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Racing the wind

Engel, Sophia Barbara

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Racing the wind

Water economy and energy expenditure in avian endurance flight

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Racing the wind

Water economy and energy expenditure in avian endurance flight

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Sophia Barbara Engel
geboren op 15 augustus 1972
te Hüttental, Duitsland

Promotores:	Prof. Dr. G.H. Visser
	Prof. Dr. S. Daan
Copromotor:	Dr. H. Biebach
Beoordelingscommissie:	Prof. Dr. P.J. Butler
	Prof. Dr. M. Klaassen
	Prof. Dr. J.J. Videler

Contents

CHAPTER 1

General Introduction

Ever since Ikarus, bird flight has been a fascinating miracle to man. Fragile birds cross deserts and overcome mountain ranges. They endure fasting, adverse climates, span the globe on the wing. What are the traits that make such a performance possible, what are the trade-offs, what the limitations? Although our knowledge of avian ecology and physiology is growing, and the miracle is disentangled step by step with each cleverly-designed and carefully executed experiment, the fascination remains.

This same fascination has been the driving force behind this thesis, which is meant as a contribution to an integrated understanding of the challenges associated with avian flight, and the ways in which animals cope with them both physiologically and behaviourally. We have chosen an approach based on empiry rather than theory. There are valuable modelling attempts available that describe the consequences of changes in physiology or behaviour and make predictions about optimal solutions to ecological, physiological or aerodynamic problems. We feel that the empirical background is not growing at the same rate as the body of theory. The experiments presented here deal with the different sometimes opposing demands on a bird's energy and water budget during sustained flight periods under controlled ambient conditions in a wind tunnel.

The challenges of avian long distance migration

Powered flight as a means of locomotion is a salient characteristic of birds, and migratory endurance flight is outstanding in its high sustained metabolic demands compared to other activities in a bird's life. The physiological mechanisms and adaptations that enable some species to cross the globe on their way from wintering to breeding grounds and back again, traversing vast inhospitable areas like deserts or oceans without eating or drinking, are part of the mystery that still waits to be solved. Three factors have been suggested as shaping the physiology and behaviour of migrants: time, energy and safety (Alerstam and Lindström 1990). For the flight period itself, energy is generally considered to be the most important factor of the three, due to the very high sustained metabolic rates (see *e.g.* Berger and Hart 1974). A fourth factor that has not received much attention but might be equally important is water. Long migratory flights may sometimes be limited by dehydration rather than by energy depletion. Energy and water management are closely interlinked (Carmi et al. 1992, Klaassen 1995) and to assess the physiological challenges of long distance flights neither factor can be studied in isolation.

During their natural migratory flights, birds change their position in a three dimensional world, thereby choosing from a continuum of ambient conditions, temperature, air pressure, humidity, wind direction or speed, to name only a few. All of these have an effect on flight performance (Fig. 1.1), sometimes in conflicting directions for energy metabolism or water flux: Ambient temperature is dependent on geographic location, season, time of day and flight altitude. Through its effect on thermoregulation, temperature is one of the main factors affecting evaporative water loss. While birds can in principle choose a preferred temperature during their flights by adjusting flight altitude, air pressure changes concurrently. This has effects on flight costs (negatively related with air density), but also on respiration (via decreasing oxygen partial pressure) and evaporation (via decreasing ambient humidity, which increases the driving force for evaporation). To complicate a bird's decision even further, wind direction and wind speed are also dependent on geographic location, season and altitude. In principle, tail or head winds do not affect flight costs *per se*, as a bird is moving with its surrounding air, like a balloon carried by a breeze. Active flight leads to movement of the bird relative to the air, irrespective of the air's movement. Wind conditions affect the bird' s movement with respect to the ground. This is what matters for a migrating bird, especially if it is under time pressure to arrive at the breeding grounds in time. Therefore, a bird may choose its flight speed according to wind conditions (increase its flight speed to offset a head wind, for instance). Flight speed is a factor assumed to determine energy requirements during flight. Flight costs, migration speed, flight route and flight altitude might therefore be affected by prevalent wind conditions.

The factors affecting energy costs of flight

During flight, birds can sustain metabolic rates of more than ten times basal metabolism for several hours or even days (Norberg 1996). This is about twice the maximum sustained metabolic rate of similarly-sized running mammals (see Thomas 1975, Butler 1980, Butler and Woakes 1990). What are the physiological adaptations of birds to these high sustained metabolic demands, and which factors determine flight costs in birds? Body mass is an important factor determining energy costs of flight: Interspecific comparisons have generated allometric equations that allow an estimate of flight costs for any species based on body mass (*e.g.* Schmidt-Nielsen 1972, Butler 1980, Masman and Klaassen 1987, Butler 1991). Intraspecifically or intraindividually, the scaling of flight costs and body mass is less clear. It appears, though, that flight costs increase less dramatically than predicted by interspecific allometry (Kvist et al. 2001, Hambly et al. 2004a, Schmidt-Wellenburg et al. in prep.). Aerodynamic theory has led to mathematical models designed to predict mechanical costs of flight for any bird and under different

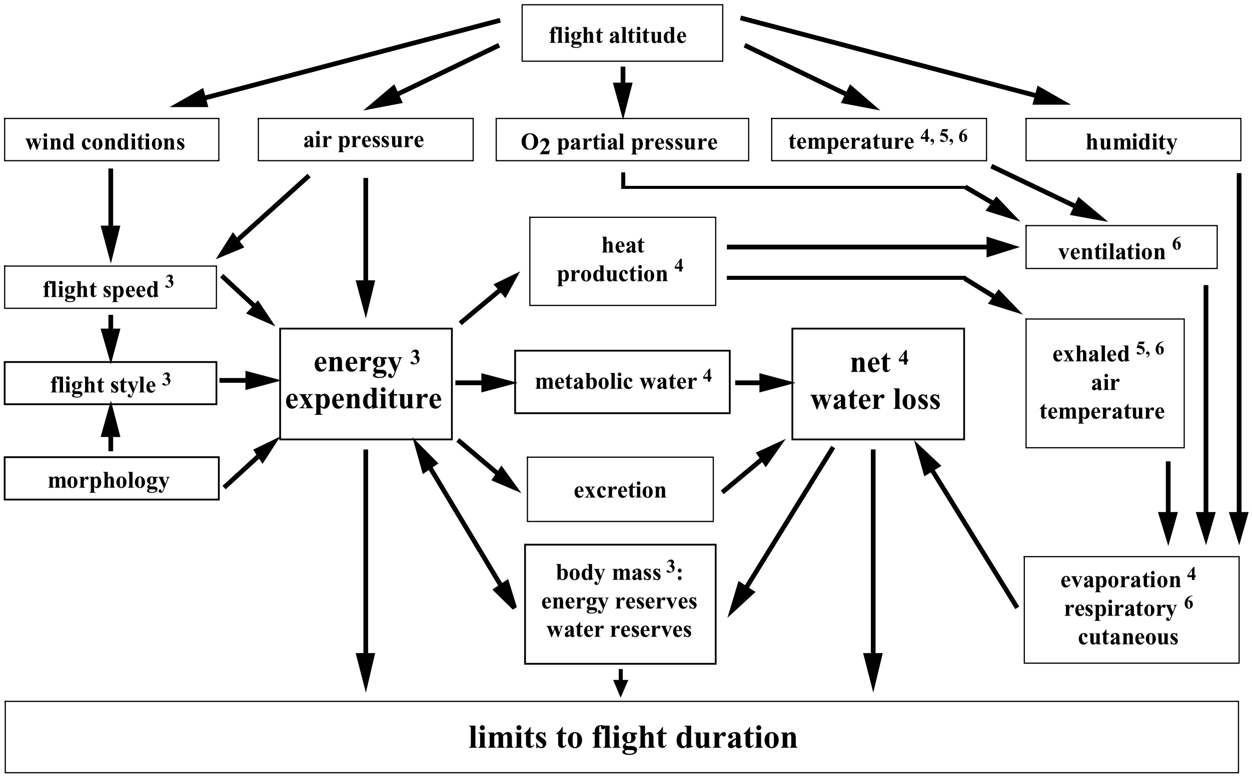

Fig. 1.1 Model of the interactions between environmental factors and morphological traits of a bird affecting energy cost of flight and water fluxes. Flight duration can be limited by fuel depletion or by dehydration and depends on the interaction of all related parameters. The superscript numbers refer to the chapters that explore the respective aspects.

ambient conditions (Tucker 1973, Greenewalt 1975, Rayner 1979b, Pennycuick 1989). Following the elementary physical principles that the power to generate lift is inversely proportional to flight speed, and the power needed to generate thrust increases with the speed cubed, flight costs as the sum of both are predicted to depend on flight velocity in a U-shaped curve (Fig. 1.2; see *e.g.* Videler 2005). This mechanical power output is only a fraction of the total metabolic power expended during flight (Nachtigall 1998, Ward et al. 2001). The major part of the metabolic energy ends up as heat and has to be dissipated (Ward et al. 1999). Further energy is required for respiration and circulation to supply the tissues with metabolites and oxygen, and for some basic physiological processes that are not directly involved in the generation of flight muscle work. The whole-body efficiency at which metabolic power is transformed into mechanical work remains poorly understood (see *e.g.* Ellington 1991, Pennycuick 1998). We also do not know whether muscle efficiency is a fixed factor irrespective of body mass or flight velocity (Kvist et al. 2001, Ward et al. 2001).

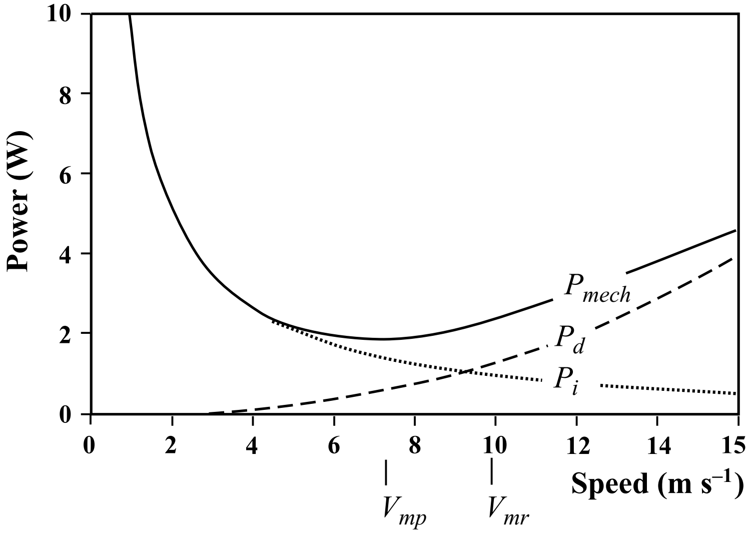

Fig. 1.2 Hypothetical mechanical power curves as a function of speed predicted by theory. The power required to generate lift (P_i) is inversely proportional to flight speed, and the power needed to to overcome drag and generate thrust (P_d) increases with the speed cubed. The total power (P_{mech}) follows a U-shaped trajectory. Minimum power speed (V_{mp}) requires minimum power per unit time. Maximum range speed (V_{mr}) is characterized by minimum power requirements per unit distance.

Calculations of metabolic power requirements on the basis of aerodynamic modelling can be only tentative for these reasons. The application of aerodynamic theory for fixed wing aircraft, which is the basis of all available models, gets complicated further by the fact that most birds usually use flapping flight instead of gliding, thereby changing the shape of the wings continuously by stretching or flexing them. They can vary the angle of attack, wing beat frequency, and wing beat amplitude, as well as using intermittent flight and changing many other parameters that might affect flight costs (Videler 2005). Although the predicted U-shaped power curve applies well to aircraft, experimental evidence for birds is weak and controversial (see *e.g.* Alexander 1997, Dial et al. 1997, Rayner and Ward 1999, and chapter 3 of this thesis). Most studies report flight costs and power-to-speed relationships only for short flights. Migratory flights differ substantially from short flights both in energetic costs (see *e.g.* Nudds and Bryant 2000) and in physiological processes underlying these costs. Short flights are powered mainly by muscular and hepathic carbohydrate stores (George and Berger 1966, Rothe et al. 1987). Endurance flight is fuelled by the combustion of extramuscular fat reserves (Rothe et al. 1987, Ramenofsky 1990, Jenni and Jenni-Eiermann 1998, Klaassen et al. 2000, Jenni-Eiermann et al. 2002), accompanied by a small but important fraction of protein catabolism (Jenni-Eiermann and Jenni 1991, Bairlein and Totzke 1992, Jenni-Eiermann et al. 2002). Therefore, to understand the physiology of bird migration we need more studies on long duration flight under controlled environmental conditions.

The factors affecting water fluxes during flight

Water gain through metabolic water production is the only positive part in the water balance equation of a flying bird, standing against water loss through excretion and evaporation. High metabolic rates as they occur during bird flight are associated with a high production of metabolic water. The exact amount of water formed in the chemical process of fuel oxidation depends not only on the metabolic rate but also on the fuel type (carbohydrates, fat or protein) and the end product of protein metabolism: urea or, in the case of birds, uric acid (Schmidt-Nielsen 1997). Apart from other possible physiological functions of protein catabolism (see *e.g.* Battley et al. 2000, Biebach and Bauchinger 2003), it could help to counteract dehydration during flight because it releases more water per unit energy than lipid combustion (Klaassen 1996).

Water loss via excretion and evaporation is inevitable, and both processes are affected by physiological requirements other than the need to conserve water. The primary function of excretion is the elimination of metabolic waste products, which must be accompanied by water. This obligatory water loss – albeit limited by the excretion of non-soluble uric acid - and the high metabolic rate during flight could conceivably constrain the bird's ability to reduce excretory water loss (Giladi and Pinshow 1999). Evaporation is a basic physical process following the water vapour pressure gradient between the bird's body (skin and respiratory surfaces) and the environment (Gates 1980). Still, resting birds can regulate their evaporation rates within certain limits by changing the lipid composition in the skin, thereby regulating cutaneous evaporation (*e.g.* Marder and Raber 1989, Wolf and Walsberg 1996, Tieleman and Williams 2002, Haugen et al. 2003) or by cooling the respired air below body temperature upon exhalation, thereby reducing respiratory evaporation (Schmidt-Nielsen et al. 1970, Tieleman et al. 1999, Geist 2000). The effect of exhalation temperature on respiratory water loss is schematically depicted in Fig. 1.3.

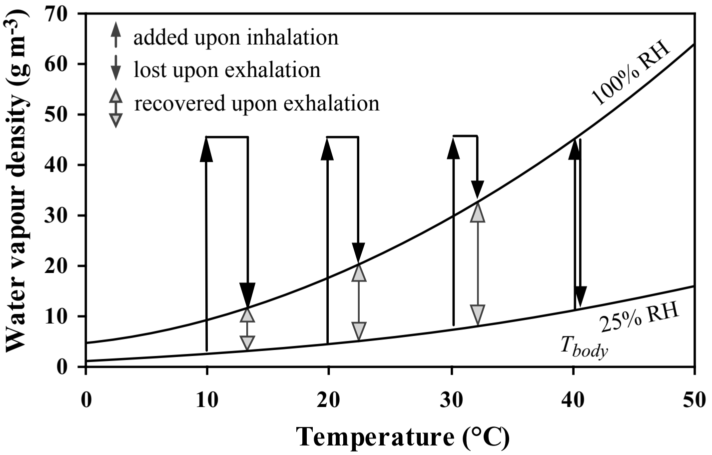

Fig. 1.3 The effect of exhaled air temperature (T_{exh}) on respiratory evaporation. Solid arrows pointing upwards represent the amount of water added to the air upon inhalation when respired air gets water saturated at body temperature (T_{body}). The solid arrows pointing downwards represent the amount of water lost upon exhalation. It depends on the temperature of T_{exh}. The grey arrows represent the amount of water recovered through exhalation of the respired air at $T_{exh} < T_{body}$. Relative humidity of ambient air is 25 % in our example.

During flight and its associated high levels of heat production (Hudson and Bernstein 1981, Ward et al. 1999), evaporation plays an important role in thermoregulation, especially at high ambient temperatures (Tucker 1968, Bernstein 1976, Torre-Bueno 1976, 1978b, Giladi and Pinshow 1999, Michaeli and Pinshow

2001). Although a powerful means of heat dissipation, cooling by evaporation implies the risk of dehydration. Heat balance and water fluxes during flight, both in and out, are still poorly quantified.

Experimental approaches to assess energy costs and water fluxes during flight

Study species

Wigeon (*Anas penelope*)

To obtain data that are reliable and transferable to natural migratory flights, we chose undomesticated migratory species for our experiments. These birds undergo typical seasonal changes in physiology. They accumulate large amounts of body fat before onset of migration and show migratory restlessness during the migration period, indicating a high motivation to perform flights of long duration. Our first study was conducted with wigeons (*Anas penelope)* which were chosen for their relatively large body size to minimise the influence of measuring equipment attached to the animal. Birds should be able to fly in a wind tunnel if their wingspan is smaller than 70 % of the width of the test section (Rothe and Nachtigall 1987, Pennycuick et al. 1997). With a wingspan of 71-85 cm, the wigeon is the largest bird to fly in the wind tunnel used in our experiments. In another experimental session the smaller teal (*Anas crecca*; wingspan 53-59 cm) were used. These can manoeuvre more freely in the wind tunnel and are therefore less prone to stress and accidents than the wigeons. Both species are swift and able flyers and are familiar with long-distance flight under different climatic conditions (Bezzel 1985).

Rose Coloured Starlings (*Sturnus*

Teal (*Anas crecca*), equipped with a thermistor for the measurement of T_{exh} (chapter 5).

Rose Coloured Starling (*Sturnus roseus*)

roseus) are long distance migratory birds with a breeding distribution from Eastern Europe to Central Asia. They are about the same size (wing span 33-39 cm) as European Starlings (*Sturnus vulgaris*). Our birds originated from a breeding population on the Crimea peninsula (Ukraine) that migrates over about 5000 km between their wintering quarter in Northern India and their breeding grounds (Hudde 1993). The birds were hand raised in our institute in Seewiesen, and were thus very tame, familiar with the wind tunnel from the beginning, and cooperative during experiments. As flock-living birds they appear more relaxed when flying in the wind tunnel in small groups than when flying alone. European Starlings (*Sturnus vulgaris*) are common breeding birds in Europe. There are sedentary and short-distance migrant populations (Bezzel 1993). Our birds originated from a local short-distance migratory population in Southern Germany. Although no long distance migrants, European Starlings are well suited for wind tunnel experiments and have been used in a number of previous wind tunnel studies (Torre-Bueno 1976, 1978b, Torre-Bueno and Larochelle 1978, Nachtigall 1998, Ward et al. 1999, Ward et al. 2001, Ward et al. 2004), providing us with a valuable basis for comparison of physiological data.

European Starling (*Sturnus vulgaris*)

Wind tunnel design

To assess the energetics and water economy of bird migration, studies on free flying birds under natural conditions would be desirable. There are major technical difficulties in obtaining such measurements. Furthermore the ambient conditions during natural migratory flights are hardly constant nor comparable between different locations. This limits the feasibility and value of such measurements.

To control and manipulate effects of ambient conditions on bird flight performance, we conducted experiments in a large wind tunnel, schematically depicted in Fig. 1.4. This closed-circuit wind tunnel was constructed in 1999 at the Max Planck Institute for Ornithology in Seewiesen, Germany. It resembles the wind tunnel at the University of Lund, Sweden, in design and technical performance (Pennycuick et al. 1997). The flight chamber is 2 m long and 1.2 m wide and is made of transparent plastic and glass to allow continuous observation of the birds. Air speed can be set from 0 up to 45 m s^{-1} with an accuracy of 0.1 m s^{-1}. We display and record the "equivalent" air speed (Pennycuick et al. 1997), which takes into account changes in air density that determine the forces on the wings and body of a bird. This ensures that experimental conditions at a given velocity are similar for all birds. Ambient temperature can be set and controlled within 1 °C by a heat exchanger. The recirculating design, which creates a low turbulence air flow, a low-noise motor equipped with silencers, and the transparent flight section, all make this wind tunnel especially suitable for studies of bird flight.

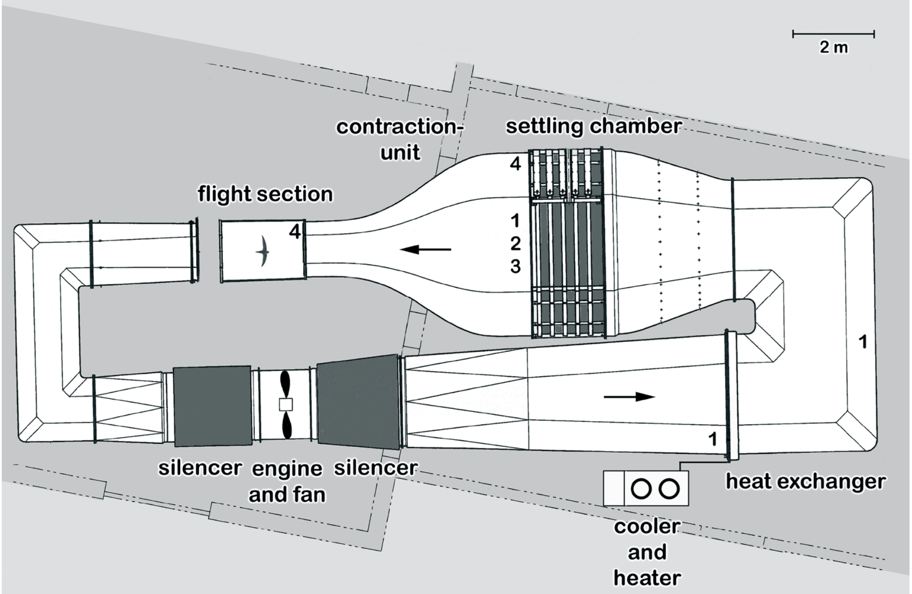

Fig. 1.4 The wind tunnel at the Max Planck Institute for Ornithology, Seewiesen, Germany. The settling chamber is equipped with five honeycomb grids to reduce turbulences in the air stream. A variety of sensors monitors ambient conditions 1) temperature sensors; 2) humidity sensor; 3) camera for the obeservation of the experimental bird in the flight section; 4) pressure sensors for the calculation of air speed (in combination with temperature).

Research questions and outline of the thesis

We chose a few key aspects of flight physiology to study in the wind tunnel, which define the scope of this thesis. We focused on the water economy of birds flying at a range of ambient temperatures, on energy metabolism - since metabolic water production is the only source of water influx during flight – and on the two main factors that determine the amount of water lost with the ventilated air: exhaled air temperature and respiratory air flow.

In **chapter 2** we present energy requirements and evaporation rates of resting Rose Coloured Starlings as determined in a temperature-controlled metabolic chamber. These data allow a comparison of resting energy expenditure and total evaporative water loss with flight measurements, as determined in the following chapters.

In **chapter 3** we measure energy expenditure during flight in relation to flight velocity, using the doubly labelled water method (DLW). Although the power-to-speed relationship has been investigated in a number of species before, the measurements for Rose Coloured Starlings presented in this thesis are the first for sustained flight periods of several hours duration of a migratory species.

In **chapter 4** we combine data on total body water and water fluxes (derived from DLW measurements) during flight in Rose Coloured Starlings, with mass balance calculations of net water loss. These data allow the estimation of total evaporative water loss and the modelling of heat balance during flight.

In **chapter 5** we present data on exhaled air temperature of flying and resting ducks. Exhaled air temperature was measured with a microbead thermistor at the nostril of the birds which was connected to a recording system via thin copper leads. A thermistor changes electrical resistance with temperature and can therefore be used as thermometer.

In **chapter 6** we combine measurements of exhaled air temperature in European Starlings with simultaneous measurements of respiratory air flow during and after flight. These data enable us to calculate respiratory water loss. Temperature of exhaled air was again measured with a microbead thermistor in the nostril of the bird. Respiratory air flow was measured with a flow probe implanted into the trachea of the bird. This flow probe consisted of a microbead thermistor as well, connected to an electronic feedback circuit which heated the probe to a constant temperature. The respiratory air flow increased convection and thereby the current needed to maintain the constant temperature. A picture of a flow probe and a schematic drawing of the experimental setup is presented in Fig. 1.5.

Chapter 7 synthesizes the experimental results presented in this thesis. Our data are integrated into available literature data on short and long flights. We discuss these results also in the context of field studies to draw a picture as complete as possible of the energy and water requirements during long distance flight.

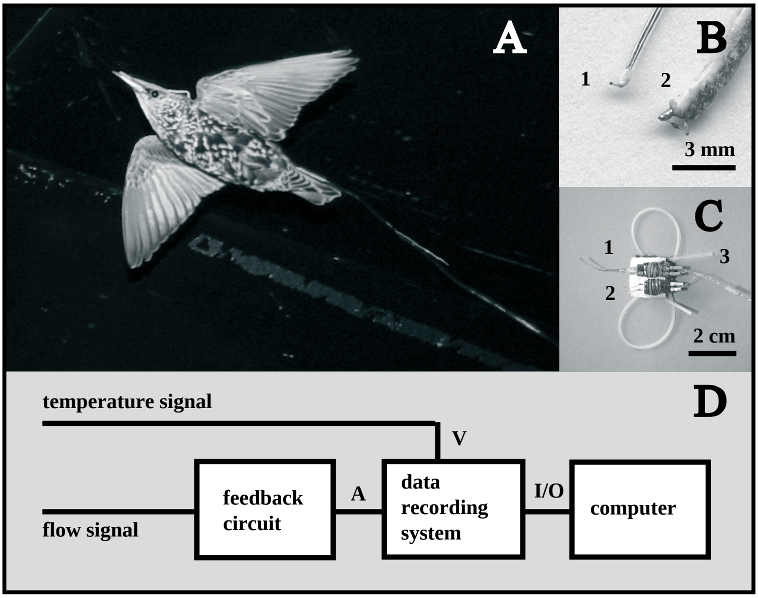

Fig. 1.5 Experimental setup for the measurement of respiratory airflow and the temperature of respiratory air. **A)** European Starling, equipped with an implanted flowprobe and a temperature sensor fixed at the nostril **B)** Flow probe (1) before implantation into the Starling’s trachea and temperature sensor (2) before attachment to the bird’s beak. **C)** Backpack for the connection of the bird to the recording system with (1) connection to the flow probe, (2) connection to the temperature sensor and (3) connection to the feedback circuit and the recording system **D**) Wiring diagram for the measurement of tracheal air flow and exhaled air temperature.

CHAPTER 2

Evaporative water loss and resting metabolic rate in the Rose Coloured Starling (*Sturnus roseus*) exposed to different environmental temperatures

Sophia Engel and G. Henk Visser

Abstract

Total evaporation rates and resting metabolic rate of eight Rose Coloured Starlings (*Sturnus roseus*) were measured in a temperature-controlled metabolic chamber at temperatures between 7 °C and 30 °C. The relationship between total evaporation ($TEWL_r$) and ambient temperature (T_{amb}) was best described by two linear regressions. At temperatures below 21.9 °C, $TEWL_r$ was on average 6.73 ± 1.07 g d^{-1} and increased only slightly with T_{amb}, following the equation $TEWL_r = 0.09 \cdot T_{amb} + 5.28$ [g d^{-1}]. At higher temperatures ($T_{amb} > 21.9$ °C), the relationship was much steeper, following $TEWL_r = 0.37 \cdot T_{amb} – 0.68$ [g d^{-1}].

Resting metabolic rate (RMR) was positively correlated with body mass and was therefore expressed as mass-specific metabolic rate. The relationship between RMR and T_{amb} was best described by two linear regressions. At temperatures above 12.9 °C mass specific RMR was constant at 0.021 ± 0.001 W g^{-1}. At lower temperatures, RMR was negatively correlated with T_{amb} following $RMR = 0.0011 \cdot T_{amb} + 0.0366$ [W g^{-1}].

Introduction

Intuitively, we assume avian physiology to be at maximum performance during flight. This is generally considered to be true for energy metabolism (*e.g.* Bishop 1997), but also evaporation rates may be substantially increased due to the high respiratory demands and internal heat loads imposed by flight (Tucker 1968, Torre-Bueno 1978b, Biesel and Nachtigall 1987, Adams et al. 1997). Indeed, measurements of flight costs in birds have impressively demonstrated sustained metabolic rates twice as high as those of similarly sized running mammals (Butler and Bishop 2000). This is impressive by itself, but to really assess what it takes to fly we need to compare flight physiology to the resting state of a bird. Often, flight costs are presented as a multiple of basal metabolic rate (see *e.g.* Masman and Klaassen 1987). Basal metabolic rate (BMR) is defined as the minimum metabolism of inactive, postabsorptive, non-productive (no moult, reproduction etc.) endotherms while in their rest-phase and thermal neutral zone (see Aschoff and Pohl 1970). Since our experimental flights all took place during daytime, the active phase in the circadian cycle of our experimental birds, and because we were interested in the effect of ambient temperature on evaporation rate, the concept of BMR does not apply. We therefore decided to make resting metabolic rate (RMR) the basis for comparison with our flight measurements. RMR is the metabolic rate of a resting, undisturbed, non-productive and postabsorptive animal during the active phase.

Birds can lose water in evaporation from the respiratory tract and through the skin, and additionally, though at much lower rates, through excretion (Lee and Schmidt-Nielsen 1971, Dawson 1982). Under fasting conditions only metabolic water can potentially offset this water loss. However, most species expend more water in evaporation than they produce by the oxidation of fuel stores already under resting conditions (Dawson 1982, Williams 1996).

In this study we measured resting metabolic rate (RMR [W]) and total evaporation rates ($TEWL_r$ [$g\ h^{-1}$]) during rest in the Rose Coloured Starling (*Sturnus roseus*) at a range of temperatures from 7 °C to 30 °C to provide a basis for comparison with measurements of flight physiology at comparable ambient temperatures that are presented later in this thesis.

Materials and Methods

Animals and housing

Our experimental birds, Rose Coloured Starlings (*Sturnus roseus*), were taken in June 2001 as nestlings from a breeding colony on Crimea Peninsula (Ukraine) and were hand raised and held at the Max Planck Institute for Ornithology, Germany. The birds were housed in large indoor aviaries at a room temperature of about 18 °C and with a light schedule that simulated natural conditions in the wild: During winter they had the photoperiod of their wintering quarters in northern India (27.5° N), which then gradually changed to the natural light conditions of the location of our institute (47.5° N, corresponding to their breeding grounds) during summer and back again. The birds were given standard food consisting of insects, heart, rusk and egg, supplemented with minerals and vitamins (AviConcept®) *ad libitum*, some mealworms and fresh fruit. They had access to fresh water and the opportunity to bathe at any time.

Evaporation rates and resting metabolic rate

The experiments were performed in February 2003 with eight individuals during the active phase of the birds (between 07:00 and 17:00h). Total evaporative water loss of resting birds ($TEWL_r$ [ml h^{-1}]) was calculated from measurements of water vapour pressure (P_W [hPa]) in a positive pressure flow-through system and a temperature-controlled transparent metabolic chamber (Fig. 2.1). Resting metabolic rate (RMR [W]) was measured simultaneously by indirect calorimetry, calculated from CO_2 production (V_{CO2} [ml h^{-1}]).

The metabolic chamber had a volume of 22.5 l and was made from perspex. It had a double-walled design, to control the temperature within the chamber by a Lauda RE120 circulating oil bath. The use of acrylic plastics may cause errors in open-flow systems due to water absorption or release (Bernstein 1971a, Welch 1980), therefore we coated the inner walls with glass. Outdoor air was dried with silica gel and pushed through the metabolic chamber at a rate of 181 ± 7 l h^{-1}, measured and controlled before entering the metabolic chamber with a Brooks mass flow controller (model 5850E) to an accuracy of 0.5 %. The excurrent air from the metabolic chamber was led through an infrared gas analyser (ADC 2250 Gas Analyser; ADC BioScientific Ltd.) that measured H_2O partial pressure and CO_2 concentration to an accuracy of 0.01 %. The analyser was equipped with a separate reference channel that allowed the simultaneous recording of water vapour pressure and CO_2 concentration in reference dry outside air to control for any drift in the system. The high flow rate ensured that CO_2 levels never exceeded 2000 ppm, the highest level that our CO_2 analyser could measure accurately. The ADC 2250 Gas Analyser was calibrated with pure Nitrogen gas at a daily basis to adjust zero.

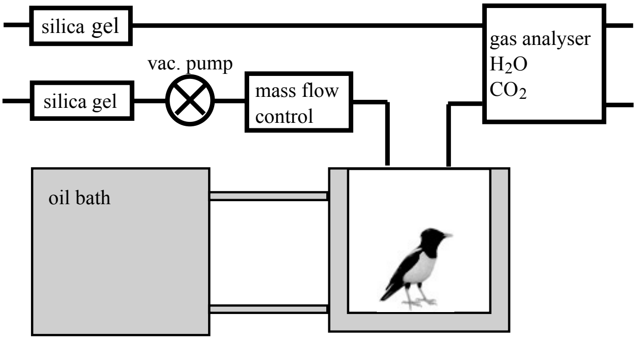

Fig. 2.1 Experimental setup for the measurement of evaporation and resting metabolic rate at controlled ambient temperatures.

Before a trial, birds had free access to drinking water but were deprived of food for at least two hours. Individual birds were placed in the metabolic chamber where they could rest on a stick or on the wire mesh floor above a mineral oil layer that trapped their excreta. The bird's behaviour was monitored and recorded with a video camera while the metabolic chamber was covered with a white cloth to prevent any disturbance by visual cues. An equilibration period of forty minutes allowed the bird to calm down and the gas measurements to stabilize. After this, a period of five minutes duration with the bird sitting completely quiet was used for the analysis of total evaporation and resting metabolic rate, if the bird had not been active for at least ten minutes immediately before this period. The temperature in the metabolic chamber was constant within 0.8 °C during each trial and ranged from 7 to 30 °C.

Total evaporation ($TEWL_r$ [g d^{-1}]) was calculated from the average water vapour pressure of a period of 5 min of stable readings. It equals the absolute humidity in the excurrent air, since the incurrent air was dry. It can be calculated from

$$TEWL_r = \left(\frac{P_{H_2O}}{T_{cell} \cdot R} \right) \cdot Flow_{ex} \cdot M_{H_2O} \quad [\mathrm{g\ d^{-1}}] \qquad \text{(eqn. 2.1)}$$

where $TEWL_r$ = evaporation rate [g d^{-1}], P_{H2O} = partial pressure of water vapour [Pa], T_{cell} = temperature within the analyser cell [K], R = gas constant [J K^{-1} mol^{-1}], $Flow_{ex}$ = flow rate of outlet air [l d^{-1}], M_{H2O} = molecular mass of water [g mol^{-1}]. In our experimental setup, flow rate was measured as Flow_{in} upstream of the metabolic chamber. Because of the high flow rate and associated low CO_2 concentration in the excurrent air, the volume difference between incurrent and excurrent air is less than 0.2 %, assuming the respiratory quotient RQ = 0.72 (see below). We consider this difference as negligible and do not apply any correction in our calculations.

CO_2 production (V_{CO2} [ml h^{-1}] STPD) was calculated from the average CO_2 concentration of a period of 5 min of stable readings as

$$\mathrm{V}_{\mathrm{CO}_2} = Flow_{in} \cdot (F_e - F_i) \text{ [ml h}^{-1}] \qquad \text{(eqn. 2.2)}$$

where Flow_{in} is the flow rate of incurrent air (STPD) [ml h^{-1}], F_i is the fractional concentration of CO_2 in incurrent air, and F_e is the fractional concentration of CO_2 in excurrent air. The ADC 2250 Gas Analyser corrects CO_2 measurements internally for the dilution through humidity in the sample.

Since the animals had been fasting before the measurements we assume an RQ of 0.72 and an energy equivalent of 27.5 J ml^{-1} CO_2 (Schmidt-Nielsen 1997) and calculate RMR [W] as

$$RMR = \frac{V_{CO_2} \cdot 27.5}{3600} \text{ [W]} \qquad \text{(eqn. 2.3)}$$

Statistical analysis

Results are presented as means ± 1 standard deviation (SD). Significance was generally accepted at the 0.05 level. We applied a continuous biphasic linear regression model (Koops and Grossman 1993, Kwakkel et al. 1993) to describe the relationship of metabolic rate (RMR [W], respectively evaporative water loss ($TEWL_r$ [ml h^{-1}]) to ambient temperature (T_{amb} [°C]), and to determine the breakpoint temperature at which the relationships changed. The general model for the calculation of a two- or multiphasic regression is

$$Y = \alpha + \beta_i X - [r(\beta_i - \beta_{i+1}) Ln(1 + e^{(x-y_i)/r})] \qquad \text{(eqn. 2.4)}$$

Where Y is the dependent variable, X is the independent variable, α is the intercept, β is the slope, y_i is the estimated breakpoint between phase i and i + 1, and r is a smoothness parameter which was set at 0.05, a rather abrupt transition (Koops and Grossman 1993). All curves were fitted using the nonlinear regression algorithm procedures from the NONLIN package (shareware program, P.H. Sherrod). The significance of adding a second phase to the model was assessed by a F-test to verify the biphasic nature of the relationship (Kwakkel et al. 1993).

For further analysis, the data sets were divided in two parts, one below the breakpoint and one above. These parts were analysed separately with mixed models in SPSS 12.0 (SPSS Inc.) that tested for the effect of T_{amb} [°C], and M [g] on CO_2 production and evaporative water loss. These models allowed the analysis of repeated measurements on the same individual (*e.g.* at different temperatures) and associated differences in variances.

Results

Evaporation rates

During 43 trials at temperatures (T_{amb} [°C]) ranging from 7 to 30 °C, eight Rose Coloured Starlings (average body mass M [g] = 68.3 ± 4.8 g) had an average evaporation rate of 7.64 ± 1.66 g d^{-1} (Fig. 2.2).

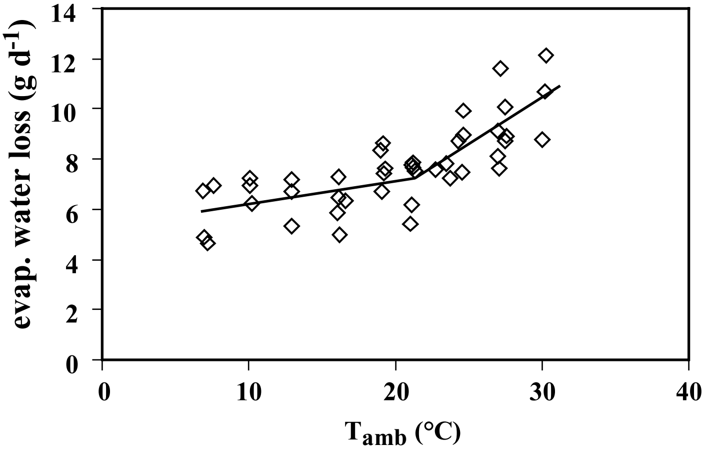

Fig. 2.2 Evaporation rates of eight Rose Coloured Starlings, resting at temperatures between 7 and 30 °C.

