

## LIMITS TO FLIGHT ENERGETICS OF HUMMINGBIRDS HOVERING IN HYPODENSE AND HYPOXIC GAS MIXTURES

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### Summary

Hovering hummingbirds offer a model locomotor system for which analyses of both metabolism and flight mechanics are experimentally tractable. Because hummingbirds exhibit the highest mass-specific metabolic rates among vertebrates, maximum performance of hovering flight represents the upper limit of aerobic locomotion in vertebrates. This study evaluates the potential constraints of flight mechanics and oxygen availability on maximum flight performance. Hummingbird flight performance was manipulated non-invasively using air and gas mixtures which influenced metabolism *via* variable oxygen partial pressure and/or altered flight mechanics *via* variable air densities. Limits to the locomotor capacity of hovering ruby-throated hummingbirds (*Archilochus colubris*) were unequivocally indicated by aerodynamic failure in either air/helium or air/heliox mixtures. Air/helium mixtures are hypodense and hypoxic; failure to sustain hovering flight occurred at 63% of the density of sea-level air and at an oxygen concentration of 12%. Air/heliox mixtures are

hypodense but normoxic; failure in hovering occurred at 47% of sea-level air density. Thus, hummingbirds demonstrated considerable power reserves in hovering flight as well as hypoxic tolerance. In air/helium mixtures, hovering was limited by oxygen supply and not by flight mechanics. Birds hovering in air/helium mixtures increased their mechanical power output but not their rate of oxygen consumption. By contrast, birds hovering in air/heliox mixtures increased both mechanical performance and metabolic expenditure. Under hypoxia, hovering hummingbirds demonstrated non-negligible, but still limited, capacities for anaerobic metabolism and/or oxygen storage. Depending on the physical context, hummingbird flight performance can therefore be limited by oxygen availability or by flight aerodynamics.

Key words: air density, heliox, helium, hovering flight, hummingbird, *Archilochus colubris*, hypoxia, muscle power, oxygen consumption.

### Introduction

Analysis of maximum performance during locomotion can reveal physical constraints acting on animal design. Locomotor performance and the associated limits to power output have recently received considerable attention (Josephson, 1993), but appropriate measurements can be difficult to obtain for natural movements. The symmetrical and repetitive wing motions of hovering hummingbirds can be analyzed using contemporary aerodynamic modelling for hovering flight (Ellington, 1984a–f). The unique specializations of hummingbirds in structure and function provide an exemplary vertebrate system with which to examine *in vivo* locomotor performance and the limits to energetic expenditure (Weis-Fogh, 1972; Suarez, 1992; Wells, 1993a; Hochachka, 1994).

Air density is a major determinant of the aerodynamic power output of a flying animal (Norberg, 1990). Gradual replacement of sea-level air (density  $1.2 \text{ kg m}^{-3}$ ) with heliox (79% He/21% O<sub>2</sub>, density  $0.4 \text{ kg m}^{-3}$ ) reduces the density of

the medium within which an animal hovers. This reduction, in turn, necessitates alteration of wingbeat kinematics to match the increased mechanical power requirements. Oxygen partial pressure is, however, maintained at normal values, ensuring that diffusive limitations on metabolic power production cannot interfere with flight performance. Chai and Dudley (1995) non-invasively manipulated the flight performance of hovering ruby-throated hummingbirds (*Archilochus colubris*) through the use of such variable-density gas mixtures. Limits to the locomotor capacity of hovering hummingbirds were unequivocally indicated by the failure to sustain hovering during 2–4 s feeding bouts at gas densities less than half that of normal air (average  $0.54 \text{ kg m}^{-3}$ ).

While hovering, hummingbirds demonstrated considerable power reserves (Wells, 1993b; Chai and Dudley, 1995). Modulation of power output was attained primarily through variation in wing-stroke amplitude, with aerodynamic failure

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occurring near 180°. Wingbeat geometry of hovering animals may thus constrain maximum power output. Mechanical limitations may not, however, exclusively constrain flight performance. The dependence of hummingbirds on aerobic metabolism and their very high oxygen consumption rates at low air densities suggest physiological constraints due to oxygen supply. The high mass-specific rates measured by Chai and Dudley (1995) are in close agreement with maximal oxygen consumption rates measured from hummingbirds hovering under strenuous situations, e.g. with moulted or damaged flight feathers (Epting, 1980) or during load-lifting (Wells, 1993b).

To differentiate better between the potential constraints of flight mechanics and oxygen availability, this study reports hovering experiments in which sea-level air was partially replaced by pure helium. Thus, both the oxygen concentration and the density of the experimental flight medium were simultaneously reduced; heliox was used as the control. Both treatments induced aerodynamic failure, indicating limits to hovering capacity. We compare the mechanical performance, metabolic expenditure and efficiency of hummingbird flight muscles during hovering in the two types of flight media. The effects of helium replacement as experienced by flying animals parallel high-altitude flights in three ways – in addition to the reduction in air density and oxygen partial pressure, heat-flux pattern is also similar to that at the low ambient temperatures of high altitudes as a result of the higher thermal conductivity of helium relative to air (Reid *et al.* 1987; López-Calleja and Bozinovic, 1995). Thus, the present results are comparable to those obtained from hovering hummingbirds at simulated high altitudes achieved through pressure reduction (e.g. Berger, 1974), albeit *via* a much simpler manipulation. Also, normobaric helium manipulation should require less ventilatory compensation by birds compared with hypobaric exposure (see Berger, 1978).

## Materials and methods

### *Experimental procedures*

Ruby-throated hummingbirds (*Archilochus colubris* L.) were mist-netted in the vicinity of Austin, Texas, USA, and were housed in screen cages of dimensions equal to those of the acrylic cube used in the experiments (90 cm×90 cm×90 cm). Hummingbird care was in accordance with federal and state guidelines. Two birds were housed together within one cage; this arrangement maintained the aggressive behaviour characteristic of hummingbirds. By selectively exchanging cagemates, the extent of aggression could be manipulated. Interference with feeding by aggressive cagemates prevented the potentially adverse body mass increases that tend to occur in captivity with *ad libitum* food provision (Wells, 1993a). Birds were fed a commercial hummingbird diet (Roudybush, Templeton, CA, USA) and were trained to feed through a cylindrical mask (24 mm long and 12.5 mm inner diameter) attached to a hanging syringe (10 ml) also used in experiments. The feeder was hung at the cube center approximately 30 cm from the top. A perch

branch was provided at the side, and birds had to fly 40 cm or more in order to feed. Five hummingbirds with intact flight feathers (three males and two females) were used in experiments. However, bird 5 had not completed its moult by the time of experimental measurements. The second-longest primary (the ninth), which is the last primary to be replaced, did not grow to full length and left a slight notch (<2% of wing area) along the trailing edge of the wing. Each bird was subjected to four percentage oxygen/density reduction trials conducted over three consecutive days (two trials were conducted on the second day). The experimental helium treatment had three replicates (trials), whereas the control heliox treatment was conducted only once because an earlier study (Chai and Dudley, 1995) found no trial effect. The study sequence for each bird was composed of two initial helium treatment trials, one heliox treatment trial and one final helium trial.

