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Evaporative and excretory water loss during free flight in pigeons

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Abstract Body water conservation is important in flying birds because the very high metabolic demands and heat dissipation requirements during flight depend on plasma-volume integrity. Wind tunnel experiments and theoretical model predictions show that evaporative water loss (EWL) depends on air temperature (T_a) and water vapor density (ρ_a), but these relationships have not been examined in free-flying birds. The contribution of excretory water loss to the total water loss of a flying bird is thought to be negligible but this assumption is untested. To study the dependence of water losses on environmental conditions in free-flying birds and to quantify the contribution of excretory water loss to total water loss, we estimated evaporative and excretory water losses in 16 trained, free-flying tippler pigeons (*Columba livia*, 250–340 g). We collected excreta by attaching a light latex, water-impermeable receptacle around each bird's vent. By gravimetry, we measured evaporative and excretory water losses of birds for eight flights at different T_as and compared flying to resting (control) birds for two of these flights. EWL was constant with respect to T_a when less than 15 °C, and increased with increasing T_a above 19 °C, indicating that evaporative cooling was invoked when the heat load increased. EWL increased with increasing ρ_a, possibly due to the strong correlation between ρ_a and T_a. Excretory water loss was independent of ρ_a or T_a and averaged almost 10% of the total water loss. Measurements of EWL made on pigeons during wind tunnel experiments and previous free-flight studies are consistent with our free-flight measurements made at similar T_as.

Key words Cutaneous evaporation · Excretory water loss · Respiratory evaporation · Evaporative cooling · Pigeon

Abbreviations *EWL* evaporative water loss · *EXWL* excretory water loss · ρ_a water vapour density · T_a air temperature · *TWL* total water loss

Introduction

Long-duration flapping flight is a prominent feature in the lives of hundreds of bird species, especially migrants [see Alerstam (1990) and Berthold (1993) for extensive reviews]. During flapping flight, a bird's metabolic rate may be 10–15 times that at rest (e.g., Berger et al. 1970; Rayner 1982; Bernstein 1987; Butler and Woakes 1990; reviewed by Norberg 1996). Between 7% and 25% of this energy input is transformed into flight power while much of the remainder is an internal, surplus heat load (Pennycuick 1989; Rayner 1990). Although a small amount of this excess heat can be stored by increasing body temperature (Hart and Roy 1967; Torre-Bueno 1976; Hudson and Bernstein 1981; Adams et al. 1995), thereby also increasing the gradient for non-evaporative heat loss to the environment, the amount stored is negligible compared with the total heat production during extended flight (Pearson 1964; Hirth et al. 1987). Therefore, in long duration flight, virtually all of the excess heat load must be dissipated by non-evaporative and evaporative means. Consequently, evaporative water loss (EWL) during free flight may be substantially elevated over that at rest, especially at higher air temperatures (T_as) (e.g., Berger et al. 1971; Torre-Bueno 1976; Hudson and Bernstein 1981; Biesel and Nachtigall 1987; Adams et al. 1997).

Although metabolic water produced during flight may compensate for some of the water lost, the quantity

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produced is not great enough to alleviate dehydration except at low T_as (Berger et al. 1971; Torre-Bueno 1978; Adams et al. 1997). Because the demands for gas exchange, metabolite transport, and heat dissipation greatly increase during flight, and because all these functions depend on plasma volume maintenance, water conservation is likely to be of paramount importance to flying birds (Carmi et al. 1993).

Except for the early experiments of Pearson (1964) in which he sealed the cloacas of tippler pigeons, the relationships between EWL and ambient conditions in free-flying birds are not documented, and in flight excretory water loss has not been previously measured. However, a theoretical attempt to assess total in-flight water loss at different T_as and water vapor densities (ρ_as) was made by Carmi et al. (1992). Their model emphasized the importance of respiratory water loss and predicted that exhaled T_a is an important variable in the water loss equation. Following Berger et al. (1971), Carmi et al. (1992) assumed that exhaled T_a in flying birds depends linearly on T_a. Thus, the model predicted that the dependence of respiratory water loss on T_a is also linear. However, the actual driving force for evaporation from any of the bird's evaporative surfaces (skin, eyes and respiratory tract) is the ρ_a gradient between a given surface and the environment (Gates 1980). Because these surfaces may vary in temperature, evaporation does not necessarily have a simple dependence on a single temperature. A further assumption of the model is that in-flight excretory water loss (EXWL) is negligible. Although the model has been examined and modified (Klaassen 1995), it was not tested with physiological data from free-flying birds.

