

RESEARCH ARTICLE

Respiratory mechanics and morphology of Tibetan and Andean high-altitude geese with divergent life histories

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ABSTRACT

High-altitude bar-headed geese (*Anser indicus*) and Andean geese (*Chloephaga melanoptera*) have been shown to preferentially increase tidal volume over breathing frequency when increasing ventilation during exposure to hypoxia. Increasing tidal volume is a more effective breathing strategy but is also thought to be more mechanically and metabolically expensive. We asked whether there might be differences in the mechanics or morphology of the respiratory systems of high-altitude transient bar-headed geese and high-altitude resident Andean geese that could minimize the cost of breathing more deeply. We compared these two species with a low-altitude migratory species, the barnacle goose (*Branta leucopsis*). We ventilated anesthetized birds to measure mechanical properties of the respiratory system and used CT scans to quantify respiratory morphology. We found that the respiratory system of Andean geese was disproportionately larger than that of the other two species, allowing use of a deeper breathing strategy for the same energetic cost. The relative size of the respiratory system, especially the caudal air sacs, of bar-headed geese was also larger than that of barnacle geese. However, when normalized to respiratory system size, the mechanical cost of breathing did not differ significantly among these three species, indicating that deeper breathing is enabled by morphological but not mechanical differences between species. The metabolic cost of breathing was estimated to be <1% of basal metabolic rate at rest in normoxia. Because of differences in the magnitude of the ventilatory response, the cost of breathing was estimated to increase 7- to 10-fold in bar-headed and barnacle geese in severe hypoxia, but less than 1-fold in Andean geese exposed to the same low atmospheric P_{O_2} .

KEY WORDS: Air sac morphology, Andean goose, Bar-headed goose, Barnacle goose, *Chloephaga melanoptera*, *Anser indicus*, *Branta leucopsis*, Compliance, High-altitude hypoxia, Respiratory mechanics, Work of breathing

INTRODUCTION

Obtaining sufficient oxygen across the respiratory surfaces can become challenging when the demands for oxygen are very high, such as during exercise. Avian flight is the most energetically expensive of all forms of vertebrate locomotion per unit time

(Thomas, 1975; Withers et al., 2016), and it has been hypothesized that the basis of the enhanced capacity for oxygen transport that supports flight in birds is the evolution of the parabronchial lung air sac system (Maina, 2005). Flight is even more challenging at high altitude where O_2 is limiting and recent reviews have summarized many adaptations found in high-altitude species (Scott et al., 2015). One aspect that has been little explored is the cost associated with various respiratory strategies (York et al., 2017). However, a complete understanding of the respiratory adaptations that enable cardiorespiratory performance requires knowledge of the mechanical properties of the respiratory system, as well as the volumes of the different morphological components of the respiratory system, such as the lungs and air sacs.

In the wild, barnacle and bar-headed geese migrate in the autumn from their northern breeding grounds to southern wintering grounds, and return in the spring – often flying continuously for hours (Hawkes et al., 2012; Butler et al., 1998). The bar-headed goose migrates primarily at high altitudes, over the Himalayas between breeding grounds in northern China and Mongolia (at about 2300 m) and wintering grounds in India (sea level; Hawkes et al., 2013). While a previous anecdotal account (Swan, 1961) of bar-headed geese flying over Mount Makalu (8485 m) is frequently cited as their ability to fly over Mount Everest (8848 m), recent research using satellite transmitters indicates that bar-headed geese primarily fly through the passes (generally 5000 m) and reach a maximum altitude of about 7200 m (Hawkes et al., 2013). This is still an impressive feat, as the oxygen available at these altitudes is approximately 40% that at sea level, and bar-headed geese increase oxygen demand 15- to 20-fold from rest to flight (J. U. Meir, J.M.Y., B. Chua, W. Jardine, L. A. Hawkes and W.K.M., unpublished; Ward et al., 2002).

Barnacle geese breed in Greenland, Svalbard, the Scandinavian Peninsula, and the Kanin Peninsula of Russia, and spend the winters in the northern United Kingdom, The Netherlands and Germany (Jonker et al., 2013), flying primarily over the ocean and along coastlines at low altitudes.

Andean geese are residents of the Andes Mountains in South America. They do not migrate but spend their entire lives in wetlands at altitudes greater than 3000 m (Storz and Moriyama, 2008). Andean geese are not true geese but part of a clade called sheldgeese, more closely related to ducks than true geese (McCracken et al., 2010). Thus, unlike the bar-headed and barnacle geese, which are somewhat closely related, the Andean goose is from a phylogenetically distant lineage.

