

Respiratory water loss in free-flying pigeons

Gilead Michaeli and Berry Pinshow*

Jacob Blaustein Institute for Desert Research and Department of Life Sciences, Ben-Gurion University of the Negev, Midreshet Ben-Gurion, 84990 Israel

*Author for correspondence (e-mail: pinshow@bgumail.bgu.ac.il)

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Summary

We assessed respiratory and cutaneous water loss in trained tippler pigeons (*Columba livia*) both at rest and in free flight. In resting pigeons, exhaled air temperature T_{ex} increased with ambient air temperature T_{a} ($T_{\text{ex}}=16.3+0.705T_{\text{a}}$) between 15 °C and 30 °C, while tidal volume V_{T} ($V_{\text{T}}=4.7\pm 1.0$ ml, mean \pm S.D. at standard temperature and pressure dry) and breathing frequency f_{R} ($f_{\text{R}}=0.46\pm 0.06$ breaths s^{-1}) were independent of T_{a} . Respiratory water loss, RWL, was constant over the range of T_{a} ($\text{RWL}=1.2\pm 0.4$ mg g^{-1} h^{-1}) used. In flying pigeons, T_{ex} increased with T_{a} ($T_{\text{ex}}=25.8+0.34T_{\text{a}}$), while f_{R} was independent of T_{a} ($f_{\text{R}}=5.6\pm 1.4$ breaths s^{-1}) between 8.8 °C and 27 °C. Breathing frequency varied intermittently between 2 and 8 breaths s^{-1} during flight and was not always synchronized with wing-beat frequency. RWL was independent of air temperature ($\text{RWL}=9.2\pm 2.9$ mg g^{-1} h^{-1}), but decreased with increasing inspired air water vapor density (ρ_{in}) ($\text{RWL}=12.5-0.362\rho_{\text{in}}$), whereas cutaneous

water loss, CWL, increased with air temperature ($\text{CWL}=10.122+0.898T_{\text{a}}$), but was independent of ρ_{in} . RWL was 25.7–32.2 %, while CWL was 67.8–74.3 % of the total evaporative water loss. The data indicate that pigeons have more efficient countercurrent heat exchange in their anterior respiratory passages when at rest than in flight, allowing them to recover more water at rest at lower air temperatures. When evaporative water loss increases in flight, especially at high T_{a} , the major component is cutaneous rather than respiratory, possibly brought about by reducing the skin water vapor diffusion resistance. Because of the tight restrictions imposed by gas exchange in flight, the amount of water potentially lost through respiration is limited.

Key words: Pigeon, *Columbia livia*, respiratory water loss, evaporative water loss, cutaneous water loss, respiratory air temperature, exhaled air temperature, breathing frequency.

Introduction

Long distance flapping flight in birds is an energetically demanding activity during which metabolic rates average nine to tenfold those at resting levels (Berger et al., 1970; Tatner and Bryant, 1986; Biesel and Nachtigall, 1987). Consequently, flying birds have extremely high internal heat loads that they must dissipate. Resting birds can enhance convective cooling by exposing more skin surface area to the air through adjustment of posture and plumage [see Dawson, for review (Dawson, 1982)]. Flying birds cannot alter their posture or feather attitude without increasing aerodynamic drag and the only other way they can enhance convective cooling without affecting flight is by increasing the surface–air temperature gradient. However, at higher ambient air temperatures, convection is a less effective cooling mechanism.

In flying birds, as ambient air temperature (T_{a}) increases, the fraction of heat lost by evaporation (EHL) increases (Tucker, 1968; Torre-Bueno, 1978; Hudson and Bernstein, 1981; Biesel and Nachtigall, 1987; Giladi and Pinshow, 1999). In free-flying pigeons, EHL was 14.5 % at 14.5 °C and 44 % at 25 °C of the total heat dissipated (Giladi and Pinshow, 1999). Thus, flying at high T_{a} may be costly in terms of water loss.

Evaporation comprises respiratory and cutaneous components. Until the early 1980s, no mechanisms for the control of cutaneous water loss (CWL) were known (Marder and Ben-Asher, 1983) and the respiratory avenue was considered the main path of evaporation in birds (Dawson, 1982). This idea was challenged and several studies showed that in pigeons resting at high ambient temperatures, cutaneous evaporation is the predominant avenue of water loss (Marder and Ben-Asher, 1983; Webster and King, 1986; Withers and Williams, 1990). To date there is but one study of flying birds in which both respiratory and cutaneous evaporation were investigated, owing to technical difficulties. Hudson and Bernstein (Hudson and Bernstein, 1981) found that, for white-necked ravens (*Corvus cryptoleucus*, mean mass 0.48 kg) flying in a wind tunnel, total in-flight evaporative water loss (EWL) at T_{a} in the range 18–27.8 °C was 8.2–13.9 g h^{-1} , and the cutaneous component decreased from 42 % to 24 % of the total. In flying birds, the ventilation rate (V_{T}) may be 20-fold higher than that of resting birds [for review, see Bernstein (Bernstein, 1987)]. This increase in ventilation implies an obligatory increase in respiratory water loss (RWL).

Regulation of the rate of RWL in birds is achieved through adjustment of the amount of air expired, i.e. the minute volume, and its water content. Minute volume may be varied through modulating the V_T and through the breathing frequency (f_R). At rest, control of minute volume becomes apparent when birds are heat stressed: they pant (Calder and Schmidt-Nielsen, 1968; Bucher, 1985; Withers and Williams, 1990). However, in flight, ventilation is constrained by the continuous, high demands for gas exchange. The expired air is usually saturated with water vapor in birds and mammals (Welch and Tracy, 1977; Welch, 1984), so that its temperature, T_{ex} , determines its vapor content. The greater the difference between the expired vapor density (ρ_{ex}) and the inspired ambient air vapor density (ρ_{in}), the higher the RWL. Temporal countercurrent heat exchange in the upper respiratory tract together with complex nasal turbinates allows for a considerable reduction of the T_{ex} below body temperature (T_b) (Schmidt Nielsen et al., 1970; Welch, 1984).

Increasing blood flow to the nasal mucosa can raise T_{ex} (Murrish, 1973), thereby buffering the effect of T_a on T_{ex} . This should reduce the effectiveness of countercurrent heat exchange, and enhance RWL. There is evidence for such modulation in resting penguins (Murrish, 1973). However, T_{ex} in pigeons has not been measured, so its role in regulating RWL is unknown, and there is no information about regulation of T_{ex} through active processes in flying birds.

In light of the above, we investigated the relative contributions of the respiratory and cutaneous components of EWL in free-flying pigeons. Specifically, we tested the hypothesis that pigeons are limited in their ability to increase EWL through respiration, but increase cutaneous evaporation in times of need. To achieve this, we measured T_{ex} and breathing frequency of tippler pigeons at rest and in flight and calculated respiratory, cutaneous and total EWL.

Materials and methods

Experimental animals

Tippler pigeons were used in both 'rest' and 'flight' experiments. Tippler pigeons are a breed of rock pigeon (*Columba livia*) selected for their propensity to fly for hours at a time in large circles above their loft. Birds were kept at the Jacob Blaustein Institute for Desert Research in the central Negev Highlands (34°47'N, 30°52'E, elevation 470 m above sea level) where they lived in a shaded outdoor loft exposed to prevalent ambient conditions. The pigeons were hatched and raised in the loft and trained 3–5 times a week to fly for 3–4 h at a stretch according to a protocol established in our laboratory (Carmi et al., 1993; Adams et al., 1997; Adams et al., 1999; Giladi and Pinshow, 1997; Giladi et al., 1998). During training flights, 6–10 pigeons flew in tight formation in large circles within visual range of the loft. The pigeons were trained to land and enter the loft at the sound of a whistle or a bell. Food was offered inside the loft for 20 min immediately after the sound of the landing cue. The birds usually landed and entered the cage within 5 min of the cue. On non-flight days, birds were

fed once a day for 20 min after the cue was sounded to habituate them to it. The pigeons were fed a mix of grains (wheat, barley, corn, sunflower and sorghum) containing 2.5 % protein, 5 % fat, 2.5 % ash and 60 % carbohydrates. Water was provided *ad libitum*.