In this study, we examined the dependence of water loss in free-flying birds on ambient ρ_a and T_a, and we tested the assumption that EXWL during flight is negligible. We achieved these by measuring EWL and EXWL during free flight in trained tippler pigeons.

Materials and methods

Experimental animals

We used 16 trained tippler pigeons (282 ± 6 g, mean ± SE). The tippler pigeon is a breed of rock pigeon (*Columba livia*) selected for its predilection to fly for hours in large circles above the loft that, in this study, was located outdoors at Midreshet Ben-Gurion, Israel (34°47′N, 30°52′E, 470 m above sea level). Our training protocol involved simultaneous release of the birds from the loft 3–5 times a week for flights of 2–4 h. Birds aloft remained in view of their trainer for the duration of the flight and were conditioned to return to the loft at the sound of a whistle. Food was presented while the whistle was blown and then removed after 20 min. On training days, birds were fed immediately upon their return to the loft at the end of a flight. On non-flight days, birds were fed once a day. Water was offered ad libitum.

Flight experiments

We did eight flight experiments under different weather conditions between January 1995 and February 1996. For each experiment we randomly chose 6–12 birds from the available trained pigeons at the time of the experiment. Experimental flights lasted at least 3.5 h (Table 1) and began about 1 h after sunup when atmospheric conditions were stable and T_a was not rising rapidly. Concurrently with two flights (three and six), we made measurements on resting (control) birds that were deprived of food and water during the experiment. We flew birds over as wide a range of T_as as we could, thus T_a and ρ_a for a given experiment were dependent on the prevailing weather.

Food was withheld from the birds for at least 16 h prior to flight or experimental rest. Each bird was weighed to ±0.1 g (Ohaus balance, CT1200-S) before and immediately after flight. To capture in-flight excreta, we made receptacles from rolled, water-impermeable latex surgical glove fingers. To test for water impermeability of the receptacles we filled six glove fingers with water, tied them off, weighed them to ±0.0001 g (Precisa balance, 40SM-200A), hung them outdoors (in the shade at 20–25 °C) for 6 h and reweighed them. No significant change in mass was detected. After cleaning the skin surface prior to flight, we weighed the empty receptacles to ±0.0001 g and attached one around each bird's vent with cyanoacrylic glue. Receptacles were completely buried in the contour feathers. Thus, although the retained mass must have affected the bird's total induced power input, we assumed it had negligible effect on drag. After flight, receptacles were removed carefully from the skin, weighed (full receptacle wet mass), dried to a constant mass at 60 °C, and reweighed (full receptacle dry mass).

We are mindful of the fact that voiding cloacal contents is not a continuous process, and that a bird might defecate upon take-off to reduce weight in flight. However, as the birds did not eat for at least 16 h prior to flight, it is likely that their gut contents were minimal and that there was little excess to dump. This assumption is based on the premise that food retention time for a 300-g seed-eating bird is about 2 h and gut emptying time is four times that (Karasov 1990; W.H. Karasov personal communication). In addition, we found little difference between total voided excreta mass in the flying birds and controls that rested while the birds flew (see Table 2). These observations suggest the lack of a

Table 1 Average weather conditions and flight duration of eight tippler pigeon flights. Dry air and wet bulb temperatures were measured on a 9-m tower under the birds' flight path. From these we calculated relative humidity and vapor density. Dry bulb and wet bulb air temperatures were sampled every half-hour. n = flight duration × 2, rounded to next whole number