Flight experiments were carried out within an airtight acrylic cube (90 cm×90 cm×90 cm). Data were initially collected from birds hover-feeding in normal unmanipulated air. Air within the cube was then gradually replaced by filling with helium or heliox while allowing the cube contents to escape from an additional port. Hover-feeding flight was then recorded approximately every 15–20 min when the bird was hungry and came to feed (this period reflected the crop emptying time; see Karasov *et al.* 1986). The duration of hover-feeding flight *t* was later timed from video recordings. At low percentage oxygen and/or density levels, the bird could not hover for long and often made multiple feeding bouts from the perch (the bird rested for several seconds between bouts). Only the first bout (usually the longest) was used to represent the hover-feeding duration at that percentage oxygen/density level. For the helium treatment, the bird was allowed to feed and was filmed at each percentage drop in oxygen concentration (monitored by an Applied Electrochemistry S-3A/I oxygen analyzer; see below) until it failed while hovering, at which point helium-filling was terminated and reverse-pumping of ambient air was initiated. The bird was then allowed to feed and filmed at each percentage rise in oxygen concentration. The experiment generally ended at oxygen concentrations of at least 16%, at which point the hover-feeding duration increased substantially. The procedure for the heliox treatment followed that of Chai and Dudley (1995). Air within the cube was gradually replaced with heliox for 2–3 h until aerodynamic failure; the experiment was then terminated.

Density reduction associated with replacement of normal air by helium or heliox was monitored acoustically. Prior to each filmed episode of hover-feeding, a tube whistle within the cube was remotely activated, generating an acoustic monotone. Because the resonant frequency of a simple tube increases in helium and heliox, the change in dominant whistle frequency following helium or heliox substitution can be used to derive the air density within the flight chamber (Beranek, 1949). Fundamental frequency was recorded and determined using a 12-bit A/D converter (MacAdios, GW Instruments) and sound analysis software (Soundscope, GW Instruments). Initial chamber temperature, humidity and barometric pressure were

used to determine ambient air density. The dynamic viscosity of gas mixtures was calculated following Reid *et al.* (1987).

#### Oxygen consumption

Metabolic power input ( $P_{\text{input}}$ ) during hovering was estimated from measurements of rates of oxygen consumption ( $\dot{V}_{\text{O}_2}$ ). Oxygen consumption was determined using open-flow mask respirometry (Berger and Hart, 1972; Bartholomew and Lighton, 1986; Wells, 1993a). During hover-feeding, air and expired gases were drawn through the feeder mask. To remove water vapour, the gases were passed through silica gel within a small removable column designed for rapid replacement. Carbon dioxide was not removed as this operation would have adversely increased the response time. Downstream flow rate was continuously recorded using a turbine flowmeter (McMillan Co., model 110) which was necessarily calibrated with a gas volume meter (American Meter Co.) because the nominal output of the flowmeter referred only to normal air composition. After passing through the flowmeter, respiratory gases were analyzed using an Applied Electrochemistry S-3A/I oxygen analyzer. Because the bird typically had fed several times prior to the experiment, metabolic power expenditure was determined from the rate of oxygen consumption by assuming a conversion factor of  $21.1 \text{ J ml}^{-1} \text{ O}_2$  for carbohydrate utilization (Brobeck and DuBois, 1980) and a respiratory quotient of 1 (Suarez *et al.* 1990). This may result in errors if there is anaerobic metabolism and/or oxygen storage, especially at the beginning of the flight. Gas flow rates and oxygen concentrations were sampled at 10 Hz and were recorded using a 12-bit A/D converter (MacAdios, GW Instruments) and software (Superscope, GW Instruments) running on a Macintosh IICI computer. Only hover-feeding durations longer than 5 s with continuous asymptotic traces of oxygen consumption longer than 2 s were used (the system reached full response in 1.5 s); hover-feeding events that were short or composed of numerous intermittent feedings were not used.  $\text{O}_2$  consumption was corrected to STP. Mainly due to the presence of the filter and silica gel, the pressure at the flowmeter was approximately 933 Pa below ambient barometric pressure, and this difference was accounted for in the oxygen estimates.

#### Flight kinematics and morphological variables

A mirror tilted at  $45^\circ$  to the horizontal and positioned above the experimental cube allowed us to film horizontal projections of wingbeat kinematics for each hover-feeding sequence using a video camera (Sony CCD-FX420). Filming and subsequent analysis were at  $60 \text{ fields s}^{-1}$ , with a high-speed shutter of  $1/4000 \text{ s}$ . Wingbeat kinematics recorded at each hover-feeding sequence, together with morphological parameters for individual birds, were used to estimate the mechanical power requirements of flight using a detailed aerodynamic model of hovering flight (Ellington, 1984a–f). Wingbeat kinematics were determined from field-by-field video analysis. Wingbeat kinematics measured for each hovering sequence included wingbeat frequency  $n$  and stroke amplitude  $\Phi$ . Wingbeat

frequency was derived from video recordings, based on the interaction frequency between wing motion and filming rate. It was averaged over the last 2 s of a hovering flight in which the bird was continuously feeding through the mask. The last 2 s were chosen because hover-feeding duration prior to aerodynamic failure was very brief. For normal hover-feeding durations ( $>20 \text{ s}$ ), the wingbeat frequency at the beginning of a sequence was not noticeably different from that at the end. Stroke amplitude represents the angular extent of wingtip motion in a stroke plane to which wing motions are nominally confined, and was derived from video images in which the wings were located at the extreme positions of the wingbeat. Mean values of stroke amplitude were determined from 3–5 separate measurements. Lateral views of hovering flight were obtained only twice during each experimental trial: once for hover-feeding in normal air, and once after the bird exhibited aerodynamic failure. These data were used to derive the stroke plane angle  $\beta$ .

Morphological parameters used in the aerodynamic calculations were determined for all filmed birds (Ellington, 1984b; see Table 1). These parameters included body mass  $m$ , relative wing mass  $m_w$  of both wing pairs expressed as a percentage of body mass, wing length  $R$ , total wing area  $S$  (the area of both wing pairs), wing loading  $p_w$  ( $\text{mg}/S$ , where  $g$  is gravitational acceleration) and aspect ratio  $\mathcal{A}$  ( $=4R^2/S$ ). Body mass was taken as the average value of the body mass measured shortly before and after each experiment. Wing area was determined by filming the restrained bird with spread wings on graph paper, and then using image analysis software (NIH Image) to measure the area. Non-dimensional parameters describing distributions of wing area and virtual mass were also derived [see methodology described in Ellington (1984b) and Wells (1993a)]. One bird of each sex was killed for *post mortem* analysis to determine wing mass and its distribution. Non-dimensional equivalents of wing mass parameters were used for the other three birds. Flight muscle was assumed to equal 25% of the body mass, given published data for ruby-throated and other hummingbird species (Hartman, 1961; Greenewalt, 1962; Wells, 1993a). Instead of *post mortem* determination of muscle mass, the use of 25% body mass for all birds is appropriate given: (1) general similarity in muscle proportions across hummingbird species, (2) variable activation of motor units during locomotion, and (3) probable temporal variance in muscle mass at high metabolic rates (captive hummingbirds are capable of a 10% body mass change in one day; P. Chai, unpublished data).