A two phase regression described the relationship between evaporation rate and ambient temperature better than one linear regression ($F_{2,43} = 3.60$; $p < 0.05$). The breakpoint that divided the two phases was 21.9 °C (S.E. = 2.35). We subdivided the data set at this temperature and tested both parts separately in a mixed models analysis for the effects of T_{amb} [°C] and body mass M [g] on $\mathrm{TEWL_r}$ [g $\mathrm{d^{-1}}$]. It turned out that M had no effect on $\mathrm{TEWL_r}$ in neither subset ($p > 0.05$).

At temperatures below 21.9 °C, $\mathrm{TEWL_r}$ was on average 6.73 ± 1.07 g $\mathrm{d^{-1}}$ and increased slightly with T_{amb} (effect$_1$ T_{amb} = 0.09; S.E. = 0.04; $F_{1,23} = 5.02$; $p < 0.05$). At higher temperatures ($T_{amb} > 21.9$ °C), TEWL was on average 9.03 ± 1.44 g $\mathrm{d^{-1}}$ and the effect of T_{amb} was much stronger (effect$_2$ T_{amb} = 0.37; S.E. = 0.12; $F_{1,14} = 8.14$; $p < 0.05$). Simple linear regressions between T_{amb} and TEWL [g $\mathrm{d^{-1}}$] for the two subsets results in the equation $\mathrm{TEWL_r} = 0.09 \cdot T_{amb} + 5.28$ [g $\mathrm{d^{-1}}$] if $T_{amb} < 21.9$ °C. At temperatures above 21.9 °C, $\mathrm{TEWL_r}$ increased according to $\mathrm{TEWL_r} = 0.37 \cdot T_{amb} - 0.68$ [g $\mathrm{d^{-1}}$].

Resting metabolic rate

Carbon dioxide production (V_{CO2} [ml $\mathrm{h^{-1}}$]) was on average 194 ± 18 ml $\mathrm{h^{-1}}$ (Fig. 2.3A), corresponding to a metabolic rate (RMR [W]) of 1.5 ± 0.1 W.

In a regression analysis that tested for the effect of $\log_{10}M$ on $\log_{10}\mathrm{RMR}$, a significant relationship was detected (coefficient = 0.79; S.E. = 0.30; $p < 0.05$). Because the scaling coefficient is not significantly different from one, we corrected for this effect by dividing metabolic rate by body mass (Fig. 2.3B).

A biphasic regression described the relationship between mass specific metabolic rate and ambient temperature better than one linear regression ($F_{2,43} = 3.61$; $p < 0.05$). The breakpoint that divided the two phases was 12.9 °C (S.E. = 2.81). We therefore subdivided the data set at this temperature and tested both parts separately in a mixed models analysis for the effects of T_{amb} [°C] and M [g] on V_{CO2}. At low temperatures ($T_{amb} < 12.9$), we found a strong negative effect of T_{amb} on V_{CO2} (effect$_1$ T_{amb} = -10.65; S.E. = 4.02; $F_{1,7} = 7.00$; $p < 0.05$), while there was no significant effect of M. A simple linear regression between T_{amb} and mass specific RMR [W $\mathrm{g^{-1}}$] for the low temperature range is $\mathrm{RMR} = 0.0011 \cdot T_{amb} + 0.0366$ [W $\mathrm{g^{-1}}$]. At temperatures above 12.9 °C, V_{CO2} was on average 188.7 ml $\mathrm{h^{-1}}$, corresponding to a metabolic rate of 1.4 W or 0.021 W $\mathrm{g^{-1}}$.

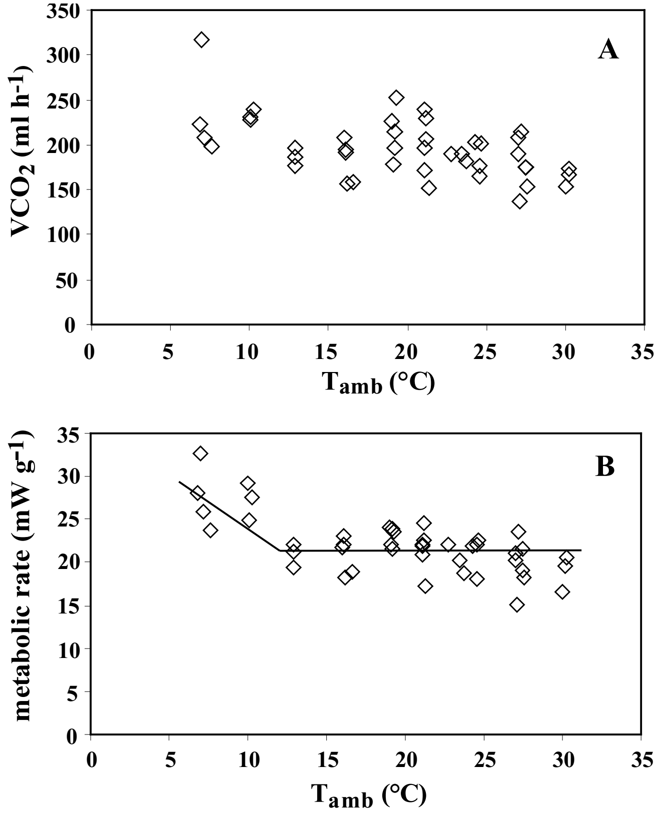

Fig. 2.3 CO_2 production [ml h^{-1}] **(A)** and mass specific resting metabolic rate [W g^{-1}] **(B)** of eight Rose Coloured Starlings at ambient temperatures between 7 and 30 °C.

Discussion

Evaporation rates

A number of adaptations have evolved that help birds reduce their evaporative water losses, many of them behavioural. Birds in hot environments typically reduce their activity in the middle of the day and seek shelter in the shade of vegetation or rocks, huddle in close contact with the cooler soil (Marder 1973b) or use cavities as thermal refuge (Williams 1996). At the same time, dry heat dissipation can be augmented, for instance by the compression of contour feathers, a behaviour that was also observed in our birds at the highest temperatures, by the exposure of thinly feathered body parts (such as the sides of the thorax) through changes in body posture, or by increasing the blood flow to the legs and feet (*e.g.* Bernstein 1974, Dawson and Whittow 2000) and exposing them to the air. In addition, several physiological mechanisms have been proposed to reduce evaporation rates in birds: hyperthermia (Calder and King 1974, Weathers 1981, Dawson 1984), a counter-current heat exchange in the nasal passages that lowers respiratory evaporation (Schmidt-Nielsen et al. 1970), and the adjustment of the skin resistance to water vapour diffusion (Marder and Ben-Asher 1983, Hoffman and Walsberg 1999, Haugen et al. 2003, McKechnie and Wolf 2004a) to reduce cutaneous evaporation.

Hyperthermia, the elevation of body temperature (T_{body}) by 2-4 °C, appears to be common in heat-stressed birds (Weathers 1981, Dawson 1984, Withers and Williams 1990), facilitating dry heat dissipation at ambient temperatures below T_{body} (Calder and King 1974), or by reducing heat gain at higher temperatures (Dawson 1982). The overall effect of hyperthermia on the rate of evaporative water loss, though, is still under discussion (see Tieleman and Williams 1999).

The temperature of exhaled air is generally regarded as the main factor determining the rate of respiratory water loss. The lowering of exhaled air temperature below T_{body} leads to a considerable reduction of respiratory evaporation (Murrish 1973, Tieleman et al. 1999, Geist 2000, Michaeli and Pinshow 2001). A counter-current heat exchange in the upper respiratory tract has been proposed to be the underlying mechanism (Jackson and Schmidt-Nielsen 1964, Schmidt-Nielsen et al. 1970).

The rate of cutaneous evaporation can be regulated by the adjustment of the lipid structure in the skin, which affects the skin resistance to water vapour diffusion (Menon et al. 1989, Menon et al. 1996). This mechanism seems to be relevant for acclimation (Tieleman and Williams 2002, McKechnie and Wolf 2004a) as well as acute responses to thermoregulatory demands (Hoffman and Walsberg 1999).

Despite the described behavioural and physiological mechanisms for the reduction of evaporative water loss, the rapid increase of evaporation rates, both, cutaneous and respiratory, above a certain critical temperature indicates the need for evaporative thermoregulation (Dawson 1982, Wolf and Walsberg 1996, Tieleman and Williams 2002, this study). Still, also in the zone of thermal neutrality, birds generally loose more water in evaporation than they produce in metabolism (Dawson 1982). When water loss via urine and feces are added to those from evaporation, the situation is even less favourable. This illustrates the importance of water economy already for resting birds, and more so during flight.

Allometric predictions of resting evaporation rates within a species' thermal neutral zone range from 5.12 g d^{-1} (Crawford and Lasiewski 1968) to 5.95 g d^{-1} (Williams 1996) for a starling-sized bird. The evaporation rates measured at the lower temperature range in our study ($TEWL_r$ = 6.73 ± 1.07 g d^{-1}) are close to the predicted values. A direct comparison, though, is not possible because allometric relationships do not take ambient conditions like humidity or radiation into account. The high flow rate of dry air in our experiments probably led to a relatively low humidity in the metabolic chamber and thereby to a high driving force for evaporation. Our evaporation rates may therefore be high compared to natural conditions.

Resting metabolic rate

The typical relationship between metabolic rate and T_{amb} in a resting animal is described by the threephasic “Scholander curve” (Scholander et al. 1950): In a moderate temperature range, metabolic rate is constant at a low level. This is the thermoneutral zone. At lower or higher temperatures, metabolic rates increase, reflecting increased demands for active thermoregulation. Because our measurements did not comprise the high temperature range, they result in a biphasic relationship. At temperatures below 13 °C, metabolic rate was negatively correlated with T_{amb}, while in the higher temperature range ($T_{amb} > 13$ °C), metabolic rate was relatively constant at a low level. Given the conceptual framework of the “Scholander curve” (Scholander et al. 1950), we consider the slight decrease of metabolic rate within the higher temperature range as biologically not significant and assume that the average energy consumption at temperatures above 13 °C (1.4 ± 0.1 W) represents resting metabolic rate in the thermoneutral zone.

Because metabolic rate is affected by the circadian rhythm of an animal (Aschoff and Pohl 1970, Wiersma et al. 2003, McKechnie and Wolf 2004b) and our measurements were taken during the active phase of the birds, most allometric equations for the prediction of energy requirements in the form of BMR do not apply. An allometric prediction of the metabolic rate during the active phase is provided by Aschoff and Pohl (1970), separated for passerine and non-passerine species. The equation for passerines predicts a resting metabolic rate of 1.1 W for birds with the body mass of our Rose Coloured Starlings. This is close to the 1.2 W that Wiersma et al. (2003) found at the beginning of their nocturnal metabolic measurements in European Starlings. Although their birds were about ten grams heavier than ours, we still consider our data in good agreement with Wiersma’s measurements, given the fact that our measurements were taken at daytime and in a metabolic chamber that was not darkened. The metabolic rates during our measurements represent the average energy consumption during five minutes of rest.

Although the birds had been quietly sitting for at least 10 minutes before this period, our data do not necessarily represent minimum evaporation and metabolic rate during the rest phase. It was not possible, however, to standardize our measurements further. Still, we consider these data as representative for an average resting phase in a bird’s day.

CHAPTER 3

Metabolic costs of avian flight in relation to flight velocity: a study in Rose Coloured Starlings (*Sturnus roseus*)

Sophia Engel, Herbert Biebach, and G. Henk Visser

Abstract

The metabolic costs of flight at a natural range of speeds were investigated in Rose Coloured Starlings (*Sturnus roseus*) using doubly labelled water. Eight birds flew repeatedly and unrestrained for bouts of six hours at speeds from 9 to 14 m s^{-1} in a low-turbulence wind tunnel, corresponding to travel distances between 200 and 300 km, respectively. This represents the widest speed range where we could obtain voluntarily sustained flights. From a subset of these flights data on the wing beat frequency and intermittent flight behaviour were obtained. Over the range of speeds that were tested, flight costs did not change with velocity and were on average 8.17 ± 0.64 W. Body mass was the only parameter with a significant (positive) effect on flight costs, which can be described as $EE_f = 0.741\ M^{0.554}$. Wing beat frequency changed slightly with speed, but correlated better with body mass. Birds showed both types of intermittent flight, undulating and bounding, but their frequencies did not systematically change with flight speed.

Introduction

Millions of birds migrate twice a year between their wintering quarters and breeding grounds. They cover thousands of kilometres in a relatively short period of time. Avian flight is among the energetically most expensive forms of behaviour (Schmidt-Nielsen 1972, Norberg 1990). Precise assessment of the energetic costs of flight is important for the estimation of daily energy expenditure (Bryant 1997) and for the calculation of potential flight ranges (Gudmundsson et al. 1991, Biebach 1992, Klaassen 1995). Interspecific allometric relationships allow us a first rough estimation of flight costs for any bird species on the basis of body mass (Schmidt-Nielsen 1972, Kendeigh et al. 1977, Butler 1980, Masman and Klaassen 1987, Butler 1991). Given the variance around the regressions, however, they offer no more than an approximation. They can not explain intraspecific variation in flight costs using body mass as a predictor (Kvist et al. 2001, Hambly et al. 2004a), nor do these relationships consider ambient conditions that influence flight performance and flight costs. The relationship of flight costs to flight speed is of particular interest and has marked ecological implications: optimum flight speed (Hedenström and Alerstam 1995) as well as optimal migration strategies (Hedenström and Alerstam 1997, Weber et al. 1998) depend heavily on trading off flight costs with other factors, such as time constraints.

Different methods for estimating metabolic power (P_{met}) during flight have been developed, each with particular advantages as well as drawbacks. Mask respirometry is the only direct way to measure the rate of gas exchange, allowing the calculation of P_{met}, and has been widely used in wind tunnel studies (for a review see Butler and Woakes 1990). However, while precise, this technique has the disadvantage of overestimating the costs for unrestrained flight by 3-30 % due to the drag of the mask and the tubing (Tucker 1972, Rothe et al. 1987, Ward et al. 2001, Ward et al. 2002). Flight costs have also been calculated from mass loss rates (Kvist et al. 1998). This method probably imposes the least stress on the birds and is the easiest to apply, when compared with other methods, but the results depend on accurate estimates of fuel composition and evaporation rates (Butler et al. 1998, Kvist et al. 1998, Battley et al. 2000, Klaassen et al. 2000). Heart rate measurements can potentially be used as indicator for metabolic rate, having the advantage of a very high temporal resolution which allows the monitoring of instantaneous changes (*e.g.* at the onset of flight). This method requires careful calibration of heart rate against metabolic rate which is not readily available for many species (Butler et al. 1998, Weimerskirch et al. 2001, Ward et al. 2002). Infrared thermography can be used to estimate heat transfer by radiation (Ward et al. 1999), and therefrom metabolic power requirements in flying birds (Ward et al. 2004), making use of the fact that only a relatively small proportion of the metabolic power is converted into muscle work, the major proportion being lost as heat (Hill 1938). The method therefore is comparably insensitive to errors in the assumed efficiency at which metabolic power is converted into muscle work. Fractional turnover rates of stable isotopes can be used to calculate energy metabolism, either for short time scales with labelled bicarbonate (Hambly et al. 2004a, Hambly et al. 2004b) or for flights of long duration with doubly labelled water (DLW, Speakman 1997, Kvist et al. 2001, Wikelski et al. 2003, Ward et al. 2004). The DLW technique determines CO_2 production, and therefrom energy expenditure, from blood samples before and after a flight. It has the advantage of not restraining the birds with measuring devices. This allows the investigation of long flight durations that might otherwise be difficult to obtain in a wind tunnel. On the other hand, these long flight durations are also required for a satisfactory accuracy of the method.

In principle, metabolic power (P_{met}) can be calculated from mechanical power (P_{mech}) assuming the efficiency with which an animal performs the mechanical work required for flight. Under the assumption that flight muscle efficiency is a fixed value, the metabolic power curve should approximate a fixed multiple of P_{mech} as a function of speed (Tucker 1973, Pennycuick 1989, Norberg 1996). Direct measurements of mechanical power have been made by Dial et al. (1997) and Tobalske (2003), but often P_{mech} is calculated from aerodynamic models (Tucker 1973, Greenewalt 1975, Pennycuick 1975, Rayner 1979a, Rayner 1979b, Pennycuick 1989, Rayner 1999). These models generally predict a U-shaped dependence of P_{mech} on flight speed, with high power demands at both low and high velocities and lowest power requirement at intermediate speeds. In a U-shaped relationship of metabolic power to velocity two characteristic speeds are of particular ecological significance: minimum power speed (V_{mp} [m s^{-1}]) and maximum range speed (V_{mr} [m s^{-1}]). V_{mp} is the speed that requires minimal energetic costs per unit time, whereas V_{mr} is the speed that requires minimal costs per unit of distance covered (Pennycuick 1989). Migrating birds are assumed to chose V_{mp} to minimize the instantaneous flight costs, or V_{mr} to maximise the distance that can be flown with a given fuel budget. The optimal solution depends on whether migration is energy- or time-constrained (Hedenström and Alerstam 1995).

Actual measurements of metabolic rates during flight at different speeds are equivocal and result in curves that are either U-shaped (Tucker 1973) more J-shaped (Berger 1985, Ward et al. 2001, Ward et al. 2004), L-shaped (Dial et al. 1997) or even flat (Torre-Bueno and Larochelle 1978, Ward et al. 2002). Despite the number of studies, we still lack a clear picture of how flight speed affects the energetic costs of flight. The duration of these experimental flights has usually been short (mostly up to one hour). However, measurements of the energetic costs of very short flights resulted in metabolic rates up to three times higher than expected from allometric predictions (Westerterp and Drent 1985, Tatner and Bryant 1986, Nudds and Bryant 2000). The take-off and the starting phase of a flight is powered by carbohydrate rich fuel (George and Berger 1966, Rothe et al. 1987), followed by a gradual transition to lipid combustion, that contributes approximately 93-96 % of total energy expenditure during endurance flight (Jenni and Jenni-Eiermann 1998). It takes about 1-2 hours of continuous flight until this metabolic switch is completed (Tucker 1972, Rothe et al. 1987, Schwilch et al. 1996, Jenni-Eiermann et al. 2002). Only very few measurements of metabolic costs during wind tunnel flights of at least one hour duration are available (Torre-Bueno and Larochelle 1978 [1.5 h], Klaassen et al. 2000 [12 h], Kvist et al. 2001 [6 to 10 h], Ward et al. 2004 [two times 1 h]). Therefore, to assess flight costs and the power-to-speed-relationships that are relevant in the context of long-distance migration, measurements of P_{met} during longer flight periods of different species are clearly needed.

For our study species, the Rose Coloured Starling (*Sturnus roseus*), a long distance migrant, theoretical calculations (Penncuick, 1989, "flight", version 1.10) predict a metabolic power curve that is relatively flat with flight costs between 5.9 W at V_{mp} (10.1 m s^{-1}) and 7.5 W at V_{mr} (17.8 m s^{-1}). Using stable isotopes (DLW), we measured the metabolic costs of sustained (6 h) voluntary flights under controlled conditions in a low-turbulence wind tunnel at the widest range of flight speeds that the birds accepted for prolonged periods of flight. The aim of this study was to measure flight costs during prolonged flights in a migratory species and to test whether the predicted relationship between energetic costs of flight and flight speed holds true, and is relevant for long distance flight.

Materials and methods

Wind tunnel

Experimental flights were conducted in a wind tunnel situated in the Max Planck Institute for Ornithology in Seewiesen, Germany. This closed circuit wind tunnel closely resembles in design and technical performance data the wind tunnel in Lund, Sweden (Pennycuick et al. 1997). Air speed can be set from 0 up to 45 m s^{-1} with an accuracy of 0.1 m s^{-1} and is monitored and recorded with an accuracy of 0.001 m s^{-1}. We display and record the "equivalent" air speed (Pennycuick et al. 1997), which takes into account changes in air density that determine the forces on the wings and body of a bird. This ensures that experimental conditions at a certain velocity are similar for all birds.

The flight chamber is 2 m long and 1.2 m wide and is made of transparent plastic and glass to allow continuous observation of the birds. Wind speed is constant within 2 % of the mean in the horizontal and 1.5 % in the vertical. Birds were prevented from leaving the flight chamber upstream by a fine net, made of braided nylon cord, 0.25 mm in diameter, with a square mesh 18 x 18 mm, fixed to the wind tunnel wall with adhesive tape. Downstream, ca. 1.5 m from the test section, there was a net made of braided nylon cord, 1.0 mm in diameter, with a mesh of 12 x 12 mm in a wooden frame.

Birds, housing and training

Rose Coloured Starlings have a breeding distribution from central Asia to the Balkans and their wintering quarter in northern India. They are long distance migrants and cover their migration route mostly during day time flights. In the wild, Rose Coloured Starlings feed mainly on insects and fruit, foraging mostly on the ground where they hunt for grasshoppers and other prey (Hudde 1993).

One of the most westerly regular breeding colonies is found on Crimea peninsula (Ukraine). This population makes a flight to and from India of at least 5000 km twice a year, and the birds thus should be capable of long periods of flight in a wind tunnel. We used eight Rose Coloured Starlings in our experiments. Four of these were purchased in 1999 as nestlings from a breeder and were adult at the time of the experiments, the others were taken in June 2001 as nestlings from a breeding colony on the Crimea peninsula and were juvenile but fully grown during the experimental phase. All birds were hand-raised in our institute. The four adult birds had been flying in the wind tunnel before and were ready for the experiments after three weeks of regular training. The juvenile birds were given regular training flights 8 weeks prior to the experiments. During the last two weeks prior to the experiments, all birds were trained daily for periods of 10 to 120 minutes at variable wind speeds, so that a total of 6 hours of training per week was achieved.

The range of flight speeds was chosen such, that the birds could sustain flights for prolonged periods of time. Minimum training speed was 8 m s^{-1} and maximum speed was 14 m s^{-1}, the widest range that the birds accepted. Attempts to fly the birds at faster or slower velocities failed because after a short flight duration of mostly only a few minutes, the birds landed frequently and would not stay aloft even with continuous chasing. At very fast flight speeds (above 14 m s^{-1}) the birds seemed to struggle against the wind and sometimes were blown against the rear net.

Our birds flew more steadily and with fewer attempts to land when they were led into the wind tunnel in groups. We therefore gave them training flights either in pairs or in groups of three. The birds changed their position within the group frequently and there was no indication of them using any particular flight formation that might save energy. None of the birds would fly systematically close to the floor or ceiling of the wind tunnel. Therefore, boundary layer effects (Rayner 1994) are unlikely.

The birds were housed in groups of three and four in aviaries (ca. 1x2x2 m) adjacent to the wind tunnel, and were given standard food consisting of insects, heart, rusk and egg *ad libitum*, supplemented with minerals and vitamins (AviConcept®), some mealworms and fresh fruit. They had access to fresh water and the opportunity to bathe at any time. The light schedule maintained during the year simulated natural conditions for the birds: During winter they had the photoperiod of their wintering quarters in northern India (27.5° N). In spring it changed gradually to the natural light conditions of the location of our institute (47.5° N, corresponding with their natural breeding grounds) during summer and in the autumn back again. This kept the birds in their annual rhythm and in migratory disposition during the experimental phase, indicated by prior fattening and by increased willingness to fly. All birds were in good condition and had intact flight feathers during the entire experiment.

Wing area and wing load (body mass divided by wing area) have a major impact on aerodynamic capabilities of a bird (Pennycuick 1989). We therefore measured the wing area of each individual prior to the experimental period by spreading one wing sideways to its fullest extent and tracing its outline on a paper with a 5 mm square grid. We then counted the squares, multiplied by two and added the approximate area of the part of the body between the wings, following the method of Pennycuick (1989).

Experimental flights

Eight Rose Coloured Starlings flew repeatedly (two to six times) at different wind speeds. Individuals had no more than one experimental flight per week. In total, 27 flights were performed successfully.

All experimental flights were conducted during migration periods, which were from March to May and from October to December 2001. The date of each experiment was included into the statistical tests as day number, starting with the first experimental flight per season as day 1. Ambient conditions were recorded automatically by a data monitoring system (ARGUS® by Sorcus) with a sampling rate of 1 Hz. Ambient temperature was kept constant at 15.0 °C during all flights. Air pressure and humidity could not be controlled for. Mean air pressure during experiments ranged from 919.0 mbar to 957.5 mbar with an average of 938.8 mbar, and relative humidity was on average 60.65 %, ranging from 48.4 to 76.8 %.

Equivalent air speed changed between flights and ranged from 9 to 14 m s^{-1} during the successful flights, with an intra-experiment standard deviation (calculated over all measurements taken at 1 Hz sampling rate) below 0.12 m s^{-1}. The succession of air speeds was chosen randomly in order to level possible seasonal effects on the measurements.

Birds were flying in pairs during the experiments. Usually, they had been food-deprived since the evening before to have them in a postabsorptive state during the flights. In eight cases flights were conducted without removal of their food the evening before. In these cases, the birds then had access to the remaining food from lights on until the beginning of the experiment at 7 a.m., *i.e.*, for an episode lasting between 60 and 90 min. Feeding during this time might have had a minor effect on the energy measurements through a somewhat longer transition period from carbohydrate combustion to fat metabolism at the beginning of the flight (Rothe et al. 1987).

Body mass was determined directly before the flight to the nearest 0.01 g. Flight behaviour was recorded by an observer during all flights. The observer also prevented the experimental birds from landing or from leaving the wind tunnel. In most cases the mere attendance of the observer was sufficient for this. Sometimes, however, a bird decided to land frequently within a short period of time, in which case the observer waved at the bird to prevent it from landing. If an individual showed signs of exhaustion it was given a rest or was taken out of the experiment. We took it as an indicator for exhaustion if a bird landed frequently, if it refused to start flying again, or if it was flying close to the exit of the wind tunnel, trying to escape. A resting episode occurred in four flights and lasted between 15 and 33 min. Only flights with an apparently natural flight behaviour were included in the analysis: a total of 27 flights, lasting on average 5.8 hours (range 4.3 - 6.3 h, SD = 0.5 h).

Energy expenditure

Energy expenditure was measured using the doubly labelled water (DLW) method (Lifson and McClintock 1966, Speakman 1997, Kvist et al. 2001, Ward et al. 2004). After a first blood sample was taken (henceforth referred to as sample 0) for the determination of the background concentration of the isotopes, the birds were injected intraperitoneally with about 0.2 g of a DLW mixture (exact quantity assessed by weighing the syringe before and after administration with a 4-decimal balance; ^{2}H and ^{18}O enrichments of the dose were 30, and 60 atom percent, respectively). An equilibration period of one hour allowed the isotopes to mix completely with the bird's body water pool, before the initial blood sample (sample 1) was taken and the bird released into the wind tunnel for the flight. After six hours of flight another blood sample (sample 2) was taken and the bird was subsequently reinjected with about 0.1 g of DLW (the quantity of the dose also assessed gravimetrically). A last blood sample (sample 3) was taken one hour after administration of the second dose. This sampling protocol enabled us to calculate energy expenditure and to monitor changes in the sizes of the body water pool in each bird during the flight. The blood samples were always taken from the jugular vein to avoid haematoma at the brachial vein, which could disturb the bird during flight. Per sampling period, a total of about 75 microlitres of blood was taken, which was subdivided over about 5 microcapillary tubes which were flame-sealed immediately, and immediately stored at 5 °C for isotope analysis at the Centre for Isotope Research in Groningen, Netherlands. The isotope analyses were done blindly, without knowledge of the corresponding flight speed. Samples were analysed in triplicate (background samples), or quadruplicate (all other samples).

The general procedure for analysis of ^{18}O and ^{2}H determination has been described in detail by Visser and Schekkerman (1999), and for wind tunnel measurements in particular by Kvist et al. (2001). Briefly, for the determination of isotope enrichment, the blood from one was distilled in a vacuum line. Thereafter, the water vapour was cryogenically trapped in a quartz tube using liquid air. ^{18}O and ^{2}H enrichments were determined with the CO_2 equilibration method and the uranium reduction method, respectively (Speakman 1997). To obtain the carbon dioxide gas for isotope ratio mass spectrometry, 2 ml of CO_2 gas was added quantitatively to the distilled water sample to equilibrate for at least 48 h in a water bath at 25.0 °C. The vial with water and equilibrated CO_2 gas was placed then in a dewer with a dry ice-ethanol mixture (to keep the distilled water frozen) and connected to the vacuum line to trap the CO_2 gas cryogenically in another quartz vial positioned in liquid air. The remaining water was reduced in an uranium oven at 800 °C, and the H_2 gas was cryogenically trapped in a quartz vial with active charcoal, using liquid air. At the end of the transfer process, the pressure of the H_2 gas was assessed to determine the amount of water vapour of the original distilled water sample. The $^{2}H/^{1}H$ and $^{18}O/^{16}O$ isotope ratios of the H_2 and CO_2 gas were determined with a SIRA 9 isotope ratio mass spectrometer (IRMS). During the sample preparation internal water standards that covered the entire enrichment range of the blood samples were applied. In addition, the IRMS was calibrated daily with internal gas standards for ^{2}H and ^{18}O at low and high enrichments. To verify the isotope enrichment of the original DLW mixture, a dilution was made with tap water (with known isotope enrichments) which was analysed in the same batches as the blood samples.

For each bird, the average initial and final enrichment of both isotopes was determined. From this, fractional turnover rates per day for ^{2}H and ^{18}O (abbreviated as k_d [d^{-1}] and k_o [d^{-1}], respectively) were calculated:

$$k_d = [\ln(C_{iH} - C_{bH}) - \ln(C_{fH} - C_{bH})] / t \qquad \text{(eqn. 3.1)}$$

$$k_o = [\ln(C_{iO} - C_{bO}) - \ln(C_{fO} - C_{bO})] / t \qquad \text{(eqn. 3.2)}$$

where C_{bH} and C_{bO} represent the average background concentrations of samples 0 for ^{2}H and ^{18}O, respectively [atom percent], C_{iH} and C_{iO} the average ^{2}H and ^{18}O concentrations of blood sample 1, and C_{fH} and C_{fO} the average ^{2}H and ^{18}O concentrations of sample 2, and t the elapsed time-interval between taking these samples.

The body water pool prior and after each flight was determined from the samples 0 and 1, and 2 and 3, respectively on the basis of ^{18}O dilution with the plateau method (Visser et al. 2000). These results will be described in chapter 4.

The rate of CO_2 production (rCO_2, [l d^{-1}]) was calculated using the following equation:

$$rCO_2 = 22.4 \cdot [N/2.078 \cdot (k_o - k_d) - rG \cdot 0.0249 \cdot WP \cdot k_d] \qquad \text{(eqn. 3.3)}$$

where WP stands for the average size of the body water pool during the measurement [mol]. The factor of 22.4 converts the volumes from mol to litre. k_d and k_o are calculated with eqns. 1 and 2, respectively. The parameter rG is the assumed fraction of water flux lost through evaporative pathways, here taken as 0.5 (Lifson and McClintock 1966). As a last step, the calculated rate of carbon dioxide production was converted to energy expenditure with a conversion factor of 27.8 kJ l^{-1} (Schmidt-Nielsen 1997), assuming that 4 % of the energy was derived from protein and 96 % from fat. To obtain values for flight costs, total energy expenditure values during the 6 hour measurement periods were corrected for resting and handling phases by subtracting the resting metabolism during the active phase, calculated according to Aschoff and Pohl (1970). These phases had a mean duration of 26 min and accounted for less than 0.5 % of the total energy expenditure (see also Kvist et al. 2001). Only the corrected value, energy expenditure during flight (*EEf* [W]), is used for further analysis.

Wing beat frequency and intermittent flight behaviour

Wing beat frequency (WBF [Hz]) and intermittent flight behaviour were analysed for a subset of 14 flights by six individuals at speeds ranging from 9 m s^{-1} to 13 m s^{-1}, all of which took place in autumn 2001. These flights were video-taped with a small highly light sensitive monochrome camera (Watec WAT-902H) installed upstream of the flight section in the settling chamber of the wind tunnel and connected to a video recorder. The recording rate of the videos was 25 frames s^{-1}. Filming started at time zero, when the bird was released into the wind tunnel, lasted for 10 min and was repeated at one hour intervals. Within these video sequences WBF was visually evaluated by counting wing beats from periods of stable flight at a fixed position that were not interrupted by gliding and lasted at least 10 s. For every hour of flight, the mean WBF of 30 s of flapping was calculated.

We distinguished between two forms of intermittent flight, undulating and bounding. Undulating is characterised by gliding phases of up to one second with the wings kept spread out, while bounding involves short ballistic phases, usually of less than 0.25 s duration, characterised by folded wings and a torpedo-like body posture. The durations [s] and frequencies [gliding bouts min^{-1}] of gliding or ballistic phases were analysed from the video tapes during the first five minutes of each sequence.

Statistical analysis

Results are presented as means ± 1 standard deviation (SD). Flight energy expenditure (EE_f [W]), wing beat frequency (WBF [Hz]), undulating and bounding (frequency [bouts min^{-1}] and gliding duration [s]) were analysed separately. For all four parameters, we used mixed models with random intercept in SAS 8.02 (SAS Institute Inc.). This model allowed the analysis of repeated measurements on the same individual (*e.g.* at different wind speeds), assigning the same regression slope of the considered relationship, but different intercepts to the different individuals. Parameters that were included into the models for both, linear and quadratic effect (speed and day number) were, in a second step, combined in a contrast. Here, we calculated the overall significance of the parameter by testing with an F-test the Null-hypothesis that either the linear or the quadratic effect is not different from zero. When random intercepts were estimated as zero (*i.e.*, the individuals' intercepts did not differ), the general linear model (GLM) procedure was chosen. ANOVAs were performed in SPSS 12.01 (SPSS Inc.). Significance was accepted at $p \leq 0.05$ (two-tailed).

Results

Energy expenditure

The energy expenditure of eight Rose Coloured Starlings during 27 flights was on average 8.17 ± 0.64 W (N = 8) (Fig. 3.1A). To test whether the observed variance could be explained by changes in flight speed or by other parameters we applied the mixed models procedure with random intercept. For $\log_{10}(EE_f)$ [W] as dependent variable we introduced speed (average: 11.19 m s^{-1}; range: 8.95 to 13.91) and speed^2 as independent variable, generation and season (spring or autumn) as fixed factor and $\log_{10}(M)$ (average body mass: 71.6 g; range: 56.14 to 86.50), day number (starting with the first experiment per season as day 1), day number^2, WBF (average: 9.78 Hz; range: 8.91 to 10.45) and wing area (average: 0.021 m^2 ; range: 0.019 to

0.023) as covariates. Parameters that did not significantly contribute to the explained variance were excluded stepwise, except speed and speed^2 because this was our manipulated variable and model calculations predicted a dependence of flight costs on speed. The final model (table 3.1A) contained speed, speed^2 and $\log_{10}(M)$.

It turned out that flight costs were not correlated with speed, either in a quadratic or in a linear relationship ($F_{2,16} = 1.50$; $p > 0.25$). The effect of body mass, however, contributed highly significantly (coefficient = 0.523; S.E. = 0.110; $t = 4.77$; $p < 0.001$) to the explained variation in flight costs. No other significant parameters were found. This means that the relationship between body mass and flight costs is the same for all individuals.

A mixed model with only $\log_{10}(M)$ as predictor, ignoring all other parameters that might have an effect on flight costs, scales body mass to EE_f as

$$\log_{10}(EE_f) = 0.554 \log_{10}(M) - 0.130 \qquad \text{(eqn. 3.4), or}$$

$$EE_f = 0.741 \cdot M^{0.554} \qquad \text{(eqn. 3.5),}$$

see also Fig. 3.1B. To visualise how far the results presented in Fig. 3.1A were obscured by body mass differences, we recalculated the data with a partial correlation that corrected the EE_f measurements to the average body mass during all flights (Fig. 3.1C). We therefore plotted $\log_{10}(EE_f)$ against $\log_{10}(M)$ and calculated a linear regression that described best the relationship between both variables (Fig. 3.1B). The residual of each data point to the regression was calculated. We then applied a correction that tilted the regression line around the average body mass (71.6 g) to the horizontal, but maintained the residual of each data point to this corrected regression that no longer shows any correlation with body mass.

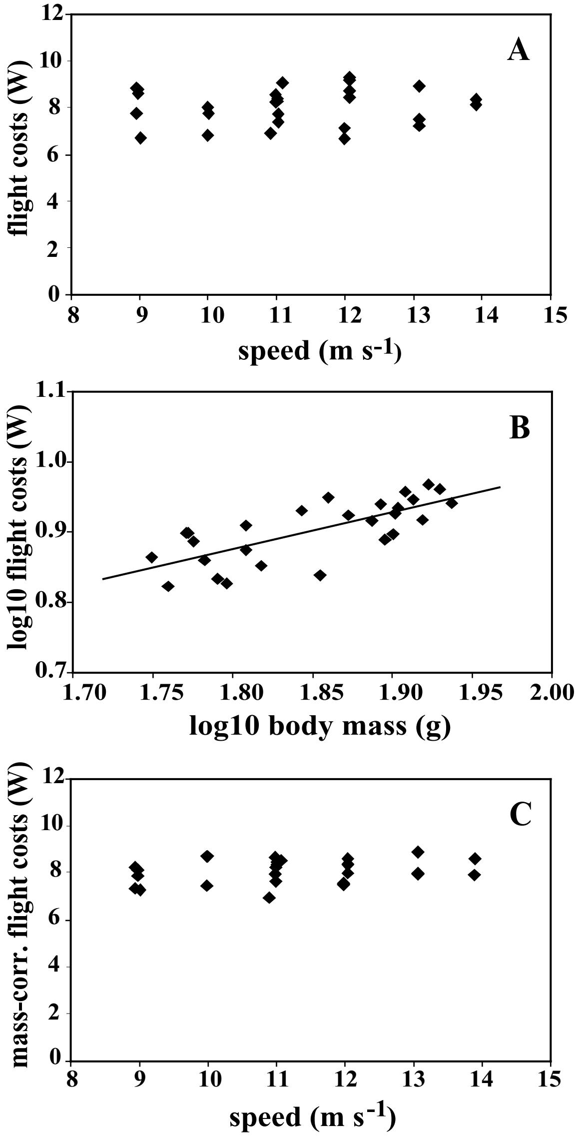

Fig. 3.1 Variation in energy costs of flight in the Rose Coloured Starling **(A)** during voluntary prolonged flights (N = 27 flights by eight individuals) of six hours duration at various flight speeds and **(B)** in relation to natural changes in body mass in the same experimental flights. Flight costs scale to body mass to the power of 0.55 (eqn. 3.4). **(C)** Mass-corrected relationship (see text) of flight speed and energy expenditure. Flight costs are normalized to the average body mass during all flights (71.6 g).

