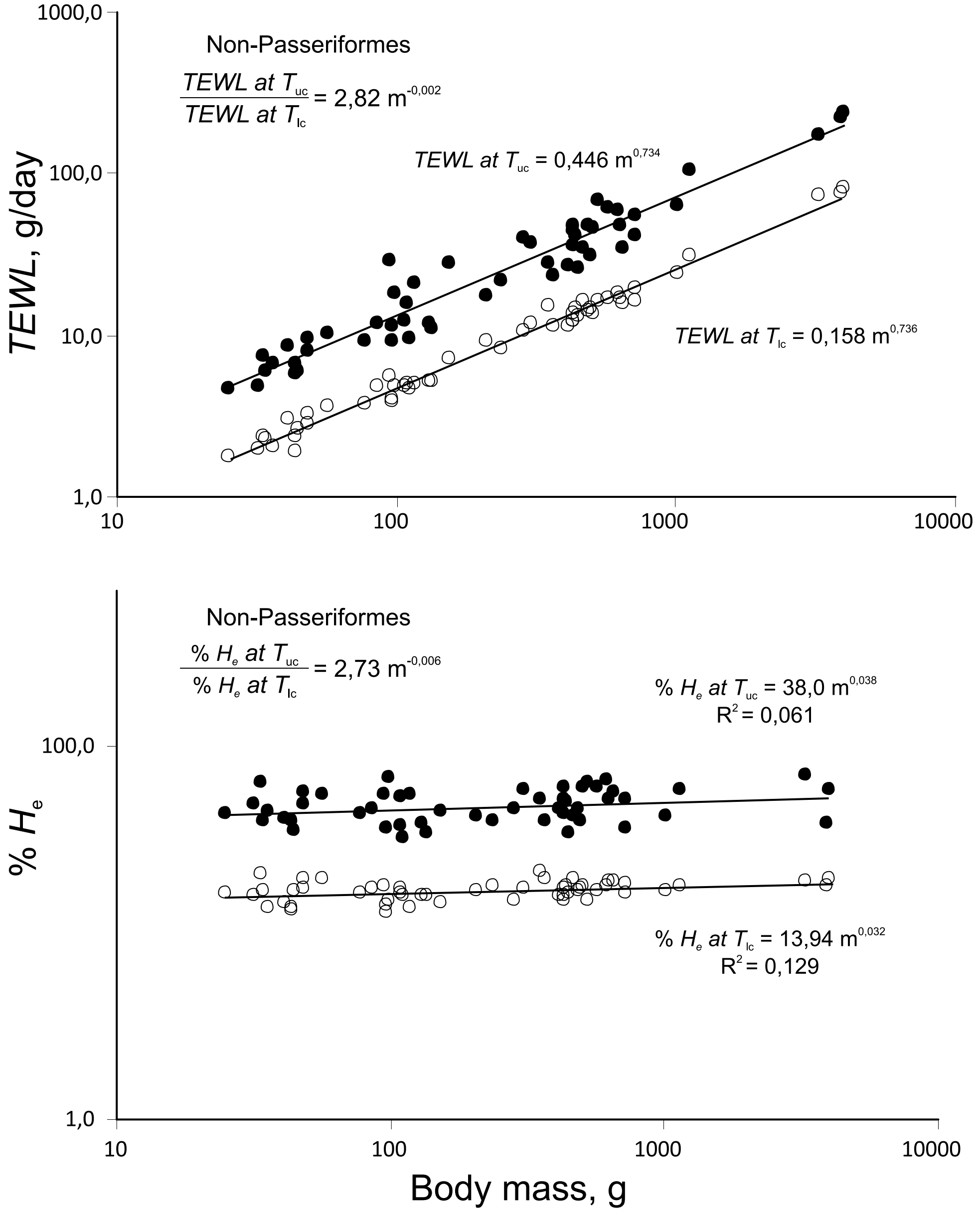

Fig. 5. Total evaporative water loss (*TEWL*) and share of heat dissipated by evaporation (%H_e) in non-Passeriformes at T_A $T_A = T_{lc}$ and at T_A $T_A = T_{uc}$ as function of body mass.

the substrates ingested with foods (lipids, carbohydrates, and proteins) and transformed them within their bodies.

Thus, the stoichiometric approach provides adequate data for *TEWL* compared with conventional methods. The initial postulates remain the same as in the work of Lasiewski et al. (1966b), but we now include the energy expenditures of birds.