Amongst the physiological traits of both Andean and bar-headed geese that underlie their high-altitude success (see Scott et al., 2015; Dzal et al., 2015, for review) are increased lung mass and vascularity relatively to body size (Scott et al., 2011; Maina et al., 2017), hemoglobin with an increased oxygen affinity (McCracken et al., 2010; Natarajan et al., 2015), and an ability to increase total ventilation to a greater extent than other species, especially by increasing tidal volume (Black and Tenney, 1980; Scott and

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Milsom, 2007; Lague et al., 2016). As a ventilation strategy, increasing tidal volume is generally more metabolically expensive than increasing breathing frequency (Otis, 1954), but it is more 'effective' compared with an equal increase in breathing frequency because the effective ventilation, or the volume of air that reaches the gas exchange surface, is increased as the result of a reduction in the proportion of dead space air. We specifically use this definition of effective for this study.

Bird lungs lie flush against the dorsal thoracic ribs; they are relatively small and rigid, expanding and contracting very little during normal breath cycles (Jones et al., 1985; Ponganis et al., 2015). In contrast, the air sacs are extensive, branching from the bronchi in the lungs and expanding to fill the body cavity of the bird. The air sacs are continuous with the pneumatic spaces of the vertebrae and long bones (Duncker, 1971), are made of thin, avascular membranes and, if punctured, do not collapse and can heal over time. It is difficult, however, to visualize the highly compartmentalized morphology of the air sacs or measure basic morphometric parameters such as air sac volume because they are immediately ruptured by any invasive procedure to the chest or abdomen.

Previous studies used terminal methods to study air sac morphology, such as occluding the airways on inspiration and filling the respiratory system with paraffin, resin, silicone or latex (Zeuthen, 1942; Akester, 1960; King and Payne, 1962; Duncker, 1972, 1977; Dubach, 1981; Jaensch et al., 2002). These fluid filling techniques can only measure volumes determined by the researcher to be relevant and are subject to material shrinkage. Other studies have used inert gas washout techniques to estimate air sac volumes (Dehner, 1946; Scheid and Piiper, 1969), but these can only measure ventilated volumes and are subject to error from gas dissolving in the blood. More modern, non-destructive imaging techniques such as computerized tomography (CT) scans can provide morphometric measurements not only of the respiratory system but also of other structures and organs simultaneously, and they also allow the same individual to be measured under multiple conditions (such as at various respiratory volumes). CT scans have been commonly used by veterinarians to study respiratory disease in birds (Orosz and Toal, 1992; Krautwald-Junghanns et al., 1993; Newell et al., 1997; Gumpenberger and Henninger, 2001), and are becoming more popular as a research tool to investigate avian respiratory physiology and morphology (Krautwald-Junghanns et al., 1998; Malka et al., 2009; Petnehazy et al., 2012; Ponganis et al., 2015).

In this study, we used CT scans to compare respiratory morphometry of three species of geese: the transient high-altitude bar-headed goose [*Anser indicus* (Latham 1790)], the resident high-altitude Andean goose [*Chloephaga melanoptera* (Eyton 1838)] and the resident low-altitude barnacle goose [*Branta leucopsis* (Bechstein 1803)]. We measured the lung mass, respiratory system volumes and mechanics of the respiratory system in these three species. We hypothesized that bar-headed and Andean geese would have larger air sacs and would have reduced the metabolic cost of breathing with a higher tidal volume by increasing the compliance and reducing the resistance of the respiratory system to air flow relative to that of barnacle geese.

MATERIALS AND METHODS

Respiratory mechanics

Six birds of each species were used in the respiratory mechanics experiments. Bar-headed and barnacle geese were captive raised at sea level and were 3 years of age (first year of sexual maturity) at the

time the experiments were conducted. Andean geese were captured and raised in San Pedro de Casta, Perú, at 3180 m and were 2 years old. Mean (\pm s.e.m.) body mass was 2.77 \pm 0.14 kg for bar-headed geese, 2.38 \pm 1.6 kg for barnacle geese and 2.29 \pm 0.15 kg for Andean geese. Experimental procedures were performed according to UBC Animal Care Committee protocols A12-0013 and A16-0019 under the guidelines of the Canadian Council on Animal Care. Wild animals were collected under authorization from the Ministerio de Agricultura del Perú (376-2012-AG-DGFFS-DGEFFS).

For the mechanics experiments, bar-headed and barnacle geese were anesthetized with isoflurane (1–5%), and Andean geese were anesthetized with intravenous propofol in the field as described by Mulcahy (2007). Experiments were conducted as described by York et al. (2017). Briefly, each anesthetized bird was intubated and attached to a custom-built constant-volume ventilator that actively inflated the respiratory system (inhalation) and allowed for passive deflation (exhalation). For the dynamic measurements, birds were ventilated in the prone position with at least three volumes (50, 75 and 100 ml) at five frequencies (20, 30, 40, 50 and 60 min⁻¹). We limited tracheal pressure to a maximum of 30 cmH₂O, and this pressure determined the maximum limit of volume and frequency used for each individual. Flow was measured with a differential pressure transducer (model DP103-18, Validyne, Northridge, CA, USA) and a pneumotachograph (4700 series non-heated pneumotachometer, Hans Rudolph, Shawnee Mission, KS, USA) that connected the endotracheal tube to the ventilator, and tracheal pressure was measured on the lung side of the pneumotachograph with a pressure transducer (for more on these techniques, see Lumb, 2017).