Flight number	Pigeon sample size	Air temperature (°C) (range) mean ± SD	Relative humidity (%) (range) mean ± SD	Ambient water vapor density ($g \cdot m^{-3}$) (range) mean ± SD	Flight duration (h)
1	10	(5.2–13.2) 10.2 ± 2.4	(66–98) 82 ± 10	(6.5–8.5) 7.8 ± 0.5	4
2	11	(10.2–12.6) 11.3 ± 0.8	(64–84) 74 ± 7	(7.0–8.2) 7.6 ± 0.4	3.8
3	6	(10.4–17.3) 13.8 ± 2.3	(46–70) 59 ± 8	(6.8–7.4) 7.0 ± 0.2	4.8
4	5	(9.9–17.0) 14.1 ± 1.9	(62–86) 72 ± 6	(8.2–9.4) 8.7 ± 0.3	4.5
5	6	(11.6–15.6) 14.7 ± 1.2	(42–68) 52 ± 6	(5.8–7.3) 6.6 ± 0.6	3.5
6	6	(14.3–22.7) 19.6 ± 2.7	(48–80) 58 ± 9	(9–10.2) 9.8 ± 0.3	4.5
7	8	(20.5–25.0) 22.9 ± 1.5	(58–72) 68 ± 5	(12.5–14.4) 14.0 ± 0.6	3.75
8	5	(21.6–28.4) 24.9 ± 2.0	(47–85) 64 ± 11	(12.5–16) 14.7 ± 1	3.5

momentous voiding event at the onset of flight, although we can not entirely exclude this possibility. Therefore, in light of the above and for convenience, we refer to EXWL in the same terms as the other water loss components, that is, as a continuous rate. Withholding food for 16 h prior to flight assured that the birds were post-absorptive and that most of the substrate catabolized during flight was fat as discussed below. After the birds landed, access to water and food was denied until the sampling procedure was complete.

The pigeons typically flew at altitudes of 25–100 m. During flights, we measured dry and wet bulb temperatures every half-hour using a sling psychrometer (Bacharach, 12-7013) on a 9-m tower under the birds' flight path. In the standard atmosphere, T_a decreases with increasing altitude by just 0.7 °C/100 m (List 1984), thus we considered our measures of T_a representative of where the birds were flying. This was also verified by flying the same pigeons carrying T_a telemetry devices (N.J. Adams, H. Biebach and B. Pinshow personal observation). We expressed EWL as a function of ambient T_a and ρ_a that was calculated from dry and wet-bulb temperatures using appropriate tables in List (1984) based on the Goff-Gratch equation.

Calculation of water loss

The use of mass balance to measure EWL requires knowledge of mass loss or gain through gas exchange [O_2 uptake ($\dot{V}O_2$) and CO_2 release ($\dot{V}CO_2$)]. When only fat is catabolized, the respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$) equals ~0.72, a value close to 0.723 at which the mass of O_2 consumed equals the mass of CO_2 released (Norberg 1996). RQ in flying pigeons was reported to be 0.73 after less than 0.5 h of wind tunnel flight (Biesel and Nachtigall 1987) and in free flight (Bordel and Haase 1993; Schwilch et al. 1996).

To assess the sensitivity of our measures to assumptions about RQ, we assumed a steady, linear shift from carbohydrate to fat metabolism in the 1st h of flight from RQ = 0.9 to RQ = 0.73, based on Biesel and Nachtigall (1987). Under these circumstances we conservatively estimated mass loss due to gas exchange to be less than 1 g. Ignoring this mass loss will introduce an error into the calculation of EWL and total water loss (TWL), and, in the most extreme case, this error could amount to 8% of EWL, where a particular bird's total mass loss was minimal. Fasting the birds for 16 h prior to flight, along with the long duration of the flights, kept RQ close to 0.73 and minimized this error type. Thus, we assumed that in our flight experiments, mass loss through gas exchange was negligible and all mass loss was either evaporative or excretory (other minor mass changes such as loss of feathers or sloughed skin were ignored). Lasiewski et al. (1966) verified the use of the mass balance method for resting pigeons by simultaneous measurement of EWL in resting, post absorptive pigeons by gravimetry and with an open flow gas exchange system.

We calculated EXWL as the difference between full receptacle wet and dry masses, and dry excreta mass as the difference between full receptacle dry mass and empty receptacle dry mass. TWL was calculated by subtracting dry excreta mass from the total mass loss, and EWL by the difference between TWL and EXWL. We standardized our results to flight duration and body mass to obtain mass specific rates of water loss which are presented as milligrams of water per gram of body mass and hour of flight duration.