The relationship between *TEWL* and the body mass for birds in the study of Williams (1996) (n = 102) at T_A = 25°C gives the slope of the regression line equal to 0.68. A slope of 0.70 was found for all birds-combining the data of Williams with our own (n = 102 + 157 = 259) at T_A = 25°C. Separating data into groups (non-passerines and passerines) increases the exponent in the equations for *TEWL* similar to what happens with the basal metabolic

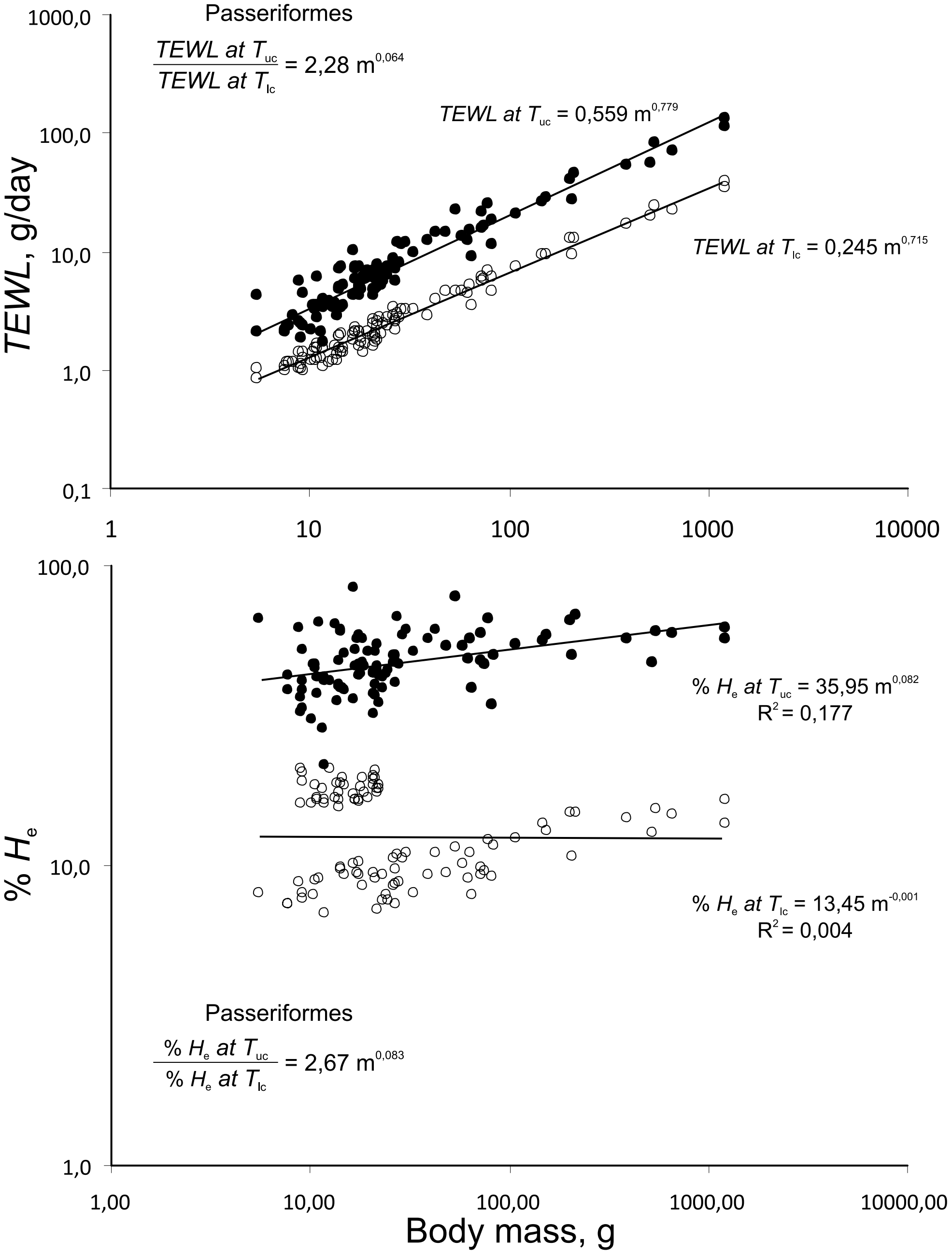

Fig. 6. Total evaporative water loss (*TEWL*) and share of heat dissipated by evaporation (%H_e) in Passeriformes at T_A T_A = T_{lc} and at T_A T_A = T_{uc} as function of body mass.

rate (Lasiewski and Dawson 1967; McNab 2009 2016; Gavrilov 2014). There are differences between Passeriformes and Non-Passeriformes at $T_A = 25°C$, $T_A = 0°C$, $T_A = T_{lc}$, and $T_A = T_{uc}$.