#### Aerodynamic analysis

To evaluate lift and power production in variable-density mixtures, a detailed model of hovering aerodynamics was used with kinematic and morphological data obtained for individual hummingbirds. Because of approximately horizontal wing motions, the downstroke and upstroke were assumed to contribute equally to vertical force production (Ellington, 1984a). For each hovering flight sequence, the mechanical power requirement of flight was estimated by evaluating its

individual components: profile ( $P_{\text{pro}}$ ), induced ( $P_{\text{ind}}$ ) and inertial power.  $P_{\text{pro}}$  represents the energetic expenditure required to overcome profile drag forces on the wings, while  $P_{\text{ind}}$  corresponds to the power necessary to impart sufficient downward momentum to the surrounding air so as to offset the body weight. The inertial power during the first half of a half-stroke ( $P_{\text{acc}}$ ) was estimated from the moment of inertia of the wing and the maximum angular velocity, assuming simple harmonic motion of the wings in the stroke plane. Total inertial power requirements through the wingbeat will be zero if the kinetic energy of the oscillating wing mass and virtual mass can be stored elastically during deceleration of the wing stroke and subsequently released to re-accelerate the wings. Thus, total mechanical power requirement assuming perfect elastic energy storage,  $P_{\text{per}}=P_{\text{pro}}+P_{\text{ind}}$ . Alternatively, assuming zero elastic energy storage, additional power will be required to accelerate the wing during the first half of a half-stroke. However, the power required for wing deceleration during the second half of the half-stroke is negligible, while aerodynamic power requirements can be met by the kinetic energy of the decelerating wings. Thus, total mechanical power requirements assuming zero elastic energy storage,  $P_{\text{zero}}=(P_{\text{pro}}+P_{\text{ind}}+P_{\text{acc}})/2$ . For comparison with published values, muscle mass-specific power expenditure for a flight sequence was calculated for the two cases of zero ( $P_{\text{zero}}$ ) and perfect ( $P_{\text{per}}$ ) elastic storage of wing inertial energy, representing maximum and minimum estimates of required mechanical power, respectively. However,  $P_{\text{per}}$  represents a more accurate estimate of power expenditure because hummingbirds can probably store kinetic energy elastically during the deceleration phase of the wing stroke (Wells, 1993a; Chai and Dudley, 1995). Muscle mechanical efficiency  $\eta_m$  was estimated for  $P_{\text{per}}$  only as  $P_{\text{per}}/(0.9P_{\text{input}})$ , where  $P_{\text{input}}$  is metabolic power, assuming 90% direct energy expenditure by flight muscle (Lasiewski, 1963).

#### Statistical analyses

The effects of percentage oxygen/density reduction on kinematic, metabolic and aerodynamic variables were tested for statistical significance using repeated-measures analysis of variance (ANOVA) modelling with trial and percentage oxygen/density effects as within-subject sources of variation

(SAS Institute, 1989). For the helium treatment, each model included trial and percentage O<sub>2</sub> as independent factors as well as potential interactions between these factors (see Table 2). To achieve a balanced statistical design, data points were the mean value for each trial and percentage O<sub>2</sub> combination attained by each bird. There were three helium trials (on consecutive days) and ten percentage O<sub>2</sub> levels from 21% (normal air) to 13%, with aerodynamic failure (between 11 and 14% O<sub>2</sub>) designated as the final percentage O<sub>2</sub> level (see Table 1; Fig. 1). For  $\dot{V}_{\text{O}_2}$  and  $\eta_m$  ( $P_{\text{per}}$ ), the last two percentage O<sub>2</sub> levels were excluded from the analysis because oxygen consumption rates near to failure could not be reliably obtained, given the short duration of hover-feeding. Only density effects were assessed for the heliox control treatment because there were no trial replicates. There were seven density levels from 1.2 kg m<sup>-3</sup> (normal air) to 0.6 kg m<sup>-3</sup>, plus failure densities (between 0.5 and 0.6 kg m<sup>-3</sup>) as the final density level (see Table 1; Fig. 1). Hover-feeding events were grouped by rounding air densities at which feeding events occurred. For  $\dot{V}_{\text{O}_2}$  and  $\eta_m$  ( $P_{\text{per}}$ ), the last density level at failure was excluded owing to the short hover-feeding duration. Values of hover-feeding durations were log-transformed for statistical analysis because the mean and variance became much smaller towards failure (see Fig. 1). Logarithms were applied to minimize correlation between the mean and the variance of the data. Since both the oxygen and density levels of repeated measures were quantitative, polynomial comparisons were performed in the statistical modelling to identify linear and quadratic trends along the oxygen and density gradients.

It was informative to compare results over the density range for which the two gas treatments overlapped. Owing to aerodynamic failure at higher densities in the helium treatment (Table 1), the results of the two treatments could be compared only across four higher density levels (1.2, 1.0, 0.9 and 0.8 kg m<sup>-3</sup>). Results from the helium treatment were first re-grouped according to density levels by rounding. Differences in mean values (i.e. heliox minus helium) for each bird  $\times$  density combination between the two treatments were then used in a repeated-measures ANOVA that modelled density effect as the within-subject source of variation (see Table 2). No difference in mean value should appear if helium and

Table 1. Mean morphological parameters for five individuals of *Archilochus colubris* and the oxygen concentration and air density at aerodynamic failure due to partial helium or heliox replacement of normal air

Bird, Sex	$m$ (g)	$R$ (mm)	$\mathcal{AR}$	$p_w$ (Nm <sup>-2</sup> )	$S$ (cm <sup>2</sup> )	$m_w$ (%)	O <sub>2</sub> (He) (%)	$\rho$ (He) (kg m <sup>-3</sup> )	$\rho$ (HeO <sub>2</sub> ) (kg m <sup>-3</sup> )
Bird 1, M	3.40	44	8.13	35.0	9.5	3.9	12.0	0.75	0.54
Bird 2, M	3.45	44	8.03	35.1	9.6	3.9	12.0	0.74	0.59
Bird 3, M	3.46	41	7.41	37.4	9.1	3.9	13.7	0.83	0.60
Bird 4, F	3.53	48	8.52	31.9	10.8	4.2	11.0	0.69	0.46
Bird 5, F	3.55	48	8.10	30.5	11.4	4.2	11.7	0.72	0.55

$m_w$ , body mass;  $R$ , wing length;  $\mathcal{AR}$ , aspect ratio;  $p_w$ , wing loading;  $S$ , total wing area of both wing pairs;  $m_w$ , relative wing mass of both wing pairs as a percentage of body mass; O<sub>2</sub> (He), oxygen concentration at failure in air/helium mixtures;  $\rho$  (HeO<sub>2</sub>), air density at failure in air/helium mixtures;  $\rho$  (He), air density at failure in air/heliox mixtures.

heliox treatments yielded similar effects; conversely, a trend towards greater differences across the density levels should emerge if the two treatments differed in effects.