Wing beat frequency

The average wing beat frequency (WBF) during a subset of 14 flights filmed in six individuals was 9.78 ± 0.39 Hz (N = 6) (Fig. 3.2A). To test whether speed was a predictor for WBF we applied the mixed model procedure and included the average WBF per flight as dependent variable; speed (average: 10.88 m s^{-1}; range: 8.95 to 13.08), $speed^2$, as independent variables; and $\log_{10}(M)$ (average body mass: 77.15 g; range: 56.14 to 86.50), day number (starting with the first experiment per season as day 1), day $number^2$ and wing area (average: 0.021 m^2; range: 0.019 to 0.023) as covariates. Parameters that did not significantly contribute to the explained variance were excluded stepwise, except our manipulated variable speed (and $speed^2$) until the final model (table 3.1B) contained speed, $speed^2$, $\log_{10}(M)$, day number and day $number^2$.

Of all variables, body mass had the most pronounced effect on WBF ($\log_{10}(M)$: regression coefficient = 11.367; S.E. = 2.271; t = 5.01; $p < 0.02$). There was a small but significant effect of speed on WBF ($F_{2,3} = 10.41$; $p < 0.05$), both linear (coefficient = -1.330; S.E. = 0.298) and quadratic (coefficient = 0.062; S.E. = 0.014), which results in an overall decrease of WBF with increasing flight speed in the range of speeds that was measured. Day number had an effect on WBF as well ($F_{2,3} = 10.74$; $p < 0.05$), but the effect was very small (coefficient of day number = -0.023; S.E. = 0.005; coefficient of day $number^2$ = 0.001; S.E. = 0.000). Given the effect of body mass on WBF, one would expect WBF to decrease over flight duration due to body mass loss. This was tested with a One Way ANOVA with WBF [Hz] as dependent variable and flight time [h] as independent variable. WBF did not change systematically over time (ANOVA; $F_1 = 0.005$; $p > 0.94$).

For comparative purposes we isolated the effect of body mass on WBF (Fig. 3.2B) by applying a mixed model with only $\log_{10}(M)$ as predictor, ignoring all other parameters that might have an effect. This scales body mass to WBF as

$$\mathrm{WBF} = 2.504 \cdot \log_{10}(M) + 4.986 \qquad \text{(eqn. 3.6)}$$

With an average body mass loss of 5.08 g (range: 3.72 to 8.13) during an experimental flight, the predicted decrease in WBF would be 0.1 Hz at the most. This lies within the standard deviation of the measurements and can not be detected in a statistical test. To visualise how far the results presented in Fig. 3.2A were obscured by body mass differences, we recalculated the data with a partial correlation (see above) that corrected the WBF measurements to the average body mass during all flights (Fig. 3.2C).

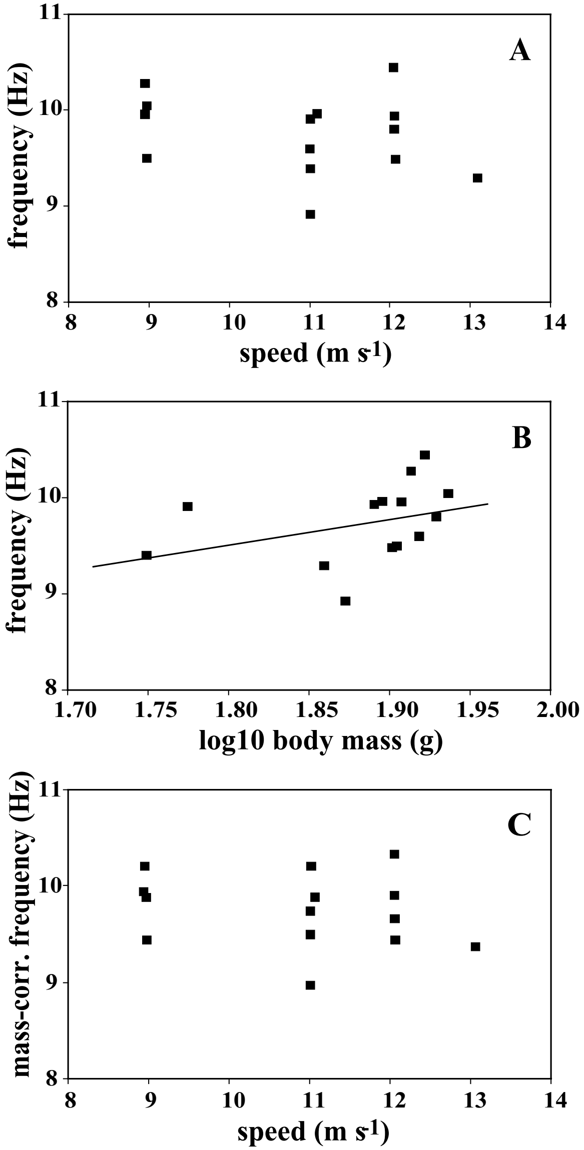

Fig. 3.2 Variation in wing beat frequency (WBF) in the Rose Coloured Starling **(A)** during voluntary prolonged flights (N = 14 flights by six individuals) of six hours duration at various flight speeds and **(B)** in relation to natural changes in body mass in the same experimental flights. Drawn line represents least squares regression (eqn. 3.6; see text). **(C)** Mass-corrected relationship (see text) of flight speed and WBF, normalized to the average body mass during these flights (77.15 g).

Gliding phases

Intermittent flight behaviour was evaluated from the same subset of flights analysed for WBF. Gliding durations are based on the number of video frames each gliding bout lasted. The first measurements of “B” (number of gliding bouts, [bouts min^{-1}]), “D” (mean duration per bout [s]) and ”G” (total duration of gliding per sample $= N \cdot D$ [s]), being made during the first minutes of flight, appeared unstable and variable and were therefore excluded from the analysis.

From the second measurement onwards, *i.e.*, after one hour of flight, we checked for changes within a flight. There was no significant change over flight duration (ANOVA; $p > 0.05$). We therefore took the mean of the following G samplings (duration of gliding per sampling) of undulating or bounding, respectively, as dependent variable and introduced speed, speed^2, $\log_{10}(M)$, day number, day number^2, wing area, and WBF as covariates. Parameters that did not contribute significantly to the explained variance were excluded stepwise. For undulating, the random intercept was estimated as zero (*i.e.*, individuals did not differ in their intercepts), therefore the final model was performed with the GLM-procedure (table 3.1C) and contained speed, speed^2, day number and day number^2. For bounding, the final model was calculated with the mixed models procedure and contained speed, speed^2 $\log_{10}(M)$, day number, day number^2 and wing area (table 3.1D).

Undulating Starting after one hour of flight, gliding events in undulating flight occurred with an average frequency (B_U) of 2.95 ± 2.70 bouts min^{-1} and an average duration (D_U) of 0.06 ± 0.03 s. The duration of gliding per 5 min sampling interval was 6.12 ± 6.82 s, which corresponds to a fraction of 2.0 % of flight time. There was no significant change in B_U, D_U or G_U over flight time (ANOVA; $F(B_U)_1 = 1.48$; $p > 0.05$; $F(D_U)_1 = 0.00$; $p > 0.05$; $F(G_U)_1 = 0.86$; $p > 0.05$). An analysis of covariance with the GLM procedure revealed that none of the parameters had a significant effect on gliding duration G_U during undulating flight.

Bounding Starting after one hour of flight, ballistic phases in bounding flight occurred with an overall average frequency (B_B) of 1.70 ± 2.93 bouts min^{-1}. The average duration (D_B) was 0.01 ± 0.01 s, summing up to a mean duration per sampling interval of five minutes (G_B) of 0.58 ± 0.90 s, which corresponds to a fraction of 0.2 % of flight time. B_B, D_B and G_B did not change with flight time (ANOVA; F(BB)1 = 1.54; $p > 0.05$; $F(D_B)_1 = 0.28$; $p > 0.05$; $F(G_B)_1 = 0.00$; $p > 0.05$). Applying a mixed model with random intercept, we found no effect of any parameter on the proportion of bounding per sampling interval.

Table 3.1 Parameters describing the variation in **(A)** flight energy expenditure ($\log_{10}(EEf)$), **(B)** wing beat frequency (WBF), **(C)** the duration of gliding in undulating or **(D)** bounding flight. Effect estimates were calculated using mixed models with random intercept (**A, B, D**) or a general linear model (**C**). Significance was accepted if $p \leq 0.05$ and is indicated with an asterisk. For parameters that were tested for both, linear and quadratic effect ("speed" and "day number") significance was tested with F-tests in a contrast that combined linear and quadratic effects

	Parameter	estimate	S.E.	t-value	F-value	df	significance	
(A)	random intercept	-0.08	0.36	-0.24			n.s.	
	speed	-0.003	0.048					
	speed^2	0.0004	0.002					
	speed contrast				1.5	2,16	n.s.	
	$\log_{10}(M)$	0.523	0.109	4.77			< 0.001	*
(B)	random intercept	-4.8	4.5	-1.08			n.s.	
	speed	-1.33	0.30					
	speed^2	0.06	0.01					
	speed contrast				10.41	2,3	< 0.05	*
	$\log_{10}(M)$	11.4	2.3	5.01			< 0.02	*
	day number	-0.02	0.01					
	(day number)^2	0.0007	0.000					
	day number contrast				10.74	2,3	< 0.05	*
(C)	intercept	-6795	3359	-2.02			n.s.	
	speed	1298	636					
	speed^2	-59.7	29.7					
	speed contrast				2.26	2,3	n.s.	
(D)	random intercept	1160	1073	1.08			n.s.	
	speed	70	1073					
	speed^2	-3.27	3.40					
	speed contrast				0.49	2,3	n.s.	
	$\log_{10}(M)$	-723	485	-1.49			n.s.	
	day number	2.13	1.04					
	(day number)^2	-0.05	0.03					
	day number contrast				2.08	2,3	n.s.	
	wingarea	6836	8080	-0.85			n.s.	

Table 3.2 Comparison of flight costs (P_{met}) of the Rose Coloured Starling (this study) with measurements of P_{met} in the European Starling using different methods: mask respirometry (Ward et al. 2001), wind tunnel respirometry (Torre-Bueno and Larochelle 1978), labelled bicarbonate (C^{13}; Hambly et al. 2004b) and doubly labelled water (DLW; Ward et al. 2004, Westerterp and Drent 1985). DLW was used in combination with time-energy budgets that allowed an extrapolation to 100% flight time (Westerterp and Drent 1985), with indirect calorimetry in a metabolic chamber for the time that was not spent flying (Ward et al. 2004) and as sole method (this study). The study marked with one asterisk allowed the calculation of P_{met} for M = 71.6 g, the average body mass during our experiments; flight time was estimated from flight distance: "short flights" = 7 m; "long flights" = 10-30 km. The studies marked with two asterisks allowed the calculation of flight costs for a flight speed of 11 m s^{-1}, the average flight speed during our experiments.

Author	Speed [m s^{-1}]	Average P_{met} [W]	Range P_{met} [W]	Average M [g]	Flight duration [min]	Method
Hambly et al. (2004b)		20.6	16.5−23.1	71.1	< 1	^{13}C
Westerterp and Drent (1985) *		31.9	15.0−48.9	71.6	< 1	DLW, time energy budget
Westerterp and Drent (1985) *	10	9.9	9.7−10.2	71.6	10	DLW, time energy budget
Ward et al. (2001) **	11	11.0	10.4−14.9	85.5	12	Mask respirometry
Ward et al. (2004) **	11	11.4	7.5−14.0		30	Thermography
Ward et al. (2004) **	11	10.3	5−19	82.0	2 x 60	DLW, metabolic chamber
Torre-Bueno and Larochelle (1978)	11	8.9	8−10	72.8	90	Wind tunnel respirometry
This study	11	8.2	6.7−9.3	71.6	360	DLW

Discussion

Average flight costs

The quantification of avian flight costs has been approached from empirical perspectives (Schmidt-Nielsen 1972, Kendeigh et al. 1977, Torre-Bueno and Larochelle 1978, Butler 1980, Masman and Klaassen 1987, Butler 1991, Ward et al. 2001, Hambly et al. 2004b, Ward et al. 2004) and theoretical (Tucker 1973, Greenewalt 1975, Pennycuick 1975, Rayner 1979a, Pennycuick 1989, Norberg 1996, Rayner 1999). We are not aware of any published measurements of flight costs in the Rose Coloured Starling that could be used for comparison with our results, but there are several studies on European Starlings (table 3.2). This species is closely related to the Rose Coloured Starling and similar in size and weight, but does not exhibit long-distance migration. Estimates of P_{met} during flights of more than 10 minutes duration for the European Starling lie in the range of 9 - 11 W. Our measurements of 8.2 W are in accordance with these, given the slightly lower body mass of our birds. A direct comparison of different methods (mask respirometry, thermography and DLW) in the same individuals showed that the results for P_{met} do not differ, although it appeared that the DLW method had a lower discriminative power than the other methods (Ward et al. 2004). Because of the high metabolic rates during flight, resulting in a high k_o / k_d ratio of 2.9 (see also Kvist et al. 2001, and Ward et al. 2004), combined with a long flight duration in our experiments (6 h), resulting in large differences between ^{2}H and ^{18}O concentrations, we could increase the discriminative power of our measurements. Therefore, DLW-based errors in our wind tunnel study are at the same level as for measurements on daily energy expenditure in free living Rose Coloured Starlings during the breeding season (Schmidt-Wellenburg, unpublished data). It was calculated that a 2 % error in either ^{2}H or ^{18}O resulted in a 3 % error in the estimate for flight costs. Given the fact that all isotope analyses were executed in quadruplicate we expect the average uncertainty in our data to be less than 10 %. Flight costs of very short flights as those studied by Hambly et al. (2004b) or Westerterp and Drent (1985) are substantially higher than those of longer flights (see table 3.2). This observation has been made also for other species (Tatner and Bryant 1986, Nudds and Bryant 2000) and stresses the importance of steady state measurements during prolonged flights. Take-off and the starting phase of a flight is powered by carbohydrate rich fuel (George and Berger 1966, Rothe et al. 1987), while endurance flight is fuelled mainly by lipids (Jenni and Jenni-Eiermann 1998). The metabolic switch is completed after about 1-2 hours of continuous flight (Tucker 1972, Hudson and Bernstein 1983, Rothe et al. 1987, Schwilch et al. 1996, Jenni-Eiermann et al. 2002).

The DLW method gives no estimate for instantaneous metabolic rate but an average over the whole measurement period. Long flight durations minimise the effect of metabolic transitions during the starting phase of a flight.

An allometric equation that scales flight costs to body mass and morphological measurements, derived from 23 published studies on flight costs in birds (Masman and Klaassen 1987), predicts flight costs of 10.1 W for our Rose Coloured Starlings (average body mass = 71.6 g, average wing span = 0.369 m and average wing area = 0.0214 m^2). The average flight costs in our experiments were 8.2 W or 19 % lower. Pennycuick's (2001) theoretical prediction ("flight" version 1.10) with the same morphological parameters and the additional input of WBF (9.8 Hz), a proportion of bounding flight of 2 % (the approximate proportion of intermittent flight in our measurements), a flight altitude of 688 m a.s.l. (the altitude of our wind tunnel) and a flight muscle efficiency (η) of 0.18, as recommended by Ward et al. (2001) for starling-sized birds, predicts flight costs of 6.1 W at 11.2 m s^{-1} which was the average flight speed in our experiments. Our measurements of 8.2 W in the Rose Coloured Starling are thus 34 % higher than predicted by this model and closer to the allometric prediction. It should be noted in this respect that predictions of P_{met} from aerodynamic models depend very much on the assumed efficiency (η) at which metabolic power is converted into mechanical power. Direct measurements of mechanical efficiency do not exist for birds in forward flapping flight, but there are some indirect estimates (Tucker 1968, Bernstein et al. 1973, Tucker 1972, Hudson and Bernstein 1983, Dial et al. 1997, Ward et al. 2001), which lie in the range of 0.18 (Ward et al. 2001) to 0.20-0.25 (Norberg 1990). Often a value of 0.23 is taken for calculations of P_{met} (Pennycuick 1989) although simultaneous estimates of P_{mech} and P_{met} indicate that η might be substantially lower (Masman and Klaassen 1987, Norberg et al. 1993, Chai and Dudley 1995, Kvist et al. 2001, Ward et al. 2001). Since P_{mech} forms only a relatively small proportion of P_{met}, any inaccuracy in the estimate of P_{mech} or η will be magnified in the estimated P_{met}.

Body mass is the most important predictor for flight costs in our analysis, and various models scale flight costs to body mass. Theoretical predictions based on aerodynamics usually result in scaling exponents of $^7/_6$ or larger (Pennycuick 1975, Rayner 1990, Norberg 1996) whereas direct measurements (Masman and Klaassen 1987, Norberg 1996, Butler and Bishop 2000) tend to result in lower exponents. In an interspecific analysis of flight costs with body mass as sole predictor, Masman and Klaassen (1987) find a scaling exponent of 0.79. There is evidence that intra-specifically, flight costs increase with body mass at an even lower scaling exponent. The only published study addressing this issue that we are aware of reports an exponent of 0.35 (95 % confidence interval: 0.08 to 0.62, Kvist et al. 2001). In the present study, flight costs scale to body mass to the power of 0.55 (95 % confidence interval: 0.36 to 0.75), when no other parameters are taken into consideration. This is in accordance with Kvist et al., given that the confidence intervals overlap, and supports their conclusion that carrying large fuel loads during migration is less costly than previously thought.

The power-to-speed relationship

Because we focused on long distance flights of 6 h duration, it appeared that our measurements could only be performed over the 9 to 14 m s^{-1} speed range. Energy expenditure in Rose Coloured Starlings did not significantly change with increasing flight speed despite a 55 % increase in flight speed. This result is in accordance with other measurements of flight costs in birds that usually do not show the whole U-shape predicted by aerodynamic modelling. Generally, metabolic power changes little, if at all, over a wide range of flight speeds (for a review see Rayner and Ward 1999). Most studies result in curves that are rather shallow and may be better described as J (hummingbirds: Berger 1985, Alexander 1997) increase linearly (Ward et al. 2004), or the power-to-speed relationship is even flat (Starlings: Torre-Bueno and Larochelle 1978, geese: Ward et al. 2002). For only one single species, the budgerigar, has a pronounced U-shaped power curve been reported (Tucker 1968, Bundle and Dial 2000), which therefore appears rather as an exception instead of a rule.

Constant flight costs, as in our study, might be the result of a limited speed range. As a potential explanation for the limitation of speed range in our study we investigated the possibility that changes in flight speed are associated with changes in flight kinematics, in particular wing beat frequency (WBF) and the use of intermittent flight (Ward-Smith 1984, Rayner 1985, Tobalske 1995). For European Starlings, Tobalske (1995) reports an increase in WBF and bounding with increasing flight speed. In the Rose Coloured Starlings, bounding as well as gliding occurred, but the use of these flight styles was variable, and we did not find any systematic change with wind speed. WBF slightly decreased with increasing speed but was better correlated with body mass. The absence of a velocity dependence of intermittent flight behaviour in our study renders it impossible to attribute the accepted speed range to variable amounts of intermittent flight behaviour in the Rose Coloured Starling. However, there are many other potentially relevant aspects of flight kinematics that might change with speed and that could not be measured with our method, like changes in rotational movements of parts of the wing, angles of attack, wing span or wing beat amplitude (Videler 2005).

A comparison of our data on the power-to-speed-relationship with studies in the European Starling is equivocal. An early study by Torre-Bueno and Larochelle (1978) did not find any relationship between flight costs and velocity, while Ward and her coworkers (2004) found an increase with speed, the magnitude depending on the method used. Their DLW measurements resulted in a U-shaped relationship with minimum costs at about 10 m s^{-1} and an increase in metabolic power of more than 40 % (from 10 to 14 W) between flights at 9 m s^{-1} and 14 m s^{-1}. Measurements by mask respirometry or thermography showed a linear increase in flight costs with speed of 18 %, respectively 28 %, but the differences between results obtained with different methods were statistically not significant and the authors attributed the different shape of the DLW curve to a lower discriminative power of their DLW measurements.

The observation that most studies including the present one do not show a clear U-shaped metabolic power curve, as predicted from aerodynamic models, could have several reasons. First, the speed range studied does not cover the slow and fast velocities that are associated with increased flight costs. The studies that report a flat or even missing power-to-speed relationship (Torre-Bueno and Larochelle 1978, Berger 1985, Dial et al. 1997, Ward et al. 2001) possibly show truncated U-curves, where an extension of the velocity range on either side might reveal increased costs (Rayner and Ward 1999). However, most authors state that they measured the widest range of speeds that their experimental birds would accept (Budgerigar, 5-13 m s^{-1}: Tucker 1968; Laughing Gull, 6-13 m s^{-1}: Tucker 1972; Starling, 8-18 m s^{-1}: Torre-Bueno and Larochelle 1978; Starling, 8-18 m s^{-1}: Tobalske 1995; Starling, 6-14 m s^{-1}: Ward et al. 2001). Also in the present study, flights were conducted in the maximum range where we obtained voluntarily sustained flights in the Rose Coloured Starlings. This range might not be as wide as it would be for short flights (Budgerigar, 20 min: Tucker 1968, Starling, 1 min: Tobalske 1995), but it probably represents the whole natural range of speeds for long distance flight. We are not aware of any measurements of flight speeds in the wild for this species. For European Starlings, Bruderer and Boldt (2001) report a flight speed of 12.2 ± 2.6 m s^{-1} in migratory flights. We assume that this speed range can be transferred to Rose Coloured Starlings and therefore that our birds flew in their natural range of speeds. Birds might simply avoid to fly at costly speeds for longer distances.

Second, the metabolic power curve might not reflect the mechanical power curve because of a variable flight muscle efficiency. The assumption that the efficiency η, converting P_{met} into muscle work, is a fixed value over the whole speed range or with respect to body mass, and that metabolic power therefore approximates a fixed multiple of P_{mech} as a function of speed, has been questioned recently (Kvist et al. 2001, Ward et al. 2001).

Third, the mechanical power requirements of flight are not correctly predicted by aerodynamic models. These models (Tucker 1973, Greenewalt 1975, Pennycuick 1975, Rayner 1979a, Rayner 1979b, Pennycuick 1989, Rayner 1999) calculate P_{mech} on the basis of fixed wing theory, as used in aircraft design (Videler 2005). Knowledge of the aerodynamics of flapping flight, the prevailing flight style of many bird species including Starlings, is still very limited but crucial for the understanding of the interaction between the bird and the air. The wake behind a flying bird (as the result of this interaction) has been studied by flow visualisation as an attempt to understand how birds generate lift during flapping flight (Kokshaysky 1979, Spedding et al. 1984, Spedding 1986, 1987, Spedding et al. 2003). It appears that the vortex wakes behind a bird change gradually with flight speed from ring structures at slow speeds to a concertina vortex trail at high speeds (Spedding et al. 2003). Recently, Videler et al. (2004) succeeded for the first time to show the vortices directly at a swift wing model in a water channel. During gliding, they found that lift is generated by leading-edge vortices over the hand wing and suggested that these might also be involved in flapping flight. At the moment being our knowledge of the aerodynamics of bird flight is still far from established. Although the nature of the limitations of our birds to their certain velocity range remains unclear, we see that within the natural range of flight speeds the energetic costs of long distance flight are primarily determined by body mass rather than by flight speed.

CHAPTER 4

Water and heat balance during flight in the Rose Coloured Starling (*Sturnus roseus*)

Sophia Engel, Herbert Biebach, and G. Henk Visser

Abstract

Water imbalance during flight is being considered to be a potentially limiting factor for flight ranges in migrating birds, but empirical data are scarce. We studied flights under controlled ambient conditions with Rose Coloured Starlings in a wind tunnel. In one experiment, we measured water fluxes with stable isotopes at a range of flight speeds (9 to 14 m s^{-1}) at constant temperature (15 °C). In a second experiment we measured evaporation rates at variable ambient temperatures (5 to 27 °C) but a constant flight velocity of 12 m s^{-1}.

During all flights, the birds experienced a net water loss. Water influx was on average 0.98 g h^{-1} and water efflux 1.29 g h^{-1}, irrespective of flight speed. Evaporation was related to temperature in a biphasic pattern. At temperatures below 18.2 °C net evaporation was constant at 0.36 g h^{-1}, rising at higher temperatures with a slope of 0.11 per degree to about 1.5 g h^{-1} at 27 °C. We calculated the relative proportion of dry and evaporative heat loss during flight. Our data suggest that for prolonged flights Rose Coloured Starlings should adopt behavioural water saving strategies and that they can not complete their annual migration without stopover to replenish their water reserves.

Introduction

Flying birds have among the highest metabolic rates known (Schmidt-Nielsen 1972, Norberg 1996, Butler and Bishop 2000) of which only a minor part is converted into mechanical work (Masman and Klaassen 1987, Biewener et al. 1992, Butler and Bishop 2000) whereas most of the energy ends up as heat (Hill 1938). A little of excess heat can be stored by allowing body temperature to increase, as has been reported for resting birds under thermal stress (Dawson 1984, Withers and Williams 1990, Tieleman and Williams 1999) as well as for flying birds (Torre-Bueno 1976, Hudson and Bernstein 1981, Hirth et al. 1987, Adams et al. 1999). Given the high metabolic rates during flight, hyperthermia can buffer only a small proportion of the excess heat (Pearson 1964, Hart and Roy 1967, Craig and Larochelle 1991) and heat dissipation by dry heat transfer (convection, conduction, radiation) or evaporation is therefore of utmost importance for thermoregulation. At low ambient temperatures, *i.e.* at a high temperature gradient between the bird and the surrounding air, dry heat transfer is very effective (Ward et al. 1999), but the conditions for dry heat transfer become increasingly unfavourable as ambient temperature rises. Birds can increase dry heat transfer behaviourally by decreasing their insulation and exposing well vasculated body regions to the cooler environment. For example, flying birds may expose their feet to the air stream to increase the area of heat dissipation (Biesel and Nachtigall 1987, Ward et al. 1999, Kvist 2001, this study). At higher ambient temperatures, birds rely increasingly on evaporative cooling to maintain their body temperature at the expense of body water (Berger et al. 1971, Torre-Bueno 1978b, Hudson and Bernstein 1981, Biesel and Nachtigall 1987, Adams et al. 1997, Dawson and Whittow 2000). During flight, they may open their beak to a variable degree or duration, exposing the moist inner side of the beak and thus promoting both evaporation and convection, a behaviour that has been observed repeatedly during wind tunnel flights at higher temperatures (Biesel and Nachtigall 1987, St-Laurent and Larochelle 1994, Ward et al. 1999, Kvist 2001, this study).

We address the balancing of the water budget at variable wind speeds and ambient temperatures in the Rose Coloured Starling (*Sturnus roseus*), a long distance migrant. In a bird's water budget, water loss stands against metabolic water production, the only appreciable source of water gain during flight. Metabolic water production depends directly on the metabolic rate and the substrate that is metabolized (Jenni and Jenni-Eiermann 1998, Willmer et al. 2000). The influence of ambient temperature on flight costs, and thus heat production and metabolic water production, is assumed to be negligible in a moderate range of temperatures (Hudson and Bernstein 1981, Rothe et al. 1987). We therefore expect water influx to depend mainly on flight velocity (Pennycuick 1989, Rayner 1999). We measured water influx (metabolic water production plus possibly uptake of humidity from the air) and efflux (total water loss) as well as the energetic costs of flight and heat production in relation to flight speed with doubly labelled water (DLW).

Evaporative water loss, in contrast, should be directly linked to ambient temperature and the water vapor pressure deficit (VPD [hPa]) between the evaporating surfaces (skin, eyes and respiratory tract) and the surrounding air (Gates 1980). We calculated evaporation rates from mass loss rates during flights at constant velocity but different ambient temperatures. From the evaporation rates we calculated evaporative cooling and the relative importance of dry heat transfer in flying Rose Coloured Starlings. These data allow a tentative calculation of the maximum flight duration to assess if migration distance is limited by fuel or water depletion.

Material and Methods

Wind tunnel

All experimental flights were conducted in a closed-circuit wind tunnel situated in the Max Planck Institute for Ornithology in Seewiesen, Germany, which closely resembles in design and technical performance data the wind tunnel in Lund, Sweden (Pennycuick et al. 1997). Wind speed and temperature can be controlled, and ambient conditions (air pressure, humidity, temperature, speed) are automatically recorded by a data monitoring system (ARGUS by Sorcus) with a sampling rate of 1 Hz. We display and record the “equivalent air speed” (Pennycuick et al. 1997), which takes into account changes in air density that determine the forces on the wings and body of a bird. This ensures that experimental conditions at a certain velocity are comparable for all birds. During this study, temperature was controlled either by a heat exchanger and a cooler installed in the air stream or, to achieve high temperatures, by electrical heating fans that were introduced into the air stream upstream of the test section.

A fine net, made of braided nylon cord, prevented the birds from leaving the flight chamber upstream. Downstream, ca. 1.5 m from the test section, there was a net made of braided nylon cord, 1.0 mm in diameter, with a mesh of 12 x 12 mm in a wooden frame.

Birds, housing and training

Rose Coloured Starlings (*Sturnus roseus*) have a breeding distribution from central Asia to the Balkans and their wintering quarter in northern India. They are long distance migrants and cover their migration route mostly during day time flights. In the wild, Rose Coloured Starlings feed mainly on insects and fruit, foraging mostly on the ground where they hunt for grasshoppers and other prey (Hudde 1993). One of the most westerly regular breeding colonies is found on Crimea peninsula (Ukraine). This population makes a flight to and from India of about 5000 km twice a year, and the birds should be capable of long periods of flight also in a wind tunnel.

The experiments were performed with 14 Rose Coloured Starlings, four of which were purchased in 1999 as nestlings from a breeder, the others were taken in June 2001 as nestlings from a breeding colony on Crimea peninsula, Ukraine. All birds were hand-raised in our institute. During the experimental periods the birds were housed in groups of three and four in aviaries (ca. 2 x 1 x 2 m) adjacent to the wind tunnel, and were given standard food consisting of insects, heart, rusk and egg, supplemented with minerals and vitamins (AviConcept®) *ad libitum*, some mealworms and fresh fruit. They had access to fresh water and the opportunity to bathe at any time.

The light schedule was varied to simulate natural conditions for the birds: During winter they had the photoperiod of their wintering quarters in northern India (27.5° N), which then changed gradually to the natural light conditions of their breeding grounds (47.5° N) during summer and back again. This kept the birds in their annual rhythm and in migratory disposition during the experimental phase, indicated by prior fattening and an increased willingness to fly. All experimental flights were conducted during the migration period. The birds were in good condition and had intact flight feathers during the entire experimental phase.

Prior to the DLW experiments we gave the birds an intense training at varying flight speeds to achieve a flight duration of six hours, necessary for a good accuracy of the isotope analysis. The four adult birds had been flying in the wind tunnel before and were ready for the experiments after three weeks of regular training. The juvenile birds were given regular training flights 8 weeks prior to the experiments. During the last two weeks prior to the experiments, all birds were trained daily for periods of 10 to 120 minutes at variable wind speeds, so that a total of 6 hours of training per week was achieved.

The range of flight speeds that the birds could sustain for prolonged periods of flight was from 9 m s^{-1} to 14 m s^{-1}. Attempts to train the birds at lower or higher velocities failed because after a short flight duration of mostly only a few minutes, the birds landed frequently and refused to start flying again. At very fast flight speeds (above 14 m s^{-1}) the birds seemed to struggle against the wind and sometimes were blown against the rear net.

Our birds flew more steadily and with fewer attempts to land when they were led into the wind tunnel in groups. We therefore let them fly in pairs or in groups of three, also during the experiments. The birds changed their position within the group frequently and there was no indication that they used any particular flight formation that might save energy. None of the birds would fly systematically close to the floor or ceiling of the wind tunnel. Therefore, boundary layer effects (Rayner 1994) are unlikely.

Experimental flights

Experiments were conducted in two series. In Experiment 1 flights were conducted at constant ambient temperature (15 °C) but different flight speeds (9 to 14 m s^{-1}) and lasted six hours each. In this set, energetic costs of flight (EE_f [W]), total body water content before (TBW_i [g]) and after the flight (TBW_f [g]) and water fluxes (rH_2O_{in}, rH_2O_{out} [g h^{-1}]) were measured with stable isotopes. In Experiment 2 flights lasted one hour each. Flight speed was held constant (12 m s^{-1}) but ambient temperature differed between flights (5 to 27 °C). Evaporation rates were calculated from mass loss rates. Heat balance was calculated from the combined data of both experimental series.

Experiment 1. Total body water (TBW_i, TBW_f [g]), water flux rates (rH_2O_{in}, rH_2O_{out} [g h^{-1}]) and energetic costs of flight (EE_f [W]) were investigated using the doubly labelled water (DLW) method (Lifson and McClintock 1966, Speakman 1997, Kvist et al. 2001). Eight birds, four adults and four juveniles, flew repeatedly (two to six times) for six hours at different wind speeds during the spring and autumn migration periods in 2001. Ambient temperature was kept constant at 15.04 °C (SD = 0.09 °C). The standard deviation within a flight never exceeded 0.54 °C. Air pressure and humidity could not be controlled for. Mean air pressure during the experiments ranged from 919.0 hPa to 957.5 hPa with an average of 938.8 hPa, and relative humidity was on average 60.65 %, ranging from 48.4 % to 76.8 %. This corresponds to a water vapour pressure of 1.04 ± 0.18 hPa. The water vapour pressure deficit (VPD), the difference between the maximum and the actual vapour pressure at a given temperature, was on average 0.67 ± 0.18 hPa, ranging from 0.39 to 0.87 hPa.

Wind speed changed between flights and ranged from 9 m s^{-1} to 14 m s^{-1} with an intra-experiment standard deviation (calculated over all measurements taken at 1 Hz sampling rate) smaller than 0.12 m s^{-1}. The succession of wind speeds was chosen randomly in order to avoid possible effects of time of the year on the measurements. Individuals had no more than one experimental flight per week. Only flights with an apparently natural flight attitude were included in the analysis. In total, 27 flights were performed successfully.

Usually, the birds had been food deprived since the evening before to have them postabsorptive during the flights. In eight cases, flights were conducted without the removal of the food the evening before. The birds then had had access to the remaining food from light on till the beginning of the experiment at 7 a.m., an episode lasting between 60 and 90 min. Even if the birds had been feeding during this time, it would have had only a minor effect on the accuracy of the energy and TBW measurements (Speakman 1997).

Body mass was taken directly before and after the flight to the nearest 0.01 g. Flight behaviour was continuously recorded by an observer for all flights who also prevented the experimental birds from landing or from leaving the wind tunnel. In most cases the mere attendance of the observer was sufficient for this. Sometimes, however, a bird decided to land frequently within a short period of time, in which case the observer waved at the bird to prevent it from landing. If an individual showed signs of exhaustion it was given a rest or was taken out of the experiment. We took it as an indicator for exhaustion if a bird landed frequently, even when being chased up immediately after landing, if a bird ignored the observer and refused to start flying again, or if a bird was flying close to the exit of the wind tunnel, trying to escape. A resting episode occurred in four flights and lasted between 15 and 33 min.

Before each flight, an initial blood sample (sample 0) was taken for the determination of the background concentration of the isotopes in the bird. The bird was then injected intraperitoneally with 0.2 g of a DLW mixture of known isotope concentration. The quantity was assessed to the nearest 0.0001 g by weighing the syringe before and after the injection on an analytical balance (Sartorius BP121S). An equilibration period of one hour without access to food or water allowed the isotopes to mix completely with the bird's body water, before the initial blood sample (sample 1) was taken and the bird released into the wind tunnel for the flight. This blood sample allowed the calculation of the initial body water (TBW_i [g]). After six hours of flight another blood sample (sample 2) was taken and the bird reinjected with 0.1 g of DLW (the exact quantitiy assessed gravimetrically, as described above). After an equilibration of one hour, again without access to food and water, a last blood sample (sample 3) was taken that allowed the calculation of the final body water (TBW_f [g]).

The blood samples were taken from the jugular vein to avoid haematoma at the brachial vein, which could disturb the bird during flight. All blood samples were flame sealed immediately in glass capillaries, and stored at 5 °C for isotope analysis at the Centre for Isotope Research in Groningen, Netherlands. The samples were analysed blindly, without knowledge of the flight conditions, and in quadruplicate to give a maximum of accuracy. For details of analysis see Visser et al. (2000) and Kvist et al. (2001).

Total body water: The size of the body water pool at the start (TBW_i [g]) and at the end (TBW_f [g]) of each experimental flight was calculated by the principle of isotope dilution, *i.e.*, on the basis of the determination of the hydrogen dilution space. The quantities (Q_{d1}, resp. Q_{d2} [mol]) and the ^{2}H concentrations (C_{d1}, respectively C_{d2} [atom percent]) of the doses are known, as well as the ^{2}H concentration in the bird's body water pool prior to the initial DLW administration (C_b, from sample 0 [atom percent]), respectively prior to the DLW reinjection (C_r, from sample 2 [atom percent]) and the measured ^{2}H concentration after the initial (C_i, from sample 1 [atom percent]), respectively the second (C_f, from sample 3 [atom percent]) administration

$$TBW_i = 18.02 \cdot Q_{d1} \cdot (C_{d1} - C_i) / (C_i - C_b) \text{ [g]} \qquad \text{(eqn. 4.1)}$$

$$TBW_f = 18.02 \cdot Q_{d2} \cdot (C_{d2} - C_f) / (C_f - C_r) \text{ [g]} \qquad \text{(eqn. 4.2)}$$

This method has been referred to as the plateau method (Speakman 1997, Visser et al. 2000).

Water flux rates: Because none of the animals were in a steady state with respect to body mass (*i.e.*, body water), water efflux rates (uncorrected for fractionation effects; rH_2O_{unc} [g h^{-1}]) were first calculated following Nagy and Costa (1980), who provided an equation which takes changes in the size of the water pool into account:

$$rH_2O_{unc} = t \cdot (TBW_f - TBW_i) \frac{\ln\left(\frac{(C_i - C_b) \cdot TBW_i}{(C_r - C_b) \cdot TBW_f}\right)}{\ln\left(\frac{TBW_f}{TBW_i}\right)} \text{ [g h}^{-1}\text{]} \qquad \text{(eqn. 4.3)}$$

where t is time expressed in hours. Second, a correction was made for isotope fractionation effects due to evaporative water loss, which takes into account a proportionally higher evaporation of non-labelled molecules compared to heavy isotopes. Corrected water efflux rates (rH_2O_{out} [g h^{-1}]) are calculated as

$$rH_2O_{out} = rH_2O_{unc} / (x \cdot f_1 + 1 - x) \text{ [g h}^{-1}\text{]} \qquad \text{(eqn. 4.4)}$$

(Speakman 1997), where x represents the proportion of the water flux lost through evaporative pathways (taken as 0.5 as proposed by Lifson and McClintock 1966), and f_1 is the fractionation factor (taken as 0.94, as recommended by Speakman

(1997, p.107). Water influx rates (rH_2O_{in} [g h^{-1}]) were calculated using eqn. 4 and the fractionation adjusted water efflux rates

$$rH_2O_{in} = rH_2O_{out} + (TBW_f - TBW_i) / t \ \ [g\ h^{-1}] \qquad \text{(eqn. 4.5)}$$

again, t is time expressed in hours. rH_2O_{in} represents metabolic water produced from the combustion of substrate and an unknown fraction of the humidity in the inhaled air that might have been taken up in the respiratory tract.