Flow curves were integrated to produce volume (ambient temperature and pressure dry, ATPD), and pressure–volume loops were generated using LabChart software (ADInstruments, Sydney, Australia). Dynamic compliance was measured as the slope of the line connecting the points of zero flow on the pressure–volume loops (the points of maximum and minimum volume). Work to overcome elastic forces (elastic work) was measured as the area of the triangle made between the two points of zero flow and the coordinate (0, maximum volume). Work to overcome resistive forces (resistive work) was measured as the area enclosed by the compliance line and the curve of the loop during the inflation phase. Total work per breath is the sum of these two work components (elastic and resistive; Otis, 1954). The resistive work performed to move the air through the endotracheal tube alone, as measured by repeating all experiments with only the endotracheal tube attached to the system, was subtracted from the total resistive work per breath. The minute work (or power) of breathing was calculated by multiplying the total work per breath by the breathing frequency (f_R).

To produce the static pressure–volume curves, the birds were hyperventilated and then disconnected from the ventilator, and a 200 ml glass syringe was used to inflate the respiratory system to a maximum of 30 cmH₂O and deflate to a minimum of –30 cmH₂O in a step-wise fashion (25 ml per step). This was repeated 2–3 times. The volume of air displaced between 30 and –30 cmH₂O was taken to be vital capacity (VC), while inspiratory and expiratory capacities were taken as the volume required to bring the system from 0 cmH₂O (atmospheric) to 30 or –30 cmH₂O, respectively. Animals were then either allowed to recover or killed and dissected for measurements of lung mass and volume of the extrapulmonary airways (details below). Static compliance was measured as the slope of the static deflation curve at its steepest point (between –5 and 5 cmH₂O).

Measurements of dynamic compliance, τ (the passive time constant of the respiratory system, calculated as the product of compliance and resistance), elastic and resistive work at each pump frequency were plotted versus tidal volume (V_t) using Origin[®] 2016 software (OriginLab, Northampton, MA, USA). Linear fits were used to place frequency isopleths on these plots, and these lines were used to estimate parameter values at selected combinations of pump volumes and frequencies (see Table S1 for equations). The selected combinations were either the same for all birds [low V_t/f_R : $f_R=20$ breaths min^{-1} , $V_t=7\%$ VC; high f_R : $f_R=40$ breaths min^{-1} , $V_t=7\%$ VC; high V_t : $f_R=20$ breaths min^{-1} , $V_t=14\%$ VC] or equal to the values measured in the same individual animals while resting and awake in normoxia and hypoxia (*in vivo* V_t/f_R values from C. M. Ivy, J.M.Y., S. L. Lague, B. A. Chua, L. Alza, K.G.M., W.K.M. and G. R. Scott, submitted, and Lague et al., 2016). Values were compared using one-way ANOVA with *post hoc* Tukey tests.

We may be underestimating total resistive work in our calculations as we subtracted the resistive work to move the air through the endotracheal tube from our estimates. The resistive work done to move the air through the endotracheal tube was approximately 1% of total resistive work at low frequencies, but reached as high as 10% at higher frequencies. We chose to exclude the work to move the air through the endotracheal tube as these tubes were smaller (they fit inside the trachea) and stiffer than the trachea. In doing so, however, we also subtracted the resistive work done to move air through the trachea.

For the Andean geese, experiments were conducted in the field at the altitude where the birds were captured and raised. Thus, we might overestimate compliance and underestimate the work of breathing in the Andean geese because of the low atmospheric pressure. To correct for this, comparisons were made at volumes expressed as a percentage of the VC defined as the volume change between respiratory pressures of 30 and -30 cmH_2O measured at altitude.

Lung mass and tracheal volume

To measure lung mass, three barnacle, three bar-headed and all six Andean geese were killed with an overdose of either pentobarbital (bar-headed and barnacle geese) or propofol (Andean geese). Their lungs were immediately extracted and weighed. In the Andean geese, extra-pulmonary airway volume was measured from the glottis to the entrance of the primary bronchi into the lungs by slowly filling the dissected trachea with known volumes of water.

CT scans

We used CT scanning to further investigate differences in morphology between the three species. We selected two geese of

each species (one of each sex; Andean geese were provided for CT scans by Sylvan Heights Waterfowl Park, Scotland Neck, NC, USA, and housed at SeaWorld, San Diego, CA, USA). The birds were anesthetized (1–5% isoflurane) and intubated, and whole-body scans were performed in the prone position at resting lung volume (glottis open to the atmosphere). The birds were then hyperventilated, inflated to a tracheal pressure of 30 cmH_2O and scanned again (Toshiba model Aquilion CT scanner). Quantitative CT analysis was performed, and images were rendered using Amira 3D software (FEI, Hillsboro, OR, USA; helical 1 mm acquisition; chest acquisition with reconstruction using soft tissue algorithm; kVp 120). Values were compared using two-way ANOVA comparing effects of species and sex with *post hoc* Tukey tests.

