

Heart rate and the rate of oxygen consumption of flying and walking barnacle geese (*Branta leucopsis*) and bar-headed geese (*Anser indicus*)

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Summary

We tested the hypotheses that the relationship between heart rate (f_H) and the rate of oxygen consumption (\dot{V}_{O_2}) differs between walking and flying in geese and that f_H and \dot{V}_{O_2} have a U-shaped relationship with flight speed. We trained barnacle geese *Branta leucopsis* (mean mass 2.1 kg) and bar-headed geese *Anser indicus* (mean mass 2.6 kg) to walk inside a respirometer on a treadmill and to fly in a wind tunnel with a respirometry mask at a range of speeds. We measured f_H and \dot{V}_{O_2} simultaneously during walking on the treadmill in five individuals of each species and in one bar-headed goose and four barnacle geese during flight in the wind tunnel. The relationships between f_H and \dot{V}_{O_2} were significantly different between flying and walking. \dot{V}_{O_2} was higher, and the increment in \dot{V}_{O_2} for a

given increase in f_H was greater, for flying than for walking geese. The relationship between f_H and \dot{V}_{O_2} of free-living barnacle geese during their natural migratory flights must differ from that measured in the wind tunnel, since the f_H of wild migratory birds corresponds to values of \dot{V}_{O_2} that are unrealistically low when using the calibration relationship for our captive birds. Neither f_H nor \dot{V}_{O_2} varied with flight velocity across the range of speeds over which the geese would fly sustainably.

Key words: flight, exercise, heart rate, oxygen consumption, bird, goose, metabolic power, *Branta leucopsis*, *Anser indicus*, migration.

Introduction

Flight is the most energetically costly form of sustained activity performed by animals (Schmidt-Nielsen, 1972; Norberg, 1990). The metabolic rate during flight is, therefore, of great importance in the energy budget of all flying animals (Bryant, 1997). The energy requirement for flight is of particular importance for migrating birds since it determines every aspect of migration strategy such as flight range, the appropriate level of fuel loading, necessary stopover times and flight speed (e.g. Rayner, 1990; Walsberg, 1990; Carmi et al., 1992; Lindström and Alerstam, 1992; Weber et al., 1994, 1998; Hedenström and Alerstam, 1995, 1997; Klaassen, 1995, 1996; Butler et al., 1998; Pennycuik, 1998).

There are several approaches to the calculation of the energy expenditure of free-flying migratory birds. Average data for a population can be obtained from the difference in body mass before and after migratory flights, although the difficulty in catching birds immediately before departure and after arrival (Nisbet et al., 1963; Butler et al., 1998; Battley et al., 2000) and in interpretation of mass loss data in terms of energy consumption (Kvist et al., 1998) means that these estimates may be inaccurate. The doubly labelled water (DLW) technique (Lifson and McClintock, 1966; Speakman, 1997) would, in theory, allow the measurement of the cost of

migratory flight by individual birds, but this approach is impractical because of the difficulty in capturing an individual bird at the appropriate times. The DLW technique can be used to measure metabolic rate during simulated migratory flights in a wind tunnel (Lindström et al., 1999; Klaassen et al., 2000; Kvist et al., 2001) or during free flight in homing pigeons (LeFebvre, 1964; Gessaman and Nagy, 1988). Energy expenditure during flight can be estimated for a given bird mass from an allometric relationship with the rate of oxygen consumption for birds flying in wind tunnels wearing masks from which expired gases are collected (e.g. Butler and Bishop, 2000). However, this approach cannot currently take into account morphological variation among species because of the relatively small number of species that have been trained to fly in wind tunnels.

Flight costs can also be predicted from aerodynamic models, particularly that described by Pennycuik (1989). This approach has the advantage that the effects upon the mechanical power for flight of variation in mass, flight speed, wing morphology and altitude can be calculated (e.g. Lindström and Alerstam, 1992; Klaassen, 1995, 1996; Hedenström and Alerstam, 1995, 1997; Pennycuik, 1998). However, aerodynamic models have the disadvantage that they

predict mechanical power, which represents only 10–23% of total metabolic power. Because the efficiency of the flight muscles is not well known (Kvist et al., 2001; Ward et al., 2001), it is currently difficult to make accurate predictions of metabolic flight costs from aerodynamic models. One of the predictions of most aerodynamic models is that flight costs should form a U-shaped curve with respect to flight speed (Rayner, 1979; Pennycuick, 1989), so examining the shape of the power–speed curve is one way of testing the accuracy of the predictions of aerodynamic models (e.g. Dial et al., 1997; Rayner and Ward, 1999; Kvist et al., 2001; Ward et al., 2001). The form of the metabolic power–speed curve has not previously been examined in relatively large birds such as geese.

Data on heart rate (f_H) have been collected from free-living barnacle geese (*Branta leucopsis*) during natural migration (Butler et al., 1998) and from captive-bred birds trained to fly behind a truck (Butler and Woakes, 1980). Although there is significant variation in f_H during migratory flights, there was no change in f_H , respiratory frequency or wingbeat frequency over a wide range of airspeed in the geese flying behind the truck. This raises the possibility that power input (determined by measuring the rate of oxygen consumption, \dot{V}_{O_2}) may also be constant over a range of flight speeds in these birds. A preliminary study of barnacle geese (1.7 kg body mass) flying in front of a wind generator succeeded in measuring mean f_H and mean \dot{V}_{O_2} of 2–3 birds flying at a single speed (13 ms⁻¹), demonstrating the feasibility of training this species to fly in a suitably large wind tunnel (Butler et al., 2000). The relationship between \dot{V}_{O_2} and f_H has previously been established for walking barnacle geese (Nolet et al., 1992), but this relationship does not predict realistic values of \dot{V}_{O_2} from measurements of f_H made during natural migratory flights (Butler et al., 1998). To determine whether the relationship between \dot{V}_{O_2} and f_H differs between walking and flying, or between captive and wild geese (or both), we measured the f_H and \dot{V}_{O_2} of barnacle geese during walking on a treadmill and flight in a wind tunnel. We also studied bar-headed geese (*Anser indicus*) because the ability of this species to fly at high altitude during migration (up to 9 km, where the partial pressure of oxygen is 30% of that at sea level; Swan, 1961) and its different form of haemoglobin (Rollema and Bauer, 1979; Perutz, 1983) suggest that this species may have an unusual flight physiology. These are the first data of which we are aware on the detailed relationship between f_H and \dot{V}_{O_2} during flight for any animal.