The final statistical analysis compared results from the heliox treatment of this study with those obtained by Chai and Dudley (1995). These two studies shared identical heliox treatment procedures, but the present study had only one trial which was conducted 5–6 months after that of Chai and Dudley (1995), after the birds had completed their pre-migratory moult. A repeated-measures ANOVA incorporating an extra variable for between-subject experimental effects was performed to determine whether prolonged captivity influenced flight performance.

*Hovering while breathing only helium*

After finishing the experiments described above, we developed a simple method to evaluate hovering performance while the bird was breathing pure helium. This was carried out

by pumping helium into the feeder mask used previously in respirometry and revealed the capacities of hummingbirds for anaerobic metabolism and/or oxygen storage. Six different birds (three males and three females) were each tested at least twice. Hover-feeding duration prior to failure was timed from video recordings.

**Results**

Both helium and heliox replacement of normal air resulted in declining duration of hover-feeding (Fig. 1). Initial percentage oxygen/density reduction resulted in a slow decline, but below a critical percentage oxygen/density level (approximately 18% O<sub>2</sub> and 1.0 kg m<sup>-3</sup> in air/helium mixtures and 0.9 kg m<sup>-3</sup> in air/heliox mixtures), the duration of feeding bouts exhibited a sharper decline. As a result, both linear and quadratic terms of the regression trends along declining percentage oxygen/density levels were significant (Table 2).

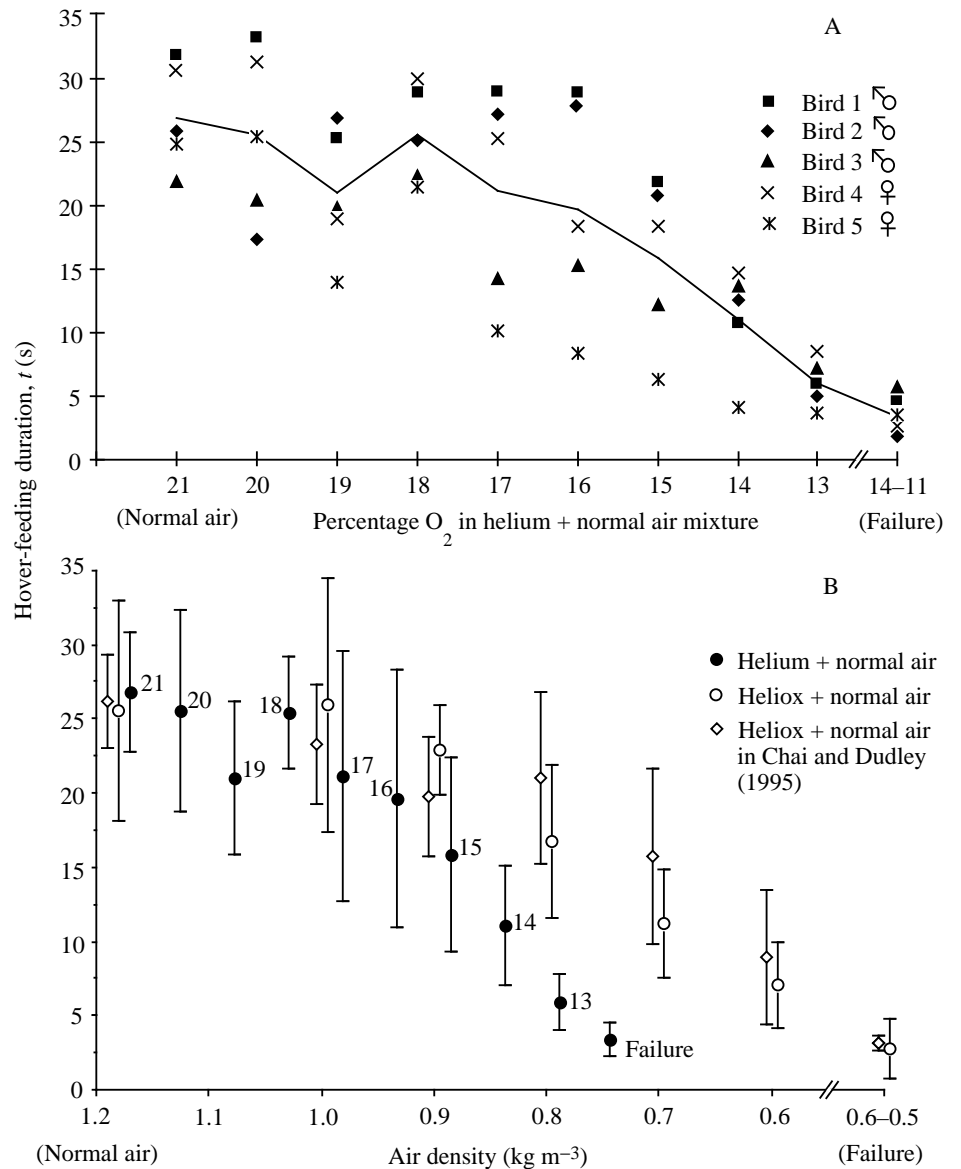


Fig. 1. Hover-feeding duration (*t*) of five individual hummingbirds in relation to reduction in oxygen concentration and air density. (A) Partial replacement of normal air with helium. Values are means for each individual at each oxygen level. (B) Partial replacement of normal air with helium and heliox. Values are means ( $\pm 1$  S.D.) of mean values from five individual birds at each density level (only four birds in Chai and Dudley, 1995). Numbers beside filled circles indicate the respective percentage O<sub>2</sub> values.

Table 2. Results of repeated-measures ANOVA for kinematic, metabolic and mechanical variables in variable oxygen/density gas mixtures for five ruby-throated hummingbirds