Cost of breathing

To estimate the metabolic cost of breathing, we converted basal metabolic rate (BMR) using oxygen consumption and CO_2 production values provided by Lague et al. (2016) and Ivy et al. (submitted), collected in the same individual birds, and the equation given by Romijn and Lokhorst (1966) to obtain energy expenditure (in kJ):

$$\text{Energy expenditure} = 16.19 \times V_{\text{O}_2} + 5.00 \times V_{\text{CO}_2}, \quad (1)$$

where the volume (V) of O_2 and CO_2 is in liters. We then corrected our work of breathing values for respiratory muscle efficiency by multiplying by 10 (Otis et al., 1950), and then doubling those values to account for active expiration as well as inspiration (Lee and Milsom, 2016).

RESULTS

Respiratory capacity

While the body mass of the birds did not differ (Fig. 1A; $F_{2,16}=3.1$, $P=0.072$), VC differed significantly, being largest in the Andean goose and smallest in the barnacle goose (Fig. 1B; $F_{2,12}=24.8$, $P<0.001$). This was also the case for inspiratory capacity, but the expiratory reserve volume was larger in the bar-headed goose than in the other two species (significantly larger only in comparison to the barnacle goose; see Table 1). Extracted lung mass (not adjusted to body size) did not differ significantly between the three species ($F_{2,4}=27$, $P=0.18$).

CT scans

Images of CT scans for a representative individual of each species are shown in Fig. 2. Neither body mass nor body volume differed significantly among species for the birds used in the CT scans (see Table 1). Lung volume was significantly larger in Andean geese than in barnacle geese ($F_{2,2}=21$, $P=0.045$) whereas bar-headed

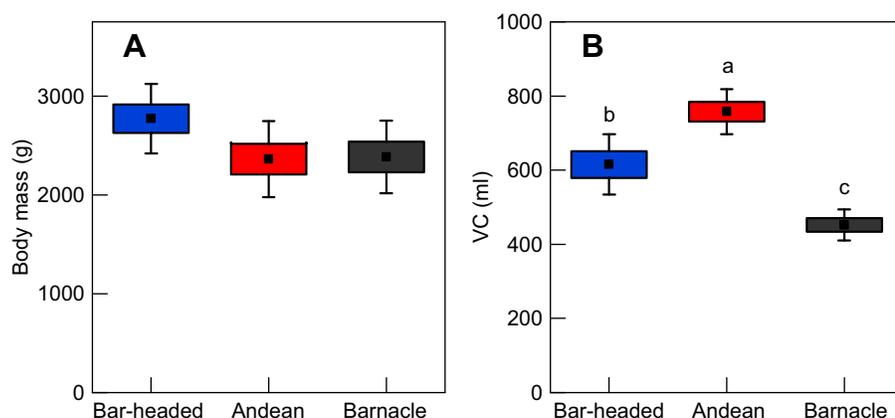


Fig. 1. Comparison of body size and size of the respiratory system in bar-headed, Andean and barnacle geese. (A) Comparison of body mass. (B) Comparison of vital capacity (VC). Different letters indicate significant differences between species (one-way ANOVA, $P<0.05$). Black squares indicate means, colored boxes indicate s.e.m. and whiskers indicate 95% confidence interval.