The aims of the present study were to measure f_H and \dot{V}_{O_2} during walking and flying in two species of goose, to calibrate the relationship between f_H and \dot{V}_{O_2} during these two forms of exercise and to test the hypotheses (i) that the relationship between f_H and \dot{V}_{O_2} differs between flying and walking (Nolet et al., 1992; Butler et al., 2000) and (ii) that f_H and \dot{V}_{O_2} show U-shaped relationships with flight speed that reflect the changes in mechanical power predicted from most aerodynamic theories (Rayner, 1979; Pennycuick, 1989).

Materials and methods

Birds and training

Eggs of barnacle geese [*Branta leucopsis* (Latham)] and bar-headed geese [*Anser indicus* (Bechstein)] were purchased from wildfowl collections in England and hatched in an incubator. The goslings (ten barnacle geese and eight bar-headed geese) were imprinted on their human foster parent (S.W.) (Lorenz, 1970; Butler and Woakes, 1980).

The geese were flown in the wind tunnel during times that approximated to the migratory periods of their wild conspecifics in the autumn (in September and October, when the barnacle geese were 12–20 weeks old and the bar-headed geese were 14–22 weeks old) and in the following spring (in April and May, when the birds were 9–10 months old). We measured f_H and \dot{V}_{O_2} during flights by both species in autumn and f_H during flights by barnacle geese in spring. The geese were lifted into the air stream by the trainer at the start of each flight in the wind tunnel. Initially, the trainer had to stand in the flight section to stop the goslings from landing, but during flights in the spring it was possible to do this while standing out of the air stream at the side of the flight section. The position in which the goose flew relative to the trainer was categorised as position A (with the tail of the bird at least 1 m upwind of the trainer), position B (with the tail less than 1 m upwind of the trainer) or position C (with the trainer standing out of the air stream). Each bird was flown over as wide a range of sustainable flight speeds as was possible (14–20 ms⁻¹ for barnacle geese and 16–21 ms⁻¹ for bar-headed geese). The range of flight speed of barnacle geese in the wind tunnel was similar to that recorded during natural migratory flight by barnacle geese (M. Green, personal communication). We measured the \dot{V}_{O_2} of four barnacle geese (mean mass 1.98±0.10 kg) and two bar-headed geese (mean mass 2.66±0.15 kg) and the f_H of six barnacle geese (mean mass 2.07±0.10 kg) and two bar-headed geese (mean mass 2.56±0.20 kg) during flight in the wind tunnel.

Five barnacle geese (mean mass 1.99±0.12 kg) and five bar-headed geese (mean mass 2.75±0.09 kg) (including the individuals that were flown in the wind tunnel) were trained to walk on a treadmill enclosed in a respirometry chamber. The treadmill experiments were conducted when the birds were 7–8 months old. All procedures followed Home Office guidelines for the use of animals in experiments in the UK.

Heart rate

We recorded f_H using custom-built loggers implanted into the abdomen (which logged the mean f_H over 30 s intervals in barnacle geese and over 10 s intervals in bar-headed geese) or radio transmitters (which transmitted the electrocardiogram from two subcutaneous electrodes) taped to feathers on the back of the bird (Woakes et al., 1995). The procedure for abdominal implantation of heart rate loggers followed that used by Stephenson et al. (1986).

Walking and resting respirometry

Measurements of gas exchange during walking were made

by placing the goose inside a Perspex box (74 cm high \times 58 cm long \times 47 cm wide) that rested on a wooden frame on a variable-speed treadmill (Powerjog, Sports Engineering Ltd). The air in the chamber was mixed by three fans in a side compartment. Brush-style draught excluders minimised air leaks between the wooden frame and the treadmill belt. The flow rate that we used should replace 95% of the air in the chamber in 12 min. Data from the training sessions confirmed that stable levels of gas exchange were reached after 10–13 min of walking at each speed. Mean \dot{V}_H and gas exchange data from the final 5 min of the 18 min spent walking at each speed (i.e. after 13 min of walking at that speed) were taken to be representative of that walking speed. The geese walked in the treadmill respirometer at 8–12 speeds at approximately 0.07 m s⁻¹ increments between 0.07 and 0.92 m s⁻¹. Only one bird would walk at the full range of speeds. The order of the speeds at which each bird walked was determined randomly.

The extent to which the treadmill respirometer leaked when the belt was moving was determined by supplying oxygen-free dry nitrogen gas (BOC) into the chamber at 1 l min⁻¹ (measured to ± 0.005 l min⁻¹ with a 1.21 min⁻¹ KDG 1100 rotameter) while air was pumped through the chamber at a rate similar to that used during the experiments with the geese (Fedak et al., 1981). The calculated values of gas exchange were adjusted to compensate for the loss of chamber gas.

Measurements of \dot{V}_H and the rates of gas exchange from resting birds were made overnight in the treadmill respirometer. Chamber air temperature was 19–21°C, within the thermoneutral zone for geese (Calder and King, 1974). Resting rates of gas exchange and \dot{V}_H were calculated from the lowest values when averaged over 5 min.

Mask respirometry

Flying birds were fitted with a mask made from vacuum-formed polyurethane (of mass 4.4 g for barnacle geese and 8.1 g for bar-headed geese) that covered the beak and forehead, but not the eyes, of the bird. The mask was attached by an elastic or lightweight leather strap round the back of the head that passed just below the base of the skull. Air was extracted from above the nostrils through a polyurethane tube (internal diameter 6.5 mm, external diameter 9 mm; 46 g m⁻¹) that ran over the back of the bird from the top of the head. The mask had four round holes (each 1.5 mm in diameter) at the front and an oval hole (10 mm \times 5 mm for barnacle geese and 15 mm \times 8 mm for bar-headed geese) underneath to allow air to enter. The trainer supported the tube 1–1.5 m from the head of the bird during flight to counteract most of the drag and weight of the tube.