Measurements of narial air temperature and breathing frequency

We measured narial air temperature with a micro-bead thermistor (Thermometrics model AB6B4-BR11). The thermistor's diameter was less than 0.4 mm and did not apparently interfere with the bird's normal breathing. The thermistor bead was hermetically sealed in glass and its platinum leads covered with a polyamide sleeve glued with epoxy over the weld joints, up to the bead, rendering it impervious to water that may accumulate in the nares of a bird. To estimate if the thermistor's response time was fast enough to measure the breathing frequency of a pigeon, we simulated a breathing pigeon in the laboratory with a variable-speed diaphragm pump and ascertained that the thermistor's response time to a step-change in temperature was less than 0.016 s. This response time is more than adequate to measure a breathing frequency of 8 breaths s^{-1} .

Pigeons at rest

Measurements of narial temperature and of tidal volume

A thermistor was inserted 3 mm into one of the pigeon's nares. The thermistor's leads were held in place on top of the bird's beak with narrow strips of medical adhesive tape. Resistance of the thermistor was measured with a digital voltmeter whose output was fed to an A/D board (Strawberry Tree, Dynares acquisition board). Before experiments, the thermistor's resistance was calibrated to 0.01 k Ω (0.1 °C) in a controlled temperature bath against a mercury-in-glass thermometer with calibration accuracy traceable to the US National Institute of Standards and Technology. Tidal volume was measured with a Statham pressure transducer connected to a port in a whole body plethysmograph (diameter 14 cm, length 29 cm). The plethysmograph used has been completely described (Bouverot et al., 1976).

When in the plethysmograph, the pigeon's head protruded through a round hole at the top of the chamber. A collar, made of a latex sheet (dental dam) with a hole in its center, was placed around the bird's neck and was fitted tightly into the chamber opening. The pigeon's neck was smeared with saline gel, and a rigid rubber collar made of two adjoining parts was placed around the bird's neck over the gel. This prevented leakage of air and minimized expansion of the neckpiece when the pressure in the body chamber increased. All openings in the plethysmograph were sealed for the duration of the sampling period. Measured changes in pressure were due to changes in body volume resulting from inhalation and expiration of air by the bird. Thus, a change in pressure corresponded to a change in V_T . We verified that, during the sampling periods, there was no drift of pressure over time. Since the measurements lasted less than 1 min, the effect of

temperature or an accumulation of water vapor in the chamber on the pressure was negligible. The pressure transducer was calibrated using a glass syringe to inject air into, and withdraw air from, the chamber at 15, 20, 25 and 30 °C. The volume of air was 2–10 ml injected in increments of 1 ml at different frequencies. During calibration, the bird was replaced by a 300 ml, sand-filled plastic bag.

Experimental protocol for resting birds

Before participating in an experiment, each pigeon was deprived of food for 20 h, but water was available *ad libitum*. The pigeon was placed in a climate room at the experimental ambient conditions for 1 h and then it was weighed to the nearest 0.1 g. Then the pigeon's beak was lightly closed with a small rubber O-ring, to restrict it to breathing through its nares, and it was placed, standing, in the plethysmograph while a plastic platform under the breast kept it upright. The O-ring caused no apparent discomfort, as was found for the flight experiments reported below, and respiration patterns with and without its use were not discernibly different. A thermistor was inserted into the nares of the pigeon, and the plethysmograph head-chamber cover, which had openings for incurrent and excurrent air, was closed. The body compartment had a detachable airtight door on one end. Except during data-sampling intervals, the door and another port in the side of the plethysmograph remained open, providing the bird with sufficient air for convective cooling. The pressure transducer was attached to the remaining port of the chamber.

Room air was drawn into the head chamber through a desiccating column of Drierite®. The temperature of the incoming air was monitored with a thermocouple in the inlet air-stream. Air flowed through the head compartment at a rate of 500 ml min⁻¹ at 15 °C and 25 °C and at 600 ml min⁻¹ at 25 °C and 30 °C, respectively. The airflow was monitored with a Rotameter calibrated with a Brooks Vol-U-meter flow calibrator. Each experimental trial lasted 60 min. Nasal air temperature, temperature of the incoming air and V_T were sampled every 5 min for 30–60 s at 10 breaths s⁻¹ using the A/D board and data acquisition software. After each trial, the pigeon was reweighed to an accuracy of ±0.1 g.

Six tippler pigeons, body mass 269.6±13.0 g (mean ± s.d.), were used in the experiments. Each experiment was repeated with the same individuals at air temperatures of 15, 20, 25 and 30 °C. These temperatures covered the range of air temperatures of the flight-RWL experiments. Measurements were also attempted at 35 °C, but the pigeons showed signs of discomfort and free water was visible in the beaks of some. It may be that at 35 °C pigeons began to pant and the restriction imposed by closing their beaks interfered with their breathing. Therefore, experiments were not carried out at air temperatures above 30 °C.

Pigeons in flight

Training birds for experiments

For several weeks before an experiment, several pigeons, randomly chosen from the trained flyers, were trained to carry

a load during flight. Initially each pigeon was flown several times with a body harness. The harness was cut from a nylon stocking and weighed 0.4 g. When mounted on the bird, the harness stretched around the base of the wings and did not compress the feathers. The harness fitted the birds well and we observed no visible changes in flight performance due to it. Next, each pigeon was trained to fly with a dummy transmitter (same mass and size as the actual transmitter) attached with Velcro® to the harness. Those pigeons that responded adversely to flying with the load were not used. The last step before experiments was to train the pigeon to fly with the load and with a rubber O-ring around its beak. Seven pigeons of mean mass 279.2±20.3 g (± s.d.) were used in experiments.

During experiments, the thermistor was attached to a custom-made FM transmitter (B.T.A., Israel). Three transmitters were used that had carrier frequencies of 150–155 MHz and the information (thermistor resistance) was frequency-modulated in the audio range 1–4 kHz. The transmitter had a resolution of 15 Hz that corresponded to an accuracy of ±0.1 °C. It was powered by a 3 VDC lithium battery (Toshiba 2025), and the current dissipation was 15–20 mA. The effect of self-heating on the thermistor was negligible. This was verified by exposing the thermistor (attached to a powered transmitter) to still air and then to a stream of air, both at constant room temperature, while monitoring the thermistor's voltage. If there had been a self-heating effect, a voltage change would have occurred in response to the alteration in forced convection caused by the air stream. However, no such change was detectable. The thermistor was calibrated to an accuracy of ±0.1 °C in a controlled temperature bath as described above.

Experimental protocol for flying birds

A thermistor was inserted 3 mm into one of the pigeon's nares. Its 15 cm leads were secured on top of the bird's beak by narrow strips of medical adhesive tape. The leads passed over the top of the head and were held in place by the hooks of a small piece of adhesive Velcro® that was stuck to the feathers, then connected to the transmitter that was enclosed in a plastic sleeve and attached to the harness on the bird's back. The total load carried was 10 g and was approximately 3 % of the bird's mass. Once the transmitter and thermistor were in place, an O-ring was placed around the pigeon's beak.