Table 1. Volume and mechanics measurements for the three goose species

Variables	Bar-headed	Andean	Barnacle	Significance
Mechanics values				
<i>n</i>	6	6	6	–
Body mass (kg)	2.77±0.1	2.29±0.2	2.38±0.2	n.s. ($P=0.072$, $F=3.1$)
Inspiratory capacity (ml)	372±22	551±21	297±19	AG ($P<0.001$, $F=31.8$)
Expiratory capacity (ml)	243±18	206±12	159±16	BH–BG ($P=0.003$, $F=9.6$)
VC (ml)	615±36	758±27	452±19	*($P<0.001$, $F=24.8$)
Static compliance (ml cmH ₂ O ⁻¹)	29.4±1.9	32.3±1.5	23.0±1.5	BG ($P=0.006$, $F=8.0$)
Static compliance (% VC cmH ₂ O ⁻¹)	4.77±0.2	4.28±0.2	5.07±0.2	AG–BG ($P=0.03$, $F=4.4$)
In vivo values				
<i>n</i>	6	7	6	–
V_t (ml BTPS)	56±3	35±3	32±3	BH ($P<0.001$, $F=18.5$)
V_t (ml STPD)	45±2	33±2	25±3	BH ($P<0.001$, $F=14.2$)
f_R (min ⁻¹)	15±1	18±1	22±3	n.s. ($P=0.075$, $F=3.1$)
Dissection values				
<i>n</i>	3	5	3	–
Lung mass (g)	27±0.7	24±1	21±2	n.s. ($P=0.18$, $F=2.7$)
Heart mass (g)		21.7±0.9		
Tracheal volume (ml)		11.4±1		
CT values				
<i>n</i>	2	2	2	–
Body mass (kg)	2.62±0.3	2.40±0.6	2.12±0.1	n.s. ($P=0.54$, $F=0.85$)
Body volume (cm ³)	1755±113	1888±457	1430±36	n.s. ($P=0.47$, $F=1.1$)
Inflated lung volume (ml)	88±7	121±17	52±2	AG–BG ($P=0.045$, $F=21$)
Resting lung volume (ml)	78±6	102±24	48±0.2	n.s. ($P=0.18$, $F=4.5$)
Inflated air sac volume (ml)	681±5	668±26	478±19	BG ($P=0.02$, $F=49$)
Resting air sac volume (ml)	334±27	323±13	181±3	BG ($P=0.02$, $F=51$)
Inflated cranial air sac volume (ml)	246±3.4	276±18	188±17	n.s. ($P=0.07$, $F=13.6$)
Resting cranial air sac volume (ml)	125±7	139±37	83±7	n.s. ($P=0.26$, $F=2.9$)
Inflated caudal air sac volume (ml)	435±2	392±7.4	290±1.7	*($P=0.004$, $F=251$)
Resting caudal air sac volume (ml)	209±20	184±24	99±3.6	n.s. ($P=0.13$, $F=6.8$)
Tracheal volume (ml)	12±1.7	8.5±1.2	8.0±1	BH ($P=0.023$, $F=43$)
Pneumatic volume, long bones (ml)	11±0.3	14.4±3	7.3±2	n.s. ($P=0.2$, $F=4.0$)
Pneumatic volume, spine (ml)	5±0.4	13.6±7.4	1.9±0.5	n.s. ($P=0.33$, $F=2.0$)
Bone (cm ³)	166±0.3	200±29	126±0.8	n.s. ($P=0.159$, $F=5.3$)
Inflated dorsobronchi (ml)	6.1±0.2	19.6±5.8	5.7	n.s. ($P=0.36$, $F=3.4$)
Resting dorsobronchi (ml)	1.5±0.1	10.5±3.3	2.2±0.2	n.s. ($P=0.14$, $F=6.4$)
Inflated ventrobronchi (ml)	15.3±2.4	20.8±7.0	7.7	n.s. ($P=0.32$, $F=4.3$)
Resting ventrobronchi (ml)	11.8±0.7	14.1±3.3	6.3±0.5	n.s. ($P=0.19$, $F=4.3$)
Fat (cm ³)	89±20	128±110	245±2	n.s. ($P=0.35$, $F=1.9$)

Values are reported as means±s.e.m. Two-way ANOVA was used for statistical comparison. Mechanics values were obtained from the static inflation/deflation curves. Static compliance values were measured as the slope of the static compliance curve at its steepest point (from curves in Fig. 3). *In vivo* values for V_t and f_R were taken from Lague et al. (2016). Dissection values were obtained from birds post-mortem. CT values were obtained from the CT scans.

VC, vital capacity; V_t , tidal volume; f_R , breathing frequency; BTPS, body temperature and pressure saturated; STPD, standard temperature and pressure dry. Significance is represented as follows: n.s. indicates a non-significant difference; BH–BG indicates that bar-headed geese differ significantly from barnacle geese; AG–BG indicates that Andean geese differ significantly from barnacle geese; BH, AG and BG indicate that bar-headed geese and barnacle geese differ significantly from the other two groups, respectively; asterisks indicate all three groups differ from one another.

geese had an intermediate lung volume. Total air sac volumes also were significantly larger in the Andean and bar-headed geese as compared with the barnacle geese ($F_{2,2}=49$, $P=0.02$). When cranial and caudal air sac volumes were compared, only the inflated caudal air sac volume differed significantly between species, the bar-headed geese having the largest and the barnacle geese the smallest ($F_{2,2}=251$, $P=0.004$). The lung volumes changed between functional residual capacity (FRC) and 30 cmH₂O pressure by 19% in Andean geese, 13% in bar-headed geese and 8% in barnacle geese. The air sacs approximately doubled in size for the Andean and bar-headed geese for the same pressure change (107% and 104% change, respectively), whereas the air sacs of the barnacle geese increased in volume 164%. According to the CT scans, tracheal volume generally scaled with body mass and therefore was highest in the bar-headed geese ($F_{2,2}=43$, $P=0.023$). The pneumatic volume of the long bones and spine, the total bone volume, dorsobronchial and ventrobronchi volume, and fat volume did not differ significantly between the three species (Table 1).

Static mechanics

The static compliance of the total respiratory system was greater in Andean and bar-headed geese than in barnacle geese (Fig. 3A, Table 1; $F_{2,12}=8.0$, $P=0.006$), but these differences were directly related to respiratory volume. When compliance was normalized to VC (percentage change in VC for a given change in pulmonary pressure; Fig. 3B), the static compliance of barnacle geese was actually significantly higher than that of Andean geese (Table 1; $F_{2,12}=4.4$, $P=0.03$). Thus, Andean geese and bar-headed geese of the same body mass as the barnacle geese had increased compliance as a result of their increased VC, not because of mechanical changes in the respiratory system.