Respirometry equipment

The air flow rate was 48.1 \pm 0.41 min⁻¹ STPD (mean \pm S.E.M., $N=112$) for walking and resting birds, 71.0 \pm 0.91 min⁻¹ STPD ($N=30$) for flying barnacle geese and 75.9 \pm 1.01 min⁻¹ ($N=23$) for flying bar-headed geese (KDG 2000 150 l min⁻¹ rotameter, B105 Charles Austen pump). The rate of air extraction from the mask was great enough to prevent loss of exhaled gases because calculated \dot{V}_{O_2} did not change when the flow rate was decreased

by 10% during a sample of flights (paired t -tests, barnacle goose, $t=0.29$, $P=0.8$, $N=8$; bar-headed goose, $t=2.2$, $P=0.2$, $N=3$). Electronic sensors were used to measure the barometric pressure (Prosser) and the temperature and relative humidity (Farnell Electronic Services) of the air flow. A subsample (1.5 l min⁻¹) of air from the mask or chamber was pumped (Charles Austin Dymax 30) through silica gel to dry the air before passing through a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Servomex 1440). The output signals from the gas analysers, and the pressure, humidity and temperature sensors were passed through a purpose-built interface box to an analog-to-digital converter unit (DAQ 500 or AT-MIO-16L card, National Instruments) and then to a computer (Dell Inspiron 7000 or Dell Dimension XPS P60). A customised computer program (written for LabVIEW, National Instruments) sampled the signals from the digital converter unit at 1000 Hz and saved the mean values to a file every 10 s. The gas analysers were calibrated at the start of each day by drawing oxygen-free dry nitrogen (BOC) at 1 l min⁻¹ through the oxygen analyser or carbon dioxide (BOC) at 1 l min⁻¹ through the carbon dioxide analyser (measured to the nearest 0.005 l min⁻¹, 1.21 min⁻¹ rotameter, KDG 1100) into the system together with the flow of atmospheric air to be used during the experiment (Fedak et al., 1981). The lag between gases leaving the respirometry mask and reaching the gas analysers was 20 s, at which time there was a step change in gas concentration because the volume of the mask was so small.

Respirometry calculations

The rate of carbon dioxide production (\dot{V}_{CO_2}) was calculated from:

$$\dot{V}_{CO_2} = \dot{V}_{STPD}(F_{CO_2,Out} - F_{CO_2,Amb}), \quad (1)$$

where \dot{V}_{STPD} is the equivalent flow rate of dry air through the mask or chamber at standard temperature and pressure and $F_{CO_2,Out}$ and $F_{CO_2,Amb}$ are the fractional concentrations of carbon dioxide in the outlet and ambient air, respectively. The rate of oxygen consumption (\dot{V}_{O_2}) was calculated from equation 3b of Withers (1977). On four occasions during experiments with walking bar-headed geese and once during a resting measurement with a barnacle goose, the data from the carbon dioxide analyser were lost as a result of equipment failure. For these experiments, we calculated \dot{V}_{O_2} from equation 3a of Withers (1977), where the respiratory exchange ratio (RER) was assumed to be the mean value measured in the other birds (0.78). This procedure would introduce an error of less than 1% into the calculated \dot{V}_{O_2} (Koteja, 1996), given the measured variation in RER. Metabolic power (W) was calculated from \dot{V}_{O_2} using the calorific value of oxygen that corresponded to the measured RER (Brody, 1945).

Wind tunnel

The open-circuit wind tunnel in which the geese were flown was powered by a 170 kW Thrige–Scott direct-current motor driving a Kiloheat RZR 13-1600 centrifugal fan (diameter 1.6 m, with backward curved impellers). The air from the fan passed through a 4.5 m \times 4.5 m settling area, three fine-mesh screens and

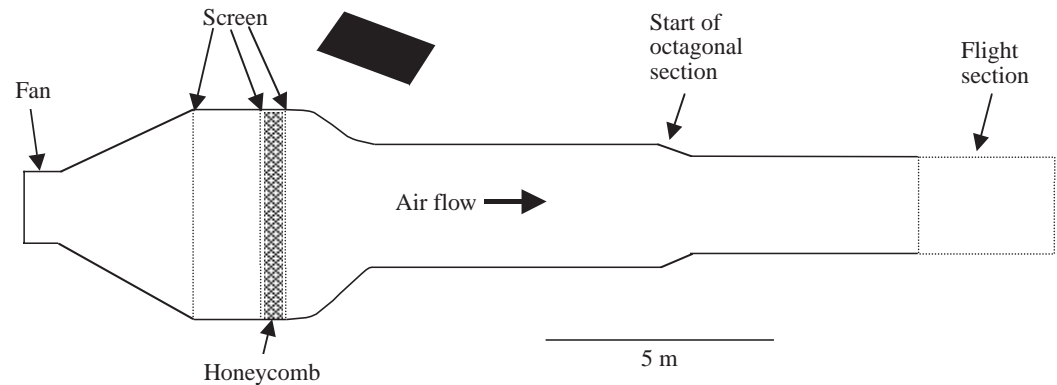


Fig. 1. Diagram of a longitudinal cross section of the wind tunnel.

a honeycomb before gradual contraction into the octagonal flight section (cross-sectional area 4.25 m^2) at the exhaust opening of the solid-walled tunnel, 19 m downwind of the fan (Fig. 1). The flight section ($3.06 \text{ m long} \times 2.10 \text{ m high} \times 2.50 \text{ m wide}$) was enclosed by nylon mesh to prevent the bird from leaving the air stream. The birds were prevented from flying upwind from the flight section by a 2 cm square plastic (0.5 mm diameter) mesh and from leaving the flight section downwind by a 6 cm square plastic (6 mm diameter) mesh. Air was drawn into the wind tunnel from outside the building, so air temperature ($14.9 \pm 2.3^\circ\text{C}$, mean \pm S.D., range 10.5 – 19.0°C , $N=187$ flights), relative humidity ($62 \pm 14\%$, mean \pm S.D., range 42 – 89% , $N=187$ flights) and barometric pressure ($100.4 \pm 1.0 \text{ kPa}$, mean \pm S.D., range 97.2 – 101.7 kPa , $N=187$ flights) in the flight section were determined by prevailing environmental conditions.

The air speed and root-mean-square longitudinal turbulence in the flight section of the wind tunnel were determined using a hot-wire anemometer (Dantec 55P01) from which the output passed through a signal-conditioning card to an eight-channel 16-bit analog-to-digital converter (Io-tech 488/ADC) and then to a Macintosh G3 computer. Turbulence and mean air speed were sampled for 40 s at 200 Hz during each measurement. The output from the hot-wire anemometer was calibrated between 10 and 19 m s^{-1} against a propeller anemometer (Schiltnecht) and the pitot tube that was used routinely to set wind tunnel air speed. The discrepancy in the cross-calibration was 0.3% (range 0–0.4%) ($N=10$ speeds at 1 m s^{-1} increments between 10 and 19 m s^{-1}), so all three instruments were assumed to provide accurate readings.

Variations in air speed and turbulence in the flight section, but without the upwind mesh in place, were measured with the hot-wire anemometer at points 0.21 m apart horizontally and 0.18 m apart vertically on a grid perpendicular to the direction of air flow. The grid was a maximum of 11 points wide and nine points deep. The centre of the grid was 1.23 m from the floor of the flight section. The longitudinal and

lateral centres of the grid were centred on the flight section, 1.53 m from either end and 1.25 m from either side. Air speed and turbulence were also measured in the centre of the grid at 2 m s^{-1} increments at speeds between 8 m s^{-1} and 20 m s^{-1} .