Food was withheld from the pigeon for at least 16 h prior to flight. This ensured that the birds were post-absorptive and that most of the substrate catabolized during flight was fat [experimentally verified by Gannes et al. (Gannes et al., 2001) and Hatch et al. (Hatch et al., 2000)]. The pigeon was weighed to an accuracy of ±0.1 g and released to fly with the flock. The transmitted signal was received with an FM radio receiver (Radio Shack Pro 49) attached to a Yagi antenna on the laboratory roof. The audio signal was recorded on a cassette tape for the duration of the entire flight. To ascertain whether wing-beat frequency and respiratory frequency were synchronized, we recorded the birds in flight with a video camera while simultaneously recording the temperature

Table 1. Summary of ambient conditions during flights for tipler pigeons

Flight date	Bird number	Mean T_a (°C)	Range T_a (°C)	ρ (g m ⁻³)	Flight time (min)
8.11.96	29	16.6	14.5–19.5	6.36	86
10.11.96	29	22.1	21.0–23.0	5.47	88
12.11.96	29	21	18.0–23.2	3.67	152
15.11.96	29	20.9	19.8–21.5	3.65	125
19.11.96	29	17.3	15.5–19.5	11.79	174
24.11.96	29	22.1	21.5–23.3	8.79	130
18.1.96	29	15.8	14.9–17.5	7.41	157
22.11.96	27	19.8	17.0–21.8	9.41	120
29.11.96	27	16.8	14.5–17.8	10.02	143
1.12.96	27	19.6	18.0–21.7	6.42	151
3.12.96	27	21.4	20.7–22.0	8.07	116
6.12.96	27	16.7	16.0–17.5	9.24	151
15.12.96	27	14.7	13.0–15.5	7.56	162
17.12.96	27	15.3	13.2–17.3	8.50	150
27.12.96	19	16.7	15.5–18.0	5.69	137
29.12.96	19	15.8	14.5–17.2	9.43	170
31.12.96	19	14.7	12.0–16.5	6.93	154
20.01.97	19	18.2	17.8–18.8	7.78	112
12.01.97	19	19	17.9–19.6	6.85	134
6.02.97	16	8.8	8.2–9.2	3.92	134
9.02.97	16	12.9	12.9–12.9	4.51	140
11.02.97	16	13.2	12.5–13.8	6.89	123
13.02.97	16	14.2	13.0–15.5	6.11	145
11.09.97	3	26.6	24.0–29.0	10.59	134
22.08.97	250	26	23.5–29.0	15.85	151
26.08.97	250	22.75	21.0–25.2	15.26	157
30.08.97	250	25.1	22.5–26.8	16.23	134
16.09.97	3	20.8	19.0–22.5	13.59	135
19.09.97	3	27	26.0–28.0	14.95	135
17.09.97	252	19	16.7–21.3	13.05	132
20.09.97	252	27.6	25.5–28.0	14.39	142

In each experiment, ambient air temperature T_a and ambient wet bulb temperature T_w were measured every 30 min for the duration of the flight. In all trials, T_a increased with time, or remained constant. Average T_a and T_w were calculated for each flight and the mean vapor density ρ was determined from meteorological tables (List, 1984). Mean T_a and ρ were used in all analyses.

transmission on the audio track of the videotape. This allowed us to view wing beats and the respiratory frequency curve together on the video screen.

Dry- and wet-bulb air temperatures were measured with a sling psychrometer (Bacharach, 12-7013) on a 9 m tower under the pigeons' flight path every 30 min while the pigeons were flying. Immediately upon landing, the pigeon was caught, the thermistor placement checked, and the bird reweighed. Experiments were done with the same pigeon, at intervals of 2 or more days, at different air temperatures. In a few experiments, for purposes of verification described below, pigeons flew without an O-ring beak restraint. Data were used only when the pigeon remained flying with the flock for the entire flight duration. Flight experiments, lasting between 1.5 and 3 h, were

done in the winter of 1996/7 and in the fall of 1997. Mean flight air temperatures ranged from 8.8 °C to 27 °C (Table 1).

Data reduction

Audio signals of the received FM transmissions were recorded on cassette tapes, digitised and saved in *.wav files on a PC using a 16-bit sound card (Creative Sound Blaster®) and sound-editing software. The signal noise was filtered and signal frequency (narial temperature) and the rate of change in signal frequency (breathing frequency) were sampled for 3 s every 3 min for the entire flight. The resistance of thermistor modulated the 1–4 kHz carrier frequency, which thus represented the narial temperature. The peaks of the waveform of the temperature signal gave breathing frequency. For every sample, T_{ex} (the rising part of the narial air temperature waveform) was determined by calculating the mean of the narial air temperature over the 3 s interval, considering only whole breath periods. We calculated mean exhaled vapor density ρ_{ex} from mean T_{ex} , assuming that the exhaled air was saturated (Schmidt-Nielsen et al., 1970; Welch, 1984), and calculated inspired vapor density ρ_{in} from the mean wet and dry bulb air temperatures measured during the flight (List, 1984). For each sample, we counted the breathing frequency over the same 3 s interval mentioned above and averaged all the samples from the flight to obtain the overall mean breathing frequency for the trial. Wing-beat frequency and breathing frequency were analyzed by replaying the audio and video signals in slow motion on a video player.

Calculation of the components of EWL

We used mass balance to measure total EWL, following the methods of Giladi and Pinshow (Giladi and Pinshow, 1999). This 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}) and knowledge of the mass of excreta. When only fat is consumed, the respiratory exchange ratio ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) is approximately 0.73 and the mass of O_2 consumed equals the mass of CO_2 released. The respiratory quotient (RQ) in flying pigeons was close to 0.73 both in wind tunnel experiments (Biesel and Nachtigall, 1987) and in free flight (Bordel and Haase, 1993; Schwilch et al., 1996). Based on the results of Biesel and Nachtigall (Biesel and Nachtigall, 1987), we assumed a steady, linear shift from carbohydrate to fat metabolism in the first hour of flight from RQ=0.9 to RQ=0.73. Biesel and Nachtigall's actual data show a sharper decline in RQ. Therefore, we conservatively calculated mass loss due to gas exchange to be less than 1 g. Ignoring this mass loss will introduce a maximum error of 4% into the calculation of EWL and TWL, where a particular bird's water and excreta mass loss is minimal. The long duration of the flights, and the 16-hour forced abstention from food beforehand, served to minimize this error. We assumed in our flight experiments that the birds' mass loss through gas exchange was negligible and all mass loss was either evaporative or excretory, while other minor mass changes such as loss of feathers or sloughed skin were ignored. Lasiewski et al. (Lasiewski et al., 1966) verified the use of this mass balance technique for resting

pigeons by simultaneously measuring EWL in resting, post-absorptive pigeons by gravimetry and by open-flow gas exchange. In our calculations, we used excreta mass values previously measured (Giladi and Pinshow, 1999) in pigeons of the same stock as those flown in the present study and flown under the same fasting regime and similar T_a conditions.

Collins et al. (Collins et al., 1971) developed a model of heat transfer in the upper respiratory tract of a small mammal. The model is based on the kangaroo rat *Dipodomys merriami* and assumes steady state flow of air, water and heat. Using only ambient conditions, they calculated the amount of heat dissipated by respiration. Welch and Tracy (Welch and Tracy, 1977) modified this model, and calculated RWL as:

$$\text{RWL} = V_T f_R \left(\frac{1}{\alpha} \rho_{\text{ex}} - \rho_{\text{in}} \right), \quad (1)$$

where V_T is the tidal volume in l; f_R is the breathing frequency in breaths s^{-1} ; ρ_{ex} is the expired vapor density in $g l^{-1}$. ρ_{ex} is calculated from the exhaled air temperature, T_{ex} , assuming that the expired air is saturated with water vapor (Schmidt-Nielsen et al., 1970; Murrish, 1973; Welch, 1984). ρ_{in} is the inspired vapor density in $g l^{-1}$ and is calculated from the ambient wet and dry bulb temperatures. α is a volume correction coefficient for expired air due to differences in volumes of oxygen intake and carbon dioxide expired. α is usually negligible in the calculation of RWL (Welch and Tracy, 1977). For technical reasons, we could not measure V_T in the flying pigeons. Therefore, as there are no other data on V_T of flying pigeons available, we used V_T as measured by Hart and Roy (Hart and Roy, 1966) for some calculations that follow. Hart and Roy (Hart and Roy, 1966) found that V_T was 5 cm^3 in pigeons (mean mass=360 g) flying for up to 14 s at an air temperature of approximately 25°C . As our birds were of significantly lower mass than those of Hart and Roy, using their value for V_T might result in an overestimate of respiratory EWL, providing a conservative estimate of cutaneous EWL. Further provisos concerning the use of Hart and Roy's values are discussed below.