Dynamic mechanics

As dynamic respiratory mechanics vary as a function of the combination of V_t and f_R , all measured combinations are plotted in Fig. 4 before normalization. The three species are then compared at common V_t and f_R when normalized to the size of the respiratory

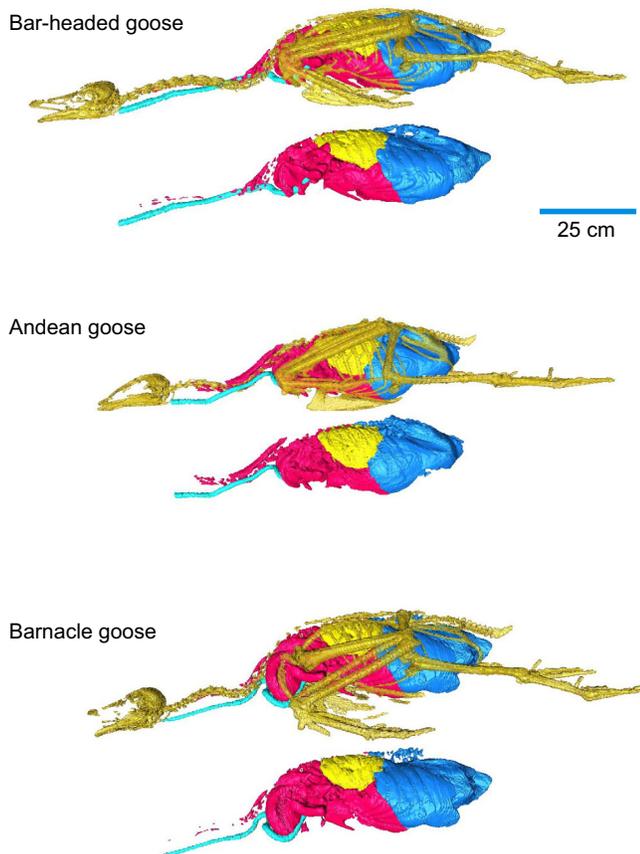


Fig. 2. CT scans of the respiratory system of each species, inflated to 30 cmH₂O. Lungs (yellow), trachea (cyan), cranial air sacs (red), caudal air sacs (blue) and bones (dark yellow) are shown. Images were taken from the lateral side of prone birds.

system in Fig. 5. In Fig. 6, normalized values are compared at the f_R/V_t combination used by each species at rest under normoxic and hypoxic conditions.

In Fig. 4, dynamic compliance and total work are plotted against V_t for each of the different ventilation frequencies. For all geese, compliance decreased with increasing V_t or f_R , whereas the total dynamic work required to ventilate the respiratory system concurrently increased. For any given combination of V_t and f_R , the Andean goose had a higher compliance and required less total work to ventilate, whereas the barnacle goose had a much lower

compliance (approximately 4-fold lower) and require more total work to ventilate (between 3- and 4-fold higher). The values of compliance and total work were intermediate for the bar-headed goose. Equations from linear fits of these relationships were used for subsequent comparisons and can be found in Table S1.

To remove the effects of the differences in VC between species, we also made comparisons at a common V_t of 7% VC and a frequency of 20 breaths min⁻¹ (roughly equivalent to intra-specific mean resting values). We also estimated how these values would change if either frequency or volume were doubled.

When compared at volumes normalized to an equal percentage of VC and the same ventilation frequencies, dynamic compliance was still always highest in Andean geese (Fig. 5A). Dynamic compliance in bar-headed and barnacle geese was equal. The time constant, τ , was longest for barnacle geese and shortest for Andean geese (Fig. 5B). These relationships were maintained when ventilation was doubled, regardless of whether it was due to a doubling of f_R or of V_t . As expected, increasing ventilation reduced dynamic compliance; however, counter to our expectations, increasing f_R reduced compliance more than increasing V_t (Fig. 5A). The power output did not differ significantly between species except during high f_R when it was significantly lower in the barnacle goose.

When we compared the values for respiratory mechanics for the combinations of V_t (as a %VC) and f_R used by each species *in vivo* at rest, we found that compliance remained highest in Andean geese, especially in hypoxia (5 kPa O₂; Fig. 6A). τ remained longest for barnacle geese and shortest for Andean geese in normoxia (21 kPa O₂ for barnacle and bar-headed geese, 14 kPa O₂ for Andean geese). In hypoxia, τ was equal for Andean and bar-headed geese (Fig. 6B). In normoxia, all three species breathed with the same power output, but in hypoxia, power did not change for Andean geese (primarily because total ventilation did not change significantly *in vivo*), but was up to 15-fold higher in both barnacle and bar-headed geese (Fig. 6C).