Variable	P value				
	% O <sub>2</sub>	Trial	% O <sub>2</sub> × Trial	Linear trend	Quadratic trend
<b>Air/helium</b>					
log( <i>t</i> )	0.001*** (9,36)	0.749 (2,8)	0.765 (18,72)	0.001*** (1,4)	0.042* (1,4)
<i>n</i>	0.156 (9,36)	0.812 (2,8)	0.039* (18,72)	0.326 (1,4)	0.303 (1,4)
Φ	0.001*** (9,36)	0.310 (2,8)	0.710 (18,72)	0.002** (1,4)	1.000 (1,4)
<i>P</i> <sub>zero</sub>	0.001*** (9,36)	0.794 (2,8)	0.001*** (18,72)	0.003** (1,4)	0.168 (1,4)
<i>P</i> <sub>per</sub>	0.001*** (9,36)	0.893 (2,8)	0.001*** (18,72)	0.001*** (1,4)	0.072 (1,4)
$\dot{V}_{O_2}$	0.335 (7,48)	0.213 (2,8)	0.999 (14,56)	0.536 (1,4)	0.167 (1,4)
η <sub>m</sub> ( <i>P</i> <sub>per</sub> )	0.001*** (7,48)	0.206 (2,8)	0.998 (14,56)	0.012* (1,4)	0.092 (1,4)
<b>Air/heliox</b>					
	Density			Linear trend	Quadratic trend
log( <i>t</i> )	0.001*** (6,24)			0.001** (1,4)	0.002** (1,4)
<i>n</i>	0.002** (6,24)			0.053 (1,4)	0.596 (1,4)
Φ	0.001*** (6,24)			0.001** (1,4)	0.301 (1,4)
<i>P</i> <sub>zero</sub>	0.001*** (6,24)			0.001*** (1,4)	0.179 (1,4)
<i>P</i> <sub>per</sub>	0.001*** (6,24)			0.001*** (1,4)	0.121 (1,4)
$\dot{V}_{O_2}$	0.001*** (5,20)			0.008** (1,4)	0.908 (1,4)
η <sub>m</sub> ( <i>P</i> <sub>per</sub> )	0.752 (5,20)			0.613 (1,4)	0.504 (1,4)
<b>Air/heliox–air/helium</b>					
	Density			Linear trend	Quadratic trend
Δlog( <i>t</i> )	0.013* (3,12)			0.036* (1,4)	0.002** (1,4)
Δ <i>n</i>	0.225 (3,12)			0.299 (1,4)	0.846 (1,4)
ΔΦ	0.513 (3,12)			0.407 (1,4)	0.835 (1,4)
Δ <i>P</i> <sub>zero</sub>	0.223 (3,12)			0.271 (1,4)	0.743 (1,4)
Δ <i>P</i> <sub>per</sub>	0.094 (3,12)			0.163 (1,4)	0.626 (1,4)
Δ $\dot{V}_{O_2}$	0.003** (3,12)			0.010** (1,4)	0.157 (1,4)
Δη <sub>m</sub> ( <i>P</i> <sub>per</sub> )	0.007** (3,12)			0.008** (1,4)	0.159 (1,4)

P values (\**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001) are from *F*-tests; degrees of freedom are given in parentheses.

Log-transformed hover-feeding duration log(*t*), wingbeat frequency *n*, stroke amplitude Φ, mechanical power output per unit flight muscle mass assuming zero (*P*<sub>zero</sub>) and perfect (*P*<sub>per</sub>) elastic energy storage, rate of oxygen consumption  $\dot{V}_{O_2}$ , and muscle mechanical efficiency η<sub>m</sub> for *P*<sub>per</sub>.

This decline eventually led to aerodynamic failure to sustain flight during a 2–4 s feeding bout; the bird dramatically descended to the chamber floor. In air/helium mixtures, the average oxygen concentration at failure was 12.1 % (equivalent to the oxygen tension at altitudes of around 4500 m) at an average air density of 0.74 kg m<sup>-3</sup> (equivalent to the air density at altitudes of around 4000 m). This density is substantially higher than the failure density in air/heliox mixtures (0.55 kg m<sup>-3</sup>, equivalent to the air density at altitudes around 6000 m).

Helium and heliox treatments altered wingbeat kinematics, oxygen consumption rate and mechanical power output during hovering in several dissimilar ways. For wing kinematics, both treatments resulted in similar increases in stroke amplitude, although the effects on wingbeat frequency differed. No significant difference in wingbeat frequency was found along declining percentage oxygen/density levels in air/helium mixtures, and wingbeat frequency stabilized at an average of 50 Hz rising to 51 Hz prior to aerodynamic failure (Fig. 2A;

Table 2). Owing to their shorter wings (Table 1), males of the sexually dimorphic ruby-throated hummingbird beat their wings faster than females. In air/heliox mixtures, the density effect was significant and the ascending linear trend in wingbeat frequency was just non-significant, with an average of 50 Hz in normal air and 54 Hz at failure (Fig. 2A; Table 2). For stroke amplitude, percentage oxygen/density levels were a significant predictor, and a linear trend with percentage oxygen and/or density levels was identified for both helium and heliox treatments (Fig. 2B; Table 2). Owing to failure at higher air densities, stroke amplitude showed an average increase of 13 % from 149° in normal air to 168° at failure in air/helium mixtures (compared with an average increase of 20 % in air/heliox mixtures, from 147 to 176°). However, no difference in wing kinematic variables was detected between the two treatments across their overlapping density levels (1.2–0.8 kg m<sup>-3</sup>, Table 2). Across treatments, stroke plane angle β remained close to horizontal, averaging 6±2° (mean of mean values for individual birds) in normal air, 6±2° at