Excretory water loss was assumed to be 0.091 of TWL, based on data from pigeons of the same stock as those used in the present study (Giladi and Pinshow, 1999) and the components of water loss were calculated as follows:

$$\text{EWL} = 0.091 \Delta m_b, \quad (2)$$

$$\text{RWL} = f_R V_T (\rho_{\text{ex}} - \rho_{\text{in}}), \quad (3)$$

$$\text{CWL} = \text{EWL} - \text{RWL}, \quad (4)$$

where Δm_b is the difference in body mass before and after flight (g), EWL is $\text{mg H}_2\text{O g}^{-1} \text{ body mass h}^{-1}$, RWL is $\text{mg H}_2\text{O g}^{-1} \text{ body mass h}^{-1}$, CWL is $\text{mg H}_2\text{O g}^{-1} \text{ body mass h}^{-1}$, f_R is breathing frequency (s^{-1}), V_T is tidal volume (cm^3), ρ_{ex} is expired water vapor density (g cm^{-3}) and ρ_{in} is inspired water vapor density (g ml^{-1}).

Data analysis

Results are presented as mass specific means ± 1 s.d. and

the number of flights (N) for which data were obtained. 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 rest *versus* flight comparisons, some of the data sets did not fulfill the assumptions demanded by parametric tests (i.e. homogeneity of variance and normality of distribution) and sometimes the power of the test was lower than acceptable. This was due to small sample size and high variance. Therefore, we used a non-parametric randomization technique (Manly, 1997) to make comparisons, using a program written by Simon (Simon, 1995). For uniformity, we used this method in all cases unless otherwise noted, and it proved more robust, with an inherently higher power, than standard non-parametric tests.

Pooling the data of different birds

The data were obtained from seven pigeons and each data point represents one experiment with one bird. Since the experiments were done outdoors where conditions were not constant, pooling the data from the different birds while treating each bird as a different individual would constitute pseudoreplication, although data may be pooled if there is no significant difference between the variance within each treatment and the variance between the treatments (Leger and Didrichsons, 1994).

Testing unrestrained beak versus restrained beak flights

Although St-Laurent and Larochelle (St-Laurent and Larochelle, 1994) advocate the possibility that ram ventilation may enhance evaporation from the buccal surfaces in flying pigeons, in the hundreds of hours that we observed and videotaped pigeons in flight, we never saw a bird fly with its beak agape. Therefore, we assumed that flying pigeons breathed mostly through their nares and that water loss enhanced by ram ventilation from the buccal surfaces was negligible. To test this assumption we repeated experiments on the same birds with unrestrained beaks and with beaks held closed by an O-ring and measured exhaled air temperature, breathing frequency and EWL. We regressed each of these against air temperature for both treatments (unrestrained and restrained beak) and compared the resulting regression equations.

Results

Pigeons at rest

Exhaled air temperature, tidal volume and breathing frequency

Exhaled air temperature increased linearly with T_a ($T_{\text{ex}}=16.3+0.705T_a$; $F_{1,13}=15.3$, $P=0.005$, $r^2=0.541$, $N=21$.) Data for individual birds are shown in Fig. 1A. Mean tidal volume for all experiments was $4.7 \pm 0.9 \text{ ml}$ at standard temperature and pressure dry (STPD). V_T was not dependent on air temperature (randomisation, $N=21$, $P=0.764$) (Fig. 1B).

Mean breathing frequency of all pigeons at all air

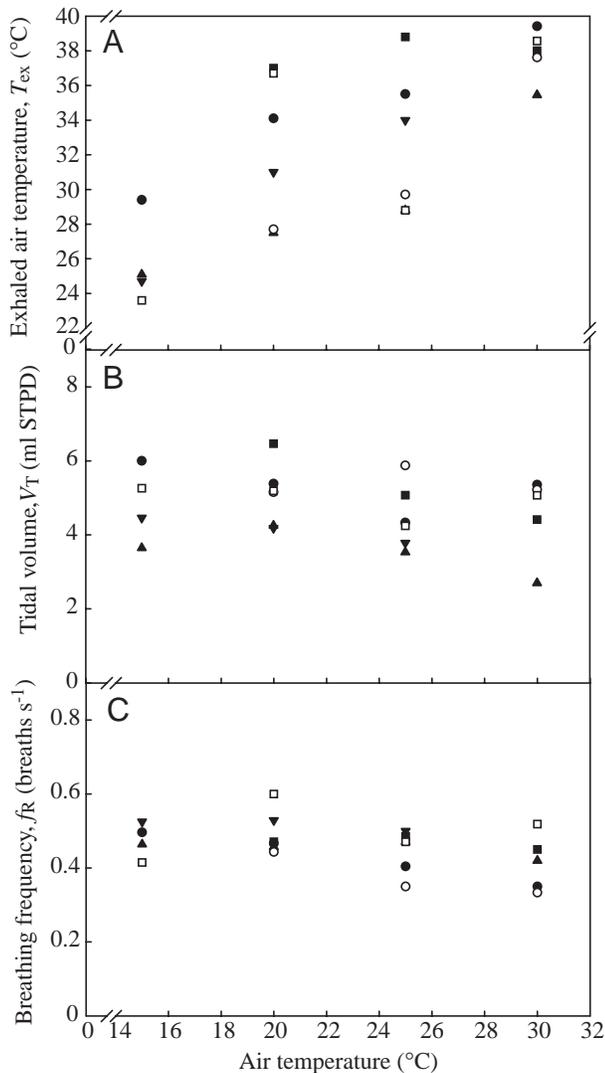


Fig. 1. Exhaled air temperature (A), tidal volume (B) and breathing frequency (C) of resting pigeons in relation to ambient air temperature. Each symbol represents a different individual, and measurements were made under conditions of standard temperature and pressure dry (STPD).

temperatures was 0.46 ± 0.06 breaths s^{-1} (Fig. 1C). Breathing frequency, f_R , decreased slightly with air temperature (randomisation, $N=21$, $P=0.001$). However, while the slope of the regression is statistically significant, it is negligibly small.

Pigeons in flight

Pooling the data from different pigeons

The variance of T_{ex} , EWL and breathing frequency for each pigeon was greater than the variance among the pigeons (F -test; for exhaled air temperature, $F=0.99$; for EWL, $F=0.88$; for breathing frequency, $F=0.466$; $P>0.5$ in all cases). Therefore, we pooled the data from the seven pigeons for subsequent analyses. Nonetheless, in the figures individual pigeons are distinguished by different symbols.

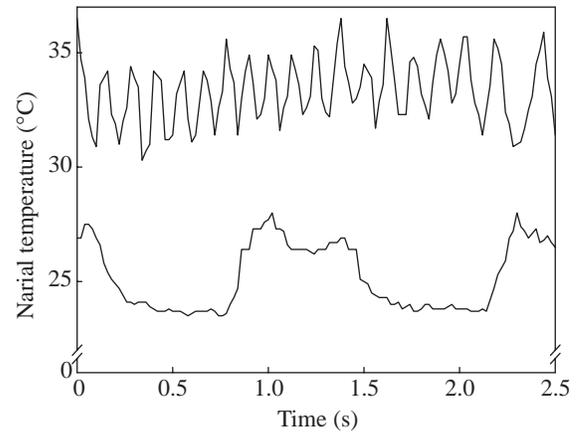


Fig. 2. Sample narial temperature from one pigeon at rest and in flight. Top waveform corresponds to mid-flight and the lower one to rest before flight. Air temperature was 12.5°C . The positive slope of the waveform corresponds to exhalation while the negative slope corresponds to inspiration.