When power output was plotted against f_R for a constant level of minute ventilation, the curves were roughly U-shaped. Thus, for any given level of minute ventilation, there is an optimal combination of V_t and f_R where power output is lowest. We plotted these curves for each species for the level of minute ventilation each used in normoxia and hypoxia and then compared the predicted optimal combinations with the actual ranges of f_R recorded *in vivo* at rest (Fig. 7). We found that barnacle geese always used the predicted optimal (lowest energy) combination but that bar-headed geese only used the predicted optimal combination in hypoxia. Andean geese used a slower, deeper breathing strategy than the predicted optimum in both normoxia and hypoxia.

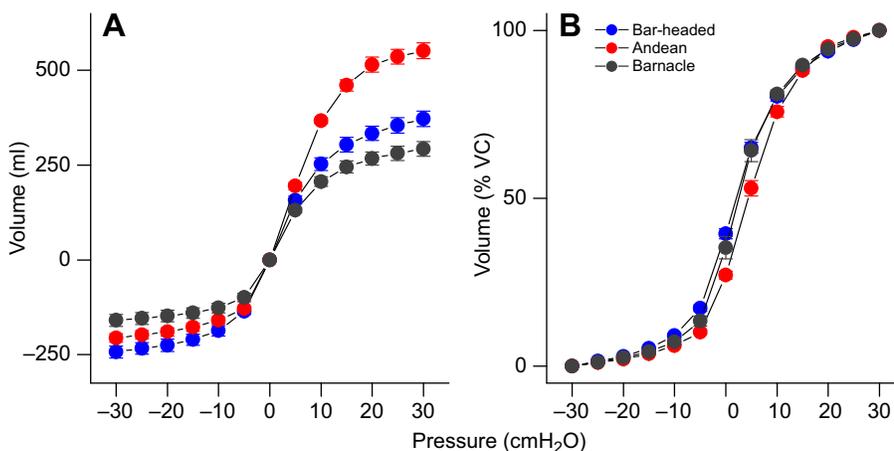


Fig. 3. Static compliance curves. Volume data are expressed as (A) volume change in milliliters for a given pressure change and (B) as a percentage of VC change for a given pressure change. Curves are means \pm s.e.m.