Air speed ($17.9 \pm 0.01 \text{ m s}^{-1}$, mean \pm S.D., $N=93$) did not vary systematically over the cross section of the flight section of the wind tunnel. Root-mean-square longitudinal turbulence at an air speed of $17.9 \pm 0.01 \text{ m s}^{-1}$ was $0.82 \pm 0.03\%$ ($N=35$ points) in the central part of the flight section in which the geese flew (Fig. 2). Turbulence increased towards the walls of the flight section to $2.46 \pm 0.18\%$ ($N=29$ points) between 0.5 and 0.3 m from the walls,

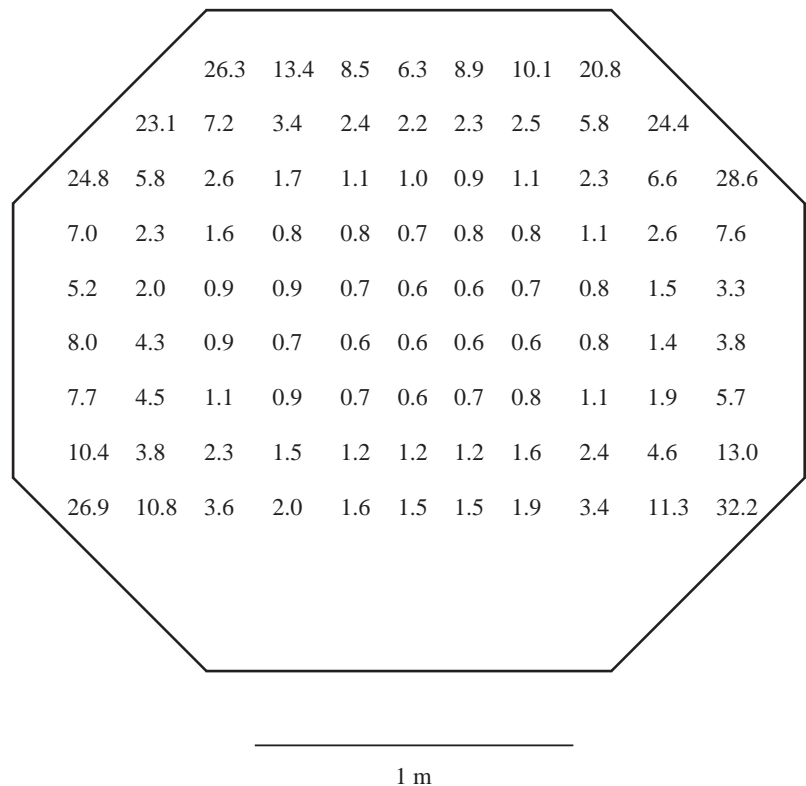


Fig. 2. The root-mean-square longitudinal turbulence (%) at each point on a cross section through the flight section of the wind tunnel. The measurements were made across an array of points centred 1.23 m from the floor and 1.53 m from either end of the flight section at an air speed of $17.88 \pm 0.01 \text{ m s}^{-1}$.

7.92±0.60% ($N=21$ points) between 0.3 and 0.1 m from the walls and 25.89±1.14 ($N=8$ points) less than 0.1 m from the walls. Turbulence was 0.6–0.9% at air speeds between 8.5 and 21 m s⁻¹ in the centre of the area in which the birds flew.

The equivalent air speed in the flight section was calculated from dynamic pressure following Pennycuick et al. (1996). Dynamic pressure was measured using a manometer connected to a pitot tube that projected 1.0 m vertically down into the air flow at the upwind end of the flight section. The pitot tube and manometer measured air speed with a precision of ±0.05 m s⁻¹ and an accuracy of 0.3±0.1% (see cross-calibration of instruments described below).

Statistical analyses

Statistical tests were carried out with the assistance of Minitab (version 12.22, Minitab Inc.) following Zar (1996). The coefficients of determination (r^2) of linear regression relationships and double-logarithmic regression relationships (equivalent to power curves) between \dot{V}_{O_2} and f_H were compared to determine which form better described these relationships. The coefficients of determination differed by less than 0.02 in all cases, with neither linear nor power relationships providing consistently greater coefficients of determination. Either linear or power relationships could therefore be used to describe the data. We present the linear relationships calculated by least-squares regression. We used mean f_H and mean \dot{V}_{O_2} measured during entire 3–45 min flights or mean f_H and mean \dot{V}_{O_2} during the last 5 min of an 18 min walking period on the treadmill at a given speed as independent data in our analyses of the relationships between f_H and \dot{V}_{O_2} . We obtained most data on the relationship between f_H and \dot{V}_{O_2} during flight from one bird for each species. We calculated the 95% prediction intervals of the relationships between f_H and \dot{V}_{O_2} during flight and the SE of the predicted \dot{V}_{O_2} for a given value of f_H during flight following equation 16.29 of Zar (1996). We assessed whether simultaneous measurements of f_H and \dot{V}_{O_2} from four flights by three additional barnacle geese were consistent with the relationship between f_H and \dot{V}_{O_2} for the individual of that species from which we had most data by determining whether the data from the additional birds fell within the 95% prediction intervals of the relationship.

We used analysis of covariance (ANCOVA) to test whether the slopes and intercepts of the relationships between f_H and \dot{V}_{O_2} during walking on the treadmill varied among birds (sections 17.4 and 17.5 in Zar, 1996). Since the slopes of the relationships did not vary significantly among birds, we used a single regression equation with a common slope and a common intercept to describe the relationship (Zar, 1996).

Analysis of variance (ANOVA) was used to examine the effects of more than one factor upon a dependent variable. Factors that did not contribute significantly to a model were eliminated until only those factors that explained a significant amount of variation in the dependent variable remained in the model. We used t -tests to analyse the effect of two alternative factors upon a dependent variable. Two-tailed tests of

statistical significance were applied in all analyses. Differences with $P<0.05$ were regarded as significant. Values are presented as means ± S.E.M. unless stated otherwise. Mean values across birds are means of the mean values for each bird.