The dependencies of *TEWL* on body mass in Passeriformes and Non-Passeriformes were analyzed concerning the differences in the *BMR* levels of these two groups (Table 1). The dependencies of the evaporative water loss on body mass at various T_A (0°C, 25°C, T_{lc}, and T_{uc}) are statistically significant for both the slope and y-intercept for passerine and non-passerine birds, as were the ratios between evaporative and non-evaporative heat dissipation (Fig. 2). The dependences of the basal metabolic rate on body mass for all birds at various thermoneutral ambient temperatures T_{lc}, 25°C and T_{uc} are the same: *BMR* (kJ/day) = $3.48m^{0.652}$ (McNab 2009). When the distinction between passerines and non-passerines is taken into account, the *BMR* McNab obtained was (kJ/day) = $2.14m^{0.724}$ for non-passerines and (kJ/day) = $3.12m^{0.713}$ for passerines. Whether or not the difference in BMR between passerines and other birds is due to “phylogeny” their mass-independent basal rates are different (McNab 2009 2016). In Passeriformes, only at T_{lc} did the evaporative water loss increase in parallel with the metabolic rate; at T_{uc}, the larger birds dissipated more heat by evaporation than the smaller birds. The slopes of the regression lines for *TEWL* increased for passerine birds, reflecting increasing difficulties in maintaining heat balance in large-bodied birds at high T_A. The calculations performed using these equations gave the following exponents: 0.68-0.71 at $T_A = 0°C$; 0.71-0.74 at $T_A = T_{lc}$; 0.73-0.78 at $T_A = T_{uc}$ (Table 1). These data indicate that the mass exponents for *BMR* and *TEWL* are approximately equal at T_{lc} but differ at other temperatures. At higher and lower temperatures, evaporative heat loss increases with body size more rapidly than metabolic rate does.

The percentage of heat dissipated by water evaporation also depends on body size (Figs. 5, 6). At T_{lc}, it was equal to 14-18% in birds of various sizes. Experiments showed that the evaporative heat loss accounts for approximately 40-60% of the heat produced by *BMR* at $T_A = T_{uc}$. This fraction increased slightly with body size ($m^{0.007}$) in passerines and showed virtually no increase in non-passerines. The water loss increased with body size ($m^{0.17}$) in passerines because of forced evaporative heat loss. In non-passerine birds, there were nearly equal increases in evaporative water loss and metabolic rate: $m^{0.73}$ in summer and $m^{0.69}$ in winter, suggesting that birds from this group maintained the heat balance even at T_{uc}.

Several studies showed that levels of energy expenditure and water loss in birds vary with the overall phenotypic variation, the sum of inter- and intra-individual environment (Klaassen 1995; Wikelski et al. 2003; Tieleman et al. 2003). Variations in *BMR* and *TEWL* are caused by inter-individual interactions (Lessells and Boag 1987) and season (Weathers and Sullivan 1993; Webster and Weathers 2000). Here we have shown that at all ambient temperatures passerine birds have to consume significantly more water to maintain their heat balance than non-passerine birds.

The high basal metabolic rate in Passeriformes involves benefits like a higher maximum metabolic power and the ability to breed at lower ambient temperatures (Gavrilov 2014), but it comes with a cost: a significant expenditure of evaporative water. The dependence of water loss on body mass at different T_A illustrates this cost. These dependencies at $T_A = 0°C$, T_{lc}, and T_{uc} vary in the same way as the relationships between the evaporative and non-evaporative heat losses (Gavrilov 1995 2014). Total amount of water evaporated in the thermoneutral zone (at T_{lc} or T_{uc}) was considerably greater in passerines than in non-passerines (by approximately 40-60%). This is consistent with the differences in *BMR* at T_{lc} between the two groups. Thus, a high expenditure of evaporative water represents the cost of a high *BMR*. In spite of the fact that a high *BMR* may significantly increases ecological opportunities of a species, this way of expanding ecological niches is possible only in the small size class.

CONCLUSIONS

We showed that the stoichiometric approach to estimating *TEWL* yields adequate results for *TEWL* in comparison to conventional methods. The equations obtained give similar values of evaporative water losses within the range of actually existing body sizes determined by the above-described equations (Crawford and Lasiewski 1968; Williams 1996).

The *BMR* and *TEWL* are two fundamental parameters of energetics that determine the level of physiological organization in the animal. *BMR* and *TEWL* are dependent on the architecture of circulatory and respiratory systems and on plumage insulation (Gavrilov 2012a b). *TEWL* is more applicable as a characteristic at the taxonomic level. It is thermal conduction that determines ecological adaptation of species to humid environments. Thus, *TEWL* and *BMR* are integrated parameters of the system that involve the assimilation of oxygen and maintenance of constant body temperature. We showed that Passeriformes need to expend more water than Non-Passeriformes because they have higher level of basal metabolic rate: by 50% at 25°C, 30% at 0°C, 39% at the lower critical temperature, and 59% at the upper critical temperature.

High *BMR* requires considerable amounts of water for evaporation to maintain a heat balance at high ambient temperatures. This requirement imposes strong limitations on the range of sizes in this group (Gavrilov 1997 2014 2015).

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Appendix 1. Thermoregulation energetics at rest at night, energetic equivalent of loss body mass at rest (*q*, kJ/g upon T_A) and total evaporative water loss (*TEWL*, g/day) at different ambient temperatures in non-Passeriformes and Passeriformes in different seasons. (download)