Unrestrained versus restrained beak flights

We tested whether or not a difference existed between flights of pigeons with their beaks closed (n_{closed}) and flights in which beaks were unrestrained (n_{open}) and we compared the slopes of the regressions of exhaled air temperature, EWL and breathing frequency against air temperature under both conditions. There were no significant differences in any of the three variables (randomisation, T_{ex} : $n_{open}=10$, $n_{closed}=21$, $P=0.531$; EWL: $n_{open}=10$, $n_{closed}=20$, $P=0.257$; breathing frequency: $n_{open}=6$, $n_{closed}=18$, $P=0.914$). We concluded that by restraining their beaks, we did not affect the pigeons' respiration and EWL. Consequently, we combined the data in the following analyses.

Sample waveform of narial air temperature

Fig. 2 shows two 2.5 s data samples taken from one experimental flight, T_{ex} of the pigeon just before flight and T_{ex} in mid-flight. The sections of the waveform with positive slopes correspond to exhalation, while the sections with negative slopes correspond to inspiration. In this example, T_{ex} at rest was $23\text{--}28^\circ\text{C}$ and during flight it was $31\text{--}36^\circ\text{C}$. Mean breathing frequency at rest was 0.8 breaths s^{-1} while during flight it was 5.6 breaths s^{-1} .

Exhaled air temperature: rest and flight

T_{ex} in pigeons at rest before flight was significantly lower than T_{ex} for the same pigeons at the same T_a during flight. $T_{ex}(\text{rest})=27.5 \pm 4.5^\circ\text{C}$ and $T_{ex}(\text{flight})=32.3 \pm 2.8^\circ\text{C}$ (paired t -test, $t=-6.985$, $P<0.0001$, $N=29$).

Breathing frequency at rest and during flight

Mean breathing frequency in pigeons at rest before flight was 1.0 ± 0.3 breaths s^{-1} whereas mean breathing frequency of flying pigeons was 5.6 ± 1.4 breaths s^{-1} . These were significantly different (paired t -test, $t=-21.360$, $P<0.0001$, $N=22$).

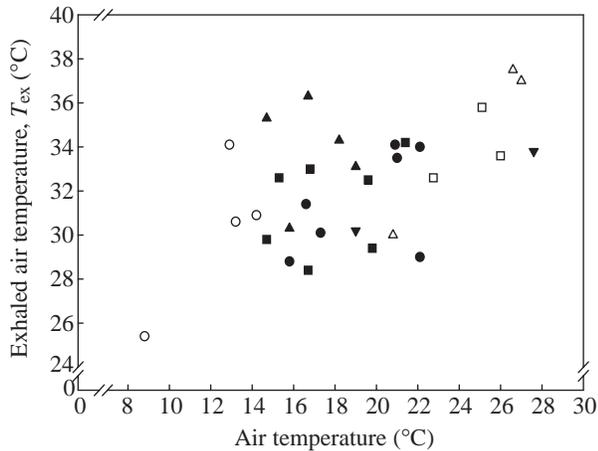


Fig. 3. Exhaled air temperature of flying pigeons in relation to ambient air temperature. Each symbol represents a different individual and is the mean of samples taken from the entire flight. The samples were taken every 3 min for the duration of the flight (2–3 h).

Effect of ambient air temperature on exhaled air temperature and on breathing frequency

Exhaled air temperature increased with air temperature ($T_{\text{ex}}=26.0+0.327T_a$; $F_{1,29}=13.2$, $P=0.01$, $r^2=0.31$, $N=31$). Data for individual birds are shown in Fig. 3. Breathing frequency of the flying pigeons was not constant within each flight nor between flights. While in most flights the mean breathing frequency was 7 breaths s^{-1} , some birds showed an intermittent slow breathing frequency of 2–4 breaths s^{-1} (Fig. 4). We did not find a pattern relating fast and slow frequencies so we did not pursue analysis of these any further. A slow breathing frequency, between 2–3 breaths s^{-1} , dominated in several flights. In most of these cases, wing-beat frequencies did not

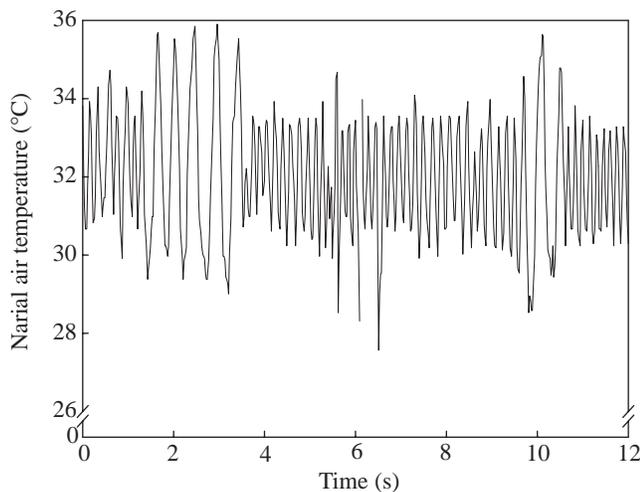


Fig. 4. A 12 s sample of narial temperature measured in a free flying pigeon. An intermittent breathing pattern is apparent with fast segments (7 breaths s^{-1}) interspersed with a slower component (3 breaths s^{-1}).

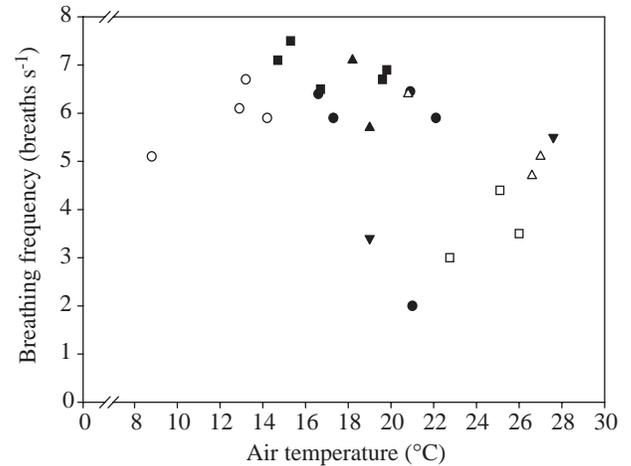


Fig. 5. Breathing frequency of pigeons in flight in relation to ambient air temperature. Each symbol represents a different individual and is the mean of samples taken every 3 min for the duration of the flight (2–3 h).

change from the normally fast pattern (see Discussion). In general, there was a tendency for breathing frequency to decrease slightly with air temperature (ANOVA, $F_{1,24}=4.969$, $P=0.036$, $N=24$), but as the slope was 0.0125, there was no meaningful difference between the breathing frequency at the low T_a and at the high T_a (Fig. 5).

Respiratory water loss

Mean RWL of flying pigeons, calculated as outlined above, was $9.2 \pm 2.9 \text{ mg g}^{-1} \text{ h}^{-1}$ over all air temperatures and T_a did not affect RWL ($N=31$, $P=0.349$). RWL ($\text{mg g}^{-1} \text{ h}^{-1}$) decreased linearly with increasing ambient air vapor density (g m^{-3}) ($\text{RWL}=12.5-0.362\rho_{\text{in}}$, $F_{1,30}=8.277$, $P=0.007$, $r^2=0.222$, $N=31$). Data for individual birds are shown in Fig. 6.

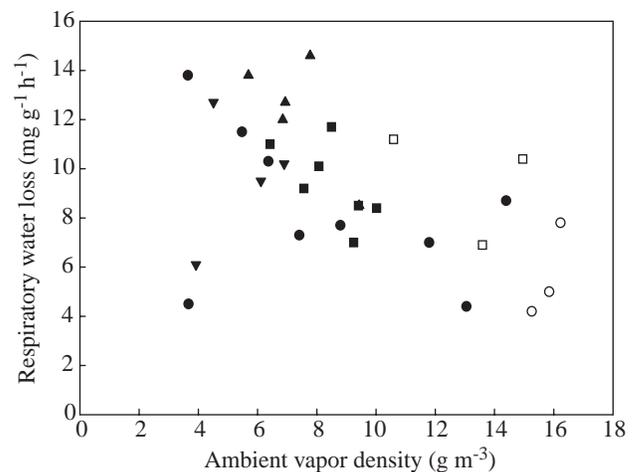


Fig. 6. Respiratory water loss of flying pigeons in relation to ambient air water vapor density. Each symbol represents a different individual. See text for method of water loss calculation.