Statistical analysis

Results, except in Table 1, are presented as mean ± 1 SE; number of birds sampled (n_b) and number of flights (n_f). For statistical comparisons, we chose $P = 0.05$ as the minimum acceptable level of significance and 0.8 ($\beta = 0.2$) as a minimum acceptable power level. In the comparison of flight to rest, some of the data sets did not fulfill the assumptions demanded by parametric tests (i.e., homogeneity of variance and normal distribution) and sometimes the power of the test was lower than acceptable. This was due to small sample size and high variance. Thus, we used a non-parametric, boot-strap randomization technique (Manly 1997) to make comparisons, with a program written by Simon (1995). For uniformity we used this method in all cases and it proved more robust, with higher power, than standard parametric tests. Briefly, data from both treatments (flight and rest) were pooled for a given set of conditions, then randomly dealt into two new "treatments", and the difference between the means of the new treatments was calculated. The procedure was repeated 1000 times. Finally, the probability of the difference between the randomly generated treatments being equal or larger than the measured difference was calculated. If that probability was less than 5%, the measured difference was considered significant. This is analogous to the parametric Students *t*-test.

Results

Flight performance

The flock of pigeons typically flew in tight formation, in a broad circle within a radius of 50 m to 500 m of the loft at an altitude of 10 m to 100 m above ground level. The birds' steady, flapping flight was interrupted by short gliding periods. Experimental flight duration was between 3.5 h and 4.8 h (Table 1). Although the flock remained visible for the duration of all flights, we were unable to determine flight speeds and therefore distances flown. We did not use data from birds that landed during a flight.

Table 2 Comparison of avenues of mass loss in tippler pigeons at two ambient temperatures during free-flight and at rest. Values are means ± SE (number of birds). Comparisons between in flight and rest values at each temperature are by resampling statistics as described in text. (*EWL* evaporative water loss, *EXWL* excretory water loss, m_{de} dry excreta mass, *TWL* = total water loss)

Flight air temperature	13.8 °C			19.6 °C		
Avenue of water loss	In flight	At rest	*P*	In flight	At rest	*P*
TWL $mg \cdot g^{-1} \cdot h^{-1}$	15.43 ± 0.88 (6)	5.23 ± 0.84 (5)	< 0.0001	19.30 ± 0.66 (6)	7.32 ± 1.24 (4)	< 0.0001
EWL $mg \cdot g^{-1} \cdot h^{-1}$	13.66 ± 0.72 (6)	4.06 ± 0.79 (5)	< 0.0001	17.68 ± 0.78 (6)	6.49 ± 1.18 (4)	< 0.0001
EXWL $mg \cdot g^{-1} \cdot h^{-1}$	1.77 ± 0.27 (6)	1.17 ± 0.06 (5)	0.03	1.63 ± 0.24 (6)	0.83 ± 0.23 (4)	0.022
m_{de} $mg \cdot g^{-1} \cdot h^{-1}$	0.49 ± 0.08 (6)	0.35 ± 0.01 (5)	0.07	0.42 ± 0.07 (6)	0.31 ± 0.08 (4)	0.15
water in excreta %	77.93 ± 1.40 (6)	76.64 ± 0.65 (5)	0.21	79.60 ± 0.51 (6)	72.40 ± 0.99 (4)	0.001

The effect of ρ_a and T_a on water loss

As indicated in the introduction, the actual driving force for evaporation from any of the bird's evaporative surfaces is the ρ_a gradient between a given surface and the environment (Gates 1980). As these surfaces may vary widely in temperature, it is not feasible to express the dependence of EWL on a single vapor density difference. Thus, to clarify the dependence of EWL on environmental conditions, we chose to express its dependence on T_a and ambient ρ_a. The dependence of EWL on T_a and ρ_a is shown in Figs. 1 and 2 respectively. Data from wind tunnel experiments by Biesel and Nachtigall (1987) are included for comparison.