Energy expenditure: Flight energy expenditure (EE_f [W]) was analysed from isotope turnover rates between sample 1 and sample 2 (see chapter 3). The calculated metabolic rate was corrected for resting and handling phases by subtracting the energy expenditure of these non-flight periods, calculated as the resting metabolism during the active phase according to Aschoff and Pohl (1970). Non-flight phases had a mean duration of 0.36 h (range: 0.20 to 1.07 h) and accounted for less than 0.5 % of the total energy expenditure. The corrected value represents energy expenditure during flight (EE_f), which is taken for the calculation of gas exchange and metabolic water production later. A detailed analysis of the flight costs in relation to flight velocity will be published elsewhere.

Experiment 2. In the second series of flights, evaporation rates (total evaporation (TEWL) and net evaporation (NEWL) [g h^{-1}]) were calculated from mass loss rates of a total of 58 flights of one hour duration by ten individuals. These flights were conducted by the same four adult birds that had been used also during the DLW flights and by six juveniles. The adults flew during the migration periods autumn 2000, spring 2001 and autumn 2001 and the juveniles during the autumn migration period 2001. An individual never had more than two experimental flights per week. The number of successful flights per bird was on average 6 and varied between 2 and 14.

Wind speed was kept constant during all flights at 11.98 m s^{-1} (SD = 0.06 m s^{-1}) while ambient temperature changed between flights and ranged from 5.1 °C to 27.3 °C with a SD < 0.58 °C within a single flight. The succession of ambient temperatures was chosen at random in order to avoid possible date effects. Air pressure and humidity could not be controlled for. Mean air pressure was 938.64 hPa (SD = 8.32 hPa), and relative humidity 51.53 % (SD = 13.24 %) for all experimental flights, corresponding to an average water vapour pressure of 0.87 hPa (SD = 0.21 hPa). The water vapour pressure deficit (VPD) is the difference between the actual and the maximum vapour pressure at a given temperature and determines the driving force on evaporation. VPD was on average 0.87 ± 0.57 hPa, ranging from 0.16 to 2.34 hPa.

The birds had been food-deprived since the evening before the experiment to have them postabsorptive, and also to reduce excretion during flight. Flights started between 8 a.m. and 10 a.m. and lasted 60 minutes (SD = 3.34 min). Before a flight the cloaca of the birds was covered with a small piece of cotton and adhesive tape to prevent excretory mass loss. These cloacal covers added less than 0.3 g to the bird's body mass and disappeared completely under the feathers, so that they did not increase the bird's drag in the air stream. The body mass of the birds including the attached cloacal cover was measured directly before and after the flight to the nearest 0.01 g. Measurements were included in the analysis only if the tape was dry at the outer surface and still well attached to the bird's body during the second weighing. This ensured that mass loss consists only of evaporation and the combustion of fuel. Total mass loss (m_t [g h^{-1}]) can be described as

$$m_t = m_{ex} + m_{ev} - m\mathrm{H_2O_{catabol}} + m\mathrm{CO_2} - m\mathrm{O_2} \quad [\mathrm{g\ h^{-1}}] \qquad \text{(eqn. 4.6)}$$

where m_{ex} [g h^{-1}] is the excretory mass loss rate, m_{ev} [g h^{-1}] is the total evaporation rate, $m\mathrm{H_2O_{catabol}}$ [g h^{-1}] is water influx from metabolic water production and the liberation of structurally bound water during catabolism of body tissue, $m\mathrm{CO_2}$ [g h^{-1}] is the carbon dioxide production and $m\mathrm{O_2}$ [g h^{-1}] the mass gain rate through oxygen uptake. Since excretory mass loss was prevented during the experimental flights, m_{ex} is zero.

Catabolic water influx ($m\mathrm{H_2O_{catabol}}$) and the mass loss rate from carbon dioxide production and oxygen consumption ($m\mathrm{CO_2} - m\mathrm{O_2}$) depend on the metabolic rate and the composition of fuel that is combusted. The metabolic rate of Rose Coloured Starlings during flight (EE_f [W]) was derived from the previously described DLW measurements. Since energy expenditure did not change with flight speed (see chapter 3), we took the average flight costs of all individuals during 27 flights, which was 8.17 ± 0.64 W (n = 8). From this value we assume 4 % to be derived from protein breakdown and the rest from fat, which is in the range of empirical estimates for migrating birds (Jenni and Jenni-Eiermann 1998, Klaassen et al. 2000, Battley et al. 2001b). Carbohydrates may contribute to the fuel composition at the onset of a flight, but are rapidly substituted by fat and protein metabolism. Previous fasting entails an even quicker transition (Rothe et al. 1987, Walsberg and Wolf 1995). We therefore assume that carbohydrate combustion is negligible over the course of one hour flight. For the assumed fuel composition we calculated an energy equivalent of 27.8 kJ l^{-1} $\mathrm{CO_2}$ according to Schmidt-Nielsen (1997) which allows us the calculation of $m\mathrm{CO_2}$ and subsequently $m\mathrm{O_2}$ from EE_f following the equation

$$m\mathrm{CO_2} = (\mathrm{MCO_2} / \mathrm{MO_2}) \cdot \mathrm{RQ} \cdot m\mathrm{O_2} \quad [\mathrm{g\ h^{-1}}] \qquad \text{(eqn. 4.7)}$$

where $\mathrm{MCO_2}$, the molecular mass of carbon dioxide, is 44.01 g mol^{-1} and $\mathrm{MO_2}$, the molecular mass of oxygen, is 32.00 g mol^{-1}. RQ depends on the fuel that is metabolized and is 0.71 for the assumed fuel composition (Schmidt-Nielsen 1997). Catabolic water ($mH_2O_{catabol}$), *i.e.*, influx through metabolic water production and the liberation of structurally bound water, can be calculated from table 1 in Jenni and Jenni-Eiermann (1998). Total evaporation rates (m_{ev} [g h^{-1}]) can now be calculated from eqn. (6). The net evaporative water loss (NEWL [g h^{-1}]) that a bird experiences is the difference between water influx (here taken as $rH_2O_{catabol}$) and total evaporation.

Heat transfer. Heat production during flight is a function of energy expenditure: The high metabolic rates necessary during avian flight are only partly transformed into mechanical work (Butler and Bishop 2000), the rest is converted into heat and has to be dissipated to maintain a constant body temperature. Heat transfer during flight at ambient temperatures below T_{body}, as in our experiments, is always in the direction from the bird's body to the surrounding air. It can take place as evaporative heat loss or as dry heat loss (mainly via convection and radiation).

Evaporative heat loss (H_e [W]) is the rate of evaporation (m_{ev} [g h^{-1}]) multiplied by the heat of vaporization of water (q [J g^{-1}]).

$$H_e = q \cdot m_{ev} / 3600 \text{ [W]} \qquad \text{(eqn. 4.8)}$$

q varies slightly with the temperature of the evaporating surface. We use an approximation of q = 2443 J g^{-1} (Schmidt-Nielsen 1997).

Dry heat loss (H_d [W]) is the total heat production (H_t [W]) minus evaporative heat loss (H_e [W]) and stored heat (H_s [W]):

$$H_d = H_t - H_e - H_s \text{ [W]} \qquad \text{(eqn. 4.9)}$$

Total heat production during flight is $H_t = P \cdot (1\text{-}\eta)$, where P is the metabolic power as derived from the DLW measurements described above, and η is the flight muscle efficiency that converts metabolic energy to mechanical energy. Estimates for η vary and usually lie between 0.23 (Pennycuick 1989) and 0.18 (Ward et al. 2001). Here, we assume a flight muscle efficiency of 0.18.

Stored heat (H_s [W]) results in an increase of the body temperature (T_{body} [°C]) and can only account for a minor fraction of the excess heat production, since T_{body} has to remain within physiological limits.

$$H_s = C \cdot M \cdot (T_{fly} - T_{rest}) / \Delta t \text{ [W]} \qquad \text{(eqn. 4.10)}$$

Where C is the specific heat of body tissue, 3.5 J g^{-1} $°C^{-1}$ (Dawson and Whittow 2000), M is body mass [g], T_{fly} is body temperature in flight [°C], T_{rest} is body temperature at rest [°C] and Δt the time spent during flight [h]. We assume a T_{rest} of 41 °C, reported for the European Starling (*Sturnus vulgaris*) (Bezzel and Prinzinger 1990), a close relative to the Rose Coloured Starling of similar size. Steady state measurements of T_{fly} range from 42 °C to 45 °C (Hart and Roy 1967, Aulie 1975, Hudson and Bernstein 1981, Hirth et al. 1987). For the European Starling Torre-

Bueno (1976) measured a T_{fly} of 42.7 °C and 44.0 °C. Here, we assume a increase of body temperature during flight by 2.3 °C to T_{fly} of 43.3 °C, the average of Torre-Bueno's measurements and slightly lower than the mean of 74 species reviewed in Prinzinger et al. (1991).

Statistical analysis

Results are presented as means ± 1 standard deviation (SD). Water flux rates (rH_2O_{in}, respectively rH_2O_{out} [g h^{-1}]) in relation to flight speed and evaporation rates (m_{ev} [g h^{-1}]) in relation to ambient temperature were analysed separately. Significance was accepted at $p \leq 0.05$ (two-tailed).

Water flux rates: For both parameters, rH_2O_{in} , respectively rH_2O_{out}, we used mixed models in the statistical package MlwiN (Multilevel Models Project; Institute of Education, London) that tested for the effect of speed [m s^{-1}], VPD [hPa] and M [g]. These models allowed the analysis of repeated measurements on the same individual (*e.g.* at different wind speeds) and associated differences in variances. Non-significant parameters were excluded stepwise, except the manipulated variable, speed.

Mass loss rates: We applied a continuous two-phasic linear regression model (Koops and Grossman 1993, Kwakkel et al. 1993) to describe the relationship of evaporation rate to ambient temperature and to determine the temperature at which the evaporation rates started to increase. The general model was

$$Y = \alpha + \beta_i \cdot X - [r \cdot (\beta_i - \beta_{i+1}) Ln(1 + e^{(x - y_i)/r})] \quad \text{(eqn. 4.11)}$$

Where Y is the dependent variable, X is the independent variable, α is the intercept, β is the slope, y_i is the estimated breakpoint between phase i and i + 1, and r is a smoothness parameter which was set at 0.05, a rather abrupt transition (Koops and Grossman 1993). All curves were fitted using the nonlinear regression algorithm procedures from the NONLIN package (shareware program, P.H. Sherrod). The significance of adding a second phase to the model was assessed by a F-test to verify the biphasic nature of the relationship (Kwakkel et al. 1993). For further analysis, the data set was divided in two parts, one below the breakpoint and one above. These parts were analysed separately with mixed models that tested for the effect of T_{amb} [°C], VPD [hPa] and M [g] on evaporation rates. Again, parameters that were least significant were excluded stepwise, except the manipulated variable, T_{amb}.

Results

Experiment 1

Total body water content (TBW [g]) was measured in eight individuals before and after 27 flights of six hours duration each at flight velocities ranging from 9 m s^{-1} to 14 m s^{-1}. Body mass before the flights was on average 71.6 ± 9.9 g (n = 8). Initial total body water content (TBW_i [g]) was on average 42.9 ± 4.0 g (n = 8) and decreased significantly during the flight period to an average final total body water content (TBW_f [g]) of 41.1 ± 4.0 g (n = 8) (paired t-test: t = 14.29; $p < 0.001$). Water efflux rate (rH_2O_{out} [g h^{-1}]), consisting of evaporative water loss and the wet part of excretion, was on average 1.29 ± 0.14 g h^{-1} (n = 8), and water influx (rH_2O_{in} [g h^{-1}]), which is metabolic water production and humidity from the inspired air that might have been absorbed in the respiratory tract, was 0.98 ± 0.16 g h^{-1} (n = 8). The difference of rH_2O_{in} and rH_2O_{out} is the net water loss that the birds experienced during flight (Fig. 4.1). It turned out that the birds suffered a net water loss in all cases, on average 0.31 ± 0.06 g h^{-1} (n = 8), ranging from 0.11 g h^{-1} to 0.58 g h^{-1} (n = 27).

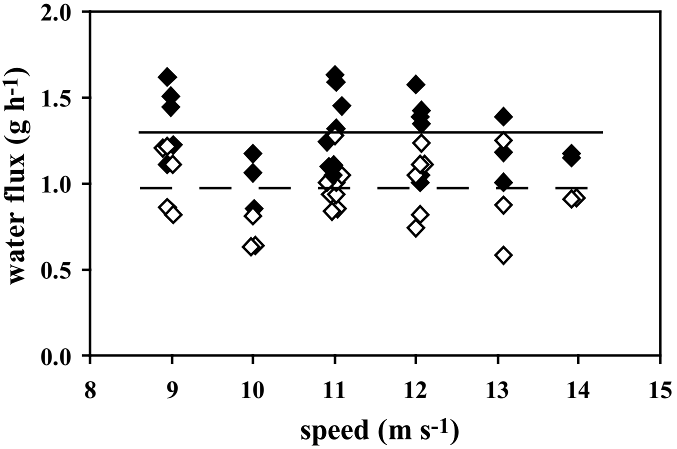

Fig. 4.1 Water fluxes during flight in Rose Coloured Starlings as measured with the DLW method. Open symbols represent water influx (average value of 0.98 g h^{-1} marked by broken line), filled symbols represent water efflux (average value of 1.29 g h^{-1} marked by black line). Flights lasted six hours each and were performed at 12 m s^{-1} and 15 °C.

To test whether part of the variance in water flux rates could be explained by differences in body mass or other parameters, we conducted a mixed models analysis, including initial body mass (M_i [g]), speed [m s^{-1}], flight costs (EE_f [W]) and water vapour pressure deficit (VPD [hPa]) as predictors for rH_2O_{in} and rH_2O_{out}, respectively. It turned out that levels of water efflux could not be explained by either of the paramenters. Water influx was dependent only on body mass (coefficient = 0.013; S.E. = 0.003; $p < 0.002$). Flight energy expenditure (EE_f) was on average 8.17 ± 0.64 W (n = 8) and did not change with speed but was dependent on body mass (see chapter 3).

Experiment 2

During 58 flights at ambient temperatures between 5.1 °C and 27.3 °C ten birds with an average initial body mass of 75.61 ± 7.05 g experienced a mass loss of 1.17 ± 0.43 g h^{-1} (n = 10, Fig. 4.2A) which corresponds to 2 % of body weight per hour. With the subtraction of mass loss due to gas exchange and mass gain due to water influx we calculated a total evaporation rate m_{ev} of 1.63 ± 0.43 g h^{-1} ranging from 1.09 to 3.42 g h^{-1} (Fig. 4.2B).

A two phase regression described the relationship between evaporation rates (m_{ev} [g h^{-1}]) and ambient temperature (T_{amb} [°C]) significantly better than one linear regression ($F_{2,55} = 7.005$; $p < 0.02$). The breakpoint that divided the two phases was 18.2 °C (S.E. = 1.33). Below this value, m_{ev} was temperature independent at 1.38 ± 0.18 g h^{-1} (slope1 = 0.014; S.E. = 0.013; $p > 0.05$). At temperatures above 18.2 °C, m_{ev} was on average 1.89 ± 0.47 g h^{-1} and increased with T_{amb} (slope2 = 0.111; S.E. = 0.019; $p < 0.001$). The data set was therefore subdivided and tested separately for the effects of T_{amb}, water vapour pressure deficit (VPD [hPa]) and initial body mass (M_i [g]). At $T_{amb} < 18.2$ °C there was no effect of T_{amb}, VPD nor M_i on evaporation rates. At $T_{amb} > 18.2$ °C there was a significant effect of VPD on m_{ev} (coefficient = 0.011; S.E. = 0.005; $p < 0.0001$).

We assume that water influx is not dependent on ambient temperature in the range studied, because flight costs are supposed to be independent of temperature (Hudson and Bernstein 1981, Rothe et al. 1987). Under this assumption, the observed biphasic pattern holds true also for net evaporation (NEWL [g h^{-1}]), which is total evaporation (m_{ev} [g h^{-1}]) minus water influx, here catabolic water ($rH_2O_{catabol}$ [g h^{-1}]). $rH_2O_{catabol}$, calculated from EE_f and the assumed fuel composition of 96 % fat and 4 % protein (Jenni and Jenni-Eiermann 1998, Klaassen et al. 2000, Battley et al. 2001b), was 1.00 g h^{-1}. This resulted in an average NEWL of 0.63 g h^{-1}. At T_{amb} below 18.2 °C NEWL was 0.38 ± 0.18 g h^{-1} and at temperatures between 18 °C and 27 °C NEWL was 0.36 to 2.42 g h^{-1} (Fig. 4.2C).

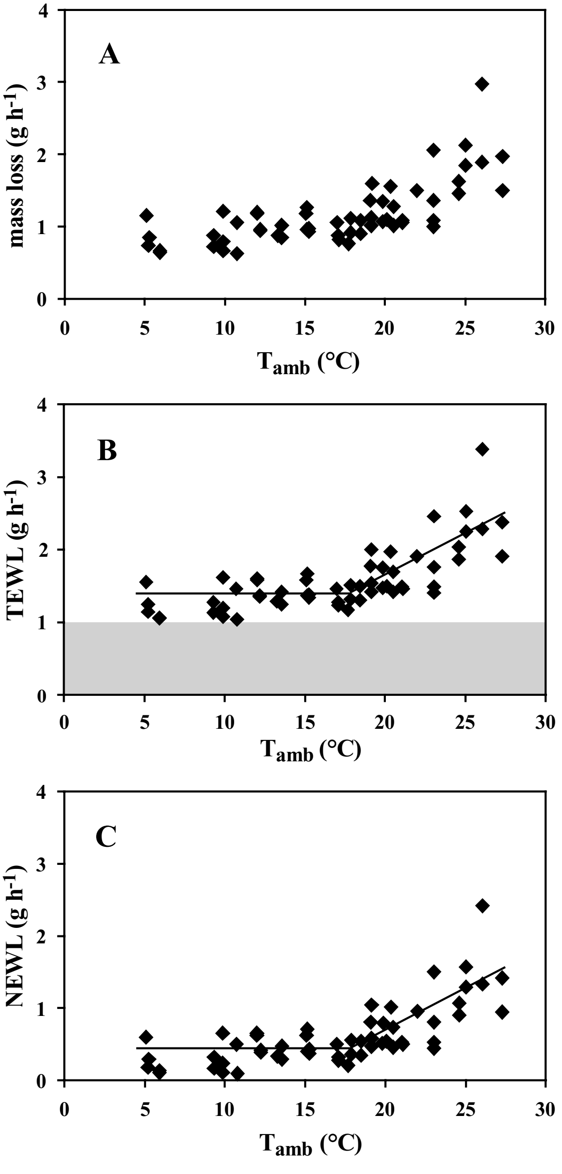

Fig. 4.2 Rates of mass loss **(A)**, total evaporation TEWL **(B)** and net evaporation NEWL **(C)** of Rose Coloured Starlings flying for one hour each time at 12 m s^{-1} at ambient temperatures between 5 °C and 27 °C. A biphasic regression (black line, B, C) described the relationship of evaporation rates to ambient temperature best. The grey bar (B) represents catabolic water production, assuming flight costs of 8.17 W, as calculated from the DLW measurements in experiment 1, and a fuel composition of 96 %fat and 4 % protein.The birds were prevented from excretion by a cloacal cover.

Heat balance

Heat production (H_t [W]) during flight is a function of energy expenditure. With average flight costs of 8.17 ± 0.64 W, as measured in the DLW flights, and an assumed flight muscle efficiency of 0.18, H_t is 6.70 ± 0.52 W. An increase in body temperature during flight by 2.3 °C results in 576 J of stored heat (H_s [W]), corresponding to 0.16 W or 2.4 % of H_t one hour. The remaining 6.54 W of heat production have to be dissipated either by convection, radiation or evaporation.

H_t and H_s are assumed to be constant over the range of ambient temperatures measured. But the relative contributions of heat loss avenues change with ambient temperature (Fig. 4.3): At temperatures below the breakpoint of 18.2 °C, total evaporation rates are on average 1.38 g h^{-1}. Since the evaporation of 1 g of water requires 2443 J (Schmidt-Nielsen 1997), this evaporation rate results in a heat loss (H_e [W]) of 0.94 W , which is 14.0 % of H_t; the rest (5.60 W or 83.6 %) has to be dissipated by dry heat transfer (H_d [W]). At higher ambient temperatures, evaporation rates increase linearly, resulting in a higher proportion of H_e relative to H_d. At 27 °C, the highest temperature that was measured, 2.5 g of water are evaporated per hour, increasing H_e to 1.70 W or 25.4 % of heat production.

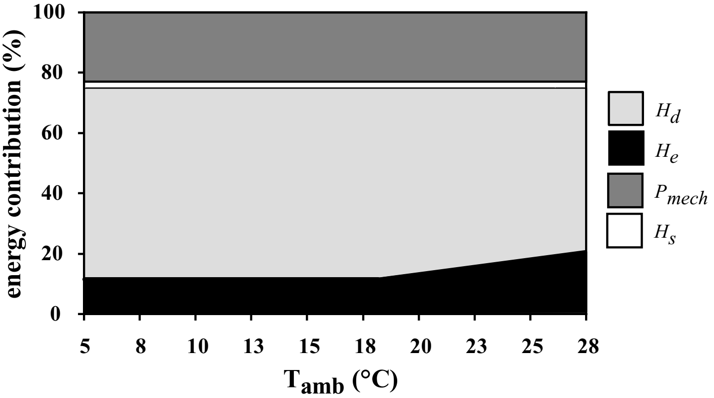

Fig. 4.3 Heat balance model for a flying Rose Coloured Starling, assuming flight costs of 8.2 W (taken as 100 % of energy expenditure), as measured with the DLW method during flights of six hours duration at 12 m s^{-1} and 15 °C. Flight muscle efficiency is assumed to be 18 %, resulting in mechanical work (P_{mech}) of 1.5 W, the rest (82 % of energy expenditure) being heat production (H_t). Hyperthermia of 2.3 °C, (a value taken from literature) results in heat storage (H_s) of 0.16 W or 2.4 % of heat production during one hour. Evaporation rates are calculated from mass loss rates during flights of one hour duration at 12 m s^{-1} and ambient temperatures (T_{amb}) between 5 °C and 27 °C and account for evaporative heat dissipation (H_e) of 0.9 to 1.7 W or 14 to 25 % of H_t, respectively, depending on T_{amb}. Dry heat transfer (H_d) is calculated as the difference of H_t and evaporative heat loss.

Discussion

Our measurements on water fluxes and evaporation rates in flying Rose Coloured Starlings revealed that the birds had a negative water balance during all flights. Water efflux during the DLW measurements was on average 1.29 g h^{-1}, standing against a water influx through metabolic water production and humidity from the respiration air of 0.98 g h^{-1}. The resulting net water loss of 0.31 g h^{-1} is statistically not different from the calculated net evaporative water loss at low ambient temperatures of 0.38 g h^{-1}, derived from the mass loss measurements ($t = 1.66$; $p > 0.05$).

Evaporation followed a biphasic pattern with relatively low and constant rates at the lower range of temperatures and an abrupt increase at ambient temperatures above 18.2 °C. A biphasic evaporation pattern has been reported also for other birds during flight (Tucker 1968, Torre-Bueno 1978b, Giladi and Pinshow 1999, Kvist 2001). European Starlings (Torre-Bueno 1978b) experienced a total evaporation rate of 1.1 g h^{-1} at 5 °C, this rate being relatively constant throughout a temperature range up to about 15 °C. At higher temperatures, evaporation rates rose to reach 3.0 g h^{-1} at 29 °C. Our results are in good agreement with these measurements. Some authors (Hudson and Bernstein 1981, Biesel and Nachtigall 1987), found constant increases of evaporative water loss with ambient temperature (minimum T_{amb} 18 °C and 7.5 °C, respectively). While the measurements in White-necked Ravens (Hudson and Bernstein 1981) may represent only the temperature range above the threshold temperature, the linear increase in evaporation rates in Pigeons (Biesel and Nachtigall 1987) is less readily to explain, more so as other measurements in Pigeons (Giladi and Pinshow 1999) revealed the expected biphasic pattern with a threshold temperature between 15 °C and 19 °C. Maybe evaporative cooling had to be initiated at a lower temperature in the former wind tunnel study compared to the latter free flight measurements due to increased metabolic demands (Giladi and Pinshow 1999).

Thermal imaging led to the calculation of a heat balance model for flying European Starlings (Ward et al. 2004). This approach allows the measurement of radiative heat transfer and the calculation of convection and metabolic power in unrestrained birds. Convection was the most important mechanism for heat transfer in that study, accounting for almost 80 % of total heat transfer, while radiation accounted for only 9 %. During these measurements, dry heat transfer, the sum of radiation and convection, was 8.9 W at 12 m s^{-1} (the flight speed of our mass loss measurements). This value is somewhat higher than our calculated value for the Rose Coloured Starling (5.6 W). Several aspects may contribute to this difference.

1) Rose Coloured Starlings may generally differ from European Starlings. To assess between-species differences we certainly need more data for comparison. 2) Body mass differences that account for different flight costs which are the basis for our model calculation. Our birds had an average body mass of 71.6 g during the DLW measurements, compared to 82.0 g in the European Starlings. Accordingly, metabolic flight costs differed (8.2 W in Rose Coloured Starlings, measured with DLW, versus 11.6 W in European Starlings, calculated from heat transfer modelling). 3) Differences in the underlying model assumptions. Our calculations are based on flight metabolic rate (measured during DLW flights), evaporation rate (calculated via mass loss rates), an assumed efficiency for the conversion of metabolic power to mechanical power of 18 % and an assumed increase in body temperature of 2.3 °C. Errors in both assumptions have a direct effect on the resulting value for dry heat transfer. If we assume an efficiency of 15 %, comparable to the calculatins of Ward et al. (2004), H_t would increase to 6.9 W. Furthermore, we assume hyperthermia during flight and thereby heat storage of 0.2 W, while Ward et al. (2004) assume body temperature to remain constant (which would increase our H_t further to 7.1 W). Despite the differences discussed above, when expressed in relative terms, both models result in very similar proportions of dry heat transfer compared to total heat flow (88.5 % in European Starlings and 82.5 % in Rose Coloured Starlings).

To assess the sensitivity of our results to the underlying assumption of fuel composition (96 % of energy derived from fat, 4 % from protein) we calculated evaporation rates from our mass loss data also under the assumption (a) that only fat is used to fuel flight, and (b) that 10 % of the energy is derived from protein breakdown. This increase in the proportion of protein catabolism results in an increase of RQ by 0.4 %. mCO_2 increases by 2 % as does mO_2. NEWL decreases by 3 % from 0.64 g h^{-1} to 0.62 g h^{-1}. In contrast to these small changes stands a huge increase in water gain from 0.85 g h^{-1} to 1.22 g h^{-1} with increasing protein use, which is mainly due to the high water content of wet protein (Jenni and Jenni-Eiermann 1998) liberated when tissue is catabolized. Accordingly, TEWL increases by 24 % from 1.49 g h^{-1} to 1.84 g h^{-1}. While net evaporation and therefore water balance during flight is relatively insensitive to errors in the assumed fuel composition, total evaporation changes dramatically, making heat balance calculations much less reliable. The actual error introduced by our assumed fuel composition is probably much smaller than in these extreme cases, as the good fit between DLW measurements and mass loss calculations suggests. However, more information on fuel composition during bird flight would be of great value to future modeling.

Our birds experienced a minimun evaporation during flight that exceeds water gain through the catabolism of body stores and possibly respiration air, resulting in a net water loss at all measured ambient temperatures. A negative water balance during flight imposes a potential limit to flight duration through water depletion, as has already been proposed on the basis of theoretical considerations (Carmi and Pinshow 1995, Klaassen 1995, Klaassen et al. 1999, Klaassen 2004). If water imbalance is a real problem, as our measurements suggest in accordance with previous studies, a bird during migration should seek to minimize water loss behaviourally and physiologically. Possible behavioural mechanisms are the choice of favourable weather conditions, especially with respect to ambient temperature, which includes the choice of flight altitude or possibly a night migration strategy. Physiological mechanisms might target excretory water loss, cutaneous and respiratory evaporation. The excretion of metabolic waste products in the form of uric acid might be an example of physiological adaptation to water economy. Measurements in free flying Pigeons show that excretory water loss still is a substantial proportion (almost 10 %) of total water loss, at least in this species (Giladi and Pinshow 1999). Cutaneous evaporation has long been thought to be of minor importance for avian water balance since birds have no sweat glands (Rawles 1960, Bartholomew and Cade 1963), but later work has shown that cutaneous evaporation is an important avenue of water loss in the thermoregulatory process, at least in resting birds (Bernstein 1969, Dawson 1982, Marder and Ben-Asher 1983, Webster and King 1987, Webster and Bernstein 1987, Wolf and Walsberg 1996, Tieleman and Williams 2002). Given the high respiration rates during flight (Berger et al. 1970, Butler 1980, Bernstein 1987) the relative contribution of respiratory evaporation to total evaporation is likely to be high, and might set a lower limit to evaporation rates. Respiration is a vital function underlying other and stronger needs than the need for water saving. It might therefore be not easily accessible to the evolution of water saving mechanisms. Measurements on flying Pigeons (Michaeli and Pinshow 2001) and ducks (chapter 5) suggest that there is a certain water saving potential in the fact that exhalation temperature is cooled below body temperature at low to intermediate ambient temperatures. The underlying physiological mechanisms remain to be investigated.

Based on our measurements on energy costs of flight, on body mass, TBW and evaporation rates we tentatively calculated maximum flight duration for our study species to answer the question whether flight duration is limited by energy or by water depletion (Biebach 1991, Carmi et al. 1992, Klaassen 1995, Leberg et al. 1996, Klaassen et al. 1999). The average body mass of our birds was 71.6 g, and TBW_i was 42.9 g. Assuming the ratio of TBW : lean dry mass is 0.7 : 0.3 (Ellis and Jehl 1991), this yields a lean dry mass of 18.4 g and a fat content of 10.3 g.

For reasons of simplicity we calculated flight duration for the case when fat is the only source of energy: Flight costs of 8.2 W or 29.5 kJ h^{-1} result in a fat loss rate of 0.75 g h^{-1}, because the energy content of fat is 39.3 kJ g^{-1} (Schmidt-Nielsen 1997). A fat store of 10.3 g thus enables them to fly for 13.7 h, which is a conservative estimate, because it does not take into account mass loss during this time period and consequently a reduction in flight costs. On the other hand, fat has the highest energy density of all tissues (Jenni and Jenni-Eiermann 1998); any combination with other substrates will result in a shorter flight duration.

To what extent flight distance is limited by water depletion depends on the rate of net water loss and the tolerable degree of dehydration during flight. Estimates of the maximum endurable amount of water loss in flying birds lie in the range of 15 % of body mass (Berger and Hart 1974, Carmi et al. 1993) or 30 % of TBW_i (Carmi et al. 1992), corresponding to a net water loss of 11 to 13 g water in Rose Coloured Starlings. The rate of NEWL depends on T_{amb}: At temperatures below 18.2 °C, we calculated a net water loss of 0.63 g h^{-1}, which would allow a flight duration of 17.5 h if we assume a maximum water loss value of 11 g. In this case, flight duration is clearly limited by fat depletion. Flights at 27 °C however, were associated with a net water loss of up to 2.42 g h^{-1}, reducing the potential flight duration to 4.5 h. The critical temperature above which water becomes the limiting factor for flight duration, compared to the estimates for fuel depletion, is about 21 °C, a temperature that certainly is exceeded on the migration route of this species, especially during day time. Our findings suggest that Rose Coloured Starlings and can not complete their annual migration without stopover to replenish their water reserves.

CHAPTER 5

Exhaled air temperature as a function of ambient temperature in flying and resting ducks

Sophia Engel, Raymond H. G. Klaassen,
Marcel Klaassen, and Herbert Biebach

Abstract

Exhaled air temperature (T_{exh}) has a paramount effect on respiratory water loss during flight. For migratory birds, low T_{exh} potentially reduces water loss and increases flight range. However, only three studies provide empirical data on T_{exh} during flight. The aim of this study was to record T_{exh} of birds during rest and flight at a range of controlled ambient temperatures (T_{amb}). One wigeon and two teal flew a total of 20 times in a wind tunnel at T_{amb} ranging from 1 to 24 °C. T_{exh} during flight did not differ between the two species and was strongly correlated with T_{amb} ($T_{exh} = 1.036\ T_{amb} + 13.426$; $R^2 = 0.58$). In addition, body temperature had a weak positive effect on T_{exh}. At a given T_{amb}, T_{exh} was about 5 °C higher during flight than at rest. The steep slope of the relationship between T_{exh} and T_{amb} during flight indicates that T_{exh} is actively regulated and not simply the result of passive heat exchange.

Introduction

Long distance flight is considered one of the most physiologically challenging events in a bird's life (McWilliams et al. 2004). Twice a year many bird species migrate thousands of kilometres and cross ecological barriers like deserts and large water bodies to reach favourable breeding or wintering grounds. Sufficient energy stores and a balanced water budget are vital for the successful completion of these journeys. The impact of energy consumption and water depletion on a bird's flight range was simulated with a computer model (Carmi et al. 1992, Klaassen 1995, Klaassen et al. 1999) that indicated that water imbalance might in fact be an important factor restricting flight range in migratory birds under a range of environmental conditions (Carmi et al. 1992, Klaassen et al. 1999). Comparing the predictions of their latest water and energy balance model for flying birds with data from studies using birds trained to fly outdoors or in wind tunnels, Klaassen et al. (1999) tentatively concluded that model predictions appear to be realistic. However, to validate the model, more physiological data on water exchange and related variables are required (Klaassen 2004).

During flight, water loss occurs by excretion and evaporation, of which the former is considered to be a relatively small portion of total water loss (Giladi and Pinshow 1999). Evaporative water loss takes place via the skin (cutaneous) and the respiratory tract (respiratory evaporation), the latter being determined by the volume of exhaled air (*i.e.*, breathing frequency times breath volume) and the difference in water vapour pressure between inhaled and exhaled air. The amount of water that air can contain increases exponentially with air temperature. Since exhaled air is assumed to be saturated with water (Schmidt-Nielsen et al. 1970, but see also Kaiser and Bucher, 1985 and Marder, 1973), the water content of the inhaled air and the temperature of the exhaled air determine respiratory evaporative water loss.

In resting birds exhaled air temperature is usually cooler than body temperature and strongly correlated with ambient temperature (Jackson and Schmidt-Nielsen 1964, Murrish 1973, Tieleman et al. 1999, Geist 2000, Michaeli and Pinshow 2001). A temporal counter-current heat exchange has been proposed to be the mechanism that determines exhaled air temperature (Jackson and Schmidt-Nielsen 1964): when air is inhaled, its temperature rises to body temperature, and the air is saturated with water vapour from the respiratory passages and the lungs. Convective heat exchange and evaporation of water in the nasal passages during inhalation presumably cool the associated membranes. Upon exhalation the air is cooled by these nasal surfaces, resulting in water condensation (Schmidt-Nielsen et al. 1970). This nasal cooling mechanism results in a decrease of respiratory water loss compared to the situation where birds breathe through the open beak, thus bypassing the nasal turbinates (Tieleman et al. 1999, Geist 2000).

Evaporative water loss may be important to avoid heat stress due to the high metabolic heat production during avian flight (Hill 1938, Hirth et al. 1987). Experiments and model calculations indicate that heat stress may occur in flying birds even at moderate ambient temperatures, and especially in large birds such as geese or swans (Klaassen et al. 1999, Bishop et al. 2002). Yet, also in birds in heat balance, model calculations (Carmi et al. 1992, Klaassen et al. 1999) indicate that water imbalance may occur.

According to model sensitivity analyses, exhaled air temperature is predicted to be the most important parameter determining water loss and ultimately flight range (Carmi et al. 1992, Klaassen et al. 1999). However, to date only three studies have measured exhaled air temperature in a flying bird. Berger et al. (1971) measured exhaled air temperatures of a black duck (*Anas rubripes*) immediately after 5 to 18 seconds of flight. Hudson and Bernstein (1981) used a respiratory mask to measure respiratory evaporation and then estimated exhaled air temperatures of flying white-necked ravens (*Corvus cryptoleucus*). More recently, Michaeli and Pinshow (2001) used temperature transmitters to measure exhaled air temperatures of free-flying Pigeons (*Columba livia*) at various ambient conditions. Given its importance for the estimation and understanding of water loss in flying birds and the limited availability of measurements of exhaled air temperature in flying birds, we measured exhaled air temperature of a wigeon (*Anas penelope*) and two teal (*Anas crecca*) at rest and during flights of 5 to 35 min duration at various controlled ambient temperatures in a wind tunnel. In addition to exhaled air temperature we simultaneously measured deep body temperature and respiratory frequency since they may influence exhaled air temperature and, thus, water loss in flying birds.

Methods

Experimental birds

Wigeons (*Anas penelope*) were chosen as experimental birds for their relatively large body size (wingspan of 71-85 cm), which minimises the influence of measuring equipment attached to the animal. Wingspan of wigeon is about 70 % of the width of the test section of the wind tunnel which is the maximum suggested size threshold for flying birds in wind tunnels (Rothe and Nachtigall 1987, Pennycuick et al. 1997). We also used smaller teal (*Anas crecca*, wingspan 53-59 cm) because they can manoeuvre more freely in the wind tunnel and are therefore less prone to stress and accidents than the wigeons. In the wild, both species regularly migrate long distances under variable climatic conditions. Wigeons used in this experiment were raised in captivity at the duck facilities of the NIOO-KNAW. The teal were also raised in captivity, but they originated from a German breeder. All birds were 6-10 months old during the experiments. During the experimental phase, body mass of wigeon was 632 g to 690 g, and body mass of teal was 288 g to 397 g.

Wind tunnel

Experiments were conducted in the wind tunnel of the Max Planck Institute for Ornithology in Seewiesen, Germany. The wind tunnel is a closed-circuit system, specially designed for the study of bird flight. In design and technical performance it highly resembles the wind tunnel at Lund University, Sweden (Pennycuick et al. 1997). The octagonal test section is 2 m long, with a diameter of 1.2 m. It is made of Perspex and glass so that flying birds can be continuously observed. Adjacent to the test section is a gap of 50 cm that gives the researcher unrestricted access to the flight section. Airflow in the test section is highly laminar and constant (speed variation within 2 % of the mean in the horizontal and 1.5 % in the vertical plane). Air speed can be set from 0-45 m s^{-1} with an accuracy of 0.1 m s^{-1} and is the same as Pennycuick et al.'s (1997) "equivalent air speed" which takes into account variations in air density.