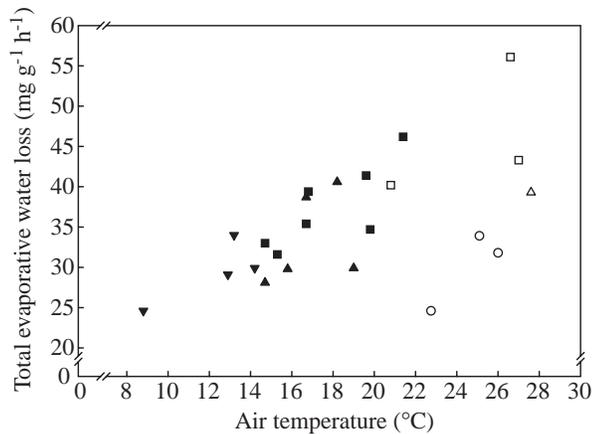


Fig. 7. Total evaporative water loss (EWL) of flying pigeons in relation to ambient air temperature. EWL was measured by mass balance, assuming that excretory water loss was 9.2% of total water loss. Each symbol represents a different individual.

Total evaporative water loss

Total EWL ($\text{mg g}^{-1} \text{h}^{-1}$) increased linearly with air temperature ($\text{EWL}=21.2+0.8T_a$; $F_{1,28}=8.073$, $P=0.008$, $r^2=0.22$, $N=30$). Data for individual birds are shown in Fig. 7. Ambient air vapor density did not affect EWL ($N=30$, $P=0.438$).

Cutaneous water loss

CWL ($\text{mg g}^{-1} \text{h}^{-1}$) increased linearly with air temperature ($\text{CWL}=10.122+0.898T_a$; $F_{1,28}=12.998$, $P=0.001$, $r^2=0.32$, $N=30$). Data for individual birds are shown in Fig. 8.

Discussion

Within the limitations of this study, the data support our working hypothesis. That is, as EWL in free-flying pigeons increased with increasing ambient temperature, and the proportional increase in CWL was greater than that of the RWL.

Pigeons at rest

Exhaled air temperature

As in other studies of birds at rest, T_{ex} in pigeons increased with T_a (Table 2). When comparing the data, one should note several differences in modes of data collection between this

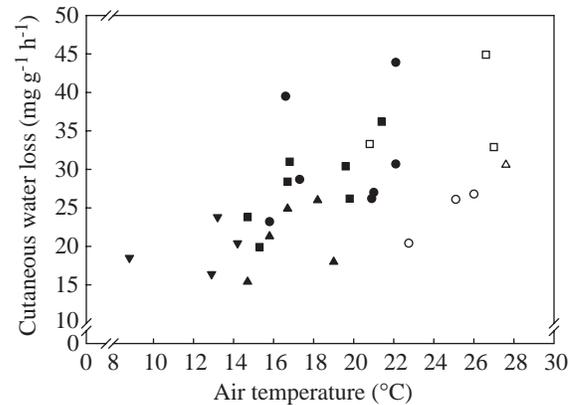


Fig. 8. Cutaneous water loss (CWL) of flying pigeons in relation to ambient air temperature. CWL was calculated from the difference between respiratory water loss and evaporative water loss. Each symbol represents a different individual. See text for details.

and previous studies. First, the depth into the nares at which T_{ex} was measured was not usually reported in previous publications, and T_{ex} generally increases with this depth. T_{ex} is also affected by T_a ; the lower the T_a , the greater the rate of change in T_{ex} with depth (Schmidt-Nielsen et al., 1970; Murrish, 1973; Getz, 1968). In addition, the length of time for which T_{ex} was recorded was not reported in these studies, so it is not clear whether T_{ex} was in steady state. To further confound comparison, in this study resting pigeons inspired dry air, whereas humidity of the inspired air was not specified in any of the other studies. With these caveats in mind, we note that the slope of the regression equation of T_{ex} versus T_a in pigeons was similar to those of other species. However, the intercept was higher by several degrees than those of all but penguins and falcons. Of the species shown in Table 2, T_{ex} of pigeons in this study was closest to that of the prairie falcon [*Falco mexicanus* (Kaiser and Bucher, 1985)].

When pigeons inhaled dry air at 15 °C, mean T_{ex} was 25.7 °C; this resulted in an estimated recovery of 53% of the water that would have been lost had the exhaled air been at T_b , calculated following the method of Schmidt-Nielsen et al. (Schmidt-Nielsen et al., 1970). This is the same water recovery rate as in the prairie falcon (Kaiser and Bucher, 1985), but less than that of the cactus wren, which was 74% (Schmidt-Nielsen et al., 1970) and of the crested lark, which was 67% (Tieleman et al., 1999), both at 15 °C.

Table 2. Exhaled air temperature (T_{ex}) at specified ambient temperatures (T_a) in birds at rest

Species	T_{ex} (°C)	T_a (°C)	Study
Rock pigeon (<i>Columba livia</i>)	$T_{\text{ex}}=16.3+0.705T_a$	15–30	Present study
Cactus wren (<i>Campylorhynchus brunneicapillum</i>)	$T_{\text{ex}}=8.51+0.756T_a$	12–30	Schmidt-Nielsen et al., 1970
Crested lark (<i>Galerida cristata</i>)	$T_{\text{ex}}=8.93+0.793T_a$	15–40	Tielman et al., 1999
Adelie (<i>Pygoscelis adeliae</i>) and Gentoo (<i>P. papuae</i>) penguins	$T_{\text{ex}}=22+0.5T_a$	10–30	Murrish, 1973
Spinifex pigeon (<i>Geophaps plumifera</i>)	$T_{\text{ex}}=11.6+0.69T_a$	2.5–37.5	Withers and Williams, 1990
Prairie falcon (<i>Falco mexicanus</i>)	$T_{\text{ex}}=15.92+0.65T_a$	0–42	Kaiser and Bucher, 1985

The upper limit of T_{ex} is constrained by T_{b} , unless T_{a} exceeds it. In resting pigeons of the same stock that was used in the present study, mean T_{b} in the thermoneutral zone was 40.5 °C (Adams et al., 1999). At a T_{a} of 30 °C, mean T_{ex} of our pigeons was 37.8 °C. This difference between T_{b} and T_{ex} would allow a recovery of only 11 % of respiratory EWL, compared to 41 % recovery of water by the spinifex pigeon *Geophaps plumifera* at 30 °C (Withers and Williams, 1990). Therefore, both at 15 °C and at 30 °C, the temporal countercurrent heat exchanger in the tippler pigeons' upper respiratory tract was evidently less effective at recovering water than in other species so far studied.

The linear dependence of T_{ex} on T_{a} does not support the idea that pigeons actively control T_{ex} through vasodilation and vasoconstriction of blood vessels in the nasal mucosa; rather it suggests that the structure of the nasal turbinates in pigeons may be relatively simple, leaving T_{ex} closer to T_{b} than to T_{a} (Schmidt-Nielsen et al., 1970; Hillenius, 1992).

Ventilation

The air temperatures to which pigeons were exposed in our experiments (15–30 °C) were lower than the known air temperatures at which birds begin to pant (Calder and Schmidt-Nielsen, 1968; Bucher, 1985; Marder and Arad, 1989; Withers and Williams, 1990). Therefore, we did not expect to see significant variation in minute volume through changes in V_{T} and/or in breathing frequency. V_{T} of resting pigeons was indeed independent of air temperature and the mean was about 20 % higher than that predicted by the allometric equation of Bech et al. (Bech et al., 1979). Breathing frequency of resting pigeons was 8 % higher than the value calculated by the equation of Lasiewski and Calder for non-passerines (Lasiewski and Calder, 1971).