Two separate lines, rather than one combining line, best describe the data. We reached this conclusion using the statstical method in Pinshow et al. (1976), i.e., the pooled mean squares for the two regressions of EWL vs. T_a that follow are smaller than that for the combined data or for any other pairs of regressions that might describe the data. EWL in free-flying birds was independent of T_a between 10.2 °C and 14.7 °C (by regression, $r^2 = 0.03$, $P = 0.73$, $n_f = 5$, $n_b = 41$), with means at each T_a ranging from 11.4 $mg \cdot g^{-1} \cdot h^{-1}$ to 17.7 $mg \cdot g^{-1} \cdot h^{-1}$ (Fig. 1). Above 19.7 °C, EWL increased linearly ($EWL = -49.46 + 3.45\ T_a$, $r^2 = 0.89$, $P < 0.001$, $n_f = 3$, $n_b = 18$). EWL increased linearly with increasing ambient ρ_a ($EWL = -7.4 + 2.7\ \rho_a$, $r^2 = 0.88$, $P < 0.001$, $n_f = 8$, $n_b = 59$) (Fig. 2). T_a and ρ_a were highly correlated, therefore, it was statistically imprudent to seek the independent affect of each on EWL.

EXWL averaged 1.71 ± 0.72 $mg \cdot g^{-1} \cdot h^{-1}$ ($n_f = 8$, $n_b = 51$) for all flights. However, although we found no dependence of EXWL on either ρ_a or T_a, the power of the regression tests that we used was low (approx. 0.3 for both). Thus the results were equivocal, i.e., we could not accept the null hypotheses (no temperature or ρ_a effects on EXWL) with certainty. The proportion of water in excreta averaged 79.2 ± 3.86% and was independent of environmental conditions with the same reservations as for EXWL. The proportion of EXWL in TWL was relatively small, averaging 9.3% over the whole temperature range.

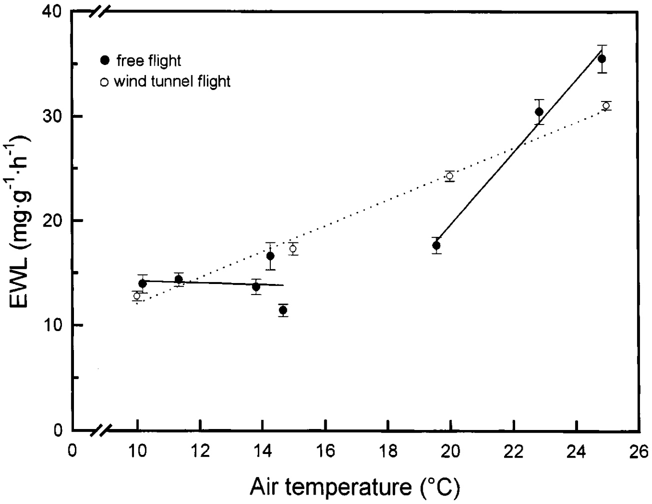

Fig. 1 Evaporative water loss (EWL) as a function of air temperature (T_a) for free-flying tippler pigeons (*filled circles*). Results are presented as means ± 1 SE. See Table 1 for numbers of birds sampled in each group and details of environmental conditions, and text for statistics. For comparison, *open circles* are data from wind tunnel flights by Biesel and Nachtigall (1987). The dependence of EWL on T_a is linear for wind tunnel experiments ($EWL = -0.32 + 1.24\ T_a$), *dashed line*