Results

Heart rate and rate of oxygen consumption in resting and walking geese

The lowest f_H and \dot{V}_{O_2} of all the birds during resting overnight in the thermoneutral zone were measured in the early hours of the morning. The mean minimum f_H and \dot{V}_{O_2} of barnacle geese ($N=5$ birds) were 113±11 beats min⁻¹ and 25.3±2.1 ml min⁻¹ (equivalent to 12.8±1.1 ml kg⁻¹ min⁻¹). The mean minimum f_H of bar-headed geese ($N=3$ birds) was 104±7 beats min⁻¹ and the mean minimum \dot{V}_{O_2} was 32.6±1.8 ml min⁻¹ (equivalent to 12.6±1.1 ml kg⁻¹ min⁻¹).

The slopes of the relationships between \dot{V}_{O_2} and f_H during walking on the treadmill did not vary among individuals of the same species for barnacle geese or bar-headed geese (Fig. 3). The relationship between \dot{V}_{O_2} and f_H during walking on the treadmill was:

$$\dot{V}_{O_2} = 0.32f_H - 11.0 \quad (2)$$

for barnacle geese and:

$$\dot{V}_{O_2} = 0.41f_H + 2.9 \quad (3)$$

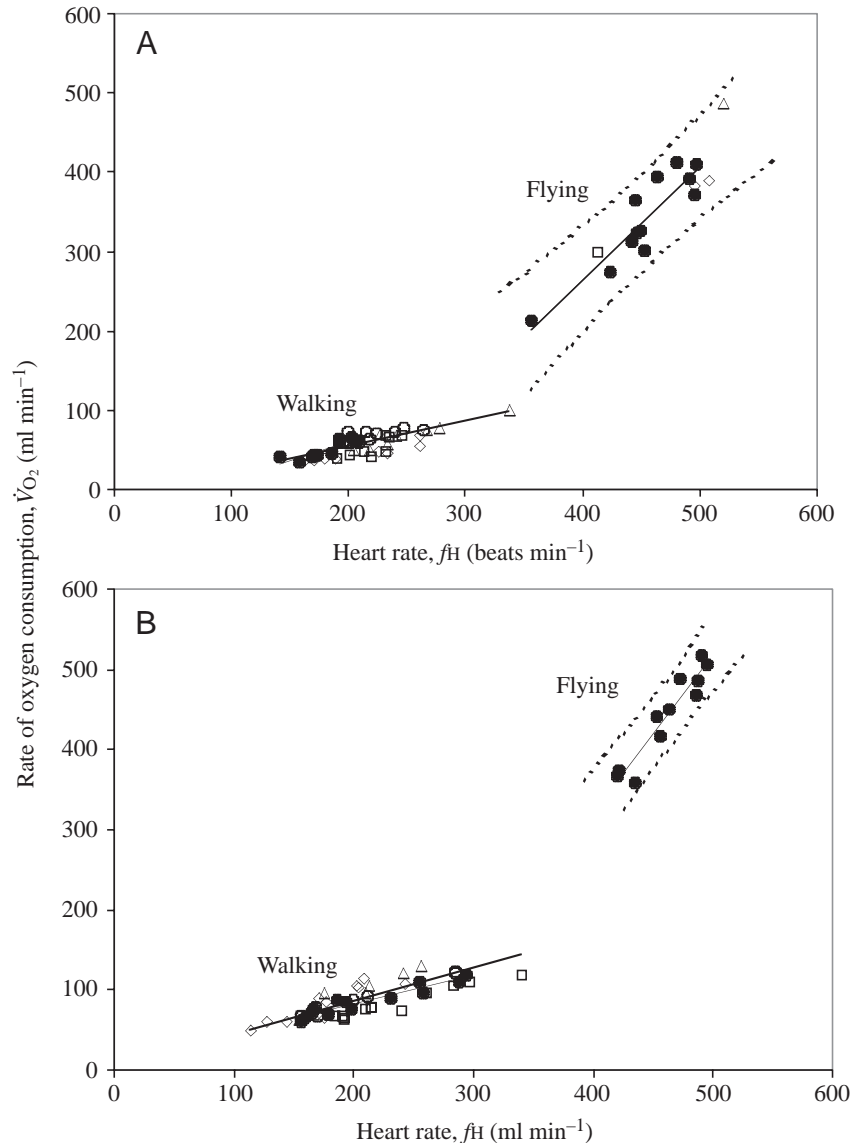
for bar-headed geese (Fig. 3). The relationship between f_H and \dot{V}_{O_2} during walking did not differ between the barnacle geese in the current study and those studied by Nolet et al. (1992) (ANCOVA, $P=0.2$).

Influence of the presence of a trainer upon heart rate and the rate of oxygen consumption during flight in the wind tunnel

The f_H of barnacle geese flying in the wind tunnel without a mask in autumn was lower when the birds flew less than 1 m from the trainer (position B) than when they flew more than 1 m from the trainer (position A) (t -test, $t_{48}=2.3$, $P=0.03$). The f_H and \dot{V}_{O_2} of barnacle geese during flight with a mask in autumn were also greater during flight in position A than in position B (ANOVA, f_H , position, $F_{1,15}=9.3$, $P=0.01$; bird, $F_{3,15}=4.4$, $P=0.03$; \dot{V}_{O_2} , position, $F_{1,29}=37.1$, $P<0.001$; bird, $F_{4,29}=11.2$, $P<0.001$). The data from barnacle geese during flight in the wind tunnel in spring, when the birds flew while the trainer stood out of the air flow (position C), demonstrate that f_H did not vary between flight in position C and that in position A (t -test, $t_{19}=0.5$, $P=0.6$). The f_H of bar-headed geese during flight without a mask and the \dot{V}_{O_2} and the f_H of bar-headed geese during flight with a mask in autumn did not vary with flight position relative to the trainer (t -tests, $P>0.5$).

Only data from flights during which costs were not reduced by flying close to the trainer (i.e. flights in position A) were used to calculate f_H and gas exchange rates typical of unassisted flight in the wind tunnel in autumn and to examine the relationship between flight costs and flight speed in