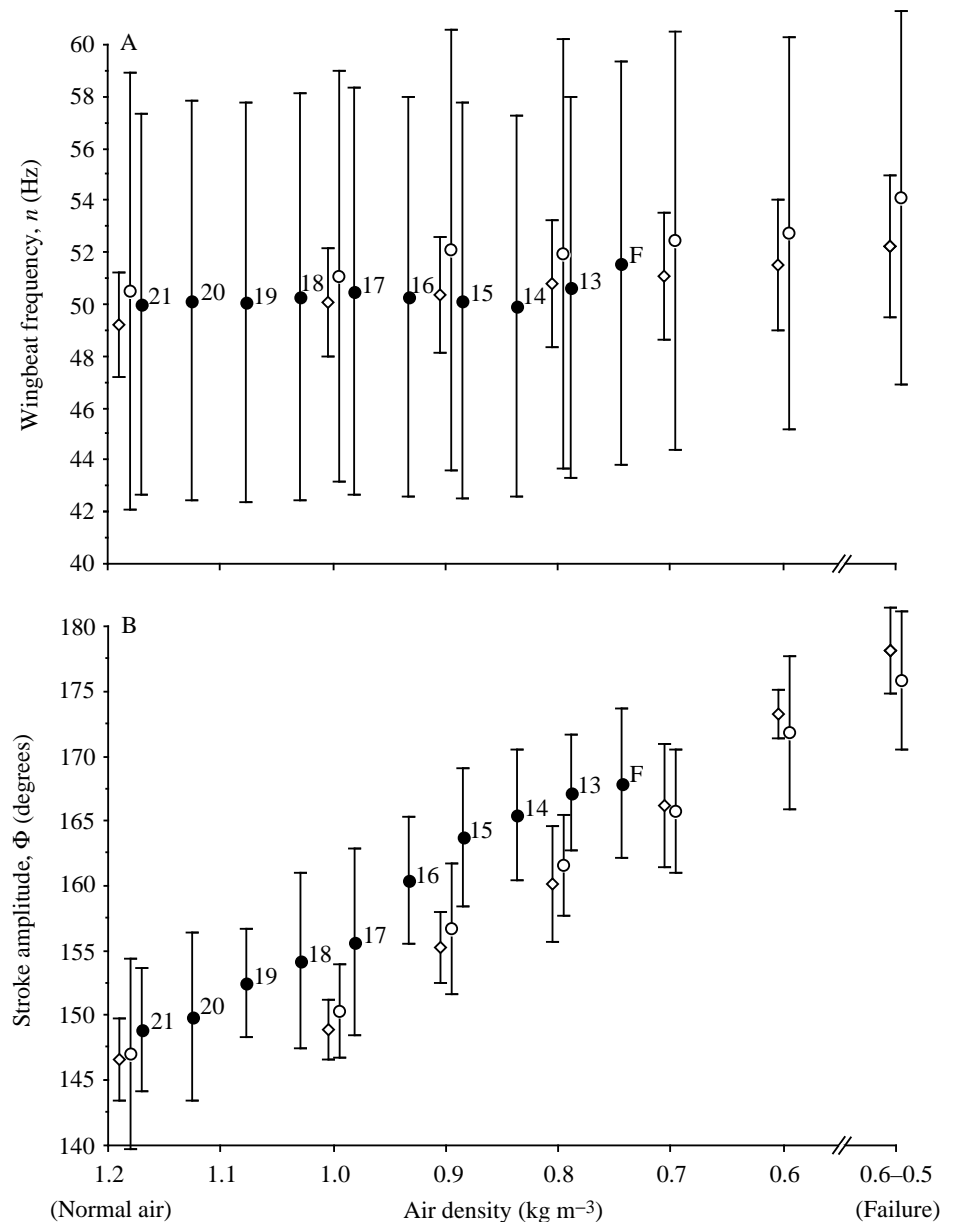


Fig. 2. Wingbeat frequency  $n$  (A) and stroke amplitude  $\Phi$  (B) in relation to reduction in oxygen concentration and air density. Filled circles, helium + normal air; open circles, heliox + normal air; diamonds, heliox + normal air (from Chai and Dudley, 1995). Numbers beside filled circles indicate percentage O<sub>2</sub> levels for helium/air mixtures (F=failure). Values are means ( $\pm 1$  S.D.) of mean values from five individuals at each density ( $N=4$  for values from Chai and Dudley, 1995).

failure in air/helium mixtures and  $7 \pm 5^\circ$  at failure in air/heliox mixtures.

For both helium and heliox treatments, muscle mass-specific power expenditure also increased linearly with decreasing percentage O<sub>2</sub> and/or density under the assumption of either zero or perfect elastic energy storage (Fig. 3; Table 2).  $P_{\text{zero}}$  in air/helium mixtures showed an average increase of 28% from  $255 \text{ W kg}^{-1}$  in normal air to  $326 \text{ W kg}^{-1}$  at failure, compared with an average increase of 52% from 261 to  $396 \text{ W kg}^{-1}$  in air/heliox mixtures.  $P_{\text{per}}$  in air/helium mixtures showed an average increase of 20% from  $96 \text{ W kg}^{-1}$  in normal air to  $115 \text{ W kg}^{-1}$  at failure, compared with an average increase of 35% from 97 to  $131 \text{ W kg}^{-1}$  in air/heliox mixtures. No difference in mechanical power output ( $P_{\text{zero}}$  and  $P_{\text{per}}$ ) was detected between the two treatments across the density levels in which they overlapped (Table 2).

Unlike mechanical power output, patterns of metabolic power input as indicated by oxygen consumption rate were quite different between the helium and heliox treatments (Fig. 4A; Table 2). The rate of oxygen consumption remained stable under helium treatment, whereas this rate increased significantly with decreasing density under the heliox treatment. Oxygen consumption rates could not be reliably obtained at failure densities owing to short hover-feeding durations. In air/helium mixtures at 14% O<sub>2</sub>, the average oxygen consumption rate was  $46 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ , almost the same as the average rate in normal air of  $45 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ . In air/heliox mixtures at air densities near  $0.6 \text{ kg m}^{-3}$ , maximum rates of oxygen consumption had increased relative to hovering in normal air by 24%, from 44 to  $55 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ . Because muscle mechanical efficiency was calculated as the ratio of mechanical power output to metabolic power input, it also