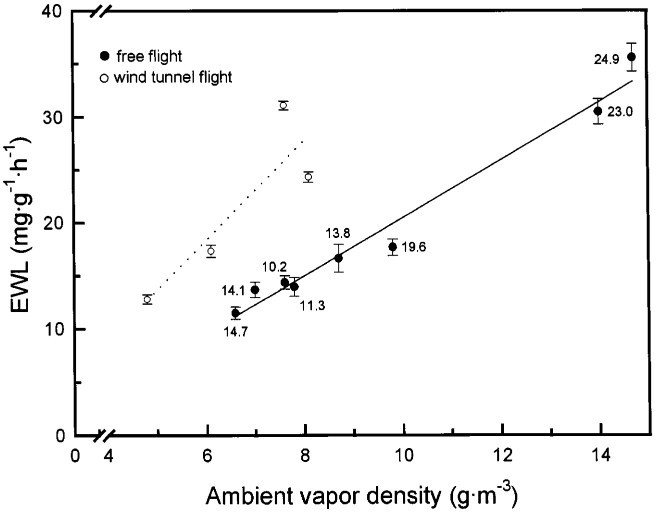

Fig. 2 Relation between EWL and ambient water vapor density (ρ_a) in free-flying pigeons (*solid circles*). The numbers adjacent to the means on the graph are the corresponding ambient temperatures (°C) at which each flight took place. Results are presented as means ± 1 SE. See Table 1 for numbers of birds sampled in each group and details of environmental conditions, and text for statistics. For comparison, *open circles* are data from wind tunnel flights by Biesel and Nachtigall (1987). The dependence of EWL on ρ_a is linear for wind tunnel experiments ($EWL = -6.91 + 5.74\ \rho_a$), *dashed line*

Comparison of flying birds to birds at rest

We compared flying to resting birds at two T_as, 13.8 °C and 19.6 °C (Table 2). TWL and EWL were significantly higher (≈300%) in flying than in resting birds at both temperatures. EXWL in flight was also higher than at rest, but dry excreta mass in flight did not differ significantly from that at rest. As a result, the proportion of water in excreta was higher in flight than at rest at $T_a = 19.6$ °C, but was not significantly different at 13.8 °C.

Discussion

The effect of flight and environmental conditions on EXWL

EXWL is affected by physiological requirements other than the need to conserve water. Metabolism in flight is much higher than that at rest, thus one might expect that in-flight production of waste metabolites, such as creatinine, urea and uric acid (Jenni-Eiermann and Jenni 1991; George and John 1993; Schwilch et al. 1996) to increase accordingly. Further, excretion of these materials is obligatorily accompanied by water and this water loss could conceivably constrain the bird's ability to reduce EXWL. Therefore, we predicted that in-flight EXWL would increase in flight compared to rest. However, since by decreasing EXWL to the minimum required for excretion a bird can save water without compromising thermoregulation, and since flight metabolic rate is independent of T_a (Rothe et al. 1987), we further predicted that in-flight EXWL would not change with T_a.

The data support these predictions; EXWL was significantly higher in flight than at rest (Table 2) and EXWL in our flying pigeons was independent of T_a or ρ_a. However, the modest increase in EXWL during flight, compared to the increase in EWL or the increase in metabolic rate, can be explained by the fact that in free-flying tippler pigeons glomerular filtration rate does not increase and renal water reabsorption is very high (Giladi et al. 1997).

Our method for measuring excreta loss precluded the birds from ridding themselves of the excreta weight. Carrying extra weight must result in increased total induced power input and water loss. Measured by the doubly labeled water method, mean metabolic power input of 12 birds from the same stock as those used in the present study and weighing 230–290 g, was 17.94 W (K. Hatch, J. Speakman and B. Pinshow personal observation). We used Pennycuick's (1989) model and Klaassen's (1995) modification of Carmi et al.'s (1992) model to estimate the increases in power input and rate of water loss that would be incurred by a 280 g pigeon carrying an extra 5 g (the maximum receptacle wet mass measured). The increase in both parameters did not exceed 2% of the measured means. This is within the range of the variance of our results and should not affect our general conclusions.

The effect of T_a and ρ_a on EWL

EWL was fairly constant at T_as between 10.2 °C and 14.7 °C and rose sharply above 19.6 °C (Fig. 1). T_a might influence EWL directly by its effect on skin temperature and therefore on skin surface ρ_a, thus changing the driving force for evaporation. In addition, ambient T_a may influence exhaled T_a and therefore respiratory evaporation. At high T_as birds may be heat stressed and respond by increasing evaporation as a means of cooling.

In resting birds, EWL is fairly constant up to a threshold above which it rises steeply; this threshold is usually above 30 °C (e.g., Lasiewski et al. 1966; Marder 1983; Roberts and Baudinette 1986; Withers and Williams 1990). In flying pigeons, EWL began to increase between 15 °C and 20 °C. As the metabolic cost of flight is not dependent on T_a (Rothe et al. 1987), we suggest that the increase in external heat load, associated with higher ambient temperature, leads to initiation of evaporative cooling at a lower T_a than in resting birds. The mechanism of increase is likely to involve both respiratory and cutaneous avenues of evaporation. Note, that under any of the flight conditions reported here, and based on the mean metabolic flight power input of 17.94 W measured by K. Hatch, J. Speakman and B. Pinshow (personal observation), EWL would not be sufficient to dissipate more than 20–25% of the metabolic heat production of a flying pigeon. Thus, pigeons apparently rely on convection and radiation for most of their heat dissipation.