Fig. 3. Linear relationships between \dot{V}_{O_2} and f_H of (A) barnacle geese and (B) bar-headed geese walking on a treadmill and flying in a wind tunnel. The filled circles show data from a single bird of each species from which most data on the relationships between f_H and \dot{V}_{O_2} were obtained during flight ($N=12$ flights by barnacle goose B-B and 11 flights by bar-headed goose BH-O). The open symbols show data from other birds. Different symbols indicate different birds (8–12 points for each of four additional individuals of each species for walking and four flights by three additional barnacle geese). The thick solid lines show the common relationships between f_H and \dot{V}_{O_2} during walking [barnacle geese, $\dot{V}_{O_2}=(0.32\pm 0.03)f_H-(11.0\pm 7.1)$, $r^2=0.70$; ANCOVA, f_H , $F_{1,49}=91.9$, $P<0.001$; slope, $F_{4,49}=1.9$, $P=0.1$; intercept, $F_{4,49}=13.1$, $P<0.001$; bar-headed geese, $\dot{V}_{O_2}=(0.41\pm 0.02)f_H+(2.9\pm 5.2)$, $r^2=0.91$; f_H , $F_{1,53}=270.5$, $P<0.001$; slope, $F_{4,53}=2.3$, $P=0.08$; intercept, $F_{4,53}=13.6$, $P<0.001$]. The thin solid lines show the relationships between f_H and \dot{V}_{O_2} during walking and flight by barnacle goose B-B [walking, $\dot{V}_{O_2}=(0.47\pm 0.08)f_H-(35.9\pm 15.0)$, $r^2=0.78$, $P<0.001$, $N=11$, obscured by thick solid line; flight, $\dot{V}_{O_2}=(1.42\pm 0.21)f_H-(304.4\pm 95.2)$, $r^2=0.82$, $P<0.001$, $N=12$] and bar-headed goose BH-O [walking, $\dot{V}_{O_2}=(0.35\pm 0.04)f_H+(12.1\pm 8.2)$, $r^2=0.88$, $P<0.001$, $N=13$; flight, $\dot{V}_{O_2}=(1.97\pm 0.22)f_H-(467.5\pm 100.3)$, $r^2=0.90$, $P<0.001$, $N=11$]. The broken lines show the 95% prediction intervals for the relationships between \dot{V}_{O_2} and f_H during flight for barnacle goose B-B and bar-headed goose BH-O.



barnacle geese. Data from flights during which barnacle geese flew less than 1 m from the trainer (position B) were used to provide greater variation in flight costs for the calibrations of f_H against \dot{V}_{O_2} during flight. The f_H value of two barnacle geese during flight in positions A or C did not vary among birds ($P=0.6$) but was greater in the spring than in the autumn (ANOVA, $F_{1,26}=20.5$, $P<0.001$).

Respiratory exchange ratio

The respiratory exchange ratio (RER) declined steadily during flights by both species. The mean RER of barnacle geese was 1.01 ± 0.02 ($N=34$ flights by five birds) during the first minute of flight and declined steadily to 0.80 ± 0.03 ($N=10$ flights by three birds) after 10 min of flight. The mean RER of bar-headed geese was 0.98 ± 0.02 ($N=23$ flights by two birds) during the first minute of flight, 0.89 ± 0.02 ($N=14$ flights by two birds) after 10 min of flight and 0.68 ± 0.01 ($N=10$) between 50 and 60 min of a flight by one bird that flew for 61 min.

Relationships between flight speed and heart rate and the rate of oxygen consumption during flight

There was no significant relationship between flight speed and f_H during flight without a mask or between flight speed and \dot{V}_{O_2} during flight with a mask in either species of goose [regression with flight speed as the independent variable, ANOVA with flight speed as a factor or multiple regression with (flight speed) $^{-1}$ and (flight speed) 2 as the independent variables (as might be expected if variation in f_H or \dot{V}_{O_2} paralleled that in mechanical power; Ward et al., 2001), $P>0.08$, Fig. 4].

Relationship between the rate of oxygen consumption and heart rate during flight

Variation in f_H followed that in \dot{V}_{O_2} within flights during which the position of the bird relative to the trainer changed during the flight (Fig. 5). We used the mean f_H and the mean \dot{V}_{O_2} from each flight by barnacle goose B-B (1.8 kg) and bar-headed goose BH-O (2.8 kg) to calculate the relationship

between \dot{V}_{O_2} and f_H during flight for the single bird of each species for which we had data from sufficient flights to perform this analysis (Fig. 3). The relationship between \dot{V}_{O_2} and f_H during flight was:

$$\dot{V}_{O_2} = 1.42f_H - 304.4 \quad (4)$$

for barnacle goose B-B and:

$$\dot{V}_{O_2} = 1.97f_H - 467.5 \quad (5)$$

for bar-headed goose BH-O (Fig. 3). Measurements of \dot{V}_{O_2} and f_H from four flights by an additional three barnacle geese (2.1 ± 0.1 kg) fell within the 95% prediction intervals of the relationship determined for barnacle goose B-B (Fig. 3A).

Rate of oxygen consumption during flight without a mask

We used the difference in f_H , measured during flight by the same bird with and without a mask to account for the effect of carrying the respirometry mask and associated tubing upon \dot{V}_{O_2} . The mean f_H of barnacle goose B-B during flight with a mask in autumn in positions in which flight costs were not reduced by proximity to the trainer was 479 ± 8 beats min^{-1} ($N=6$ flights), equivalent to a mean \dot{V}_{O_2} of 374 ± 28 ml min^{-1} (equation 4). The mean f_H of the same bird during flight under the same conditions but without a mask was 12% lower (423 ± 15 beats min^{-1} , $N=12$ flights), equivalent to a mean \dot{V}_{O_2} of 295 ± 29 ml min^{-1} (21% lower than that during flight with a mask). The mean f_H of bar-headed goose BH-O during flight without a mask was 434 ± 9 beats min^{-1} , 6% lower than that during flight with a mask (461 ± 8 beats min^{-1}). The relationship between \dot{V}_{O_2} and f_H for bar-headed goose BH-O (equation 5) predicted a mean \dot{V}_{O_2} during flight without a mask of 388 ± 21 ml min^{-1} , which is 12% lower than that during flight with a mask (441 ± 20 ml min^{-1}).

The mean \dot{V}_{O_2} of barnacle goose B-B during flight without a mask is equivalent to 168 ± 12 ml $\text{kg}^{-1} \text{min}^{-1}$, 12 ± 1 times resting metabolic rate measured at night in the same bird and 18 ± 1 times basal metabolic rate (calculated on a mass-specific basis from Nolet et al., 1992), or 102 ± 7 W ($N=12$ flights). The mean value of 388 ± 21 ml min^{-1} for the \dot{V}_{O_2} of bar-headed goose BH-O during flight without a mask is equivalent to 138 ± 6 ml $\text{kg}^{-1} \text{min}^{-1}$, 12 ± 1 times resting metabolic rate measured at night in the same bird, or 135 ± 6 W ($N=19$ flights).