Variation in minute volume among our pigeons was high; the minimum value was 88 ml min⁻¹ body temperature and pressure saturated (BTPS) (at 20 °C) and the maximum was 211 ml min⁻¹ BTPS (at 25 °C). Minute volume was independent of air temperature below 30 °C in several other bird species (Bernstein and Schmidt-Nielsen, 1974; Bernstein, 1976; Bucher, 1985; Kaiser and Bucher, 1985; Withers and Williams, 1990).

Pigeons in flight

The effect of ambient air temperature on exhaled air temperature

In the present study, T_{ex} was sampled throughout flights lasting 1.5 h or more and we consider the results to represent steady-state flight. The increase of T_{ex} with T_{a} in flying pigeons was less than for resting pigeons, suggesting that temporal countercurrent heat exchange in flight may be less effective than at rest. This smaller increase of T_{ex} with T_{a} may be due to the effect of T_{b} on T_{ex} in flight, buffering it from T_{a} , and/or it may be because of vasodilation of the blood vessels in the nasal mucosa. T_{b} of flying birds is up to several degrees higher than the value at rest (Hart and Roy, 1966; Hirth et al., 1987; Adams et al., 1999). This may result in higher temperatures in

the nasal passages, making T_{ex} less sensitive to T_{a} , thus causing temporal countercurrent heat exchange to be less efficient. Elevated T_{ex} is advantageous as it enhances respiratory cooling, either through evaporation or by convection (Murrish, 1973).

The only published data of exhaled air temperature in flying birds are from wind tunnel flights of black duck (Berger et al., 1971), starling *Sturnus vulgaris* (Torre-Bueno, 1976) and white-necked raven (Hudson and Bernstein, 1981). In the black duck, the longest flight was 14 s and T_{ex} increased linearly with T_{a} ; $T_{\text{ex}}=23+0.43T_{\text{a}}$ between air temperatures of -30 to +20 °C. In the same range of temperatures, T_{ex} of the flying pigeons was close to that of the flying black ducks.

Ventilation

Breathing frequency of flying pigeons was constant up to T_{a} of about 22 °C; from that temperature then it tended to decrease as T_{a} increased. The dependence of breathing frequency on T_{a} was previously quantified in three studies of flying birds. In the fish crow, breathing frequency was independent of T_{a} (Bernstein, 1976), whereas in the white-necked raven it increased with T_{a} (Hudson and Bernstein, 1981). In the flying budgerigar, breathing frequency was relatively high – either 5 breaths s⁻¹ below 26 °C or 16 breaths s⁻¹ above 34 °C (Aulie, 1975). Although the correlation between breathing frequency and body mass in birds in flight is usually negative (Bernstein, 1987), there are not yet enough empirical data to generalize.

Mean breathing frequency for the flying pigeons was 5.6 breaths s⁻¹. This is lower than the values reported by both [Butler et al. (Butler et al., 1977); 6.8 breaths s⁻¹ and Hart and Roy (Hart and Roy, 1966); 8.1 breaths s⁻¹]. The difference may be the result of the birds not having reached a steady state in the short wind-tunnel flights in these two studies. The dominating breathing frequencies of the flying pigeons in the present study were between 6 and 7 breaths s⁻¹, but other frequencies were observed. For example, some pigeons breathed at 2–3 breaths s⁻¹ for most of the flight in three test flights. In addition, an intermittent fast/slow breathing sequence (see Fig. 5, for an example) was observed irregularly in almost all flights.

The breathing frequencies of the flying pigeons are comparable to those of panting pigeons at rest (Bernstein, 1987). Continuous rapid breathing may induce conditions where the rate of CO₂ removal is greater than the rate of its production, leading to hypocapnia and consequent respiratory alkalosis (Calder and Schmidt-Nielsen, 1968). Aulie (Aulie, 1975) found a compound form of ventilation in the flying budgerigar, which presumably prevented it from becoming alkalotic. Bech et al. (Bech et al., 1979) demonstrated that in heat stressed, resting flamingos one or more slow, deep 'flush out' breaths periodically interrupted panting. This slow, deep breath eliminated large quantities of CO₂. The intermittent fast/slow breathing observed in the flying pigeons might therefore be a response to low concentrations of blood CO₂ as observed in panting flamingos (Bech et al., 1979).

Table 3. Respiratory water loss (RWL) in birds during flight at specified air temperatures (T_a)

Species	T_a (°C)	RWL (mg g ⁻¹ h ⁻¹)	Dependence on T_a	Method	Study
Rock pigeon (<i>Columba livia</i>)	8.8–28	4.4–14.6	No	*	Present study
Budgerigar (<i>Melopsittacus coruscans</i>)	18–38	11.9–37.3	Yes	*	Tucker, 1968
White-necked raven (<i>Corvus cryptoleucus</i>)	23–32.8	13.0–34.0	$1.23T_a - 12.17$	Dew point hygrometry	Hudson and Bernstein, 1981
Fish crow (<i>Corvus ossifragus</i>)	22.5–28.5	12.2–27.8	$8.99T_a - 13.5$	Dew point hygrometry	Bernstein, 1976
Black duck (<i>Anas rubripes</i>)	19	12.0	–	*	Berger et al., 1971

* indicates that RWL was not measured directly, but was calculated from measurements of exhaled air temperature, breathing frequency and tidal volume.

If all other variables remain constant and breathing frequency is reduced by as much as threefold, as we found, and if no compensating mechanism comes into play, the bird's oxygen supply should also be reduced by threefold. While there are no data for instantaneous oxygen consumption for birds in free flight, the oxygen consumption of birds flying in wind tunnels was 10- to 15-fold greater than the values at rest (Rothe et al., 1987; Tucker, 1968). It seems highly unlikely that the pigeons flying in the present study reduced their oxygen demand threefold when their breathing frequency decreased. To maintain a sufficient oxygen supply, the pigeons must have compensated for slow breathing frequencies, either by increasing their V_T or by elevating their rate of oxygen extraction.

Oxygen extraction was measured in birds during wind tunnel flights (Butler et al., 1977; Bernstein, 1976) [for a review, see Bernstein (Bernstein, 1987)]. In pigeons, oxygen extraction increased up to 1.8-fold the resting value (Butler et al., 1977). If, during the periods of low breathing frequency, pigeons in the present study increased their oxygen extraction by 1.8-fold, total oxygen supply would still be below their requirements. This implies that tidal volume must have increased as well.

Starlings and evening grosbeaks (*Coccothraustes vespertinus*) increased their V_T by 4.2- and fourfold, respectively, in flight (Berger et al., 1970; Torre-Bueno, 1978). Berger et al. (Berger et al., 1970) found that V_T in pigeons varied little in flight compared to other birds, and suggested that flying pigeons increase ventilation primarily by increasing breathing frequency. They further suggested that the pigeon's low tracheal resistance compared to that of the evening grosbeak and the black duck allowed them to attain greater breathing frequencies (Hart and Roy, 1966; Berger et al., 1970). It may simply be that because of the short duration of the pigeons' flights in these previous studies, high V_T was not observed.

The ratio between wing-beat frequency and breathing frequency was normally 1:1, except during the slow-frequency breathing bouts where the ratio was approximately 1:3. This is contrary to previous studies of pigeons where the ratio of wing-beat frequency to breathing frequency was consistently reported to be 1:1 (Hart and Roy, 1966; Berger et al., 1970; Butler et al., 1977). Again, we suggest that the single 1:1 ratio was always observed because of the short duration of the wind

tunnel flights in those studies, and the birds may not have had the time to respond to developing hypocapnia.