Under the experimental flight conditions experienced by the pigeons, EWL increased with increasing ρ_a. This relation may appear counter-intuitive; a higher ρ_a might be expected to reduce the vapor density gradient and lessen EWL. Keeping in mind that ambient vapor density was strongly correlated with T_a (Pearson correlation, $r = 0.90$, $n = 8$) suggests a plausible explanation. At a T_a greater than 19 °C, the need for cooling increases with rising ambient temperature. This can no longer be completely accommodated for by convection and radiation, and the bird must evaporate water. Despite the higher ρ_a occurring at the higher T_a, a sufficient vapor density gradient still exists between the bird's integument and the environment to allow an increase in EWL.

To achieve this increased EWL, the pigeons must reduce their resistance to water vapor loss from their evaporating surfaces. Biesel and Nachtigall (1987) showed that flying pigeons can regulate respiratory surface convective evaporation by beak-opening, and cutaneous evaporation in resting birds can be regulated by neurochemical transmitters (Marder and Raber 1989; Ophir 1993). It is possible that both these mechanisms contribute to the observed increases in EWL, although we never observed the pigeons used in this study to fly with their beaks agape.

Comparison of free-flight EWL with data from wind tunnel experiments

Comparisons of our results with those from the wind tunnel flights of Biesel and Nachtigall (1987) are presented in Figs. 1 and 2. There were some marked differences between our conditions of measurement and those of Biesel and Nachtigall, but by and large the results of the two studies are consistent. Biesel and Nachtigall (1987) worked in a controlled temperature environment where T_a and the radiation temperature of the wind tunnel walls were probably equal, whereas our pigeons flew outdoors. Biesel and Nachtigall (1987) interrupted flights every 10 min to weigh birds; ours flew continuously. The wind tunnel flight speed range at which EWL was minimal was 10–14 $\mathrm{m \cdot s^{-1}}$ and the minimum power speed of the same pigeons was within this range at 10–12 $m \cdot s^{-1}$ (Rothe et al. 1987). We assume that since our free-flying birds were not constrained to fly at a specific speed, they flew within that range which minimized both energy consumption and water loss, but we have no measure of this speed.

In wind tunnel experiments, EWL increased linearly both with increasing T_a (Fig. 1) and with ρ_a (Fig. 2). In free flight, EWL was relatively constant below 15 °C, but increased markedly at higher T_a, despite the high ρ_a. In both cases it was apparently necessary for the birds to enhance evaporative cooling when the heat load increased at higher T_as.

Biesel and Nachtigall (1987) used gravimetry to measure EWL directly by weighing the bird along with its "excrement bag". Unfortunately, they did not analyze the bag contents so we could only compare EWL, but not EXWL or TWL.

It should be noted that the mean metabolic power (17.94 W) measured by K. Hatch, J. Speakman and B. Pinshow (personal observation) in birds from the same stock as those used in the present study, is substantially less than that measured by respirometry in wind tunnel experiments, averaging 32 W for 380 g pigeons (Nachtigall 1995), even when body size is accounted for. This difference might be the result of natural versus wind-tunnel flight conditions as suggested by Nachtigall (1995). For a flying bird to maintain heat balance under similar environmental conditions, the higher the metabolic rate, the lower the T_a at which evaporative cooling will be initiated. Therefore, in wind-tunnel flight the increase in EWL with T_a will occur at lower temperatures than in free flight (Fig. 1).

Comparison of TWL with other free flight studies

Pearson (1964) flew tippler pigeons outdoors and measured their mass loss during flight. To prevent any mass loss by defecation he sealed the birds' cloacas, therefore the mass loss he measured represents mainly evaporation. The average mass loss in his experiment was 12 $mg \cdot g^{-1} \cdot h^{-1}$, close to the mean EWL of 15 $mg \cdot g^{-1} \cdot h^{-1}$ at $T_a < 19$ °C in the present study. Pearson's birds flew at T_as of 13–23 °C and he measured only one bird (and usually a different one) for each flight. Furthermore, he did not present any relations between mass loss and T_a. Thus, it is difficult to make rigorous comparisons between Pearson's data and our data.