Ambient temperature was controlled by a cooling system and heating fans upstream of the flight chamber. Temperature was constant within 1 °C during experimental flights. Relative humidity of the ambient air could not be controlled, but was recorded (accuracy of ± 1 %) using a HMP231 hygrometer equipped with a HUMICAP®H-Sensor located upstream of the flight chamber. During the experiments, the ceiling and the bottom of the lower end of the test section were covered with a 2 cm foam layer to protect the birds from occasional collisions. During the teal flights, a mist net was placed upstream of the test section to keep the birds from entering the air-settling compartment. All these alterations are known to influence the laminarity of the airflow (Pennycuick et al. 1997). However, it is assumed that these effects are negligible, and comparable safety constructions have been applied in other wind tunnel studies (Kvist et al. 2001).

Training

Birds were trained to fly in the wind tunnel by releasing them into the air stream and gently pushing them back into the air if they intended to land. Some individuals tried to land in the front part of the test section after a short flight. This was impeded by presenting a glove mounted on a stick under the flying birds. Birds eventually flew for longer periods without the presence of the researcher. We adjusted wind speed until the bird flew in an apparently natural and relaxed manner. The preferred flight velocity, estimated from visual observation of the flying bird differed between species. We spent 2-3 months training eight wigeon and ten teal. Only two wigeon and three teal successfully learned to fly unassisted for at least 30 min in the wind tunnel of which one wigeon and two teal provided usable data for this experiment.

Measuring exhaled air temperature

Exhaled air temperature (T_{exh}) was measured with a small (0.20 x 0.14 mm) thermistor bead (Fenwal, Small Bead BC32L1) that was soldered to copper leads (Ø 0.1 mm). The leads were attached to the side of the bird's bill (using small pieces of Hansaplast® tape, soaked in diethylether to improve adhesion) so that the tip with the thermistor was positioned in the (left) nostril opening. Thus, the temperature of the air was measured exactly at the point were it entered (inhalation) and exited (exhalation) the bird. We opted for this placement since respiration air temperature is a function of the distance to the nostril opening and this temperature gradient can be extremely steep (*e.g.*, 3 °C over the first 5 mm of the respiratory tract, Murrish, 1973). During flight, the high respiratory frequency (see results) and increased tidal volume (Bernstein 1987) produces a high flow rate of air through the nostril, so we assumed that mixing of expired and ambient air in the nostril opening was negligible.

The thermistor wires led from the bill over the head to the back and tail of the bird and then connected to the data recording system (Pico® ADC-11, sampling rate 0.1 kHz, with PicoLog for the wigeon and PowerLab® / 8SP, sampling rate 1 Hz, for the teal). Wires were attached to the body feathers on the head, back, and tail using small pieces of Hansaplast®. A 3 m section of wire between the recorder and the bird allowed the bird to move freely within the wind tunnel test section. Before the experiments, the thermistor was calibrated to 0.1 °C against a calibrated mercury-in-glass thermometer (calibrated by the State Office of Metrology of Hessen, Germany) over a range of temperatures larger than the range of respiration air temperatures. The stabilisation time of the thermistor was estimated by switching it from still air into airflows of different temperatures and flow rates and determining the time needed to fully stabilise at the new temperature. Stabilisation time (t_s [sec]) depended on the temperature gradient (ΔT [°C]; $t_s = 0.221\ \Delta T^{\,0.1161}$; $R^2 = 0.505$) and was sufficiently short to directly register T_{exh} in resting birds (Fig. 5.1A) but too long to follow the temperature changes of the inhaled and exhaled air during flight (Fig. 5.1B). We circumvented this problem by approximating T_{exh}

during flight from the average temperature of the respiration air (T_{av}) and the inhaled air or ambient temperature (T_{amb}) as

$$T_{exh} = 2 \cdot T_{av} - T_{amb} \ [°C] \qquad \text{(eqn. 5.1)}$$

assuming that inhalation and exhalation cause the same but opposite temperature change of the thermistor (see Fig. 5.1B) and that T_{av} therefore lies midway between T_{amb} and T_{exh}. For flying birds we calculated one T_{exh} value per minute by averaging the temperature reading over 5-10 sec intervals.

During flight birds sometimes breathed through their opened bills instead of their nostrils. Since in- or exhalation through the (partially) open beak affected the temperature readings of the thermistor, we used only data recorded while the bird's bill was closed, which could be judged from regular and undisturbed readings.

Measuring ambient temperature, body temperature and respiratory frequency

Respiratory frequency (f [Hz]) was determined from the T_{exh} traces by counting the number of minima or maxima over a known time period and dividing this by the duration of the counting interval. For each minute of flight the mean f over a period of at least ten seconds was determined.

T_{amb} was measured to the nearest 0.1 °C using either a calibrated thermocouple (sampling rate 0.033 Hz) or a thermistor (sampling rate 1 kHz). During flight, deep body temperature (T_{db}) was measured with a thermistor (Fenwal, Small Bead GB38J1) soldered to copper leads (Ø 0.1 mm) that were inserted into a flexible tube (Ø 2 mm, length: 10 cm). The thermistor was sealed in the tip of the tube with a drop of epoxy resin, which made it waterproof. The thermistor was calibrated to 0.1 °C against a calibrated mercury-in-glass thermometer (calibrated by the State Office of Metrology of Hessen, Germany) in a water bath. For the experiments, the probe was inserted 10 cm into the colon of the bird and fixed with small pieces of Hansaplast® tape at the body feathers, just outside the cloaca.

Experimental flights

One wigeon completed nine successful flights during April 2000. Two teal completed 11 successful flights during December 2000 and January 2001. Flight conditions are summarised in table 5.1. Experiments were conducted at an average wind speed of 17.8 and 15.5 m s^{-1} for wigeon and teal, respectively. Flight duration was 5-35 min, and T_{amb} ranged from 11.5 °C to 18.4 °C for the wigeon and from 0.8 °C to 24.4 °C for the teal. T_{amb} was kept constant (± 0.5 °C) during each experimental flight.

Measurements in resting birds

We also measured T_{exh} , f and T_{db} in the same birds while resting at T_{amb} from -13.7 °C to 30.0 °C. For these measurements the birds were placed in a cardboard box (about 40 x 30 x 30 cm) with some hay to sit on. The box was placed in a darkened temperature cabinet. Measuring devices and recording system were the same as used in the flight sessions. Measurements were taken during daytime. Only phases without activity were used in this analysis. The first 30 minutes of each recording were discarded to allow the bird time to habituate to the new ambient temperature and the box. We selected from the next 30 min of recordings a continuous period of 10 min with regular breathing. At each one-minute interval within this 10 min period we calculated the average peak temperature and f over a 10 second period.

Statistics

All statistics were conducted using STATISTICA version 6, StatSoft, Inc. We used General Linear Models to analyse the relationships between T_{exh} , f, T_{db} and T_{amb} in both resting and flying birds. If the interaction term between the dependent variable (T_{exh} , f or T_{db}) and T_{amb} was not significant, indicating the slopes of the relationship were not significantly different at the species or individual level, it was removed from the analysis.

Table 5.1 Overview of experimental flight conditions. Ambient temperature (T_{amb}) was measured with a calibrated thermocouple (sampling rate 0.033 Hz) or a calibrated thermistor (sampling rate 1 kHz) and was constant within 1 °C during each flight. Relative humidity (RH) in the wind tunnel was measured with HUMICAP®H-Sensor at a rate of 1 Hz, and was constant within 4 % during a single flight. Absolute humidity [g m^{-3}] was calculated from T_{amb} and RH using physical tables (Schmidt 1979).

Date	Individual	Flight speed [m s^{-1}]	T_{amb} [°C]	Humidity [%]	Water vapour [g m^{-3}]	Flight duration [min]
30.03.00	Wigeon	17.6	14.3	46.1	5.7	5.0
13.04.00	Wigeon	17.5	16.4	48.2	6.8	5.1
13.04.00	Wigeon	18.0	17.1	47.6	7.0	10.8
14.04.00	Wigeon	17.7	18.2			5.0
14.04.00	Wigeon	17.4	18.6			3.8
17.04.00	Wigeon	17.7	13.7	54.0	6.4	3.3
17.04.00	Wigeon	18.5	12.9	51.4	5.8	24.2
19.04.00	Wigeon	17.5	11.4	61.6	6.3	35.2
20.04.00	Wigeon	18.0	12.1	60.9	6.6	12.1
23.12.01	Teal 2	15.6	9.0	41.3	3.7	9.4
24.12.01	Teal 1	15.5	12.5	38.2	4.2	9.3
07.01.01	Teal 1	15.6	7.6	65.0	5.3	19.4
10.01.01	Teal 1	15.4	15.6	43.6	5.8	14.5
11.01.01	Teal 2	15.3	10.7	53.3	5.3	19.0
14.01.01	Teal 2	15.5	4.9	38.3	2.6	11.3
19.01.01	Teal 1	15.4	19.4	57.3	9.7	4.7
22.01.01	Teal 1	15.4	1.0	85.2	4.4	7.4
24.01.01	Teal 1	16.2	16.2	58.7	8.1	6.9
26.01.01	Teal 1	15.5	24.4	35.9	8.1	5.2
29.01.01	Teal 1	15.3	10.6	45.3	4.4	8.4

Results

General breathing pattern

In Fig. 5.1 examples of a respiration air temperature trace in a resting (Fig 5.1A) and a flying bird (Fig 5.1B) are presented. Temperature increased during exhalation and decreased during inhalation. As shown in Fig. 5.1B the temperature change during breathing is not a steady increase and decrease over time. Rather, for all individuals there is a characteristic deformation in the curve just before a maximum is reached. A similar pattern was reported by Hart and Roy (1966) who measured the respiratory air flow in flying Pigeons. They suggested it was caused by the structural organisation of the avian respiratory tract which includes air sacs.

Exhaled air temperature and breathing frequency during rest

Using the average T_{exh} measured during six trials in the resting wigeon and five and seven trials in the two teal, T_{exh} was positively related with T_{amb} ($T_{exh} = 0.776\ T_{amb} + 11.090$; $R^2 = 0.825$; $p < 0.001$; $n = 18$). Although the slopes were the same for all individuals, there was a significant difference between intercepts ($F_{2,14} = 4.139$, $p = 0.039$). This appeared to be a difference between species rather than among individuals within a species, since for the teal there was no difference in intercept between the two individuals ($F_{1,9} = 0.804$, $p = 0.393$). For teal, T_{exh} was positively related with T_{amb} ($T_{exh} = 0.746\ T_{amb} + 9.691$; $R^2 = 0.799$, $p < 0.001$; $n = 12$), T_{db} was negatively related with T_{exh} ($T_{exh} = -\ 48.848\ T_{db} + 1916$; $R^2 = 0.346$; $p = 0.044$; $n = 12$), and T_{amb} was negatively related with T_{db} ($T_{db} = -\ 0.007\ T_{amb} + 38.96$; $R^2 = 0.522$; $p = 0.008$; $n = 12$). For the wigeon, T_{exh} was positively related with T_{amb} ($T_{exh} = 0.753\ T_{amb} + 14.792$; $R^2 = 0.988$; $p < 0.001$; $n = 6$), and we detected no other significant relationships between the other measured variables. Breathing frequency *(f)* was on average 0.30 Hz for the wigeon (SD = 0.05) and 0.59 Hz for the teal (SD = 0.13) irrespective of T_{amb}.

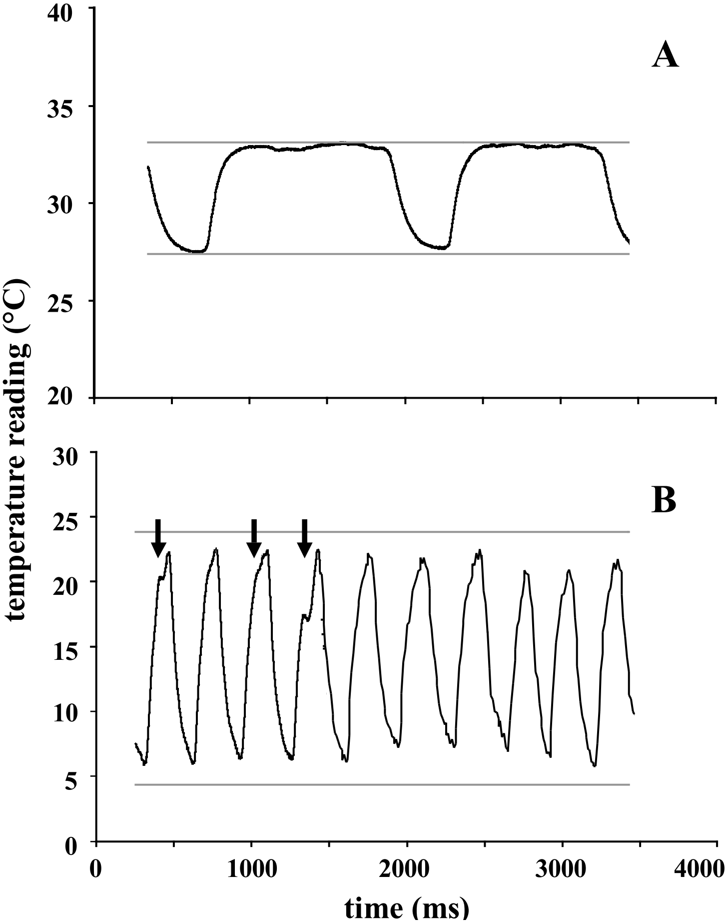

Fig. 5.1 Traces of respiration air temperatures of a teal during rest (A) and flight (B). An increase in temperature corresponds with exhalation, whereas a decrease in temperature corresponds with inhalation. Horizontal lines represent T_{amb} as measured and T_{exh} as it was calculated (see text). **A:** Rest at 27.5 °C: Breathing frequency is slow enough for the thermistor to follow the full temperature amplitude, as indicated by the plateau before the next temperature change and the fact that $T_{inhalation}$ equals T_{amb}. Calculated and measured T_{exh} = 33.1 °C. **B:** Flight at 4.9 °C: The thermistor's stabilisation time is too long to reach the actual T_{exh} and T_{amb}, respectively. Readings therefore had to be converted to obtain T_{exh} (see text). Calculated T_{exh} = 24.4 °C. Arrows indicate characteristic deformations in the curve that were observed in all individuals (see text).

Exhaled air temperature and breathing frequency during flight

Using the average T_{exh} calculated during nine flights in the wigeon and eight and three flights in the two teal (table 5.1), a positive relationship was found between T_{exh} and T_{amb} ($T_{exh} = 1.036\ T_{amb} + 13.526$; $R^2 = 0.582$; $p < 0.001$; $n = 20$; Fig. 5.2). The slope and intercept of this relationship were not significantly different between the three individuals ($F_{2,14} = 0.737$; $p = 0.496$).

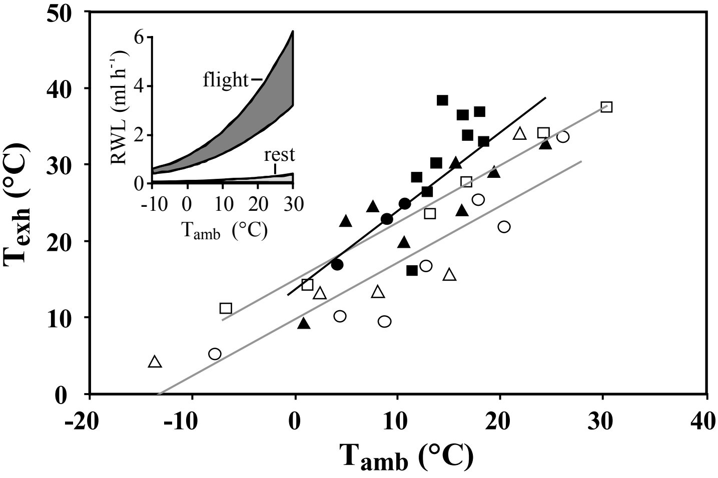

Fig. 5.2 Exhaled air temperature in relation to ambient temperature. Filled symbols represent flight measurements, open symbols resting data. Squares represent data of the wigeon, triangles and circles data from the two teal, respectively. The relationship of T_{exh} to T_{amb} during flight is best described by the black regression line $T_{exh} = 1.04\ T_{amb} + 13.5$ and is the same for both species. During rest, the relationship for teal is $T_{exh} = 0.75\ T_{amb} + 9.7$ (lower grey regression line) whereas for the wigeon it is $T_{exh} = 0.75\ T_{amb} + 14.8$ (upper grey regression line). **Inset**: To exemplify the consequences of the relationships between T_{exh} and T_{amb} for water loss, we calculated ranges of respiratory evaporative water loss (REWL, ml h^{-1}) in relation to T_{amb} for teal from the measurements of T_{exh} and f, and an assumed tidal volume of 10.0 ml during flight and 5.0 ml during rest (Bernstein, 1987) under the assumption that expired air is water saturated. Assuming all respiratory variables being constant, REWL at a given T_{amb} depends on the water vapour pressure in the ambient air, with an upper limit at 0 % and a lower limit at 100 % relative humidity, respectively.

For the wigeon only, T_{db} was positively related with T_{exh} (T_{exh} = 12.226 T_{db} + 480.704; $R^2 = 0.628$; $p < 0.02$; n = 9), and the inclusion of T_{db} and T_{amb} in a multiple regression analysis increased the explained variation in T_{exh} from 52 % to 63 %. Irrespective of the fact that there was no obvious relationship between average T_{exh} and T_{db} in teal, it became apparent that body temperature nevertheless had a significant positive effect on T_{exh} in all individuals by investigating the relationship between both variables within separate flights. For the individual flights of wigeon (Fig. 5.3), the effect of T_{db} on T_{exh} was highly significant ($F_{1,181} = 178.14$; $p < 0.001$), the slopes were indistinguishable, but the intercepts were significantly different ($F_{4,181} = 354.24$; $p < 0.001$). For the individual flights of teal, the positive effect of T_{db} on T_{exh} was highly significant ($F_{1,192} = 25.17$; $p < 0.001$), and both the slopes ($F_{9,192} = 2.78$; $p = 0.004$) and the intercepts ($F_{9,192} = 2.73$; $p = 0.005$) were significantly different between flights.

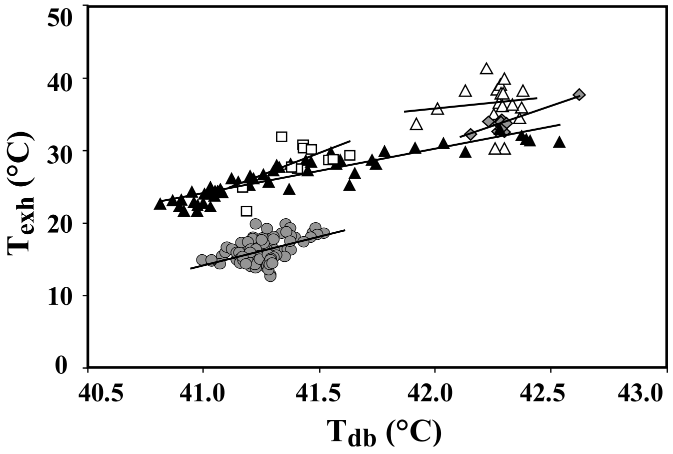

Fig. 5.3 Exhaled air temperature (T_{exh}) in relation to body temperature (T_{db}) during five flights in the wigeon. Each line represents the relationship between T_{exh} and T_{db} for a single experimental flight. The slopes and intercepts of the regression lines do not differ statistically.

f was on average 2.59 Hz for the wigeon (SD = 0.12) and 2.73 Hz for the teal (SD = 0.25). *f* was positively related to T_{amb} in the wigeon only ($f = 0.037\ T_{amb} + 2.038$; $R^2 = 0.670$; $p = 0.007$; n = 9; Fig. 5.4). This positive relationship between *f* and T_{amb} in the larger wigeon (average body mass: 662 g) compared to the smaller teal (average body mass: 316 g) may be caused by heat stress in wigeon as indicated by the decrease in (voluntary) flight duration as T_{amb} increased (duration = -241.117 T_{amb} + 4414.773; $R^2 = 0.589$; $p = 0.016$; n = 9).

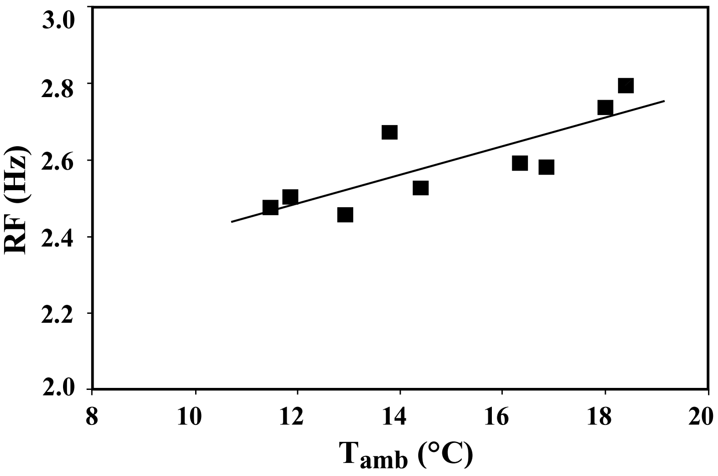

Fig. 5.4 Respiratory frequency of a wigeon during flight at different ambient temperatures. Each point represents the mean of a single flight. *f* increases with T_{amb} following the equation $f = 0.037\ T_{amb} + 2.038$.

Comparison of resting and flying birds

When comparing resting with flying birds the relationship between T_{exh} and T_{amb} ($F_{1,34}$ = 107.309, $p < 0.001$) was significantly different between species ($F_{1,34}$ = 12.052, $p = 0.001$) and type of activity (resting vs. flying; $F_{1,34}$ = 14.359, $p = 0.001$), although the difference between resting and flying was not significant for the wigeon ($F_{1,12}$ = 4.049, $p = 0.067$). For teal, T_{exh} adjusted for T_{amb} was significantly lower (ANCOVA: $F_{1,20}$ = 1.818; $p = 0.005$) when the birds were at rest (17.5 °C) compared to when flying (22.8 °C). For wigeon, T_{exh} adjusted for T_{amb} was not significantly different (ANCOVA: $F_{1,11}$ = 1.963, $p = 0.189$) although adjusted T_{exh} was lower when birds were at rest (25.5 °C) compared to when flying (29.8 °C).

Discussion

The linear relationship between T_{exh} and T_{amb} (for $T_{exh} < T_{db}$) that we documented in both resting teal and wigeon is consistent with previous studies on resting birds (Schmidt-Nielsen et al. 1970, Murrish 1973, Tieleman et al. 1999, Geist 2000, Michaeli and Pinshow 2001). For resting teal but not wigeon, T_{db} was negatively correlated with both T_{amb} and T_{exh}. This phenomenon of elevated T_{db} at low T_{amb} for the teal may occur because metabolic demands increase more rapidly as temperatures cool for the relatively small teal compared to the wigeon. We thus assume a causal relationship between T_{amb} and T_{db}. Since there also exists a causal relationship between T_{exh} and T_{amb} the negative correlation between T_{db} and T_{exh} comes as no surprise. However, we do not consider this a causal relationship.

For the cases where $T_{amb} < T_{db}$, inhaled air has a cooling effect on the respiratory surfaces. During exhalation the respired air, heated to T_{db}, loses part of its heat to these surfaces. If there were no specific regulation of T_{exh}, this passive process would result in an T_{exh} which is intermediate to T_{db} and T_{amb}. Accordingly, the difference between T_{exh} and T_{amb} would be highest at low T_{amb} and gradually decrease with increasing T_{amb}. An active regulation of T_{exh}, in contrast, would enable an animal to increase the slope of the relationship between T_{exh} and T_{amb}. At low T_{amb}, when body temperature must be conserved, regaining body heat from the breathing air would be beneficial and favour low T_{exh}. At high T_{amb}, high rates of evaporative heat loss are facilitated by high T_{exh} which can prevent overheating. Indeed, penguins actively regulate T_{exh} by changing the blood flow to nasal tissues (Murrish 1973). Measurements on desert larks suggest that passive processes may also be involved because oral T_{exh}, which is unaffected by changes in blood flow to the nasal mucosa, was only slightly warmer than nasal T_{exh} (Tieleman et al. 1999). However, passive and active mechanisms need not be mutually exclusive.

Knowledge about the mechanisms that determine T_{exh} during flight is limited, mainly because studying respiratory physiology during flight is difficult. Previous studies that report T_{exh} in flying birds are summarized in Fig. 5.5. Despite differences in methodology and study species, Berger et al. (1971) and Michaeli and Pinshow (2001) report an almost identical relationship between T_{exh} and T_{amb}. This irrespective of the fact that Berger et al. (1971) did not measure T_{exh} during flight but took their measurements directly after flights of 5 to 18 s duration. Hudson and Bernstein (1981) estimated T_{exh} in ravens using mask respirometry and found that T_{exh} was negatively related to T_{amb} although their estimates of T_{exh} are generally higher than measured in the other studies. Possibly, this difference is due to their specific method, which primarily measured respiratory evaporation. Such measurements of respiratory evaporation may overestimate T_{exh} because a high proportion of open-bill breathing leads to high evaporation rates in part because air bypasses the nasal turbinates, which are the main structure of the cooling system (Murrish 1973). A back-calculation to T_{exh} from evaporation rates therefore does not represent nasal T_{exh} but an unknown combination of nasal and oral T_{exh}. Our study was restricted to nasal T_{exh} and can therefore not readily be compared with the raven data.

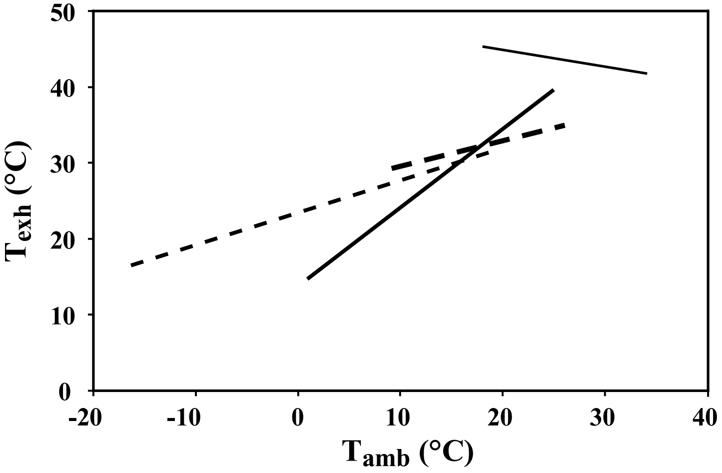

Fig. 5.5 Empirical estimates for exhaled air temperature during bird flight in relation to ambient temperature. Thin stippled line: Black ducks, measured directly after short flights (Berger et al. 1971). Thick stippled line: Free flying Pigeons (Michaeli and Pinshow 2001). Thin solid line: White-necked ravens flying with a respirometry mask in a wind tunnel (Hudson and Bernstein 1981). Thick solid line: wigeon and teal flying in a wind tunnel (this study).

In the present study, T_{exh} was positively correlated and consistently higher (ca. 13 °C) than T_{amb} and below body temperature. Cooling efficiency, indicated by the difference between T_{exh} to T_{db}, decreased with increasing T_{amb}, probably because the temperature gradient decreased between T_{amb} and T_{db}, which led to a slower heat flow from the warm body to the surroundings. In addition, a faster respiration, which is a reaction to heat stress also in flying birds (Hudson and Bernstein 1981), shortens the time of contact between breathing air and nasal tissues which further reduces heat exchange. An extrapolation of the relationship of T_{exh} to T_{amb} indicates that T_{exh} will approach T_{db} at a T_{amb} of 26 °C. Whether this is an adaptation to increase evaporation in favour of thermoregulation or a passive process remains to be shown. The relatively steep relationship between T_{exh} and T_{amb} along with the relatively constant difference between T_{exh} and T_{amb} suggests active regulation.

Body temperatures were markedly higher in the wigeon than in the teal. Measurements of core temperatures during flight in three species of bird were 42° to 45° C (Torre-Bueno 1976, Hudson and Bernstein 1981, Hirth et al. 1987, Adams et al. 1999) and were typically about 2.8 ° higher than at rest (Prinzinger et al. 1991). Similarly, the T_{db} in wigeon were 40.3 °C at rest and 41.8 °C during flight. In contrast, T_{db} in the teal were surprisingly low (38.9 °C at rest and 39.3 °C during flight). A direct comparison of body temperatures, however, is problematic because the position of the temperature sensor in the body was not fully standardized. The fact that T_{db} increased with T_{amb} in the wigeon, whereas T_{db} in teal was relatively stable over the same range of T_{amb}, suggests that the wigeon was heat stressed, but the teal were not. Also, flight duration of wigeon changed with T_{amb} whereas it did not for teal. If birds actively regulate T_{exh}, then heat stressed birds should have a higher T_{exh} at a given T_{amb} than birds that are not heat stressed. This idea is not supported by our data, although the lack of a significant difference in T_{exh} between the two species might be due to small sample size.

Although the small sample size in our study necessitates caution when generalizing the results, our data show that cooling of the exhaled air occurs during flight, and they suggest that birds may at least in part actively regulate T_{exh}. Our data support the observation of Michaeli and Pinshow (2001) that the temporal counter-current heat exchange mechanism is more efficient during rest compared to flight, since T_{exh} in our ducks was about 5 °C higher during flight than at rest. A quantification of respiratory evaporative water loss (REWL) during flight, however, requires more detailed knowledge of ventilation. Only very limited data on tidal volume (V_t) in flying birds are available (Hart and Roy 1966, Berger et al. 1970, Bernstein 1976, 1987, Banzett et al. 1992, Boggs et al. 1997a), and none of these studies used teal or wigeon. Still, we tentatively estimated REWL for teal at various ambient temperatures assuming either completely dry air (relative humidity RH = 0 %) or completely saturated air (RH = 100 %; Fig. 5.2, inset). The actual relative humidity during our experimental flights was on average 51.8 % (ranging from 35.9 to 85.2 % RH) with low temperatures being associated with lower humidity.

For the calculation of REWL we used the allometric prediction of V_t from Bernstein (1987) that was based on measurements of V_t during flight in 10 species (body mass range: 6-1000 g), and we assumed that V_t was independent of T_{amb} and that exhaled air was completely water saturated. Exhaled air is usually considered to be water saturated (Schmidt-Nielsen et al. 1970) but there are indications that this might not always be the case in birds (Marder 1973a, Kaiser and Bucher 1985), in which case our estimates for REWL would be too high.

Considering only T_{exh} and assuming all other respiratory variables remain constant, the fact alone that T_{exh} is less than T_{db} leads to a decrease of respiratory water loss, as long as T_{amb} are relatively cool. The relatively steep relationship between T_{exh} and T_{amb} suggests a strong advantage of flying at low T_{amb} because it reduces respiratory water loss even further than previous measurements suggested (Berger et al. 1971, Michaeli and Pinshow 2001), which would considerably increase flight range.

CHAPTER 6

Respiratory water loss during rest and flight in European Starlings (*Sturnus vulgaris*)

Sophia Engel, Roderick A. Suthers, Herbert Biebach, and G. Henk Visser

Abstract

We assessed respiratory water loss in European Starlings (*Sturnus vulgaris*) both at rest and during steady flight in a wind tunnel over a range of ambient temperatures using respiratory air flow and exhaled air temperature data. In resting Starlings, breathing frequency *(f)* was constant at 1.4 ± 0.3 Hz at ambient temperatures (T_{amb}) between 6 °C and 25 °C. Also tidal volume (V_t) was independent of T_{amb} with an average value of 1.9 ± SD = 0.4 ml. There was a negative correlation between V_t and f ($V_t = -1.01 \cdot f + 3.30$; n = 17; $p < 0.01$; $R^2 = 0.45$). Mean ventilation rate (V_{min}), the product of f and V_t, was 156 ± 28 ml min^{-1} (n = 17) at all T_{amb}. Exhaled air temperature (T_{exh}) during rest was strongly dependent on T_{amb} ($T_{exh} = 0.92 \cdot T_{amb} + 12.45$; n = 23; $p < 0.001$; $R^2 = 0.89$). Respiratory water loss at rest (REWL) averaged 0.18 ± 0.09 ml h^{-1} (n = 10) and was independent of T_{amb}, but showed a slight positive dependence on f and T_{exh}. In flying Starlings, f was on average 4.0 ± 0.4 Hz (n = 44) and unchanging over the range of T_{amb} measured. V_t during flight averaged 3.6 ± 0.4 ml (n = 25) and increased with T_{amb} ($V_t = 0.06 \cdot T_{amb} + 2.83$; n = 25; $p < 0.01$; $R^2 = 0.29$), as a consequence, the volume of ventilated air during flight (average V_{min} = 789.9 ± 210.0 ml min^{-1}), increased with T_{amb} as well. T_{exh} during flight was 4.6 °C higher than at rest and strongly dependent on T_{amb} ($T_{exh} = 0.85 \cdot T_{amb} + 17.29$; n = 36; $p < 0.001$; $R^2 = 0.74$). All factors together result in respiratory water loss during flight (average REWL_f = 0.74 ± 0.22 ml h^{-1}) significantly higher than at rest and increasing with T_{amb}. REWL_f correlated best with the water vapour pressure deficit (VPD) in ambient air. From our measurements and data from the literature, we conclude that respiratory evaporation accounts for most water loss in flying European Starlings and increases to a higher degree than cutaneous evaporation with rising ambient temperature.

Introduction

Avian flight is associated with enormous energetic demands (Norberg 1996) and high internal heat loads that must be dissipated (Hill 1938, Biewener et al. 1992). During flight both, the high oxygen demand and the increased heat load are likely to increase evaporation, an important means of heat dissipation, to well above resting levels. The idea of water depletion as a limiting factor in long-distance migration of birds was proposed almost 50 years ago (Yapp 1956, 1962), although few studies actually showed dehydration in birds following long periods of flight across deserts or oceans (Serle 1956, Johnston 1968, Moreau and Dolp 1969, Haas and Beck 1979, Leberg et al. 1996). Nevertheless, even in the absence of direct physiological signs of dehydration (Carmi et al. 1993), the behaviour and physiology of migrants may well be governed by the threat of dehydration, as model calculations suggest (Carmi et al. 1992, Carmi et al. 1993, Klaassen 1995, Klaassen et al. 1999, Klaassen 2004). Sensitivity analyses of these models indicated that of the model input variables, exhaled air temperature and oxygen extraction rate most strongly affect dehydration rates during flight (Carmi et al. 1992, Klaassen et al. 1999). Both variables act on the rate of respiratory water loss (REWL): oxygen extraction through its effect on the volume of ventilated air (*i.e.*, breathing frequency times tidal volume) and exhaled air temperature by modulating recondensation of water vapour in expired air on the walls of the respiratory passages (Schmidt-Nielsen et al. 1970).

Through temporal counter-current heat exchange in the upper respiratory tract (Jackson and Schmidt-Nielsen 1964, Schmidt-Nielsen et al. 1970) exhaled air temperature in resting endotherms is usually below body temperature, though strongly correlated with ambient temperature (Jackson and Schmidt-Nielsen 1964, Murrish 1973, Welch 1984, Tieleman et al. 1999, Geist 2000, Michaeli and Pinshow 2001). When the beak is closed, this anterior respiratory tract cooling mechanism indeed brings about lower respiratory water loss than when birds breathed through the open beak, thus bypassing the narial turbinates (Tieleman et al. 1999, Geist 2000). Exhaled air temperature during flight was measured in only a few studies (Berger et al. 1971, Hudson and Bernstein 1981, Michaeli and Pinshow 2001 and chapter 5). The only direct comparison of T_{exh} during rest and flight suggests that the counter-current cooling mechanism is less efficient during flight than at rest (Michaeli and Pinshow 2001), but more data are needed to solidify this.

In theory, birds can adjust their ventilation rates to metabolic needs through a modulation of respiratory frequency or tidal volume, or a combination of both (Bucher 1985). Because the temperature range that our birds were exposed to was moderate when at rest, we did not expect detectable changes in ventilation volume among measurements made at different ambient temperatures. In contrast, during flight, metabolic rate increases to about tenfold resting values (Berger et al. 1970, Tatner and Bryant 1986, Biesel and Nachtigall 1987), and, in some studies, the increased oxygen demand was reflected in a concomitant increase in ventilation (Berger et al. 1970, Bernstein 1976, Hudson and Bernstein 1981). The energy cost of flight appears to be independent of ambient temperature (Bernstein et al. 1973). We therefore hypothesised that ventilation (the product of tidal volume and breathing frequency) is also independent of ambient temperature (but see Bernstein 1976), while exhaled air temperature is positively correlated with ambient temperature (Berger et al. 1971, Hudson and Bernstein 1981, Michaeli and Pinshow 2001 and chapter 5), thereby increasing respiratory water loss with increasing ambient temperature. Because only a few studies of limited scope have been published in this area (Hart and Roy 1966, Berger et al. 1970, 1971, Bernstein 1976, Butler et al. 1977, Berger 1978, Hudson and Bernstein 1981, Michaeli and Pinshow 2001) the quantitative aspects of ventilation and respiratory evaporation during bird flight remain poorly known.

Resting birds react to increasing ambient temperatures by increasing both cutaneous and respiratory evaporation (Marder and Ben-Asher 1983, Wolf and Walsberg 1996, Hoffman and Walsberg 1999, Tieleman and Williams 2002). The relative contribution of cutaneous and respiratory water loss to total evaporation is variable and depends, among other factors, on the phylogenetic order (Wolf and Walsberg 1996). It appears that at high ambient temperatures, passerines, unlike *e.g.* Pigeons (Webster and King 1987, Withers and Williams 1990, Michaeli and Pinshow 2001) rely more on respiratory than on cutaneous evaporation (Lee and Schmidt-Nielsen 1971, Wolf and Walsberg 1996, Tieleman and Williams 2002). Given the high heat load and the increased ventilatory demands during exercise (Bernstein 1976, Brackenbury 1984), we predicted that respiratory evaporation is the major avenue of water loss for passerines also during flight.

Here we report measurements of tidal volume and respiratory frequency in European Starlings (*Sturnus vulgaris*) in relation to ambient temperature. We also made simultaneous determinations of exhaled air temperature for the birds, both at rest and during steady flight in a wind tunnel. These variables allow the quantification of respiratory water loss and of heat loss through respiratory evaporation. In combination with data from the literature on flight costs and total evaporation rates, we present a tentative calculation of the oxygen extraction rate and estimate the relative importance of respiratory and cutaneous evaporation for flying Starlings.

Methods

Experimental birds and training

Experiments were conducted with ten European Starlings (*Sturnus vulgaris*) that were taken in April 2001 as nestlings from a local population, and hand raised in the Max Planck Institute for Ornithology, Seewiesen, Germany. The birds had an average body mass of 78.5 g throughout the experimental period. They were trained to fly in the wind tunnel of the Max Planck Institute for Ornithology, described in more detail in chapter 3. Air speed in the wind tunnel can be set from 0 to 45 ± 0.1 m s^{-1}. Temperature can be controlled and kept within 1 °C by a heat exchanger in the air stream. Wind speed, ambient temperature, humidity and air pressure were recorded automatically by a data monitoring system (ARGUS by Sorcus) with a sampling rate of 1 Hz.