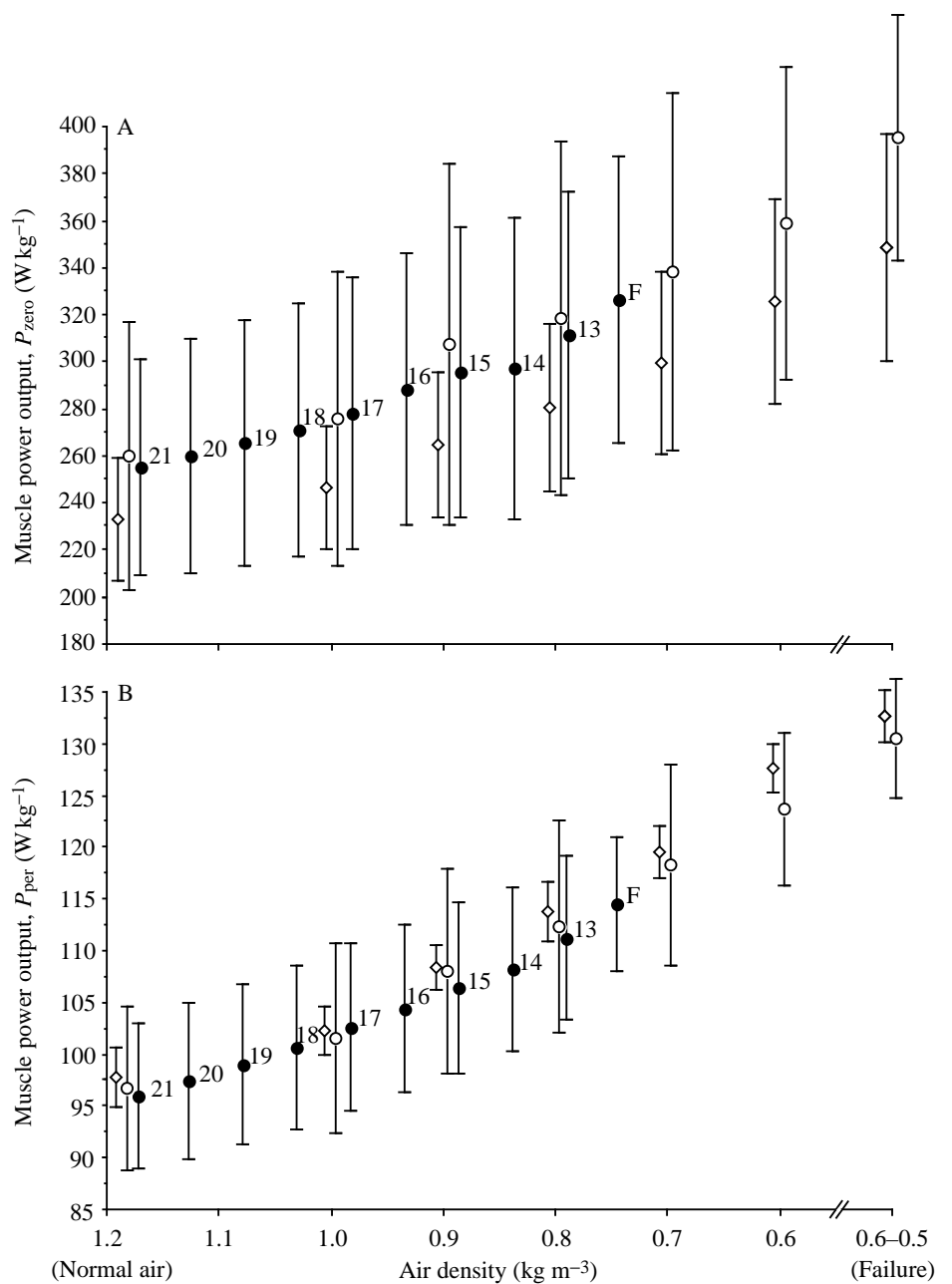


Fig. 3. Muscle mass-specific power output assuming zero ( $P_{\text{zero}}$ ) (A) and perfect ( $P_{\text{per}}$ ) (B) elastic energy storage in relation to reduction in oxygen concentration and air density. Filled circles, helium + normal air; open circles, heliox + normal air; diamonds, heliox + normal air (from Chai and Dudley, 1995). Numbers beside filled circles indicate percentage  $\text{O}_2$  levels for helium/air mixtures (F=failure). Values are means ( $\pm 1$  S.D.) of mean values from five individuals at each density ( $N=4$  for values from Chai and Dudley, 1995).

varied between helium and heliox treatments (Fig. 4B; Table 2). In air/helium mixtures, muscle efficiency under the assumption of perfect elastic energy storage increased with decreasing percentage  $\text{O}_2$ /density level. The muscle efficiency for  $P_{\text{per}}$  in air/helium mixtures exhibited an average increase from 10% in normal air to 11% at 14%  $\text{O}_2$ ; no significant trend was found in air/heliox mixtures. Thus, statistically significant differences were detected in oxygen consumption and muscle efficiency between the two treatments across the density levels for which they overlapped (Table 2).

In the repeated-measures ANOVA comparing results in air/heliox mixtures from this study with those from the earlier study of Chai and Dudley (1995), no statistically significant difference between the two studies was detected for any of the

metabolic or mechanical variables, indicating that prolonged captivity did not appreciably reduce the flight performance of these hummingbirds (Figs 1–4). Wingbeat frequency and  $P_{\text{zero}}$  were consistently (although not statistically significantly) higher in air/heliox mixtures of this study compared with those in the earlier study. This is because the two juvenile males in Chai and Dudley (1995) had produced adult male plumage with shorter and more pointed wings after moulting (and thus had an increased wingbeat frequency) by the present study.

In the manipulation that forced birds to hover-feed while breathing only helium, all birds demonstrated the capacity to hover very briefly ( $1.5 \pm 0.3$  s), followed by drastic aerodynamic failure and ballistic descent to the padded chamber floor. This failure was unlike that in air/helium or air/heliox mixtures in



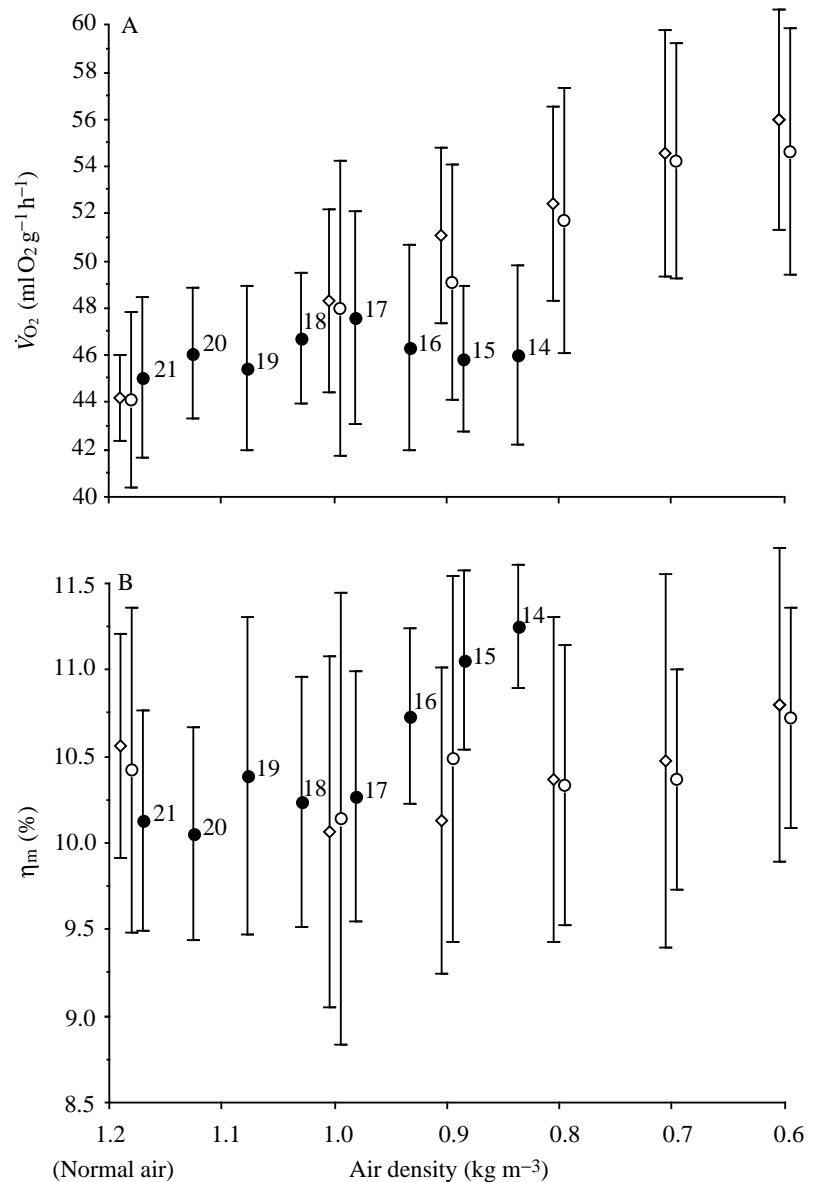


Fig. 4. Body mass-specific rate of oxygen consumption  $\dot{V}_{O_2}$  (A) and muscle mechanical efficiency  $\eta_m$  (B) assuming perfect elastic energy storage in relation to reduction in oxygen concentration and air density. Filled circles, helium + normal air; open circles, heliox + normal air; diamonds, heliox + normal air (from Chai and Dudley, 1995). Numbers beside filled circles indicate percentage O<sub>2</sub> levels for helium/air mixtures. Values are means ( $\pm 1$  S.D.) of mean values from five individuals at each density ( $N=4$  for values from Chai and Dudley, 1995).

which the birds still possessed some ability to circle or zigzag to the floor to lessen impact. Following aerodynamic failure, the birds always recovered after a period of rest in the normoxic atmosphere.