Gessaman et al. (1991) measured daily water turnover rates of two groups of tippler pigeons, one resting and one flying and resting, and used a time budget record to calculate water turnover during flight. For flying birds they calculated an average TWL of 21.5 ± 4.3 $mg \cdot g^{-1} \cdot h^{-1}$ which is similar to the mean of TWL 19.30 ± 0.66 $mg \cdot g^{-1} \cdot h^{-1}$ that we measured at $T_a = 19.6$ °C. However, because Gessaman et al. (1991) did not report T_a, we are unable to tell if the two methods yield truly similar results.

Adams et al. (1997) used tritiated water to estimate water fluxes, by measuring total body water both before and after a flight, in the same stock of pigeons and using the same flight regime that we used in our experiments. Adams et al. flew birds at T_as of 18–27 °C and ρ_a of 4.2–17.5 $g \cdot m^{-3}$ and measured means of TWL of between 19.7 $mg \cdot g^{-1} \cdot h^{-1}$ and 26.4 $mg \cdot g^{-1} \cdot h^{-1}$. At 18 °C, the mean of 19.7 $mg \cdot g^{-1} \cdot h^{-1}$ is virtually identical to the mean of 19.3 $mg \cdot g^{-1} \cdot h^{-1}$ measured at 19.6 °C in the present study. In contrast, at $T_a > 20$ °C, the mean TWL measured by Adams and his co-workers was as much as 40% lower than our measurement under comparable ambient conditions. Moreover, Adams et al. (1997) did not find a significant effect of T_a on TWL, as opposed to our findings. We have not yet determined the sources of these differences. However, there is wide variance in the values reported by Adams et al. (1997) and the labeled water technique has larger inherent variability than the use of gravimetry. It is possible that to use labeled water for accurate measurement of water fluxes during continuous flight, when there are no diluting influxes other than inhaled and metabolic water, flight times need to be longer.

Effect of water loss on flight duration

Biesel and Nachtigall (1987) calculated the maximum flight duration and range for pigeons at a T_a of 25 °C to be 90 km and 2 h, respectively, based on an estimated dehydration tolerance of 5% of initial body mass. Adams et al. (1997) noted that their study pigeons lost 8% of their initial body mass in 4 h flights, results similar to those of Carmi et al. (1993). In this study, pigeons lost water at about 4 $g \cdot h^{-1}$. If one conservatively assumes a pigeon to have the ability to dehydrate until it has lost 10% of its initial body mass, which Adams et al. (1993) suggest is the maximum loss that can be tolerated by a flying pigeon, one may calculate the maximum flight time of the pigeons in this study to be about 7 h at $T_a < 19$ °C. Assuming the maximum loss tolerated by pigeons to be 17% of initial body mass, as reported by Carmi et al. (1993) for resting pigeons, a pigeon could fly for some 12 h. In the present study, pigeons lost almost 10 g of water per h at a T_a of 25 °C. If the limits to water loss tolerance are between 10% and 17% of initial body mass, flight duration would be reduced to 3 h and 5 h, respectively. This estimate concurs with our observations of our pigeon's "unwillingness" to fly for more than 4 h at the higher T_as.

In conclusion, flying birds lost more water than their resting counterparts as might be expected, the increase was mostly evaporative. EWL in free-flying tippler pigeons was independent of T_a below 15 °C, and increased with increasing T_a above 20 °C in a way that indicated it was necessary for the birds to use evaporative cooling when the external heat load increased. That EWL should increase with increasing ambient vapor density is counter-intuitive. However, this is likely explained by the concomittant rise in T_a, which may be an overriding stimulation when evaporative cooling is necessary. Thus, these results imply that EWL is subject to physiological modulation. EXWL was independent of ambient ρ_a or temperature. EXWL averaged almost 10% of TWL, thus it should not be neglected (at least for pigeons) in estimates of in-flight water loss as was done by Carmi et al. (1993). Lastly, we found that measurements of EWL made on pigeons during wind tunnel experiments (Biesel and Nachtigall 1987) are consistent with our free flight measurements made at similar T_as.

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