Comparison of the relationship between heart rate and the rate of oxygen consumption during walking and flying

The relationships between \dot{V}_{O_2} and f_H had steeper slopes and lower intercepts during flight than during walking in the barnacle goose and the bar-headed goose for which we had sufficient data to perform the analysis (ANCOVA, barnacle goose B-B, intercept, $F_{1,22}=9.2$, $P=0.007$; slope, $F_{1,22}=7.9$, $P=0.01$; bar-headed goose

BH-O, intercept, $F_{1,23}=42.2$, $P<0.001$; slope, $F_{1,23}=87.3$, $P<0.001$; Fig. 3).

Discussion

Effects of the trainer during flight in a wind tunnel

The reduction in the \dot{V}_{O_2} and f_H of barnacle geese when birds flew less than 1 m from the trainer (position B) compared with that during flight when they were more than 1 m from the trainer or when the trainer stood out of the air stream was presumably due to areas of rising air and/or increased pressure that the geese were able to use to reduce their flight costs when they flew close to the trainer. The wingbeat amplitude of barnacle geese (measured from video images taken from downwind of the birds; S. Ward, unpublished data) was also reduced during flight in position B. Since mechanical

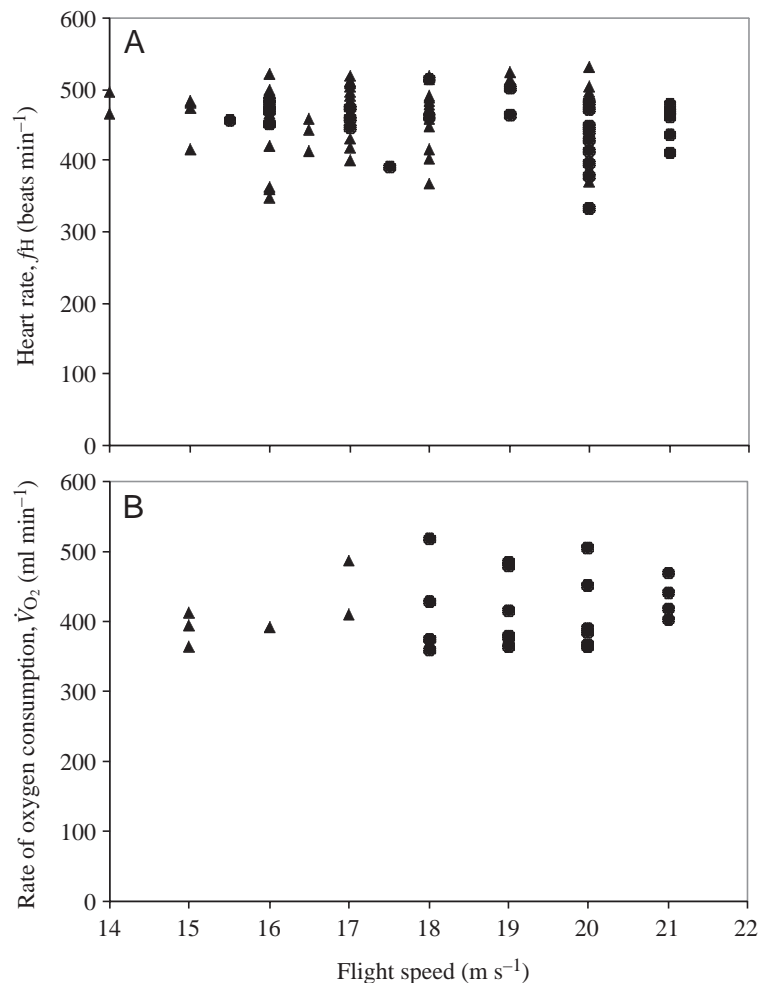


Fig. 4. Variation with respect to flight speed in (A) f_H of barnacle geese (triangles, $N=56$ flights by seven birds) and bar-headed geese (circles, $N=29$ flights by two birds) flying in a wind tunnel without a respirometry mask and (B) \dot{V}_{O_2} of barnacle geese (triangles, $N=11$ flights by two birds) and bar-headed geese (circles, $N=11$ flights by one bird) flying in a wind tunnel with a respirometry mask. The data from barnacle geese are from flights during which the f_H and \dot{V}_{O_2} were not reduced as a result of the bird flying close to the trainer.

power during flight is proportional to wingbeat amplitude (Pennycuik, 1975; Rayner, 1979, 1993), the reduction in wingbeat amplitude during flight in position B suggests a mechanism by which mechanical power (and hence metabolic power, \dot{V}_{O_2} and f_H) could be reduced during flight close to the trainer.

Relationships between heart rate, the rate of oxygen consumption and flight speed

Neither f_H nor \dot{V}_{O_2} showed the U-shaped relationship with flight speed that might be expected if the general relationship between mechanical power and speed predicted by most aerodynamic models (Rayner, 1979; Pennycuik, 1989; Rayner and Ward, 1999) were reflected in variation in metabolic power (Fig. 4). However, the predicted mechanical power based on one aerodynamic model (model 1A of Pennycuik, 1989) also had an almost flat relationship with flight speed for the speed range in which our geese were prepared to fly, so our data are consistent with the predictions from this particular aerodynamic model and with the measurements of the f_H of barnacle geese flying behind a truck (Butler and Woakes, 1980). This result suggests that further measurements of f_H in geese that aim to elucidate any changes in metabolic rate during flight with respect to flight speed in a wind tunnel may not be useful because any such changes are likely to be small in relation to other sources of variation in f_H or \dot{V}_{O_2} over the range of flight speed at which the geese would fly. Lack of dependence of aerobic metabolic rate upon sustainable flight speed in geese also leads to the prediction that migratory flights should be conducted towards the upper end of the speed range to minimise time and energy constraints. However, there is great variability in the f_H of barnacle geese during migration (Butler et al., 1998) that we are not currently able to explain in relation to possible changes in the energetic cost of flight, although in the short term they could be associated with descent or periods of gliding and in the long term with changes in body mass and/or physiology.