Water recovery

To calculate the water recovery in the nares, assuming the air was exhaled at a temperature equivalent to T_b , we used the regression equation of T_{ex} on T_a , and assumed that a pigeon's body temperature was 42 °C in flight (Adams et al., 1999). At 15 °C, T_{ex} was 30.9 °C, giving a water recovery of 44 %, while at 25 °C, T_{ex} was 34.3 °C, providing a water recovery of only 32 %. At low T_a , water recovery in flight was less than at rest because of the weaker dependence of T_{ex} on T_a in flight. Thus, we concluded that there is relatively more narial recovery of water at rest than in flight.

Respiratory water loss

RWL of the flying pigeons ranged from 4.4 to 14.6 mg g⁻¹ h⁻¹. We consider these to be conservatively low values for RWL because, in flight, V_T was probably greater than the value used in our calculations. Table 3 summarizes studies where RWL in flying birds was measured. The studies were done using different ranges of T_a . Nevertheless, RWL of free-flying pigeons was lower than in other species, and was not clearly dependent on T_a as it was in the budgerigar, white-necked raven and fish crow.

The apparent independence of RWL from T_a is surprising when one considers that T_{ex} increased linearly with T_a . The factors that offset the effect of T_{ex} on RWL were the slight decrease of breathing frequency and the substantial increase of inhaled vapor density with increasing T_a .

The ratio of inhaled vapor density to exhaled vapor density, ρ_{in}/ρ_{ex} , increased with T_a . At 8.8 °C ρ_{in}/ρ_{ex} was 0.1, whereas at 28 °C it was 0.44. Therefore, the amount of water vapor in the ambient air contributed so much that it caused RWL to be independent of T_a . RWL in flight is always higher than at rest, thus the scope for its adjustment may be more limited. The reasons for this are: (1) the high metabolic demands of birds in flight require continuously high ventilation rates, (2) in flight, as T_{ex} approaches T_b , temporal countercurrent water recovery is less efficient than at rest, and (3) inspired water vapor can have a significant input, leading to overall lower rates of net RWL.

Although we used Hart and Roy's constant value for V_T of 5 ml (Hart and Roy, 1966), it is unlikely that V_T remained constant throughout the long flights of our study. V_T may have changed with breathing frequency, and possibly with T_a . Depending on the pigeons' ability to increase oxygen extraction, V_T may have been two- to threefold greater than we assumed. That is, V_T may have been as high as 15 ml breath⁻¹ when breathing frequency was 2 breaths s⁻¹.

To incorporate the possibility that V_T did change in flight, rather than assume it was constant, we recalculated RWL assuming that ventilation remained constant while V_T and breathing frequencies are inversely proportional to one another. The most frequent in-flight breathing frequency was 7 breaths s⁻¹. If, at this frequency, V_T were 5 ml [after Hart and Roy (Hart and Roy, 1966)], then the ventilation would be 35 ml s⁻¹. Recalculating the mean RWL for all flights with this value gives a range of 7.5–17.2 mg g⁻¹ h⁻¹, with an overall mean of 11.8 mg g⁻¹ h⁻¹. This is 20% greater than the values calculated assuming constant V_T . Using the above assumptions, the bounds for mean RWL in all the flights were 9.4 mg g⁻¹ h⁻¹ (where $V_T=5$ ml) to 11.8 mg g⁻¹ h⁻¹ (where ventilation=35 ml s⁻¹).

Evaporative water loss

EWL in flying birds was measured in several studies (LeFebvre, 1964; Tucker, 1968; Torre-Bueno, 1978; Hudson and Bernstein, 1981; Biesel and Nachtigall, 1987; Adams et al., 1999; Giladi and Pinshow, 1999). In general, in-flight EWL was constant over a range of T_a below 20 °C, above which it increased, sometimes sharply. In the present study, EWL increased with T_a , although there was no T_a value at which a steep rise was clearly evident. EWL of flying pigeons in this study was approximately twice that measured by mass balance (Giladi and Pinshow, 1999) in birds of the same stock. As experimental flights were made over the same range of air temperatures and humidities by Giladi and Pinshow (Giladi and Pinshow, 1999) as by us, it is likely that the extra load carried by our pigeons caused the higher EWL rates. Gessaman and Nagy (Gessaman and Nagy, 1988) showed that metabolic rate increased by up to 50% in homing pigeons carrying a load equal to 2.5% of their body mass, in comparison with unladen pigeons. In the present study, the transmitter load was approximately 3% of a pigeon's mass; this extra mass and the increased aerodynamic drag that it caused probably gave rise to an increase in the bird's metabolism, and consequently in EWL. This increased metabolism is associated with an additional heat load that can be dissipated by convection or by evaporation. If birds cannot dissipate much more heat through convection than they already do without a load, to prevent overheating, they must dissipate most of the excess heat through evaporation. Gessaman et al. (Gessaman et al., 1991) found that tippler pigeons carrying a dorsal load weighing 5% of their body mass lost 50–100% more water than did control birds.

The high in-flight metabolic rate found in pigeons by Gessaman and Nagy (Gessaman and Nagy, 1988) may imply that loaded birds increased their ventilation rates, thereby obligatorily increasing their respiratory evaporation. In flight,

most metabolic energy is converted to heat (Tucker, 1968; Rayner, 1990; Pennycuik, 1975) and the increase in respiratory evaporation would help offset the extra heat production associated with transporting the transmitter mass, but additional cutaneous evaporation might also be required. It is unknown how this extra EWL is partitioned between the respiratory and cutaneous routes.

EWL was independent of ambient vapor density and, hence, was seemingly independent of its supposed driving force, the difference between skin and air water vapor densities. This is consistent with the results of Giladi and Pinshow (Giladi and Pinshow, 1999) who suggested that because the need for cooling increases with rising ambient temperature, which can no longer be accommodated by convection and radiation, the bird must evaporate water. The vapor density gradient between the bird's integument and the environment is negative even at higher p values, allowing for water loss. Indeed, Marder and Ben-Asher (Marder and Ben-Asher, 1983) showed that the vapor diffusion resistance of the skin decreases in heat-stressed pigeons, and Arieli et al. (Arieli et al., 2000) established that CWL increases through neurotransmitter-mediated peripheral hemodynamic and ultrastructural changes in the skin of the same birds.

The contribution of respiratory and cutaneous components to evaporation

We calculated CWL from the difference between total EWL and RWL. CWL increased with air temperature in the same manner as did EWL. To assess the relative contributions of respiratory and cutaneous water loss we used the mean values for all flights, applying an assumption of a constant value for V_T of 5 ml as one limit, and an assumption of constant ventilation of 35 ml s⁻¹ as the other. Thus, RWL accounted for an average of 25.7–32.2% of total EWL, while CWL averaged 67.8–74.3% of the total EWL. The only other flying bird in which CWL has been measured is the white-necked raven. At 22 °C, its CWL was 6.1 mg g⁻¹ h⁻¹, which was 22% of the total evaporation (Hudson and Bernstein, 1981). In the pigeons, CWL at 22 °C (from the regression for CWL *versus* T_a), was 29.9 mg g⁻¹ h⁻¹, 77% of the total.

We conclude, again with the limitations of our experimental system in mind, that pigeons are limited in their ability to increase EWL through respiration, but do increase cutaneous evaporation in times of need.

This paper is dedicated to Samuel L. Jaff on the occasion of his 90th birthday, in deep appreciation for introducing B. P. to the wonders of ornithology many moons ago. We thank Nigel Adams, Itamar Giladi and Lenny Gannes for their assistance during experiments. We also thank Eyal Shani, Andrea Shuchman and Orna Shimoni, who provided invaluable help training and maintaining birds, and Drs Marvin H. Bernstein, Ian G. van Tets and Carmi Korine, who constructively criticized versions of the manuscript. Special thanks to Yvon Le Maho at the Centre d'Ecologie et Physiologie Energetiques, CNRS, France for providing the plethysmograph. This study was supported by United States-

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