### Discussion

Although both helium and heliox treatments can induce aerodynamic failure in hovering hummingbirds, air densities at failure in air/helium mixtures were higher than in air/heliox mixtures and birds were not limited by flight mechanics. Rather, the oxygen supply could not meet the mechanical power output necessary to sustain hovering. In hypodense and normoxic air/heliox mixtures, hummingbirds demonstrated considerable power reserves, with an average increase in  $P_{per}$  of 35% (present study) and 36% (Chai and Dudley, 1995). In hypodense and hypoxic air/helium mixtures, the birds

demonstrated considerable tolerance to hypoxia while hovering: an average reduction in oxygen content of 42% from sea level values gave an increase of 20% in  $P_{per}$ . In the air/helium mixtures, the rate of oxygen consumption remained constant under hypoxia while mechanical power output increased in the hypodense air. This is a quite remarkable ability, as it is often suggested that hovering hummingbirds are operating at their aerobic limits (Diamond, 1990). Their flight muscles are highly oxygen-dependent, and hovering hummingbirds have the highest mass-specific rates of oxygen consumption among vertebrates (Suarez, 1992; Hochachka, 1994). Mitochondrial volume density in their flight muscles can account for 35% of the muscle volume (Suarez *et al.* 1991); this value may represent an upper limit because any further increase may compromise space for the myofibrils necessary for mechanical work (Pennycuik and Rezende, 1984). Hummingbirds possess hearts that are twice as large as

predicted from scaling across bird species (Hartman, 1961). Heart rates at  $1200 \text{ beats min}^{-1}$  during hovering may also approach an upper limit because hummingbirds of variable sizes all have similar heart rates (Bishop and Butler, 1995). Nevertheless, the non-invasive manipulations of the present study clearly show that, even during hovering, hummingbirds are operating with a reasonable physiological safety margin (Diamond and Hammond, 1992). These physiological reserves are actually essential for their ecological and behavioural repertoire, e.g. for ascending flight during foraging or to carry fat loads during migration (Carpenter *et al.* 1993; Wells, 1993b) and to perform acrobatic manoeuvres during courtship and territorial disputes (Wolf, 1978).

The steep decline of hover-feeding duration with decreasing percentage  $\text{O}_2/\text{density}$  has been previously observed for the South American hummingbird *Colibri coruscans* in simulated high-altitude flights at low air densities and reduced oxygen partial pressure through hypobaric manipulation (Berger, 1974). This larger and montane species was capable of hovering at altitudes over 6000 m. Like the ruby-throated hummingbird, the wingbeat frequency of *Colibri coruscans* remained stable at around 30 Hz across simulated altitudinal gradients. However, several large birds such as geese and swans are famous for high-altitude flight at over 8000 m, and birds are generally more tolerant than mammals to hypoxia (Butler, 1991; Faraci, 1991). It will be interesting to investigate these physiological adjustments and adaptations in hummingbirds and other birds flying in hypodense and hypoxic air media.

Gradual replacement of sea-level air with helium reduced both air density and oxygen tension, which parallels changes experienced during altitudinal ascent. Compared with altitudinal simulations at the same oxygen tension, the density of comparable air/helium mixtures will always be slightly higher (owing to the presence of helium). For example, an oxygen concentration of 12.9% in an air/helium mixture (12.9%  $\text{O}_2$ , 38.5% He and 48.6%  $\text{N}_2$  at 101 kPa) will exert the same oxygen pressure as atmospheric air at 4000 m above sea level (at 63 kPa), but the density of this air/helium mixture is 8% higher. Thus, the cost of flight is reduced in air/helium mixtures for the same oxygen availability. This may be one reason why the present study did not detect an increase in oxygen consumption rate with reduced air density, whereas Berger (1974) reported a slight increase of 6–8% from sea level to simulated 4000 m elevations in hovering hummingbirds (*Colibri coruscans* and *Amazilia fimbriata*, based on three birds). However, the slight increase in power input measured by Berger (1974) did not match the theoretical increase in mechanical power requirements. Thus, muscle mechanical efficiency will have increased in both studies under hypodense and hypoxic air, assuming that power input is derived solely from aerobic metabolism. These findings are in contrast to the results from the heliox treatment of this study as well as that of Chai and Dudley (1995), who found that, under normoxic conditions, the increase in oxygen uptake rate at low air densities matched the required increase in flight

power output, resulting in a constant muscle efficiency of 10–11% assuming perfect elastic energy storage.  $\dot{V}_{\text{O}_2}$  values in Chai and Dudley (1995) were overestimated by approximately 10% because values were not converted to STP.

Berger (1974) suggested that hummingbirds may go into oxygen debt under hypoxia. Since hummingbirds in the present study showed the capacity to hover for 1.5 s while breathing pure helium, this alternative explanation may be more likely than improved muscle mechanical efficiency. In both the present study and that of Berger (1974), hover-feeding duration showed a steep decrease with declining percentage  $\text{O}_2/\text{density}$  corresponding to stabilization or reduction in oxygen consumption at low air densities (14–16%  $\text{O}_2$ , see Figs 1, 4). Assuming that anaerobic metabolism and/or oxygen storage can contribute to mechanical power output during short bouts of hover-feeding, muscle mechanical efficiency may remain constant rather than increasing with declining percentage  $\text{O}_2/\text{density}$  as postulated above.

Several lines of evidence indicate that hummingbirds can utilize phosphagen for anaerobic ATP generation and also release stored oxygen to the aerobic pathway. It is now known that hummingbirds possess little capacity for glycolytic phosphorylation but that they retain the capacity for burst performance, as indicated by high activities of creatine kinase in the flight muscles (Suarez *et al.* 1986; Hochachka, 1994). Thus, anaerobic ATP supply through the creatine-kinase/phosphocreatine system, which normally provides transient maintenance of maximal power at the start of muscular work (Van Deursen *et al.* 1993), could supplement muscle energetics under hypoxic conditions. In addition, as found in diving ducks (Keijer and Butler, 1982), hummingbirds may have an enhanced oxygen storage capacity, as indicated by their high haematocrit and moderately high myoglobin concentration (Johansen *et al.* 1987). Furthermore, hummingbirds might also adopt thermoregulatory strategies such as hypothermia to reduce the basal metabolic rate (Bernstein, 1989; Wood, 1991). All of these mechanisms could alleviate oxygen dependence and thus account for stable rates of oxygen consumption in air/helium mixtures. The steep decline in hovering duration with decreasing percentage  $\text{O}_2$  and/or density for both air/helium and air/heliox mixtures presumably indicates constraints on oxygen delivery. After a short hover-feeding duration in hypodense and/or hypoxic air, hummingbirds rest and hyperventilate for at least a few seconds (but generally more than 5 s) before flying again (P. Chai, personal observations). After aerodynamic failure, hummingbirds exhibit much longer rest and hyperventilation periods (generally more than 30 s). The detailed mechanisms leading to recovery under these strenuous conditions are unclear, and further investigations are needed. The existence of brief hovering periods prior to aerodynamic failure in either air/helium or air/heliox mixtures and even in pure helium does, however, suggest transient relaxation of aerobic constraints on power production.

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