Five of our ten birds had previous experience flying in the wind tunnel, but unrestrained. All 10 birds were trained to wear measuring equipment connected to a recording system by thin copper leads (Ø = 0.3 mm). For four weeks prior to the experiments, the birds were flown daily for 10 to 60 minutes with a dummy back-pack and about one metre of thin copper wire trailing behind. When the first birds got used to wearing this equipment and were flying in an apparently natural manner, the naïve birds were trained alongside them which greatly shortened the time it took for them to learn, even when carrying a pack. To avoid habituation to a certain ambient temperature during flight we changed the flight temperature frequently in a range from 5 °C to 25 °C. During the training and experimental phase the birds were kept in groups of three in small aviaries (1 x 2 x 2 m) adjacent to the wind tunnel under a light regime simulating natural conditions at the location of the institute at Seewiesen (47.5° N). All birds were in good condition and had intact flight feathers during experiments.

Measuring exhaled air temperature, tidal volume and respiratory frequency

Experiments were conducted during autumn 2002 and spring 2003. We simultaneously measured exhaled air temperature (T_{exh} [°C]), tidal volume (V_t [ml]) and breathing frequency (f [Hz]) during and after flights at ambient temperatures (T_{amb} [°C]) of 6.0 ± 0.7 °C, 15.2 ± 0.2 °C or 25.2 ± 0.3 °C. Flight speed was kept constant at 11.6 ± 1.0 m s^{-1}. Flight duration was between 10 and 25 min depending on the bird's condition. For analysis we discarded the first five minutes of flight and the first three minutes of rest after the flight. From the following minute onwards we determined one value of each variable per minute. T_{exh} and f values were averaged each five to ten seconds, while V_t was averaged over 10 to 20 seconds intervals. For statistical analysis the mean of these values was used for each flight or resting period. During some flights, the birds did not always breathe through the nostrils but sometimes opened the beak to a variable degree for short periods. This behaviour usually resulted in a deterioration of the T_{exh} signal, and we discarded these episodes from our analysis. Thus only results collected while the birds flew with closed beaks are presented.

The birds carried a backpack made of two thin silicon tube loops connected by a small (15 x 15 mm) piece of Velcro® during the measurements. The loops were put around the bird's legs and adjusted so that the backpack was positioned snugly on the lower part of the back without disturbing the normal behaviour of the birds. Two pairs of gold mini pin connectors were fixed on the Velcro to which the sensors for the flow and the temperature measurements could be connected. A thin four-stranded copper wire (Ø = 0.3 mm), long enough to allow the bird free movement in the wind tunnel test section, was connected to the free poles of the connectors of the backpack during the experiments and to the data recording system (a TEAC model RD135T DAT-recorder in autumn 2002 or PowerLab®/8SP in spring 2003). The total mass of the backpack with measuring devices including the wire had a maximum mass of 4.0 g, which is about 5 % of the average body mass of the birds.

V_t was measured in the actual respiratory air flow with a flow transducer made of a microbead thermistor (Thermometrics B07JA202N or B07KA202N), nominally 0.18 mm in diameter, mounted on the end of an epoxy-coated wire support. The bead was positioned in the center of the tracheal lumen by inserting it through a small hole in the ventral side of the trachea and attaching its wire support to the outside of the trachea with tissue adhesive and a suture. Fine leads from the flow probe were routed under the skin to the animal's back and soldered to one pair of gold mini pin connectors fastened on the backpack of the bird (Schurr Hartley and Suthers 1989). A feedback circuit (built by Hector Engineering, Elletsville, IN, U.S.A. in 2002; Johannes Sagunsky, MPI Seewiesen in 2003) heated and maintained the implanted flow probe at a constant temperature. The current required to heat the thermistor increased with the rate at which air flowed past the bead and was recorded with a sampling rate of 10 kHz.

Before and after each flight, the output of the feedback circuit was calibrated against the rate of airflow through the trachea by tightly fitting a mask with a previously calibrated thermistor over the bird's beak so that the output voltages were recorded simultaneously from the calibrated and the implanted thermistor. The flow probe in the mask was calibrated prior to the experiments against a range of known flow rates (0.2 to 50.0 ml s^{-1}) of water saturated air at 40 °C, resembling respiratory air as much as possible. Both transducers experienced the same volume flow, but shifted in time because the calibrated thermistor was about 6 cm upstream from the implant. For analysis we therefore shifted one of the two signals in time in so that the start of each inhalation or exhalation was aligned. A plot of the internal signal against the calibrated signal revealed a linear relationship that allowed the calculation of air flow rate also from the non-calibrated implant. The flow signal recorded during experiments was later integrated to tidal volume as described by Suthers and Hector (1982), using only the expiratory signal, because the calibration conditions more closely resembled exhaled air than ambient air. All volumes are reported at body temperature and pressure, assuming saturation with water vapor (BTPS).

Exhaled air temperature (T_{exh}) was measured with a microbead thermistor (Fenwal BC32L1), 0.20 mm in diameter, soldered to a thin copper wire support that was connected to the backpack. The probe was attached to the side of the bird's bill immediately before a flight with small pieces of Hansaplast® tape so that the tip of the thermistor was positioned exactly in the opening of the left nostril. The signal was recorded at a sampling rate of 10 kHz. Before the experiments, the thermistor was calibrated to 0.1 °C against a mercury-in-glass thermometer, with accuracy certified by the State Office of Metrology of Hessen, Germany over a range of temperatures larger than the range of respiratory air temperatures.

The electronic resistance of the thermistor changed linearly with temperature. The time needed for the thermistor to fully stabilise at a new temperature (stabilisation time t_s [s]) was estimated experimentally ba exposing the thermistor to airflows of different temperatures and flow rates and measuring the time needed to fully stabilise at the new temperature. The stabilisation time of the thermistor appeared to be mainly dependent on the temperature gradient (ΔT [°C]) between initial and final temperature ($t_s = 0.221\ \Delta T^{\ 0.1161}$; $R^2 = 0.505$) and was too slow to precisely follow the temperature changes during breathing. This was obvious from the inhalation temperature, which should equal T_{amb}, but never reached this value. Accordingly, we assume that the actual T_{exh} was not attained. Therefore, we calculated T_{exh} from the average temperature (T_{av} [°C]) and the inhalation or T_{amb}:

$$T_{exh} = 2 \cdot T_{av} - T_{amb}\ \ [°C] \qquad \text{(eqn. 6.1)}$$

assuming that inhalation and exhalation cause the same response, but of opposite polarity, from the thermistor (Fig. 6.1), and that T_{av} therefore lies midway between T_{amb} and T_{exh}. Generally, during rest there was only a small difference between the minimum readings of the respiration temperature trace and the known T_{amb}. In the example in Fig. 6.1A, average minimum readings were 15.7 °C over five seconds, as opposed to the known T_{amb} of 15.3 °C. Accordingly, average maximum readings were slightly lower than calculated T_{exh} (24.3 °C average maximum versus 24.9 °C calculated T_{exh}). During flight, the durations of exhalation and inhalation were closer to equality than at rest (see Fig. 6.1B), the calculated T_{av} therefore was probably closer to the middle between actual T_{exh} and T_{amb}. Therefore, the error in our estimate is probably smaller for flight than rest, and even if our assumption that T_{av} is precisely between T_{amb} and T_{exh} was not true, the resulting error would be less than one degree.

We calculated one T_{exh} value per minute by averaging the temperature readings over a 5 to 10 second interval. The respiratory frequency was determined by counting the number of minima or maxima over a known period in the T_{exh} recording; each maximum corresponds to the start of an inhalation, and each minimum to the start of an exhalation.

In addition to the flight experiments, we also measured post-flight values of T_{exh}, V_t and f from the birds while they sat for 10 to 15 minutes on a perch in the wind tunnel with the wind still on. The first three minutes of the post-flight recordings were discarded and measurements were only taken if the bird was resting quietly and the signals were clean. If a bird pecked at the backpack when perched, it was taken out of the wind tunnel to protect the sensor probes.

The implanted flow probes functioned for a maximum of four days before the signal deteriorated, probably due to mucus secretions covering the thermistor. During the four days, experimental flights were made as long as the bird cooperated in the wind tunnel, typically for two to four flights a day. Between trials, the nasal probe was removed. After the experiments the flow probe was removed surgically. All surgery was done under anaesthesia (Isoflurane®). The birds recovered quickly from this treatment, behaved normally, and usually could fly in the wind tunnel within half an hour. Because we obtained unequal numbers of observations per bird results are given as weighted averages ± 1 SD (Sokal and Rohlf 1981). Significance was generally accepted at the 0.05 level.

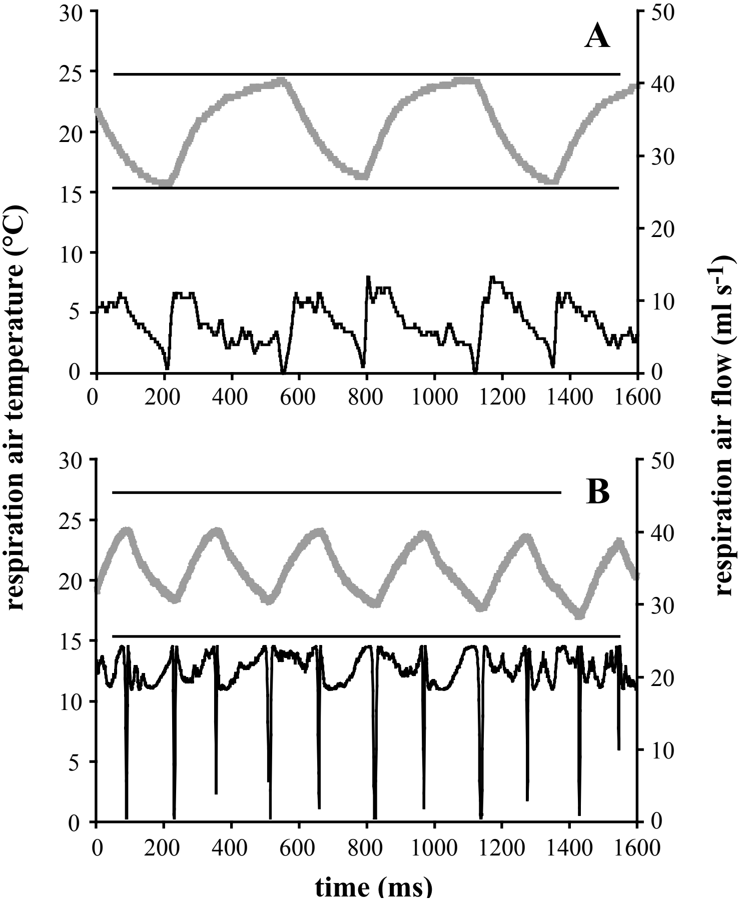

Fig. 6.1 Traces of respiratory air temperature (grey lines) and respiratory air flow (black lines) of a Starling during rest at 15.3 °C **(A)** and during flight at 15.2 °C **(B)**. The temperature of respired air increases during exhalation and decreases during inhalation. Horizontal lines represent T_{amb} as measured in the wind tunnel and T_{exh} as it was calculated (see text). The thermistor's reaction time was too slow to reach T_{amb} at inhalation or the actual T_{exh} at exhalation. Readings therefore were corrected to obtain T_{exh} (see text). Tidal volume was calculated from respiratory air flow during exhalation, because our calibration conditions (vapour-saturated at 40 °C) resembled those of the exhaled air rather than ambient air.

Results

Respiration at rest

As an example of respiration during rest, we present the recording of respiratory air temperature and respiratory air flow in Fig. 6.1A. The temperature of respired air increases during exhalation and decreases during inhalation. Average T_{exh} during 23 measurements was 25.7 ± 6.2 °C (n = 7 birds), mean f for 25 measurements at rest was 1.4 ± 0.3 Hz (n = 6 birds), and V_t during 17 measurements was 1.9 ± 0.4 ml (BTPS) per breath (n = 6 birds). To test whether part of the variation could be explained by changes in ambient conditions and to check for any effect of the day on which an experiment took place, we performed mixed model analyses. Neither the year of the experiment nor its time in the season had a detectable effect on either of the parameters. For T_{exh} as dependent variable, we included T_{amb} and day (1 being the first day of the first experiment, *i.e.* September 9th) as covariates, as well as f, assuming that f might influence T_{exh} through the time of contact between respired air and the tissue of the nasal passages. Only T_{amb} had a significant effect on T_{exh} (estimate = 0.92; S.E. = 0.07; t = 13.10; $p < 0.001$). This relationship is depicted in Fig. 6.2 and is best described by the least squares regression: $T_{exh} = 0.92 \cdot T_{amb} + 12.45$ (n = 23; $p < 0.001$; $R^2 = 0.89$).

Breathing frequency was tested for the effects of T_{amb} and day. There was a small but statistically significant negative effect of T_{amb} on f (estimate = - 0.03; S.E. = 0.01; t = -3.10; $p < 0.005$), that is best described by the least squares regression: $f = -0.03 \cdot T_{amb} + 2.02$ (n = 25; $p < 0.01$; $R^2 = 0.30$).

We predicted that tidal volume is dependent on T_{amb}, but we also assumed a correlation with f, and tested whether there is an effect of the time within the experimental season at which the experiment took place. Only f had a significant effect on V_t (estimate = - 1.01; S.E. = 0.29; t = - 3.53; $p < 0.005$; see Fig. 6.3), following the least squares regression $V_t = - 1.01 \cdot f + 3.30$ (n = 17; $p < 0.005$; $R^2 = 0.45$)

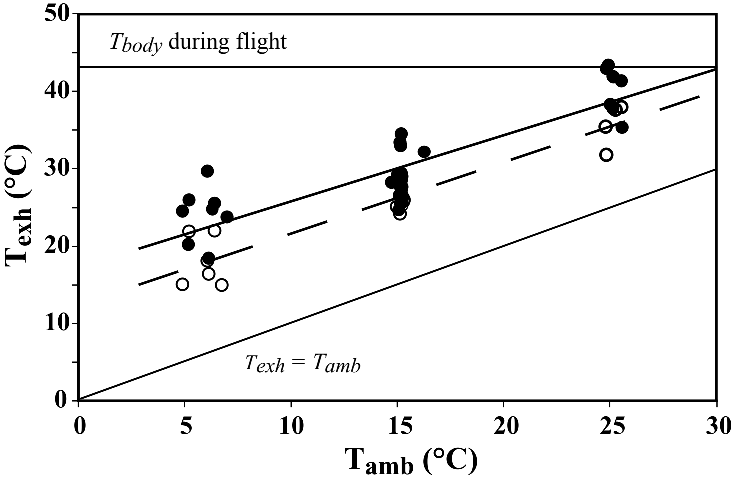

Fig. 6.2 Exhaled air temperature (T_{exh}) in relation to ambient temperature (T_{amb}). Filled circles represent flight measurements, open circles resting data. The relationship of T_{exh} to T_{amb} during flight is best described by the solid regression line $T_{exh} = 0.92 \cdot T_{amb} + 12.45$. During rest, the relationship is best described by the broken regression line $T_{exh} = 0.85 \cdot T_{amb} + 17.29$. For better orientation we also depicted body temperature (T_{body} = 43.3 °C) of European Starlings during flight (Torre-Bueno 1976) and the relationship $T_{exh} = T_{amb}$.

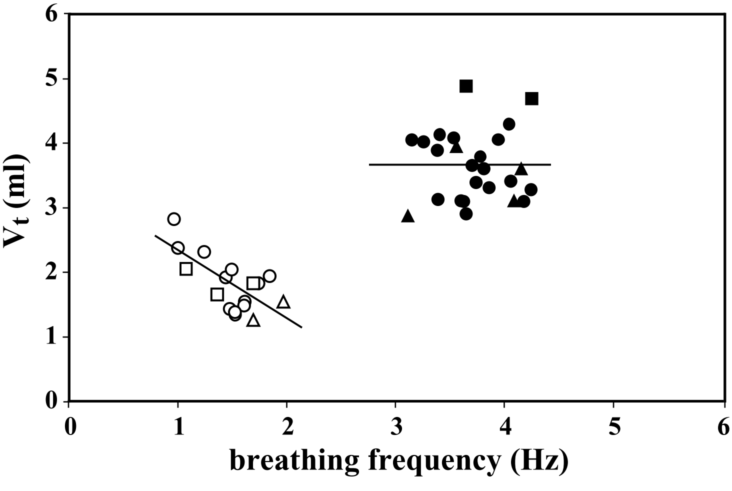

Fig. 6.3 Tidal volume (V_t) in relation to breathing frequency *(f)*. Filled symbols represent flight measurements, open symbols resting data. Triangles are measurements made at 6 °C, circles at 15 °C and squares at 25 °C, respectively. V_t does not change with breathing frequency during flight. At rest, the relationship is best described by the equation $V_t = -1.01 \cdot f + 3.3$. Regression statistics are given in the text.

Since breathing frequency decreases with rising T_{amb}, V_t is constant over T_{amb}. Minute volume V_{min}, calculated from f and V_t, was on average 156.4 ± 27.9 ml min^{-1}. There was no detectable effect of T_{amb} on V_{min} (Fig. 6.4).

We calculated respiratory evaporation during rest ($REWL_r$ [ml h^{-1}]) from V_{min} and T_{exh}, assuming the expired air to be saturated with water vapour at T_{exh}. $REWL_r$ averaged 0.18 ± 0.09 ml h^{-1}. In a mixed model analysis we tested $REWL_r$ for the effect of the water vapour pressure deficit of the ambient air VPD, T_{amb}, T_{exh}, V_t, f and date. f was positively correlated with $REWL_r$ (estimate = 0.11; S.E. = 0.03; t = 3.47; $p < 0.05$), and T_{exh} also had a small but significant positive effect (estimate = 0.01; S.E. = 0.00; t = 8.96; $p < 0.001$). We did not detect any significant effect of ambient conditions on REWL at rest (Fig. 6.5). By multiplying the average rate of respiratory evaporation by the heat of vaporization of water (estimated as 2424 J g^{-1}, following Schmidt-Nielsen 1997) we calculated a cooling capacity of 0.12 ± 0.06 W.

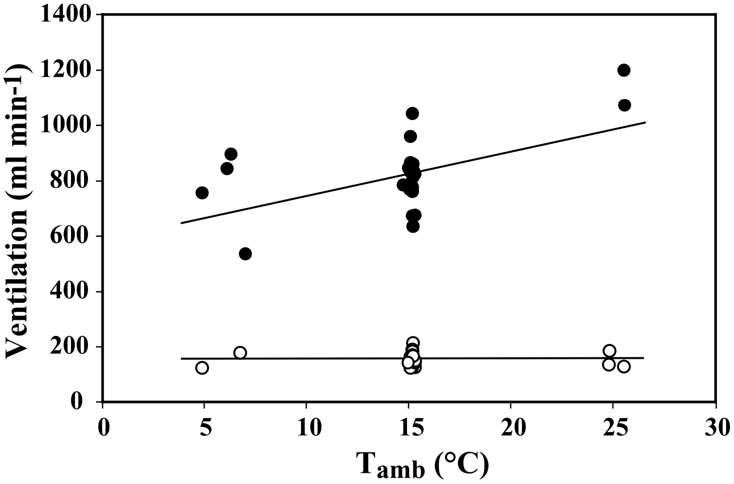

Fig. 6.4 Respiratory ventilation (V_{min}) in relation to ambient temperature (T_{amb}). Filled symbols represent flight measurements, open symbols resting data. Ventilation at rest is constant over the range of T_{amb}. During flight, the relationship of ventilation to T_{amb} is best described by the regression line $V_{min} = 15.9 \cdot T_{amb} + 586.8$. Regression statistics are given in the text.

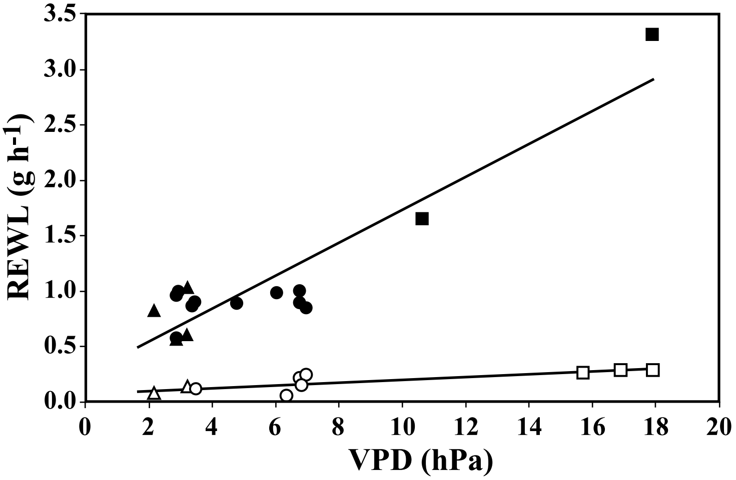

Fig. 6.5 Respiratory evaporative water loss (REWL) in relation to vapour pressure deficit (VPD) of the ambient air. Filled symbols represent flight measurements, open symbols resting data. Triangles are measurements made at 6 °C, circles at 15 °C and squares at 25 °C, respectively. The slope of the relationship between the two variables at rest is not significantly different from zero. During flight, the relationship between REWL and VPD is best described by REWL = 0.15 · VPD + 0.25. Regression statistics are given in the text.

Respiration during flight

An example of the respiration pattern during flight is given in Fig. 6.1B. T_{exh} averaged 30.3 ± 6.4 °C (36 flights; n = 8 birds). This is 4.6 °C higher than during rest (Fig. 6.2). Mean f during 45 flights (n = 8 birds) increased significantly to 4.0 ± 0.4 Hz (One-way ANOVA; $F_1 = 559.07$; $p < 0.001$), while V_t (26 measurements, n = 6 birds) increased to an average of 3.6 ± 0.4 ml (BTPS) per breath (One-way ANOVA; $F_1 = 127.38$; $p < 0.001$). To test for the effect of T_{amb} on either variable, we did mixed model analyses. We also tested for the effect of the date at which an experiment was performed and, again, found no detectable effect on any variable.

To examine T_{exh} as a dependent variable we used T_{amb} as covariate, as well as date and f during flight. The effect of T_{amb} on T_{exh} was most pronounced (estimate = 0.76; S.E. = 0.08; t = 9.39; $p < 0.001$), but there was also a positive correlation with f (estimate = 3.76; S.E. = 1.09; t = 3.43; $p < 0.005$). Since f increased with T_{amb} as well (as shown below) both effects lead to an increase of T_{exh} with increasing T_{amb} (see Fig. 6.2). A linear regression analysis with T_{amb} as sole predictor results in the equation $T_{exh} = 0.85 \cdot T_{amb} + 17.29$ (n = 36; $p < 0.005$; $R^2 = 0.74$)

To examine breathing frequency during flight as dependent variable we introduced T_{amb} and date as covariates. Only T_{amb} had a small but significant effect on f (estimate $T_{amb} = 0.03$; S.E. = 0.01; t = 3.42; $p < 0.001$), following the least squares regression $f = 0.03 \cdot T_{amb} + 3.40$ (n = 44; $p < 0.001$; $R^2 = 0.25$).

Tidal volume as dependent variable was tested for the effect of T_{amb}, date and f. In contrast to the resting values, there was no effect of f on V_t (Fig. 6.3), but only T_{amb} had a significant effect (estimate = 0.06; S.E. = 0.02; t = 3.04; $p < 0.01$), which can be described as $V_t = 0.06 \cdot T_{amb} + 2.83$ (n = 25; $p < 0.01$; $R^2 = 0.29$)

The minute volume during flight (V_{min}), calculated from f and V_t, averaged 789.9 ± 210.0 ml min^{-1} and, unlike at rest, increased with T_{amb} (Fig. 6.4; estimate = 13.55; S.E. = 5.17; t = 2.62; $p < 0.05$). As a result of increased T_{exh} and increased ventilation, respiratory evaporation during flight (REWL) was significantly higher than at rest (One-way ANOVA; $F_1 = 21.81$; $p < 0.001$) and increased with T_{amb}. At a T_{amb} of ~ 6.0 °C, REWL_f averaged 0.74 ± 0.22 ml h^{-1} increasing to 0.89 ± 0.12 ml h^{-1} at 15 °C and 2.49 ± 1.17 ml h^{-1} at 25 °C (average 1.06 ± 0.65 ml h^{-1}). However, mixed model analysis, testing for the effect of VPD, T_{amb}, T_{exh}, V_t, f and date, revealed that REWL correlated best with VPD (Fig. 6.5; estimate = 0.11; S.E. = 0.01; t = 9.58; $p < 0.001$). Also V_t had a positive effect on REWL_f (estimate = 0.35; S.E. = 0.07; t = 5.26; $p < 0.01$), while T_{amb} had a slightly negative effect (estimate = - 0.08; S.E. = 0.01; t = - 7.33; p = 0.001). We do not consider the latter a causal relationship due to the strong correlation of VPD and T_{amb} that makes it difficult to statistically distinguish between the effects of these two variables. The respiratory evaporation rates are associated with a cooling capacity of 0.50 ± 0.15 W at 6 °C, 0.60 ± 0.08 W at 15 °C and 1.67 ± 0.79 W at 25 °C, respectively.

Discussion

Within the limitations of this study, our data are in accordance with our expectations and with the available literature. Because the study aimed primarily at measuring respiratory variables during flight, the experimental setup was focused on obtaining flight measurements. Data on resting birds were collected after flight period, allowing the birds a three minute rest. None of the birds showed signs of exhaustion upon landing (panting, closed eyes, or fluffed feathers) that could indicate an abnormal physiological condition. In Pigeons, Butler et al. (1977) found an immediate drop in heart rate, O_2 consumption, CO_2 production and body temperature upon landing, with the values returning to pre-flight levels within two minutes. Respiratory frequency, in contrast, initially increased upon landing, and reached pre-flight levels only after three to ten minutes. The effect on T_{exh} is less clear. We hope to have minimized any error that may have been introduced due to previous exercise by discarding the first three minutes after landing. When the variables no longer changed with time, we assumed they represent resting conditions.

Exhaled air temperature in resting birds

We found a positive relationship between T_{exh} and T_{amb}, as found in previous studies on T_{exh} in resting birds (Murrish 1973, Kaiser and Bucher 1985, Withers and Williams 1990, Tieleman et al. 1999, Geist 2000, Michaeli and Pinshow 2001 and chapter 5). A direct comparison of the available data, however, is complicated because different methodologies were used and by the fact of that the exact position of the temperature sensors in the naris was not always reported. For example, Michaeli and Pinshow (2001) inserted the thermistor 3 mm into one of the Pigeon's nares, while temperature measurements in the present study represent the temperature directly at the opening of a naris. Temperatures of exhaled air therefore are likely to be lower in the present than in Michaeli and Pinshow's (2001) Pigeons, this difference being most pronounced at low T_{amb}, when the rate of temperature change in the anterior respiratory tract is biggest. This would result in a steeper slope in the regression of T_{exh} on T_{amb} and could explain part of the difference observed between the studies. Given the variation between the study species (Fig. 6.6A) we consider our data to be in good agreement with those of Michaeli and Pinshow (2001).

Ventilation in resting birds

In principle, V_{min} can be adjusted to metabolic or thermoregulatory needs by modulating breathing frequency, tidal volume, or both. Since the temperatures to which the starlings were exposed in our experiments (6 °C to 27 °C) were in a moderate range it is unlikely that the resting birds were heat stressed (Dawson 1982, Bucher 1985, Marder and Arad 1989, Withers and Williams 1990, Wolf and Walsberg 1996). Breathing frequency in resting, non-heat-stressed birds scales with body mass (M [kg]) according to $f = (17.2\ M^{-0.31}) / 60$ [Hz] (Lasiewski and Calder 1971), which yields a prediction of 0.6 Hz for Starlings (average M = 0.078 kg). This equation is based on non-passerine birds, which probably reduces the predictive accuracy for our passerine study species. An empirical study on European Starlings (in Groebbels 1932) reports a higher breathing frequency of 1.5 Hz, which is about the same value as observed in our birds. Generally, there appears to be no dependence of f on T_{amb} at moderate T_{amb} (Bernstein 1976). In our study, however,

f decreased slightly with increasing temperature, as was also reported for resting Pigeons (Michaeli and Pinshow 2001). Given the very low regression coefficient of only –0.03, we consider this decrease to be negligible.

Tidal volume in resting birds also scales with body mass, according to the allometric equation $V_t = 16.9\ M^{1.05}$ [ml BTPS] (Bech et al. 1979). For a starling with $M = 78$ g, this equation predicts a V_t of 1.2 ml per breath. The only empirical value for V_t in European Starlings that we are aware of reports 0.7 ml (Torre-Bueno 1978a), calculated from air sac gas composition and literature data on breathing frequency and tracheal volume (Calder 1968, Hinds and Calder 1971). The measurements in the present study of 1.9 ml (BTPS) are higher than predicted but still in the expected order of magnitude. V_{min}, calculated from f and V_t, was constant over T_{amb} in the resting starlings, which is in accord with our hypothesis and those studies that report the relationship between respiratory variables and T_{amb} below 30 °C (Bernstein and Schmidt- Nielsen 1974, Bernstein 1976, Bucher 1985, Kaiser and Bucher 1985, Withers and Williams 1990, Michaeli and Pinshow 2001).

Respiratory evaporation during rest

The fact that T_{exh} is also strongly positively correlated with T_{amb} in resting birds, indicates that respiratory evaporation also rises with T_{amb}, even with constant ventilation. In the present study, $\mathrm{REWL_r}$ was between 0.05 and 0.27 g h^{-1}. Statistical analysis revealed that the variation in $\mathrm{REWL_r}$ could be best explained by changes in vapour pressure deficit that, in turn, is positively correlated with T_{amb}. A direct comparison with other studies therefore is difficult without knowledge of the exact ambient conditions. For resting, non-heat-stressed birds, Crawford and Lasiewski (1968) presented a relationship that scales $\mathrm{REWL_r}$ to body mass as $\mathrm{REWL_r}$ [ml h^{-1}] $= 1.45\ M^{0.61}$. Thus, one would estimate respiratory evaporation of 0.3 ml h^{-1} for a Starling with a body mass of 78 g, a value similar to our measurement of 0.28 ml h^{-1} at 27 °C, which was the highest temperature in our study, but certainly does not impose heat stress on a resting starling. We conclude that within the limitations of this study, our resting measurements are in good accordance with previous investigations.

Exhaled air temperature in flying birds

Data on T_{exh} during flight from previous studies are summarized in Fig. 6.6B. In all cases, with the exception of the white-necked ravens in the study by Hudson and Bernstein (1981), T_{exh} increased with T_{amb}. Their estimates of T_{exh} are generally higher than measured in the other studies and are negatively correlated with T_{amb}.

This difference is possibly due to their specific method of mask respirometry that was primarily used to measure respiratory evaporation, but allowed a back-calculation to T_{exh} by assuming that the exhaled air was saturated with water vapour. If these measurements included a high proportion of open-bill breathing, the associated bypass of the nasal turbinates would lead to high rates of respiratory evaporation. A back-calculation to T_{exh} from evaporation rates would therefore not represent nasal T_{exh}, but an unknown combination of nasal and oral T_{exh}. Our study was restricted to nasal T_{exh} and can therefore not readily be compared with the raven data.

All other studies on T_{exh} during flight have been conducted with thermistor probes in (Michaeli and Pinshow 2001) or at the narial opening (Berger et al. 1971, chapter 5, this study). The studies of Berger et al. (1971) and Michaeli and Pinshow (2001) report a positive relationship of T_{exh} to T_{amb}, with very similar slopes. This, irrespective of the fact that Berger et al. (1971) did not measure T_{exh} during flight, but took their measurements directly after flights of 5 to 18 s duration. Our data on flying ducks (chapter 5) give a somewhat steeper relationship between T_{exh} and T_{amb}, as do the measurements of the present study. As discussed above concerning resting values, the difference in the positioning of the thermistor probe (3 mm into the nasal passage versus directly at the nostril opening) might explain part of the observed differences between Michaeli and Pinshow's measurements and the results of this study.

Our data support the observation of Michaeli and Pinshow (2001) that the temporal counter-current heat exchange mechanism is more efficient at rest than in flight, since T_{exh} in our Starlings was about 5 °C higher during flight than at rest. As in the studies of Berger et al. (1971) and Michaeli and Pinshow (2001), in the starlings T_{exh} increased with increasing T_{amb}, although the slope of this relationship is steeper for starlings than for black ducks or white-necked ravens. A steep increase of T_{exh} with T_{amb} indicates that at low T_{amb} birds are very efficient in cooling their expired air to a temperature below T_{body}, thereby decreasing respiratory evaporation. With increasing T_{amb}, however, the cooling mechanism appears to become less efficient. Torre-Bueno (1978b) studied total evaporation rates in flying Starlings over a wide range of ambient temperatures and found a steep increase of evaporation at temperatures above 15 °C. Given the extra-load of the measuring devices that starlings carried in the present study, it is likely that flight costs increased by about 3 % (Ward et al. 1997, Kvist et al. 2001, Hambly et al. 2004a and chapter 3) thereby also increasing the internal heat load. The birds therefore probably had to switch to evaporative cooling at lower temperatures than they might have if unencumbered, which might have increased the slope of the relationship T_{exh} to T_{amb} compared to unrestrained flight.

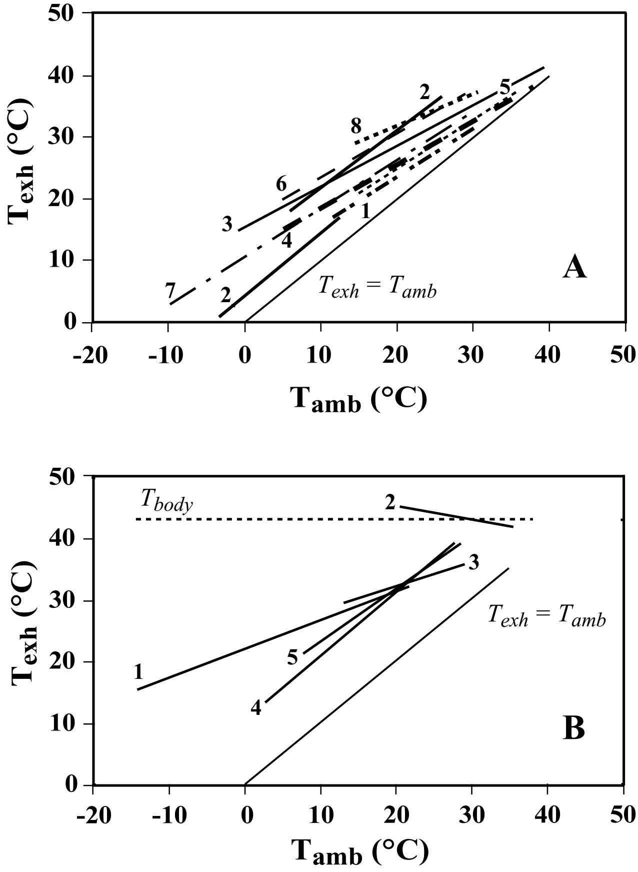

Fig. 6.6 Empirical estimates for exhaled air temperature (T_{exh}) in birds at rest **(A)** and during flight **(B)** in relation to ambient temperature (T_{amb}). For better orientation we also plotted $T_{exh} = T_{amb}$ and for the flight data a dotted horizontal line at T_{body} = 43.3 °C, following Torre-Bueno (1976) for flying European Starlings. **A**: 1 (thick doubly broken line) Cactus Wren (Schmidt-Nielsen et al. 1970), 2 (thick solid line) Penguins (Murrish 1973), 3 (fine solid line) Prairie Falcon (Kaiser and Bucher 1985), 4 (thick broken line) Spinifex Pigeon (Withers and Williams 1990), 5 (fine dotted line) Crested Lark (Tieleman et al. 1999), 6 (fine broken line) Pigeon (Michaeli and Pinshow 2001), 7 (fine slash-dotted line) Ducks (chapter 5), 8 (thick solid line) European Starling (this study). **B**: 1 Black Duck (Berger et al. 1971) measured directly after short flights, 2 White-necked Raven (Hudson and Bernstein 1981) flying with a respirometry mask in a wind tunnel, 3 Pigeon (Michaeli and Pinshow 2001) free flying, 4 Ducks (chapter 5) during wind tunnel flights, 5 European Starling (this study) during wind tunnel flights.

Ventilation and oxygen extraction rate during flight

Generally, the respiratory frequency of flying birds increases 3 to 19-fold above resting levels (Berger and Hart 1974). In starlings *f* increased threefold from 1.4 ± 0.3 Hz at rest to 4.0 ± 0.4 Hz in flight, which is the same *f* of 3 to 4 Hz reported by others for flying European Starlings (Torre-Bueno 1975, Banzett et al. 1992, from Fig. 6.2). Breathing frequency has been found to vary with flight speed (Tucker 1968, Pennycuick et al. 1996, Park et al. 2001), and body mass, although to a lesser extent than during rest (Berger and Hart 1974, Bernstein 1987, Pennycuick et al. 1996). The dependence of *f* on T_{amb} during flight has been investigated in four studies. In the Fish Crow (Bernstein 1976) and the Rock Pigeon (Michaeli and Pinshow 2001), *f* was independent of T_{amb}, while in the White-necked Raven (Hudson and Bernstein 1981) and the Budgerigar (Aulie 1975) it increased with rising T_{amb}. In the present study, *f* was positively correlated with T_{amb} during flight, but the regression coefficient of this relationship is so small (0.03 Hz $°C^{-1}$), that we consider this increase negligible.

In flying birds, tidal volume (V_t) was found to scale with body mass (Hart and Roy 1966, Tucker 1968, Berger et al. 1970, 1971, Berger and Hart 1972, Bernstein 1976, Berger 1978, Torre-Bueno 1978a, Hudson and Bernstein 1981, Banzett et al. 1992, Boggs et al. 1997b). With the allometric equation of Bernstein (1987), we predicted a tidal volume of 2.9 ml (BTPS) for a 78 g starling. This is lower than the average value (3.6 ± 0.4 ml) in our measurements, but almost the same as the 2.8 ml measured by Torre-Bueno (1978a) for Starlings flying in a wind tunnel at ambient temperatures of 10 °C to 14 °C, respectively volumes around 3 ml, reported by Banzett et al. (1992) for flights at ~ 20 °C (personal information). One should keep in mind, however, that Torre-Bueno (1978a) is one of the nine investigations forming the data base for Bernstein's (1987) analysis.

Hudson and Bernstein (1981) found a strong relationship between V_t and T_{amb}, with V_t increasing at higher temperatures, which we also found, but to a lesser degree. Minute volume was independent of T_{amb} in our measurements and on average 790 ml min^{-1} or 13.2 ml s^{-1}. At this V_{min}, the bird inhaled 2.8 ml s^{-1} of oxygen. The energy density of one litre of oxygen depends on the fuel type. We assume that the predominant fuel used in the short flights reported here was carbohydrates (Rothe et al. 1987) with an energy density of 20.9 kJ l^{-1} O_2. We assume further that flight cost is independent of T_{amb} (Bernstein et al. 1973) and 10.4 W in starlings (Ward et al. 2004, see below), corresponding to an oxygen consumption of 0.5 ml s^{-1}. The resulting oxygen extraction rate is 18.0 %, close to values reported for other birds both during flight (Bernstein 1976) and at rest (Morgan et al. 1992). If flight costs were 10 % higher than assumed the value for oxygen extraction rate would increase to 19 %. However, there is considerable variation in the oxygen extraction rates among species, ranging from 1 % to 46 % (see Bernstein 1987), which, among other factors, might be due to the indirect determination and the combination of data from different experimental series, as in this calculation as well. We therefore consider the value of 18 % as tentative.