Prediction of the flight costs of wild migratory barnacle geese

The slope of the relationship between f_H and \dot{V}_{O_2} was greater and the intercept was lower for flying than for walking geese of both species (Fig. 3). Predictions of \dot{V}_{O_2} during flight in the wind tunnel based on the relationship between f_H and \dot{V}_{O_2} for the same bird during walking would underestimate measured \dot{V}_{O_2} during flight by 38–54% for barnacle goose B-B and by 54–65% for bar-headed goose BH-O. The error in predicting \dot{V}_{O_2} during flight from the f_H/\dot{V}_{O_2} relationship that is appropriate during walking varies with f_H because the relationships between f_H and \dot{V}_{O_2} for walking and flying geese diverge with increasing f_H (Fig. 3). It is therefore not possible to calculate \dot{V}_{O_2} during flight using a relationship between f_H and \dot{V}_{O_2} derived from walking birds in combination with a

simple correction term. These data confirm the suggestion (Nolet et al., 1992; Butler et al., 2000) that the \dot{V}_{O_2} of wild migratory geese cannot be predicted from f_H and a calibration between f_H and \dot{V}_{O_2} during walking. The measurements and predictions were made in the same individuals in the current study, so our results are not confounded by potential physiological differences between individuals or among the groups of captive and free-living geese. The greater change in \dot{V}_{O_2} for a given change in f_H during flight than during walking is likely to be related to a greater cardiac stroke volume (Bishop and Butler, 1995; Butler et al., 1998). In addition, the flight muscles of barnacle geese have a much greater mass and aerobic capacity than their leg muscles (Bishop et al., 1995, 1996). Thus, flying geese would be expected to be able to extract more oxygen from the blood per heart beat than walking birds (Butler et al., 1977), leading to the observed greater \dot{V}_{O_2} , and greater rise in \dot{V}_{O_2} for a given increase in f_H , during flight.

The mean f_H of wild barnacle geese was 317 beats min^{-1} at the start of the autumn migratory flight in Svalbard, falling to a minimum of 226 beats min^{-1} when the birds were approximately 70% of the way through their migration (Butler et al., 1998). The relationship between f_H and \dot{V}_{O_2} measured during flight in a wind tunnel in the autumn in barnacle goose B-B predicted that these values of f_H would correspond to mean \dot{V}_{O_2} values of $144 \pm 40 \text{ ml min}^{-1}$ at the start of migration and $15 \pm 55 \text{ ml min}^{-1}$ when the birds are 70% of the way through migration. The latter mean value of \dot{V}_{O_2} is clearly impossible, and even the mean value from the start of the migration represents 48% of the \dot{V}_{O_2} calculated from the heart mass of migratory barnacle geese (Butler et al., 1998) or 49% of the mean \dot{V}_{O_2} predicted from f_H during flight without a mask in the wind tunnel. Our calibration of the relationship between the f_H and \dot{V}_{O_2} of captive geese flying in the wind tunnel cannot therefore be used directly to calculate the \dot{V}_{O_2} of wild migratory geese from measurements of their f_H .

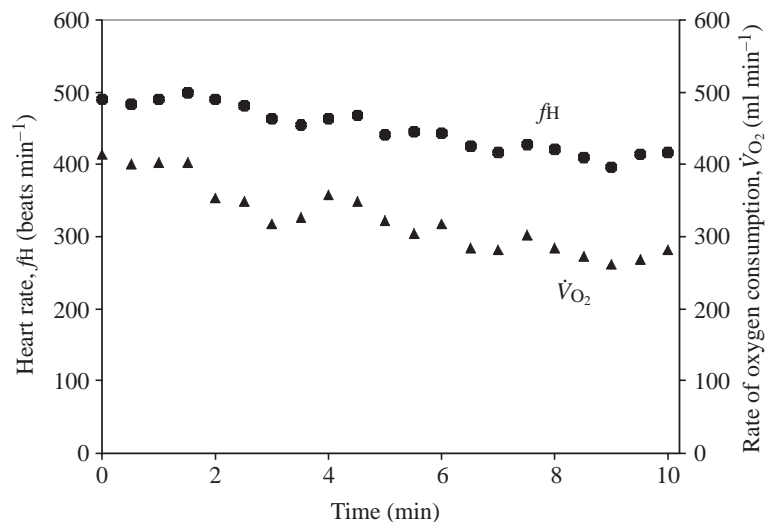


Fig. 5. f_H (circles) and \dot{V}_{O_2} (triangles) during a 10 min flight by barnacle goose B-B during which the bird flew progressively closer to the trainer during the flight.

We also show (Fig. 5) that the variation in fH recorded during migration (Butler et al., 1998) is likely to be related to variation in \dot{V}_{O_2} . Possible reasons for the difference in the relationship between \dot{V}_{O_2} and fH of barnacle geese flown in the wind tunnel and that of their free-living migratory conspecifics are discussed in Bishop et al. (2002).

Comparisons with previous studies

The barnacle geese for which we measured \dot{V}_{O_2} during flight in the wind tunnel in the current study (1.98 ± 0.08 kg, $N=4$) were heavier than those for which \dot{V}_{O_2} was measured during flight in a wind generator (1.68 ± 0.17 kg, $N=3$; Butler et al., 2000). We therefore expected that \dot{V}_{O_2} would be greater for the barnacle geese in the current study (438 ± 48 ml min⁻¹ compared with 332 ± 9 ml min⁻¹ for those flown in the wind generator) since the power required for flight increases with bird mass (Pennycuik, 1975; Rayner, 1988; Kvist et al., 2001). The \dot{V}_{O_2} of barnacle geese flying in the wind generator without a mask was estimated to be 12% lower than that during flight with a mask by Butler et al. (2000). The current study suggested that \dot{V}_{O_2} was reduced by 21% during flight without a mask. If this revised value for the difference in \dot{V}_{O_2} between flight by barnacle geese with and without a mask is applied to the birds that flew in the wind generator, their mean \dot{V}_{O_2} would be 262 ml min⁻¹ or 158 ml kg⁻¹ min⁻¹ (close to the value of 168 ± 12 ml kg⁻¹ min⁻¹ calculated for barnacle goose B-B in the present study). The fH during flight in the wind tunnel without a mask (451 ± 23 beats min⁻¹ in autumn) was higher than that measured in the wind generator (378 ± 15 beats min⁻¹) but lower than that of barnacle geese trained to fly following a truck (512 ± 4 beats min⁻¹; Butler and Woakes, 1980).

New technology is stimulating an increasing number of measurements of fH in free-living animals, such as the work reported by Bevan et al. (1994, 1995), Butler et al. (1998) and Weimerskirch et al. (2001). However, quantitative interpretation of the data from such studies in terms of the energetic consequences for flying animals is limited unless the correct relationship has been established between fH and the rate of energy consumption during flight. Without appropriate calibration of such relationships, the energetic correlates of changes in fH during flight cannot be determined. Our data clearly show that the relationship between fH and \dot{V}_{O_2} during forelimb exercise (i.e. flight) of two species of geese is substantially different from that during hindlimb exercise (i.e. walking), and this is also likely to be the case in other species of flying animal. In addition, the relationship between fH and \dot{V}_{O_2} differs between captive and wild migratory barnacle geese. This is most likely to be due to differences between the physiological condition of captive barnacle geese and their wild migratory conspecifics, but could also be influenced by variation in environmental conditions such as temperature, altitude or turbulence.

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