Respiratory evaporation during flight

As expected REWL was considerably higher in flight than at rest. By the allometric equation of Bernstein (1987) $REWL_f$ of a 78 g starling is estimated to be 2.0 g h^{-1}. This equation does not take ambient conditions into account, while those studies that investigated the dependence of respiratory evaporation during flight on ambient temperature illustrate the importance of the knowledge of T_{amb} and ρ (Berger et al. 1971, Bernstein 1976, Hudson and Bernstein 1981, Michaeli and Pinshow 2001). The predictive value of a simple allometric equation therefore is somewhat limited. Our $REWL_f$ rates lie between 0.7 g h^{-1} and 2.5 g h^{-1} and, given the mentioned limitations of the prediction, are in the expected order of magnitude. We calculated $REWL_f$ from V_t, f and T_{exh} assuming that exhaled air is saturated with water vapour (Schmidt-Nielsen et al. 1970, Welch 1984), but there are indications that for some bird species this might not always be the case, especially at high ambient temperatures during panting (Marder 1973a, Withers et al. 1981, Kaiser and Bucher 1985). Panting could potentially cause incomplete saturation through a shorter contact between the respiratory air and the inner respiratory tract. To our knowledge, only Withers et al. (1981) quantified the relative humidity of exhalation air in birds. They found a minimum relative humidity of only 75 % in ostriches that were restrained in full sun exposure at ambient temperatures between 28 and 32 °C at low ambient air humidities of 30 – 36 %. These ambient conditions are rather extreme compared to our experimental conditions. Ostriches show a number of adaptations to their desert habitat enabling them to efficiently conserve water (Cloudsley-Thompson and Mohamed 1967, Schmidt-Nielsen et al. 1969, Williams et al. 1993). The ability to expire unsaturated air is likely an adaptation to hot and dry environments as well, as it has also been reported for camels (Schmidt-Nielsen et al. 1980). Estimates as low as 75 % for the relative humidity of expiratory air are therefore probably much too low for Starlings. If expired air was not water saturated in our birds, estimates for REWL were too high, the error probably being most pronounced during flight at high ambient temperatures. Assuming that exhaled air was only 80 % saturated, REWL would be about 30 % lower than under the assumption of water saturation. On the other hand, our estimate for $REWL_f$ is restricted to the flight periods when the birds were breathing through the closed beak. Interjected intervals with the beak partly or fully opened might have been associated with higher evaporation rates because the exhalation air bypassed the nasal turbinates (Geist 2000). The opening of the beak occurred irregularly and for variable amounts of time. It was observed more often at high ambient temperatures. This fact might compensate for the possible overestimation due to the assumption of water saturation of the exhaled air.

It appears that resting passerine species rely primarily on respiratory, instead of cutaneous, evaporation (Wolf and Walsberg 1996, Tieleman and Williams 2002). Data on respiratory evaporation in flying passerines are hardly available. We are only aware of two studies addressing $REWL_f$ (Bernstein 1976, Hudson and Bernstein 1981). Measurements of cutaneous evaporation in flying passerines are not available, but for Starlings, total evaporation rates, the sum of cutaneous and respiratory evaporation, during flight over a range of ambient temperatures was 0.9 $g\,h^{-1}$ at 6 °C, rising to 1.2 $g\,h^{-1}$ and 2.4 $g\,h^{-1}$ at 15 °C and 25 °C, respectively (Torre-Bueno 1978b). In order to compare the evaporation rates in our experiments with Torre-Bueno's data, we have to account for differences in flight costs between the two studies, because differences in body weight and the extra load that our birds had to carry during their flights probably increased their flight costs and thereby the heat load they had to dissipate. There are no data on flight costs in Torre-Bueno's (1978b) study on evaporation rates, but in another series of experiments he reports flight costs of 8.9 W for his Starlings (Torre-Bueno and Larochelle 1978). We assume that these flight costs apply also to the experiments on evaporation rates. In a recent study on metabolic costs of European Starlings during wind tunnel flights, Ward et al. (2004) report flight costs of about 10.4 W for birds of 82.0 g body mass. This is comparable to the mass of our birds loaded with the measuring equipment, and we will use this value for further calculations. Our birds therefore had increased flight costs of 1.5 W, compared to Torre-Bueno's (1978b) starlings. Assuming an efficiency of 18 % for the conversion of metabolic power into muscular work (Ward et al. 2001), the birds would be left with an additional heat load of 0.27 W. If this heat was dissipated by evaporation alone, as is likely, at least in the higher temperature range, this extra load would boost total evaporation by 0.40 $g\,h^{-1}$. This would result in total evaporation rates of 1.35 $g\,h^{-1}$ at 6 °C, 1.61 $g\,h^{-1}$ at 15 °C and 2.83 $g\,h^{-1}$ at 25 °C. The relative contribution of respiratory evaporation would then be 55 % at the two lower temperatures and 88 % at 25 °C. Within the limitations of this calculation, we conclude that respiratory evaporation accounts for the major part of water loss in flying European Starlings and increases to a higher degree than cutaneous evaporation with rising ambient temperatures. This is in accordance with our prediction that also during flight, respiratory evaporation is the major avenue of water loss for passerines.

CHAPTER 7

General discussion

Methodology: flying in a wind tunnel

It is not easy to obtain physiological or kinematic data from a flying animal, and the approaches to this problem change continuously with the refinement of the technology. The earliest estimates of metabolic rates during flight were derived from the mass loss during a long flight and the assumption that fat constitutes the major part of this loss (Nisbet et al. 1963). Later the Doubly Labelled Water method became available for the measurement of energetic flight costs, sometimes in combination with time-energy budgets (LeFebvre 1964, Utter and LeFebvre 1970, Hails 1979, Westerterp and Drent 1985, Masman and Klaassen 1987, Wikelski et al. 2003). With the use of radiotelemetry it became possible to relay measurements of physiological parameters and ambient conditions from free flying birds, even over large distances, *e.g.* via satellites (Butler and Woakes 1980, Butler et al. 1998). Other studies used data loggers to store information until recapture (Benvenuti et al. 1998, Butler et al. 1998, Butler et al. 2000, Yoda et al. 2004). Despite the obvious advantages of physiological measurements on free-flying birds, these studies face considerable technical challenges and suffer from the fact that the bird's behaviour and the exact flight conditions are not known. Furthermore, it is not possible to experimentally alter single parameters, like ambient temperature or the bird's flight speed. Therefore early researchers have already considered the idea to let birds fly in a restricted space under controlled conditions. The first attempts to fly a bird in a wind tunnel were probably those of Greenewalt (1961) who put a feeder for hummingbirds directly downstream of an electric fan. The flight speed of the bird could thus be controlled by adjusting the speed of the fan. Since then a number of small wind tunnels have been built especially for bird flight experiments. The advantages of this experimental approach are obvious: birds can fly while remaining in the same place within the reach of the experimenter. They can be observed or video taped continuously, which allows the study of flight behaviour or kinematics in detail. They can be attached to stationary measuring equipment. Ambient conditions are controllable and stable. Currently, most insights into physiological and kinematic aspects of bird flight are derived from wind tunnel studies. The technique also has its drawbacks. The birds have to fly in a restricted space at a set velocity, which may affect their flight pattern. They must tolerate the noise of the fan motor and fly in an optically motionless and monotonous environment (Butler et al. 1977, Rothe and Nachtigall 1987). To what extent this has an effect on the measurements is still under debate.

Masman and Klaassen (1987) compared the measurements of flight costs available at that time. They pointed to the fact that wind tunnel measurements tend to result in higher flight costs than other studies. They concluded that allometric meta-analyses for the prediction of energy costs of flight should omit those studies that have been conducted in a wind tunnel. In their comparison, however, they do not distinguish between the exact methods used in these studies and include into their comparison both studies of relatively unrestrained flights and those where the birds had to carry heavy measuring equipment. In a later study, Liechti and Bruderer (2002) present data on wing beat frequencies and estimated flight costs in *Hirundines* both from radar observations in the wild and from wind tunnel flights. Their measurements suggest that wind tunnel flight is more expensive compared to free migratory flight, which supports Masman and Klaassen's (1987) view. Direct comparisons between free flight and wind tunnel measurements are scarce and suffer from unstandardised experimental conditions.

A comparison of our estimates for flight costs in Rose Coloured Starlings (chapter 3) with literature data on European Starlings, which have a similar body mass and morphology, shows that our measurements are at the lower end of flight cost estimates, although obtained in a wind tunnel. Also flight costs of a Thrush Nightingale and four Red Knots flying in the Lund wind tunnel were low compared to allometric predictions (Klaassen et al. 2000, Kvist et al. 2001). A systematic "wind tunnel effect" leading to a general overestimation of flight costs in wind tunnel studies therefore appears unlikely.

Possible effects of the bird's restriction to a wind tunnel's test section on flight performance are hard to quantify or even qualitatively judge. This is due to the difficulties in a direct comparison with free flight, but also to the differences in the design of the wind tunnels that have been used for studies on bird flight so far. Wind tunnel design presumably has an impact on flight performance through differences in the level of turbulence (Rayner 1994, Pennycuick et al. 1997, Simons 1999). The wind tunnel of Lund University in Sweden was designed to minimize turbulences in the air stream. This was achieved by a wide diameter of the settling chamber, a metal honeycomb and several screens at its opening (see Fig. 1.4) that serve to align the air flow with the tunnel axis, and a high contraction ratio of 12.25, accelerating the air to the desired speed in the flight chamber (Pennycuick et al. 1997). This wind tunnel is much larger than any other wind tunnel currently in use for the study of avian flight, and has excellent aerodynamic properties. Its low turbulence design has been replicated in the design of the wind tunnel at the Max Planck Institute for Ornithology in Seewiesen, Germany, which has been used in our experiments. We therefore hope to have minimized any adverse effects of turbulence on the flight performance of our birds.

Are captive birds representative for wild conspecifics?

Direct comparisons between free flight and wind tunnel measurements are further complicated by the fact that the body condition of captive birds may well be different from that of their wild conspecifics. Birds usually do not fly spontaneously in a wind tunnel but require a more or less extensive training period to cooperate in the unnatural environment. Food in captivity differs considerably from the natural spectrum, and is available in abundance. The necessary foraging time is reduced, the animal restricted in its mobility. These factors are likely to affect body mass and the general body condition (Ewenson et al. 2001, Bishop et al. 2002). Furthermore, not all birds cope well with the artificial environment of a wind tunnel. A certain selection of cooperative birds is thus likely to take place - with unknown effects on experimental outcome. In their study on wing beat frequencies of swallows and martins during wind tunnel flight and in the wild, Liechti and Bruderer (2002) minimized the environmental uncertainties by radar-tracking individual free-flying birds in three dimensions and by including data about wind conditions derived from a pilot balloon. Still, their comparison between free flight and wind tunnel measurements is hampered by the fact that free migratory flights were measured in adult birds during spring migration, whereas wind tunnel measurements were performed in summer, on juveniles outside the migratory season. It is not clear how far avian flight metabolism is influenced by physiological changes between migratory and non-migratory season (Kvist et al. 2001). Some shorebirds routinely undergo pre-migratory *pectoralis* muscle hypertrophy (Dietz et al. 1999), and in other migratory species certain catabolic key enzymes have increased activity prior to migration (George and Vallyathan 1964, Marsh 1981, Lundgren and Kiessling 1985, 1986). The juveniles had not yet reached their final wing span and were 20 to 25 % heavier than would be expected for free-living birds. These factors may explain most of the difference observed between the free flying birds and the wind tunnel measurements (Liechti and Bruderer 2002). Also Bishop et al. (2002), comparing free migration flights to wind tunnel measurements, suggest that captive birds may differ considerably from wild conspecifics. In this study, a calibration of heart rate against oxygen consumption during wind tunnel flights was used to estimate flight costs of wild Barnacle Geese during their migration on the basis of heart rate recordings. The resulting values are extremely low. A 30 % decrease in heart mass of captive compared to wild geese explains part, but not all, of the observed difference in heart beat frequency. Other factors like haematocrit (Bishop et al. 2002) or decreasing BMR during long-distance migration (Battley et al. 2001a) may contribute further to differences between flight performance of captive and wild birds.

Considering these difficulties, a direct transfer of insights from wind tunnel studies to natural free flight appears problematic. More studies comparing wind tunnel with free flight would be important, with simultaneous kinematic and physiological measurements, and preferably measuring the same individual birds under both conditions.

The ”ideal bird”

The “ideal bird” in terms of energy and water economy would fly at a low metabolic rate and thus combine low internal heat production and low metabolic waste production. The waste products should be excreted in dry form to avoid excretory water loss. Despite the low metabolic rate it would have a high rate of metabolic water production. Increased oxygen demands should be met by increasing the rate of oxygen extraction from the air by the lungs to keep ventilation and respiratory water loss at a low level. This bird should fly at low ambient temperature to easily loose excess heat. Hence it should migrate at night, at high altitude (for a low ambient temperature), high ambient humidity and high oxygen partial pressure (therefore at low altitude) and stay in temperate climate zones. This physiological and behavioural ideal bird is, of course, fictitious and self-contradictory in its traits (see Fig. 1.1). Reality is not ideal but making the best of it. Only, how?

Carmi et al. (1992) first presented a model to simulate bird flight that allowed the calculation of avian flight range limitations imposed by either water or energy depletion. This model, revised and streamlined by (Carmi and Pinshow 1995, Klaassen 1995, Klaassen et al. 1999), can be used to develop hypotheses of migration strategies and to pinpoint critical parameters affecting bird flight performance. According to model sensitivity analyses, exhaled air temperature and the rate of oxygen extraction from respiratory air are the most important parameters determining water loss (Carmi et al. 1992, Klaassen et al. 1999). The threat of dehydration appears to be highest for small trans-Saharan migrants. This predicts that they would do better flying at night and resting by day, even at places that do not provide water or food (Carmi et al. 1992). These predictions seem to be supported by field observations (Biebach 1988). Comparing the predictions of their latest water and energy balance model for flying birds with data from studies using birds trained to fly outdoors or in wind tunnels, Klaassen et al. (1999) tentatively concluded that model predictions are realistic. More physiological data on water exchange and related variables are required to validate the model (Klaassen 2004).

The physiology of flight

Energetics

Low flight costs would be beneficial for a bird by increasing the maximum flight distance powered with a given amount of fuel, or the energy available for other activities, like reproduction. Low flight costs would also reduce the internal heat load and therefore the demands on effective thermoregulation and the need for increased evaporation rates. Flight costs are determined by severe aerodynamic and physiological constraints that are not easily overcome (Videler 2005). As a rough estimate for the energetic costs of bird flight, the scaling with basal metabolic rate (BMR) is often used. This relationship provides a feeling for the impact of exercise on the energy budget of an animal. BMR of Rose Coloured Starlings with a body mass of 68 g is predicted to lie between 0.5 and 0.7 W, depending on the underlying allometric equation (Gavrilov and Dolnik 1985, McKechnie and Wolf 2004b). The average flight costs of 8.2 W (chapter 3) are thus 12 to 18 times elevated over BMR. This is six-fold over the daytime resting levels of 1.4 W (chapter 2), which may be a more meaningful comparison, since birds usually do not experience standard conditions when not flying. Given the huge variation in the relationship of flight costs to BMR (see. Fig. 7.1), and the fact that for many species BMR was not measured but estimated from allometric equations, a prediction of flight costs on the basis of BMR is not possible.

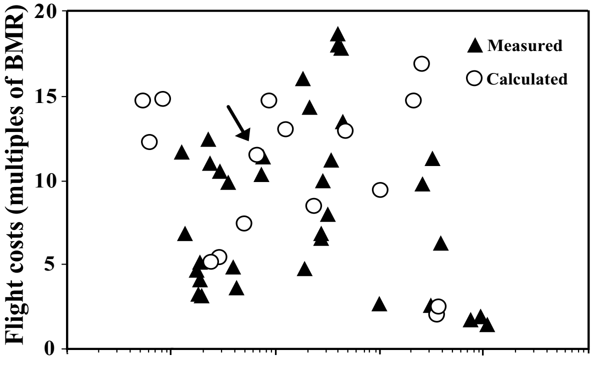

Fig. 7.1 Flight costs expressed as multiples of BMR plotted against body mass (in a logarithmic scale). Triangles are data where both, flight costs and BMR were measured. Circles are data where BMR was calculated as BMR = 4.2 $M^{0.677}$, with mass M in kg (Gavrilov and Dolnik 1985). The value for Rose Coloured Starlings (chapter 3) is indicted with an arrow. The other 52 values for 37 bird species are taken with permission from Videler (2005).

There is a strong dependence of flight metabolic rate on body mass, which allows the calculation of a regression line through the available data and the prediction of flight costs for other species, not yet experimentally studied, on the basis of body mass alone (e.g. Norberg 1996) or a combination of body mass and relevant morphological parameters, such as wing span (Masman and Klaassen 1987). Instead of drawing a regression line, Videler (2005) argues for a maximum metabolic capacity limiting flight costs at the upper end (following the equation $EE_f = 60\ M^{2/3}$, where M is body mass in kg) while allowing adaptation towards lower flight costs through flight style or morphology (Fig. 7.2).

Our measurements on Rose Coloured Starlings reveal average flight costs of 8.2 W. This is in the expected range for flapping flight (Masman and Klaassen 1987, Videler 2005). The costs are lower than the values of 9 to 11 W observed in European Starlings (by Torre-Bueno and Larochelle 1978, Westerterp and Drent 1985, Ward et al. 2001, Ward et al. 2004, see table 3.2). This may be partly explained by differences in methodology and flight duration, as our birds were flying continuously for six hours. Flights of long duration tend to result in lower estimates of flight costs because the energetically costly starting phase has less influence (Rothe et al. 1987). In contrast, an extrapolation of flight costs from time-energy budgets (*e.g.* Westerterp and Drent 1985, Ward et al. 2004) or studies on shorter flights (Westerterp and Drent 1985, Hambly et al. 2004b) are likely to result in somewhat higher estimates than measurements of continuous flight. A metabolic switch from carbohydrate combustion to fat catabolism within the first one or two hours of flight (Rothe et al. 1987, Schwilch et al. 1996, Jenni-Eiermann et al. 2002) underscores the importance of studies on long distance flight.

Flight costs should depend on velocity following aerodynamic principles (Tucker 1973, Greenewalt 1975, Pennycuick 1975, Rayner 1979a). We could not detect any such relationship in our measurements at flight speeds between 9 and 14 m s^{-1}, however (chapter 3). This is consistent with most other studies on flight costs at variable speed (reviewed in Rayner and Ward 1999) and is most probably due to the restricted range of velocities at which the birds could be made to fly. The reason for the restriction is still not clear. The kinematic aspects of wing beat frequency and variable use of intermittent flight can be excluded as a factor here. Within the natural range of speeds, flight costs are relatively constant and appear fully determined by body mass. The scaling exponent relating body mass to flight costs, though, is lower within a species than interspecifically (Kvist et al. 2001, Hambly et al. 2004a, chapter 3). This observation has not been studied systematically, and the underlying principles await future investigations.

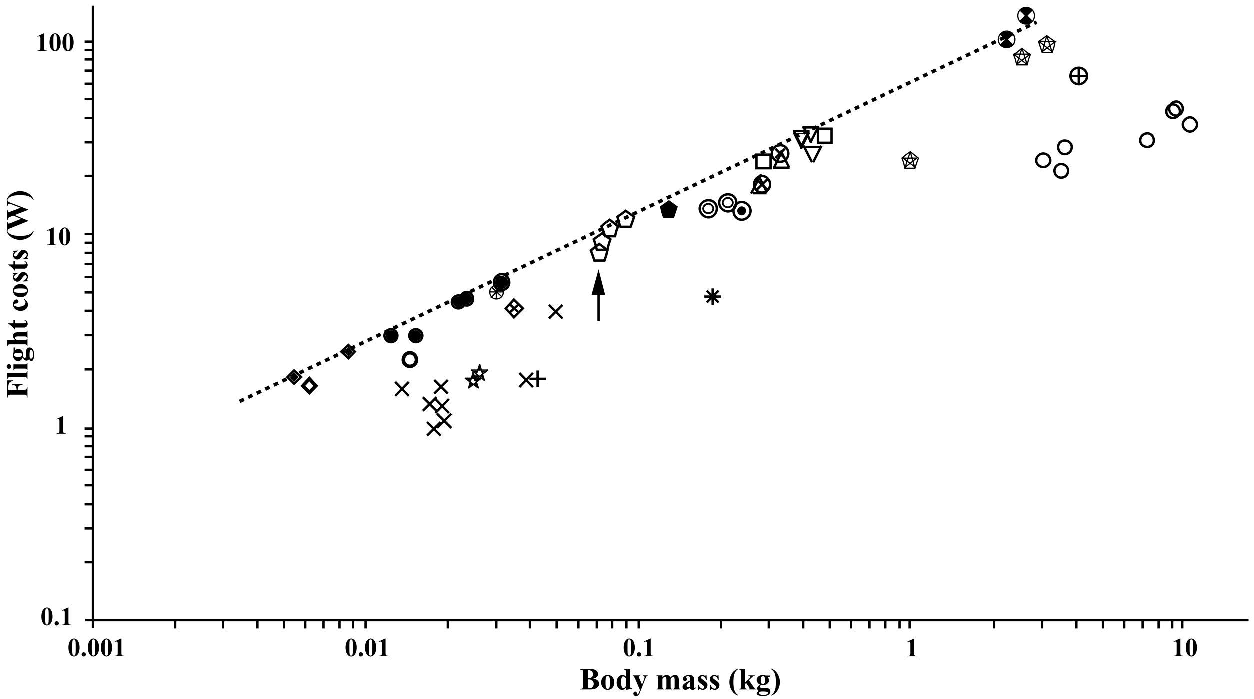

Fig. 7.2 Empirical results of flight cost measurements expressed in Watts (W) plotted against body mass in kg on logarithmic scales (taken from Videler 2005, with kind permission). The vale for Rose Coloured Starlings (chapter 3) is indicated with an arrow. The hand drawn stippled line can be interpreted as an upper limit for flight costs.

◇ Sunbird ◆ Hummingbirds ● Finches o Zebrafinch ❖ Budgerigar ⬠ Starlings ⊛ Thrushes ◎ Kestrels ⊗ Gulls △ Godwits ▽ Pigeons ✕ Aerial feeders ☆ Nightingales + Storm petrel ✱ Tern □ Corvids ⬟ Red knot ⊙ Teal ✪ Sulidae ⊕ Giant petrel ⧗ Geese ○ Albatrosses

Thermoregulation

As energy expenditure during flight increases over resting levels, internal heat load increases as well. We can roughly estimate the metabolic heat production by assuming the efficiency of the conversion of metabolic power into muscle work. We assume here 18 % efficiency (Ward et al. 2001), although there is a high degree of uncertainty in this assumption. This figure means that from the 8.2 W energy expended during flight in Rose Coloured Starlings, about 6.7 W end up as heat and need to be dissipated (chapter 4).

Dry heat dissipation has the advantage over evaporation that it reduces the risk of dehydration. Our birds were generally reluctant to fly at high ambient temperatures. It may be that birds under heat stress prefer behavioural over physiological reactions to prevent overheating. Our observation was also reported in other wind tunnel studies (Tucker 1968, Torre-Bueno 1976, Biesel and Nachtigall 1987, Ward et al. 1999). In the wigeon, we found a correlation between voluntary flight duration and ambient temperature, although the bird was probably not severely heat stressed (chapter 5). Presumably, it simply preferred to thermoregulate by avoiding the exercise instead of increasing heat dissipation. In nature, birds can adopt a night migration strategy to avoid high external heat loads. They can also fly at high altitude, where temperatures are low, although this will have its energetic drawbacks.

During flight, the thermal conductance of birds is 3- to 4-fold increased over resting values (Tucker 1968, 1972, Berger and Hart 1974). This may be partly explained by the fact that during flight parts of the body that in resting birds are usually not exposed to the surrounding air contribute to heat exchange. Thermal images of flying European Starlings revealed that the ventral brachials alone account for 21 % of dry heat loss (Ward et al. 1999). To further increase thermal conductance, birds can expose legs and feet to the air stream (Baudinette et al. 1976, Torre-Bueno 1976, Biesel and Nachtigall 1987, Ward et al. 1999) or open the beak (Torre-Bueno 1978b, St-Laurent and Larochelle 1994), thereby augmenting both convection and evaporation. These behaviours have been observed in virtually all wind tunnel studies that were conducted at a relatively high temperature range, including ours. Dry heat dissipation is very effective during flight. Ward et al. (2004) calculated on the basis of thermographic imaging and published evaporation rates of European Starlings (Torre-Bueno 1978b), that 80 % of total heat loss occurred via convection, and additional 9 % were dissipated by radiation. Evaporative cooling made up for the remaining 11 % (Ward et al. 2004). The heat balance model for our Rose Coloured Starlings results in similar proportions (chapter 4). With increasing ambient temperature the capacity for convection as well as radiation decreases (Ward et al. 2004). The increase of external heat load from the surrounding air and a simultaneous decrease in the effectiveness of dry heat dissipation illustrates the importance of evaporative thermoregulation during flight.

Evaporation

Water loss by evaporation is often higher than metabolic water production in resting birds in the absence of thermal stress, leading to a net water loss (Dawson 1982). Total evaporative water loss (TEWL), the sum of evaporative water losses through skin and from respiratory passages, as a function of ambient temperature is relatively low and near-constant to constant at a low temperature range, increasing abruptly above a certain threshold temperature. Dry heat dissipation is insufficient for thermoregulation at higher ambient temperatures and needs to be aided by actively increased evaporation (Wolf and Walsberg 1996). Our measurements in resting Rose Coloured Starlings (chapter 2) confirm this pattern: low evaporation rates (6.7 g d^{-1}) at temperatures below 22 °C and increasing rates at higher temperatures. During flight, a similar dependence of TEWL on ambient temperature applies. Evaporation rates at a low temperature range, although low, are a factor 5 higher (33.3 g d^{-1}) than in rest. The critical temperature, above which evaporation rates increase dramatically with ambient temperature, is shifted from about 22 °C in resting Rose Coloured Starlings towards 18 °C during flight (chapter 4). This is despite the fact that convection is augmented by the continuous air flow past the bird's surfaces (Biesel and Nachtigall 1987). Only few data of evaporation rates during flight are available for comparison: In most studies, including our own, evaporation rates show a biphasic relationship with ambient temperature (Fig. 7.3).

A negative relationship between TEWL and body mass makes small birds more vulnerable to dehydration than larger species (Bartholomew and Dawson 1953). Nevertheless, a large variation in TEWL remains (Crawford and Lasiewski 1968, Dawson 1982, Williams 1996) even after correcting for body mass and phylogeny. The inclusion of habitat aridity into a multi-species comparison of Larks added considerably to the explanation of variance in TEWL (Tieleman et al. 2002). The authors conclude that low TEWL is an adaptive trait in desert birds subject to strong evolutionary selection. We may also hypothesize that migratory behaviour has a strong influence on the degree of adaptation to water economy during flight. Long-distance migrants regularly face periods without the possibility of water uptake from the environment. Dehydration may be less threatening to short-distance migrants or sedentary populations with access to drinking water. Starlings belong to the large and ecologically variable genus *Sturnus,* in which the whole spectrum of migratory behaviour is represented and which inhabits temperate to tropical habitats.

This ecological variability, and the fact that Starlings are easy to keep and cooperative in wind tunnel experiments, makes this group suitable for a multi-species comparison of adaptations to long-distance flight.

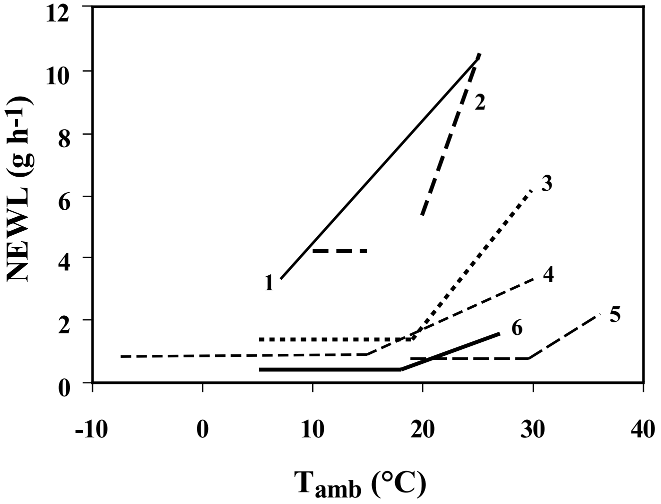

Fig. 7.3 Net evaporative water loss (NEWL) as a function of ambient temperature (T_{amb}) in flying birds. Data are for (1) Rock Pigeon in a wind tunnel (Biesel and Nachtigall 1987), or (2) during free flight (Giladi and Pinshow 1999), (3) Red Knot (Kvist 2001), (4) European Starling (Torre-Bueno 1978b), (5) Buderigar (Tucker 1968), and (6) Rose Coloured Starling (chapter 4).

Respiratory evaporation has for a long time been considered the major fraction of total water loss in birds (Bartholomew and Dawson 1953, Rawles 1960, Bartholomew and Cade 1963). The rate of respiratory evaporative water loss (REWL) depends on the volume of ventilated air (V_{min}), but also on the temperature at exhalation (T_{exh}). A modulation of V_{min} is probably not easy to achieve because ventilation must ensure sufficient oxygen supply, and hyperventilation with respect to oxygen demand can lead to hypocapnia and alkalosis (Calder and Schmidt-Nielsen 1968). T_{exh} is easier to modulate through temporal counter-current heat exchange: When air is inhaled, its temperature rises to body temperature, and the air is saturated with water vapour from the respiratory passages and the lungs. Convective heat exchange and evaporation of water in the nasal passages during inhalation presumably cool the associated membranes, and upon exhalation the air is cooled by these nasal surfaces, resulting in water recondensation (Jackson and Schmidt-Nielsen 1964, Schmidt-Nielsen et al. 1970). T_{exh} in resting endotherms, including birds, is usually below body temperature and strongly correlated with T_{amb} (Jackson and Schmidt-Nielsen 1964, Murrish 1973, Welch 1984, Tieleman et al. 1999, Geist 2000, Michaeli and Pinshow 2001). The cooling of expired air results in a decrease of respiratory water loss compared to the situation when birds are breathing through the open beak, thus bypassing the nasal turbinates (Tieleman et al. 1999, Geist 2000). The modulation of T_{exh} during flight is not well understood, because only few empirical data exist (see Fig. 6.6). It appears that the cooling mechanism is working during flight as well, although less efficiently (Michaeli and Pinshow 2001, and chapters 5 and 6), because T_{exh} is generally higher during flight than at rest. T_{exh} approaches body temperature already at moderate T_{amb} (~ 33 °C in Starlings and ~ 26 °C in Teal and Wigeon), illustrating the high heat load during flight and the necessity for evaporative cooling.

In resting birds, the relative contribution of REWL to TEWL varies between phylogenetic orders (Wolf and Walsberg 1996). Passerines seem to rely on REWL as principle means of heat dissipation at high T_{amb} (Lee and Schmidt-Nielsen 1971, Wolf and Walsberg 1996, Tieleman and Williams 2002). Flight measurements of REWL are scarce, and only two studies addressed REWL during flight of passerine species so far (Bernstein 1976, Hudson and Bernstein 1981). Hudson and Bernstein (1981) measured REWL of White-necked Ravens during flight at temperatures between 18 and 28 °C: REWL accounted for 58 % to 76 % of TEWL, the relative contribution increasing with ambient temperature. Our calculations for European Starlings also show a reliance on respiratory, rather than cutaneous, evaporation during flight, ranging from 55 % at low ambient temperatures to 88 % at 25 °C. This is consistent with the findings of Hudson and Bernstein (1981) and with the observed preference of respiratory over cutaneous evaporation of resting passerines.

Cutaneous evaporation has long been considered as negligible in the water economy of birds (Bartholomew and Cade 1963, Dawson and Bartholomew 1968). Because birds lack sweat glands, possible mechanisms for the regulation of cutaneous evaporation (CEWL) long remained enigmatic (Marder and Ben-Asher 1983). Mechanisms for the control of CEWL have later been detected (Marder and Raber 1989, Menon et al. 1996, Haugen et al. 2003). Several studies showed that cutaneous evaporation can be a considerable proportion of total water loss, accounting for up to 75 % of TEWL in resting birds (Dawson 1982, Webster and King 1987, Webster and Bernstein 1987, Wolf and Walsberg 1996, Tieleman and Williams 2002). Current data suggest that birds use two contrasting patterns of evaporative water loss as ambient temperature increases and approaches body temperature: an increasing dependence on CEWL as primary mode of heat loss (Marder and Ben-Asher 1983, Webster and King 1987, Withers and Williams 1990) or an increasing dependence on REWL (Bouverot et al. 1974, Richards 1976, Marder and Ben-Asher 1983, Wolf and Walsberg 1996, Tieleman and Williams 2002). Especially Doves and Pigeons (*Columbiformes*) seem to rely primarily on cutaneous evaporation for thermoregulation (Webster and King 1987, Withers and Williams 1990, Larcombe et al. 2003, McKechnie and Wolf 2004a). Data for passerines are scarce but suggest that CEWL is important at the lower temperature range for this group, but at high ambient temperatures, the relative contribution of CEWL decreases dramatically (Fig. 7.4). Wolf and Walsberg (1996) measured evaporation rates of Verdins (*Auriparus flaviceps*) at various environmental temperatures in a two-compartment metabolic chamber. The birds had CEWL rates of about 60 % of TEWL at low temperatures, but increased REWL over CEWL at high ambient temperatures. At the highest temperature measured (50 °C) the relative contribution of CEWL was only 15 %, compared to 85 % contributed by REWL. A similar pattern was found in Larks (Tieleman and Williams 2002).

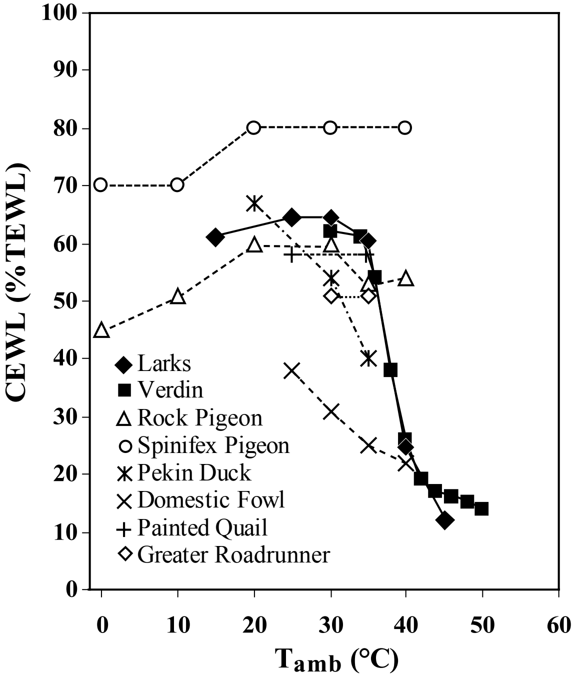

Fig. 7.4 Relative contribution of cutaneous evaporative water loss (CEWL) to total evaporative water loss (TEWL) in resting birds as a function of air temperature. Data are from the following sources: Larks, average over four species in Tieleman and Williams (2002); Verdin, from Wolf and Walsberg (1996); Rock Pigeon, from Webster and King (1987); Spinifex Pigeon, from Withers and Williams (1990); Pekin duck, from Bouverot *et al.* (Bouverot et al. 1974); Domestic Fowl, from Richards (1976); Painted Quail, from Bernstein (1971b) and Greater Roadrunner, from Lasiewski *et al.* (1971)

Direct measurements of cutaneous evaporation during flight have not been made. Some studies estimate cutaneous evaporation from other measured or estimated parameters, as TEWL, REWL and heat production. Biesel and Nachtigall (1987) measured total evaporation in flying Pigeons. They interpret low evaporation rates at low ambient temperatures as purely respiratory, because otherwise a sufficient oxygen supply would not be assured. At higher temperatures, evaporation rates increased to a higher extent than could be explained by increasing REWL. Cutaneous evaporation therefore appears to be involved in thermoregulation to a significant degree at least in the higher temperature range (Biesel and Nachtigall 1987). This conclusion was later supported by measurements on free flying Pigeons by Michaeli and Pinshow (2001). They calculated TEWL from mass loss and REWL from exhalation temperature and an estimate for tidal volume during flight at ambient temperatures between 15 and 30 °C. CEWL, the difference between TEWL and REWL, was between 68 % and 74 % of TEWL. Given the observation that during rest, *Columbiformes* seem to increase preferably cutaneous evaporation with increasing heat stress, while *Passeriformes* rely more on respiratory evaporation, we may expect the same pattern during flight. The only available study on a passerine species is that on the White-necked Raven by Hudson and Bernstein (1981). They estimated that CEWL during flight at temperatures between 18 and 28 °C accounted for 42 % to 24 % of TEWL, the relative contribution decreasing with ambient temperature. Our estimates for flying European Starlings at temperatures between 6 and 25 °C, suggest a relative contribution of $CEWL_f$ of 45 % to 12 % of $TEWL_f$ (chapter 6). These values appear in good agreement with the Raven study and support the hypothesis that respiratory evaporation is the major fraction of total water loss in flying passerines. We must heed the caveat that available calculations including our own all suffer from the fact that some relevant parameters were not measured but had to be estimated. Our understanding of cutaneous evaporation is still limited and awaiting future research.

Excretion

The primary function of excretion is the elimination of metabolic waste products. This must be accompanied by water, which precludes complete suppression of excretory water loss. Birds do have the important advantage over mammals that their nitrogen excretion involves uric acid instead of urea. With each molecule uric acid twice as much nitrogen can be excreted as in urea. Uric acid can further be excreted in a semi-solid suspension, whereas urea must be excreted in aqueous solution. In consequence, birds lose only a small amount of water by excretion, compared to mammals (Bartholomew and Cade 1963).

Because metabolism during flight is much higher than at rest, it is likely that in-flight production of waste metabolites increases accordingly. Only two studies on excretory water loss during flight are available to date. Pigeons increased their excretory water loss (EXWL) during flight by more than 50 %, compared to resting levels. EXWL was about 10 % of total water loss during flight (Giladi and Pinshow 1999). Nevertheless, birds appear to conserve water more efficiently during flight than at rest, because compared to resting control birds, glomerular filtration rate decreased and water reabsorption increased (Giladi et al. 1997). Both mechanisms are known as reaction to dehydration in birds and contribute to renal water conservation.

During flights of our Rose Coloured Starlings excretion was variable. In only 40 % of 61 flights of one hour duration (described in chapter 4) birds excreted at all. In the ten cases that could be quantified excretion averaged 0.9 ± 0.04 (SD) g h^{-1}. If we assume that the proportion of water in excreta is 79 %, as reported by Giladi and Pinshow (1999), EXWL was about 5 % of total water loss. This proportion is small, but given the meagre data base, a more thorough investigation of excretion during flight and its dependence on ambient conditions would be required.

Natural Migration

Most studies on water balance in flying birds have been conducted on captive birds in wind tunnels. These investigations and the model calculations derived from them (Carmi et al. 1992, Klaassen 1995) suggest that water depletion can be a limiting factor for flight duration. Field studies on hydration status of migratory birds are equivocal, and only few actually demonstrate dehydration after long flight periods (Serle 1956, Johnston 1968, Moreau and Dolp 1969, Haas and Beck 1979, Leberg et al. 1996). Leberg et al. (1996) found that passerine migrants had low body water after crossing the Gulf of Mexico, compared to birds recaptured or to resident species. Bar-tailed Godwits, in contrast, were found to complete a long non-stop flight without signs of dehydration (Landys et al. 2000). A normal hydration status after a long period of flight, as reported in many studies (Odum et al. 1964, Child 1969, Biebach 1991, Landys et al. 2000), can be interpreted in several ways: (1) dehydration may not occur because energetic constraints, rather than dehydration, are limiting flight range. (2) Birds are adapted to water conservation and therefore do not lose water in quantities that could cause severe dehydration. (3) Birds adjust their physiology and behaviour in favour of a balanced water budget, even at the expense of energy stores. In this case, the immediate reason for discontinuation of the flight would be fuel depletion, while the ultimate factor relates to water conservation. (4) Dehydration may not be detected because total body water may not be the right indicator to look at (see Klaassen 2004).

The biphasic relation of evaporation rate to ambient temperature, found in most studies on water economy during flight, suggests that evaporation is actively augmented above a critical temperature when dry heat loss is not sufficient for thermoregulation any more (in Rose Coloured Starlings above 18 °C). Adaptations to reduce evaporative water loss therefore will be most effective below the critical temperature. They can either affect the minimum level of evaporation or the threshold temperature, above which evaporative cooling is initiated through mechanisms that enhance dry heat dissipation, like hyperthermia (with the limit at lethal body temperatures of about 46 °C), or selectively increased temperatures of peripheral body regions (webbed feet, skin). For physical reasons, the effectiveness of these reactions to increased heat load decrease with rising ambient temperatures. Other potential adaptations that would improve the water budget in migrants are increased oxygen extraction, reduced flight costs, and increased tolerance to water loss.

To reduce external heat load, it is beneficial to fly at low ambient temperatures, which will usually be associated with high altitude. This inevitably implies higher energetic costs to reach this flight altitude, and higher ventilation rates due to low oxygen partial pressure. Air temperature decreases with altitude, roughly by 6 °C per 1000 m elevation. Radar observations on the altitude of autumnal trans-Sahara migration revealed that most migratory birds fly at an average altitude of ~ 1000 m above sea level during the day and ~ 600 m during the night (Klaassen and Biebach 2000). This is consistent with other studies on flight altitude (Kerlinger 1995, Maybee and Cooper 2004). At these altitudes, birds will experience an ambient temperature reduction of 3.5 to 6 °C, compared to temperature at ground level. There is certainly considerable variation in flight altitude, and little information is available on geographic or seasonal variation, or on species' preferences. Most small song birds are nocturnal migrants, but even species that usually migrate in daytime, such as swallows, swifts, pipits, starlings and the Yellow Wagtail, may be flexible in their behaviour and fly during the night when ambient conditions are unfavourable. At least for one species, the Yellow Wagtail, there are indications that during crossing of the Sahara desert the usual habit of diurnal migration changes into nocturnal flight (Biebach et al. 2000). For trans-Sahara migration, Biebach et al. (2000) observed distinct strategies: Intermittent migration with alternating flight and stopover periods, or non-stop flight. The majority of birds (70 %) crossed the Mediterranean Sea by night and stopped in the morning on the Egyptian coast to resume migration the next evening and cross the desert by night, presumably with a second daytime stopover north of the Sahel.

Some birds (10 %) crossed the Mediterranean Sea by night and continued their flight into the morning hours to rest later that day somewhere in the northern desert. A third group (20 %) crossed the Sea and the Sahara desert presumably in one non-stop flight of two nights and 1.5 daylight periods. It is likely that differences in migration strategy are not species-specific but that individuals use different options. Actual migration may be adjusted flexibly to the internal state, such as the extent of their fat stores or hydration status, and to external factors, like suitability of potential stopover sites or weather conditions.

Let us consider a migration scenario for our model species, the Rose Coloured Starling. This species is considered a diurnal migrant. Several populations will have to cross Iran on the way to and from the wintering quarter in India, therefore we chose average temperature conditions in Teheran as exemplary for the conditions during the migratory phase. Average daily temperatures are between 14 and 28 °C in May, during spring migration, and 12 to 24 °C in October, during autumn migration (information from the BBC Weather Centre). At an assumed flight altitude of 1000 m above ground these temperatures will be reduced to 8 to 22 °C in May, respectively 6 to 18 °C in October. These temperature ranges seem favourable to maintaining minimal evaporation levels for most of the time. Since Rose Coloured Starlings do not cross large oceans on their migration route, they probably can adjust the migration strategy to their body condition and stop the flight when exhausted or dehydrated. If the birds were flexible to fly during the night at least for part of their migration, heat stress could be avoided completely. Based on the measurements of total body water (TBW) content and the assumption that TBW constitutes 70 % of the non-fat fraction of the bird's body, we calculated a fat load of ~ 10 g for our birds. Under the simplifying assumption that only fat is used as fuel, flight costs of 8.2 W (chapter 3) correspond to a fat consumption of 0.75 g h^{-1}. Energy constraints would force the birds to land after 10/0.75 = 13.3 h. Flying at 12 m s^{-1}, the preferred flight speed during our experiments, this flight time corresponds to 575 km. We assume further that the birds can tolerate water loss of 15 % body mass, which is 11 g of body water, as shown in resting birds (Carmi et al. 1993). When flying at minimum evaporation rate of 0.6 g h^{-1} (chapter 4), they could sustain flight for 18.3 h, corresponding to a distance of 790 km, before reaching a critical dehydration level. In this case, flight range would clearly be limited by energy constraints, rather than by dehydration. However, the situation changes dramatically at higher ambient temperature: Flight costs are assumed to be independent of T_{amb}. TEWL, in contrast, is augmented drastically to 2.4 g h^{-1} at 25 °C. Dehydration would force the bird to land already after 4.6 h of flight or 200 km, under these conditions.

Perspective

Our measurements on water economy and flight energetics and the calculations on flight range limitations, coarse as they are, demonstrate that water balance during flight is always negative. Therefore, dehydration is a risk during bird migration. The results also show that birds can potentially choose a migration strategy that reduces the impact of high evaporation rates to a level that is not threatening the successful completion even of long flights. Water and energy balance models like those of Carmi (1992) and Klaassen (1995) are useful tools for the estimation of water losses during flight. The scarcity of empirical information on the physiological processes that take place during flight limits the realism of such calculations. To validate the models, more empirical data are required.

Major uncertainties in the energy part of the models awaiting future investigation are
- the efficiency at which metabolic energy is transformed to mechanical energy, as well as possible seasonal changes in the muscle composition that may affect efficiency
- the effect of body mass on flight costs intraspecifically and intraindividually

The aspects in the water-part of the models that are least understood and should be the focus of future studies are
- cutaneous evaporation during flight
- excretion
- the rate of oxygen extraction from respiration air

The latter has a huge impact on respiratory water loss and may also set an upper limit for the choice of flight altitude. Still, information is contradictory and sparse.

A validation of model predictions is complicated further by the lack of information on actual flight conditions during migration. Field studies on
- flight altitude
- flight distance per migration bout
- stopover periods
- body condition after a flight period

are highly desirable.

Since birds probably are flexible in their migratory strategy, future research in the field should focus not only at finding birds in a physiological state of dehydration, but, more importantly, on revealing behaviour directed at avoiding dehydration and optimising water balance. A multispecies comparison between long-distance and short-distance migrants or sedentary birds may be useful for the detection of physiological mechanisms relating to avoiding water imbalance. A combination of field studies and laboratory investigations, comparative studies and modelling might prove most fruitful to get to a broad understanding of physiological constraints to water saving and behavioural backdoors during migration. Wind tunnel studies on the physiology and economy of avian flight will always be an important tool for our insight in avian migration. Our study highlights in particular a surprising deviation of realized energetic costs from theoretical model predictions that eventually warrants novel theory.

Samenvatting

Vogeltrek heeft altijd een grote fascinatie op de mens uitgeoefend. Veel vogelsoorten leveren indrukwekkende prestaties door over grote afstanden van de overwinteringsgebieden naar de broedgebieden te vliegen, en vervolgens, enkele maanden later, na afloop van het broedseizoen, weer terug. Vaak gaat de trek over onherbergzame gebieden, zoals oceanen of woestijnen, die geen mogelijkheid bieden om te rusten, eten of drinken. De meeste vogelsoorten verbruiken daarom tijdens de trek hun eerder opgebouwde reserves, waarbij de energie- en waterhuishouding sterk afhankelijk is van de omgeving en het gedrag van het individuele dier.

Het doel van dit proefschrift is om een kwantitatieve analyse te presenteren van de effecten van deze langdurige trekperiodes op het energie- en waterbudget bij vogels. De in dit proefschrift opgenomen studies zijn uitgevoerd met vogels die speciaal getraind zijn om over grote afstanden te vliegen, onder de nauwkeurig gecontroleerde omstandigheden in de windtunnel van het Max Planck Institut für Ornithologie in Seewiesen (Duitsland). In **hoofdstuk 1** worden de belangrijkste parameters beschreven die invloed uitoefenen op de energie- en waterhuishouding tijdens de vlucht. De meeste omgevingsfactoren zoals temperatuur, luchtvochtigheid, windrichting en windsnelheid zijn variabel gedurende de trekperiode. Deze zijn onder andere afhankelijk van de geografische positie, tijd van het jaar, tijdstip op de dag en de vlieghoogte van het individuele dier. In de natuur kunnen vogels zich vrij bewegen in een drie dimensionale ruimte, en hebben daardoor veel speelruimte om de voor hen gunstigste omgeving te kiezen. De belangrijkste factor daarvan is wellicht de omgevingstemperatuur, die een grote invloed heeft op de warmte en waterhuishouding van het dier. De lichaamstemperatuur van vogels wordt bij hoge omgevingstemperaturen namelijk door verdamping van water gereguleerd. Om een groot waterverlies door verdamping te voorkomen, is het dus voordelig voor het dier om op grote hoogte te vliegen (aangezien de luchttemperatuur met ca. 6 °C per kilometer vlieghoogte afneemt). De vlieghoogte bepaalt echter ook de invloeden van een aantal andere factoren, zoals de richting en sterkte van luchtstromingen, luchtvochtigheid en zuurstofconcentratie in de lucht (neemt af bij grote hoogte). Op grote hoogte moet daardoor sterker geventileerd worden om het grotere zuurstofverbruik tijdens de vlucht te dekken. Er wordt dan meer lucht door de longen gepompt, wat de verdamping via de luchtwegen verhoogt. Vanuit energetisch oogpunt daarentegen is het juist voordelig om grote hoogtes te vermijden. Met afnemende dichtheid van de lucht wordt het steeds moeilijker de benodigde stijgkracht te leveren. De keuze van de meest gunstige vlieghoogte hangt dus van verschillende factoren af en kan uiteindelijk alleen maar een compromis zijn. Of de langeafstands trek uiteindelijk door watertekort of door uitputting van de energievoorraden beperkt wordt, is nog niet duidelijk. Eén van de redenen hiervoor is dat het waterbudget van vliegende vogels tot op heden nauwelijks onderzocht is.

Om een vergelijking te kunnen maken met de metingen aan vliegende vogels, wat het thema van dit proefschrift is, worden in **hoofdstuk 2** rustwaarden gepresenteerd van waterverlies door verdamping en van het metabolisme bij de roze spreeuw (*Sturnus roseus*). Deze rustwaarden zijn gemeten bij omgevingstemperaturen tussen de 7 en 30 °C, en dus vergelijkbaar met de resultaten van de later gepresenteerde vliegexperimenten. De verdamping van rustende vogels was bij temperaturen onder de 22 °C ongeveer 6.7 g d^{-1} (bijna 10 % van het lichaamsgewicht per dag) en onafhankelijk van temperatuur. Bij hogere temperaturen nam de verdamping duidelijk toe, veroorzaakt door de regulatie van de lichaamstemperatuur waarbij afkoeling wordt bewerkstelligd door verdamping, de zogenaamde evaporatieve thermoregulatie. Bij de hoogste door ons gemeten omgevingstemperatuur van 30 °C was de verdamping ongeveer 10.4 g d^{-1}. Omgekeerd lag het energieverbruik van deze rustende dieren bij omgevingstemperaturen boven 13 °C op een constant niveau van ongeveer 1.4 W. Verlaging van de omgevingstemperatuur resulteerde in een verhoging van het energieverbruik tot ongeveer 3 W bij 7 °C, vanwege het optreden van thermogene warmteproductie.

In **hoofdstuk 3** wordt bij de roze spreeuw het energieverbruik beschreven tijdens de langeafstandsvlucht als functie van de vliegsnelheid. Het energieverbruik is met de zogenaamde Dubbel Gelabeld Water (DLW) methode gemeten, aan de hand van metingen van de afnamesnelheden van de stabiele isotopen ^{2}H en ^{18}O, nadat een kleine hoeveelheid van deze isotopen aan het lichaamswater van het dier is toegevoegd. Op deze manier kan bij vrij bewegende dieren de hoeveelheid geproduceerde kooldioxide worden gemeten. Omdat kooldioxide vrijkomt bij metabole processen in het dier, kan deze waarde gebruikt worden als maat voor het energieverbruik (gemeten in Watt). Het was mogelijk de vogels tijdens een experiment van 6 uur met snelheden van 9 en 14 m s^{-1} te laten vliegen, dus over afstanden tussen de 194 en 302 kilometer. In deze periode waren de kosten van de vlucht (ongeveer 8 W, dus bijna 6 keer zo hoog als tijdens rust) en de vleugelslagfrequentie (ongeveer 10 Hz) onafhankelijk van de vlieg snelheid. Hetzelfde gold voor de manier van vliegen. Deze werd onafhankelijk van de snelheid gekenmerkt door periodes met regelmatige vleugelslag, onderbroken door korte fases met glijvluchten. Energieverbruik en vleugelslagfrequentie waren afhankelijk van het lichaamsgewicht, maar deze relatie was niet zo sterk als men zou verwachten op basis van een vergelijking tussen soorten met verschillend lichaamsgewicht.

Bij de omzetting van voedsel of lichaamsreserves in chemische energie wordt als bijproduct water geproduceerd, het zogenaamde metabolische water. Dit is de enige waterbron waarvan vogels tijdens de vlucht gebruik kunnen maken om het waterverlies door verdamping, en productie van faeces en urine te compenseren. In **hoofdstuk 4** worden metingen gepresenteerd van het waterverlies bij roze spreeuwen bij vliegsnelheden tussen de 9 en 14 m s^{-1} en bij omgevingstemperaturen tussen de 5 en 27 °C. Tijdens de langeafstands vluchten zoals hierboven beschreven was het waterverlies bij een omgevingstemperatuur van 15 °C ongeveer 1.3 g water per uur, onafhankelijk van de vliegsnelheid. In dezelfde tijdsperiode wordt ongeveer 1 g h^{-1} metabolisch water geproduceerd, dus het nettoverlies was ongeveer 0.3 g h^{-1}. Tijdens kortere vluchten bij een constante vliegsnelheid van 12 m s^{-1} en temperaturen tussen de 5 en 18 °C was de netto verdamping temperatuursonafhankelijk en bedroeg ongeveer 0.4 g h^{-1}. Bij temperaturen boven de 18 °C nam de verdamping met een factor vier toe tot 1.5 g h^{-1} bij 27 °C. Evaporatief waterverlies betekent niet alleen dat er een risico voor dehydratie bestaat, maar speelt ook een belangrijke rol bij de thermoregulatie, aangezien verdamping ook leidt tot afkoeling van het dier. Bij temperaturen beneden de 18 °C wordt door verdamping ongeveer 15 % van de warmte, die door de spieren tijdens het vliegen geproduceerd worden, afgegeven. Bij hogere temperaturen neemt dit aandeel toe tot 26 % bij 27 °C. Maar het grootste deel van de warmte wordt door convectie met de langsstromende lucht afgevoerd. Verdamping kan via de huid (transcutaan) of luchtwegen (respiratoir) optreden. De ingeademde lucht wordt in de ademwegen vochtiger en vervolgens verzadigd met water uitgeademd. Het volume van de uitgeademde lucht en de temperatuur van de lucht tijdens het uitademen (T_{exh}) bepalen het respiratoir waterverlies. T_{exh} is in de literatuur vooral bij rustende dieren onderzocht, waardoor er een grote lacune bestaat in ons inzicht bij vliegende dieren.

In **hoofdstuk 5** worden metingen van de ademluchttemperatuur van smienten (*Anas penelope*) en wintertaling (*Anas crecca*) tijdens vlucht en rust bij verschillende omgevingstemperaturen gepresenteerd. Voor beide soorten komen de waarden tijdens rust redelijk overeen met eerdere metingen aan andere soorten, T_{exh} was lager dan de lichaamstemperatuur en nam lineair toe met toenemende omgevingstemperatuur. Tijdens de vlucht was T_{exh} bij een gegeven omgevingstemperatuur met 5 °C verhoogd. De relatie met de omgevingstemperatuur was duidelijk, en de toename van T_{exh} met stijgende omgevingstemperatuur was sterker dan in eerdere studies aan vliegende vogels is gevonden.

In **hoofdstuk 6** worden metingen van T_{exh}, ademfrequentie en ademvolume van Europese spreeuwen (*Sturnus vulgaris*) tijdens vlucht en rust bij verschillende omgevingstemperaturen gepresenteerd. Deze gegevens maken een schatting van het respiratoir waterverlies mogelijk. In rust waren alle gemeten waarden onafhankelijk van de omgevingstemperatuur. Ook het respiratoir waterverlies in rust was met gemiddeld ~ 0.2 ml h^{-1} onafhankelijk van de temperatuur. Tijdens de vlucht waren ademfrequentie, ademvolume en T_{exh} verhoogd ten opzichte van de rustwaarden. Dat leidde tot een verhoging van het waterverlies ten gevolge van de ademhaling met gemiddeld 0.7 ml h^{-1}. Aangezien tijdens de vlucht het volume van de geventileerde lucht en T_{exh} met omgevingstemperatuur toenamen, werd het respiratoir waterverlies groter met toenemende temperatuur. Het respiratoir waterverlies maakte het grootste deel uit van het totale waterverlies tijdens de vlucht (55 - 88 %).

Hoofdstuk 7 geeft een afsluitende discussie op basis van de in dit proefschrift beschreven resultaten, in vergelijking met eerder uitgevoerde studies, en bespreekt de consequenties voor vrij vliegende vogels. Alle door ons bestudeerde dieren hebben tijdens de experimenten een netto waterverlies laten zien. Overwegingen met betrekking tot het warmtebudget tijdens het vliegen (Hoofdstuk 4) hebben laten zien dat een belangrijke functie van de verdamping de thermoregulatie is. Mogelijkheden om het waterverlies te beperken bestaan vooral uit het selectief vliegen in gebieden met lagere omgevingstemperaturen, waardoor er geen noodzaak is om de lichaamstemperatuur te reguleren via verdamping. Het waterverlies bij deze temperaturen is daarom ook constant en minimaal (Hoofdstuk 4). Dit waterverlies vindt ten eerste plaats als bijeffect van de afscheiding van afvalproducten van het metabolisme, ten tweede bij de ademhaling, en ten derde als passieve evaporatie bij temperatuur en/of luchvochtigheidsverschillen tussen lichaamsoppervlakte en omgevingslucht.

Vogels produceren in tegenstelling tot zoogdieren urinezuur in plaats van ureum als eindproduct van hun metabolisme. Urinezuur is niet wateroplosbaar, en kan daarom in een sterk geconcentreerde vorm afgevoerd worden. Dit maakt een uitscheiding mogelijk met een geringer waterverlies. Een andere mogelijkheid om het waterverlies te beperken is het voorkomen van verdamping via de huid. Vogels hebben geen zweetklieren, en het is aangetoond dat veel vogelsoorten bij verschillende omgevingstemperaturen de verdamping via de huid kunnen controleren. Deze transcutane verdamping is tot nu toe nog nooit tijdens de vlucht gemeten. Evaporatie via de ademwegen kan zowel via T_{exh} (Hoofdstuk 5 en 6), als via het volume van de geventileerde lucht gereguleerd worden (Hoofdstuk 6). Maar tijdens het hoge zuurstofverbruik onder het vliegen lijkt een reductie van de ventilatie geen reële optie.

Roze spreeuwen vliegen tijdens hun trek van de overwinteringsgebieden in het noordelijk deel van India naar hun broedgebieden in oostelijk Eurazië (en weer terug) vele duizenden kilometers. De in dit proefschrift gepresenteerde resultaten laten zien, dat het onmogelijk is deze afstand in één keer te vliegen. De afstand moet in meerdere kortere vliegperiodes overbrugd worden, onderbroken door rustperiodes om te eten en te drinken. Of de vliegafstand uiteindelijk beperkt wordt door uitputting van de energiereserves of door dehydratie, hangt vooral van de windomstandigheden en omgevingstemperatuur af. Roze spreeuwen trekken overdag, niet tijdens de koelere nachtelijke uren. Wanneer we de gemiddelde temperatuur in Teheran (Iran) als representatief beschouwen voor de omstandigheden op de trekroute en een vlieghoogte aannemen van 1000 m, is de temperatuur voor de vogels bijna de gehele dag lager dan 18 °C. Bij een verhoging van de temperatuur boven deze kritische waarde (Hoofdstuk 4) is evaporatieve thermoregulatie nodig. Onder deze kritische temperatuur zijn de energiereserves, ter grootte van ongeveer 10 g lichaamsvet, de beperkende factor voor de af te leggen afstand tot de volgende rustplaats (ongeveer 580 km). Door gebruik te maken van gunstige windomstandigheden kan deze afstand vergroot worden, zonder het waterbudget te beïnvloeden. Als de temperatuur hoger zou zijn zou de verdamping de vogels al tot landing dwingen voor het opraken van de energievoorraden. Om uiteindelijk te kunnen beoordelen of dehydratie tijdens langeafstands trek optreedt, zijn nu veldstudies aan deze en/of andere vogelsoorten nodig. Vooral gedetailleerde gegevens met betrekking tot vlieghoogte, klimatologische omstandigheden op de trekroute en conditie van het individuele dier aan het eind van de vlucht zijn hiervoor belangrijk. Er is derhalve nog veel onderzoek nodig om het fenomeen van de langeafstands trek goed te begrijpen.

Zusammenfassung

Vogelflug hat immer eine große Faszination auf den Menschen ausgeübt. Auch heute noch erstaunen die Leistungen, die viele Arten jährlich erbringen, um von ihren Wintergebieten zu weit entfernten Brutplätzen zu fliegen, nur um wenige Monate später den Rückweg anzutreten. Diese Wanderungen führen oft über unwirtliche Gebiete, Ozeane oder Wüsten, die keine Rastplätze mit Futter oder Wasser bieten. Während der Flugperiode selbst zehren die meisten Vögel ausschließlich von körpereigenen Reserven. Aspekte des Energie- und des Wasserhaushalts im Flug sind eng verknüpft und unterliegen Einflüssen der Umwelt und des individuellen Verhaltens. Quantitativ zu erforschen welche Folgen solche Langstreckenflüge für den Energie- und Wasserhaushalt haben, ist Ziel dieser Doktorarbeit. Die beschriebenen Studien wurden am Max Planck Institut für Ornithologie in Seewiesen mit Vögeln durchgeführt, die darauf trainiert wurden, große Entfernungen unter kontrollierten Bedingungen in einem Windkanal zurückzulegen.

Das Zusammenspiel der wichtigsten Parameter, die Einfluß auf den Energie- und Wasserhaushalt beim Flug haben, wird in **Kapitel 1** beschrieben. Viele Umweltfaktoren wie Temperatur, Luftfeuchtigkeit, Windrichtung und Windgeschwindigkeit, um nur einige zu nennen, sind variabel und unter anderem abhängig von der geographischen Lage, von der Jahres- und Tageszeit und der Flughöhe. In der Natur können sich Vögel frei in drei Dimensionen bewegen und haben damit einen großen Spielraum bei der Wahl der günstigsten Umweltbedingungen. Die Umgebungstemperatur spielt eine besonders große Rolle für den Wasserhaushalt, da die Körpertemperatur der Vögel bei hohen Temperaturen durch Verdunstung reguliert wird. Um hohe Wasserverluste durch Verdunstung zu vermeiden, ist es daher von Vorteil, in großer Höhe zu fliegen, da die Lufttemperatur um ca. 6 °C pro Kilometer Flughöhe abnimmt. Andererseits bestimmt die Flughöhe auch weitere Faktoren, wie Richtung und Stärke von Luftströmungen, Luftfeuchtigkeit und Sauerstoffgehalt der Luft. Um den hohen Sauerstoffbedarf beim Flug zu decken, muss in großer Höhe stärker ventiliert werden, also mehr Luft durch die Lungen gepumpt werden, was wiederum die Verdunstung über die Atemwege erhöht. Unter energetischen Gesichtspunkten ist es vorteilhaft, große Höhe zu meiden, da es mit abnehmender Dichte der Luft schwieriger wird, den notwendigen Auftrieb zu erzeugen. Die Wahl der günstigsten Flughöhe wird somit von mehreren Notwendigkeiten beeinflusst und kann nur ein Kompromiss sein.

Ob Langstreckenflüge letztlich durch Wassermangel oder durch Erschöpfung der Energievorräte beschränkt werden, ist noch nicht abschließend geklärt. Dies liegt unter anderem auch daran, daß der Wasserhaushalt fliegender Vögel bisher nur wenig untersucht wurde.

Als Vergleichsbasis zu den Messungen an fliegenden Vögeln, die Thema der folgenden Kapitel dieser Arbeit sind, werden in **Kapitel 2** zunächst Ruhewerte dargestellt. Bei Umgebungstemperaturen zwischen 7 und 30 °C, die denen während der Flugexperimente entsprechen, wurden der Wasserverlust durch Verdunstung und der Ruhestoffwechsel von Rosenstaren (*Sturnus roseus*) gemessen. Die Verdunstungsrate der ruhenden Vögel war bei Temperaturen unter 22 °C ungefähr 6.7 g d^{-1} (etwa 10 % des Körpergewichtes) und relativ temperaturunabhängig. Bei höheren Temperaturen stieg die Verdunstungsrate erheblich an, da die Körpertemperatur durch Verdunstungskälte, evaporative Thermoregulation, geregelt werden mußte. Bei der höchsten von uns gemessenen Temperatur von 30 °C betrug die Verdunstungsrate ~ 10.4 g d^{-1}. Umgekehrt war der Energieverbrauch bei höheren Temperaturen (> 13 °C) konstant ~ 1.4 W, stieg aber durch aktive Thermoregulation (z.B. durch Muskelzittern) bei niedrigeren Umgebungstemperaturen bis auf ~ 3.0 W bei 7 °C an.

Der Energieverbrauch während des Fluges ist im Vergleich zu den Ruhewerten etwa um das sechsfache gestiegen. **Kapitel 3** beschreibt den Energieverbrauch von Rosenstaren während des Langstreckenfluges bei verschiedenen Fluggeschwindigkeiten. Dieser Verbrauch wurde mit der sogenannten DLW Methode gemessen. "DLW" steht für Doubly Labelled Water, also durch die stabilen Isotope ^{18}O und ^{2}H doppelt markiertes Wasser. Mit dieser Methode wird die Kohlendioxidproduktion aus der unterschiedlichen Abnahmegeschwindigkeit der stabilen Isotope ^{18}O und ^{2}H im Blut errechnet. Kohlendioxid wird bei der Energieumsetzung durch die Atmung frei und kann somit als Maß für den Energieverbrauch (ausgedrückt in Watt) benutzt werden. Die Vögel akzeptierten für die langen Flüge dieses Versuchs (sechs Stunden) Geschwindigkeiten zwischen 9 und 14 m s^{-1}. Innerhalb dieser Spanne waren die Flugkosten (~ 8 W) sowie die Flügelschlagfrequenz (~ 10 Hz) unabhängig von der Geschwindigkeit. Auch der Flugstil, gekennzeichnet durch Phasen mit gleichmäßigem Flügelschlag, unterbrochen von kurzen Gleitphasen, änderte sich nicht mit der Geschwindigkeit. Der Energieverbrauch sowie die Flügelschlagfrequenz stiegen aber mit dem Körpergewicht an. Dieser Anstieg war allerdings nicht so ausgeprägt wie Vergleiche der Flugkosten zwischen Arten unterschiedlichen Körpergewichts hätten vermuten lassen.

Bei der Umwandlung von Nahrung oder körpereigenen Speicherstoffen in chemische Energie wird als Nebenprodukt Wasser gebildet. Dieses sogenannte „metabolische Wasser“ stellt während des Fluges die einzige Wasserquelle für die Vögel dar und steht gegen Wasserverluste durch Verdunstung, Kot und Urin. In **Kapitel 4** werden Messungen des Wasserverlustes während des Fluges von Rosenstaren bei Umgebungstemperaturen zwischen 5 und 27 °C und Fluggeschwindigkeiten zwischen 9 und 14 m s^{-1} präsentiert. Während der oben beschriebenen Langstreckenflüge bei 15 °C verloren die Vögel ~ 1.3 g Wasser pro Stunde, unabhängig von der Fluggeschwindigkeit. Gleichzeitig wurde aber ~ 1 g h^{-1} metabolisches Wasser gebildet. Der Netto-Verlust betrug also ~ 0.3 g h^{-1}. Während kürzerer Flüge (1 h) bei konstanter Geschwindigkeit (12 m s^{-1}) und Temperaturen zwischen 5 und 18 °C waren die Netto-Verdunstungsraten temperaturunabhängig ~ 0.4 g h^{-1}. Bei Temperaturen über 18 °C stiegen die Verdunstungsraten um das Vierfache bis auf 1.5 g h^{-1} bei 27 °C an. Evaporativer Wasserverlust beinhaltet aber nicht nur die Gefahr des Austrocknens („Dehydrierung“), sondern spielt durch die damit einhergehende Verdunstungskälte auch eine wichtige Rolle bei der Thermoregulation. Bei Temperaturen unter 18 °C wurden durch Verdunstung ~ 15 % der Wärme, die durch die Muskelarbeit beim Flug entstand, abgegeben. Bei höheren Umgebungstemperaturen erhöhte sich dieser Anteil bis auf ~ 26 % bei 27 °C. Der größte Teil der Wärme wird allerdings durch Konvektion von der vorbeistreichenden Luft abgeführt.

Verdunstung kann über die Haut (transcutan) oder über die Atemwege (respiratorisch) erfolgen. Da die eingeatmete Luft in den Atemwegen angefeuchtet wird und wassergesättigt ausgeatmet wird, definieren das Volumen der ventilierten Luft sowie deren Temperatur beim Ausatmen (T_{exh}) den respiratorischen Wasserverlust. T_{exh} wurde bisher vor allem bei Tieren in Ruhe gemessen. In **Kapitel 5** werden Messungen der Atemlufttemperatur von Pfeifenten (*Anas penelope*) und Krickenten (*Anas crecca*) im Flug und in Ruhe bei verschiedenen Umgebungstemperaturen vorgestellt. Für beide untersuchten Entenarten stimmten die Ruhewerte mit früheren Untersuchungen an anderen Arten insofern überein, als T_{exh} niedriger als die Körpertemperatur war und mit steigender Umgebungstemperatur linear anstieg. Im Flug war T_{exh} bei gegebener Umgebungstemperatur dagegen um etwa 5 °C erhöht. Die Beziehung zur Umgebungstemperatur war ausgeprägt, und der Anstieg von T_{exh} bei steigender Umgebungstemperatur steiler als in den wenigen Untersuchungen an fliegenden Vögeln, die für einen Vergleich zur Verfügung stehen.

In **Kapitel 6** werden Messungen von T_{exh}, Atemfrequenz und Atemvolumen bei Europäischen Staren (*Sturnus vulgaris*) während des Fluges und in Ruhe bei verschiedenen Umgebungstemperaturen vorgestellt. Diese Daten erlauben eine Abschätzung des respiratorischen Wasserverlustes. In Ruhe waren alle gemessenen Parameter unabhängig von der Umgebungstemperatur. Der respira-torische Wasserverlust in Ruhe war ebenfalls temperaturunabhängig und betrug im Durchschnitt ~ 0.2 ml h^{-1}. Im Flug dagegen waren Atemfrequenz, Atemvolumen und T_{exh} erhöht im Vergleich zu den Ruhewerten. Dies führte zu einer Zunahme des Wasserverlustes mit der Atmung auf durchschnittlich etwa 0.7 ml h^{-1}. Da im Flug sowohl das Volumen der ventilierten Luft als auch $\mathrm{T}_{\mathrm{exh}}$ mit der Umgebungstemperatur anstiegen, erhöhte sich auch der respiratorische Wasserverlust mit steigender Temperatur. Der respiratorische Wasserverlust stellte den Hauptanteil (55 bis 88 %) des Gesamtwasserverlusts beim Flug dar.

Die hier präsentierten Ergebnisse werden in **Kapitel 7** im Licht früherer Untersuchungen unter kontrollierten Bedingungen oder im Freiland diskutiert. Alle Tiere erfuhren während unserer Experimente einen Netto-Wasserverlust. Überlegungen zum Wärmehaushalt während des Fluges (Kapitel 4) haben gezeigt, dass eine wichtige Funktion von Verdunstung in der Thermoregulation zu sehen ist. Möglichkeiten den Wasserverlust zu verringern sind also vor allem im unteren Temperatur-Bereich zu suchen, wenn keine Notwendigkeit zur evaporativen Thermoregulation besteht. Wasserverlust bei diesen Temperaturen ist konstant und minimal (Kapitel 4). Er findet erstens als Nebeneffekt bei der Ausscheidung von Stoffwechsel-Abfallprodukten statt, zweitens über die Atmung und drittens als passiver Verdunstungsvorgang aufgrund von Temperatur- und Feuchtigkeitsunterschieden zwischen der Körperoberfläche und der Umgebungsluft. Vögel bilden, im Gegensatz zu Säugetieren, Harnsäure statt Harnstoff als Stoffwechsel-Endprodukt. Harnsäure ist nicht wasserlöslich, kann dadurch in sehr viel konzentrierterer Form ausgeschieden werden und ist daher mit weniger Wasserverlust verbunden als Harnstoff. Die Ausscheidung von Abfallprodukten ist bei Vögeln daher generell mit geringen Wasserverlusten verbunden. Weitere Möglichkeiten, den minimalen Wasserverlust zu verringern, liegen in der Beschränkung der Verdunstung über die Haut. Obwohl Vögel keine Schweißdrüsen besitzen, wurde für viele Arten gezeigt, dass sie bei unterschiedlichen Umgebungstemperaturen die Verdunstung über die Haut kontrollieren können. Transkutane Evaporation wurde bisher aber noch nicht während des Fluges gemessen. Evaporation über die Atemwege kann einerseits über T_{exh} kontrolliert werden (Kapitel 5 und 6), andererseits über das Volumen der ventilierten Luft

(Kapitel 6). Beim Flug, mit dem damit verbundenen hohen Sauerstoffbedarf, scheint es fraglich, ob eine Reduktion der Ventilation eine reale Option ist.

Rosenstare legen auf dem Zug zwischen ihrem Wintergebiet im nördlichen Indien und den Brutgebieten im östlichen Eurasien mehrere tausend Kilometer zurück. Die Ergebnisse unserer Windkanaluntersuchungen legen nahe, dass diese Strecke nicht in einem ununterbrochenen Flug zurückgelegt werden kann, sondern in kürzere Flugphasen aufgeteilt werden muss, unterbrochen von Rastperioden zum Fressen und Trinken. Ob letztlich eine Erschöpfung der Energievorräte oder Dehydrierung die unmittelbare Beschränkung der Flugstrecke darstellt, hängt vor allem von den Windverhältnissen und der Umgebungstemperatur ab. Rosenstare gelten als Tagzieher, fliegen also nicht während der kühleren Nachtstunden. Nimmt man die durchschnittlichen Temperaturen in Teheran (Iran) als exemplarisch für die Bedingungen auf der Zugroute an und geht von einer Flughöhe von ~ 1000 m aus, sind die Temperaturen, denen die Vögel ausgesetzt sind, während der meisten Tage der Zugzeit niedriger als 18 °C. Bei Überschreitung dieser in Kapitel 4 ermittelten "kritischen" Temperatur, muß evaporative Thermoregulation betrieben werden. Unterhalb der kritischen Temperatur begrenzen die verfügbaren Energievorräte, ca. 10 g Körperfett, die Flugdistanz bis zur nächsten Rast (ca. 580 km). Die Nutzung günstiger Winde würde diese Distanz vergrößern, ohne Einfluß auf den Wasserhaushalt der Tiere zu haben. Bei höheren Temperaturen dagegen dürften die hohen Verdunstungsraten die Vögel schon vor Aufbrauchen der Energievorräte zum Landen zwingen. Für eine abschließende Beurteilung, ob Dehydrierung während des Langstreckenfluges vorkommt, sind allerdings mehr Freilandstudien an dieser und anderen Arten, insbesondere zur Flughöhe, klimatischen Bedingungen auf der Flugroute und zur Körperkondition nach Beendigung eines Fluges notwendig. Es werden noch viele Forscher daran beteiligt sein, mit ihren Untersuchungen Teile ins Gesamtbild einzufügen, um das Phänomen des Langstreckenfluges von Vögeln zu erklären.

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Addresses of Co-authors

Herbert Biebach Max Planck Institute for Ornithology, Seewiesen. Von-der-Tann-Str. 7, 82346 Andechs, Germany

Marcel Klaassen Netherlands Institute of Ecology (NIOO-KNAW), Centre for Limnology. Rijksstraatweg 6, 3631AC Nieuwersluis

Raymond H. G. Klaassen Netherlands Institute of Ecology (NIOO-KNAW), Centre for Limnology. Rijksstraatweg 6, 3631AC Nieuwersluis

Roderick A. Suthers School of Medicine and Department of Biology, Indiana University, Bloomington, Indiana 47405, USA

G. Henk Visser Department of Behavioural Biology, University of Groningen, Haren, The Netherlands and
Centre for Isotope Research, University of Groningen, Groningen, The Netherlands