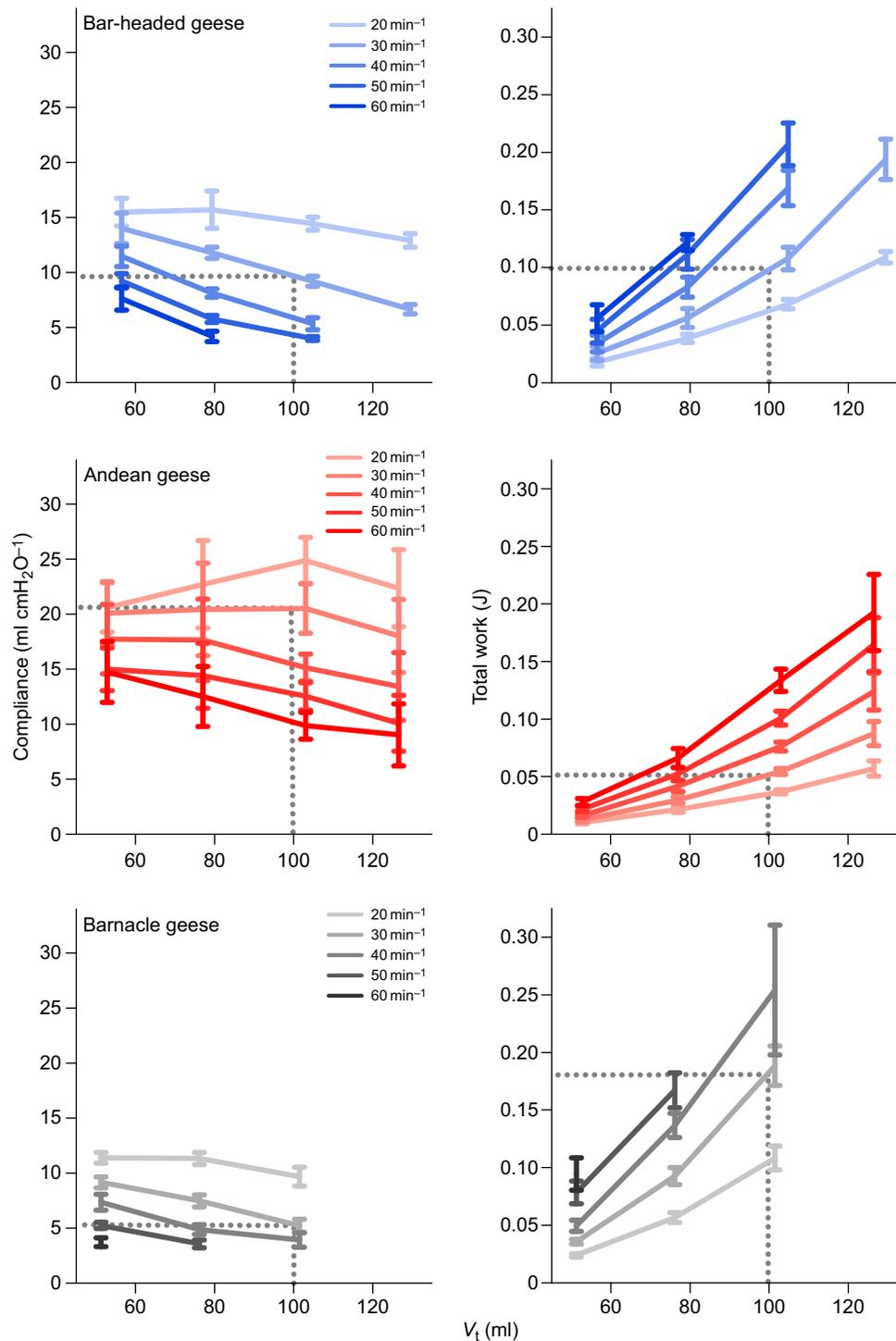


Fig. 4. Dynamic compliance (left) and total dynamic work required to ventilate the respiratory systems (right) of the study species. Top row: bar-headed geese; middle row: Andean geese; bottom row: barnacle geese. Constant frequency lines are shown, with darker shades representing higher breathing frequencies (f_R , see keys); dotted gray lines are to aid comparison between plots at an arbitrary tidal volume (V_t) and f_R combination (100 ml and 40 breaths min^{-1}).

Cost of breathing

We estimated metabolic cost of breathing to be very low in normoxia for all three species: 0.38% BMR for the bar-headed geese, 0.68% BMR for the Andean geese and 0.78% BMR for the barnacle geese. This increased by 10-fold for the bar-headed goose when breathing 5% O₂ (3.8% BMR), and by 7.5-fold for the barnacle goose (5.8% BMR). However, metabolic cost of breathing in hypoxia only increased to 0.9% BMR for the Andean goose, again reflecting the fact that neither f_R nor V_t increased greatly for Andean geese in hypoxia (Table 2).

DISCUSSION

This study examined the morphology and mechanics of the respiratory systems of bar-headed, Andean and barnacle geese and compared the overall work and cost of breathing in each. Note that the VC, compliance, resistance and work of breathing have been determined during artificial ventilation in anesthetized birds and may vary from those obtained during spontaneous ventilation. All measures were made in the same way on all species, however, providing equal comparisons between groups. We found that the respiratory system was disproportionately large in Andean geese

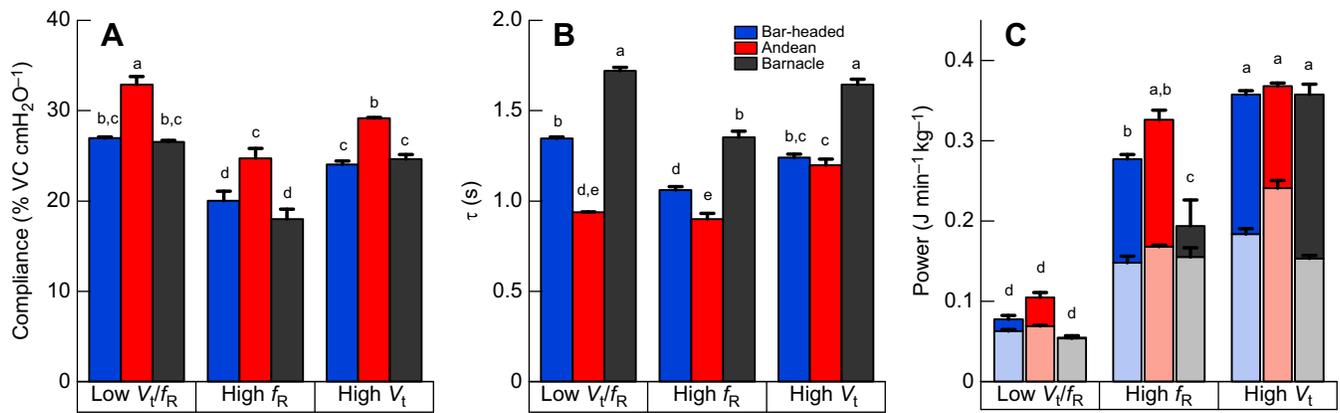


Fig. 5. Measurements and calculations of respiratory mechanics compared at equal V_t and f_R . Low V_t/f_R : $f_R=20$ breaths min^{-1} , $V_t=7\%$ VC; high f_R : $f_R=40$ breaths min^{-1} , $V_t=7\%$ VC; and high V_t : $f_R=20$ breaths min^{-1} , $V_t=14\%$ VC. (A) Dynamic compliance expressed with V_t as a percentage of VC. (B) The time constant (τ). (C) Power output required to produce these combinations. The light part of each column is the work required to overcome elastic forces, the dark part of each column is the work required to overcome flow resistance, and the whole column is total power output required. Lowercase letters in A–C indicate significant differences between groups (one-way ANOVA, $P<0.05$, *post hoc* Tukey test).

and small in barnacle geese for birds of similar body mass. Based on absolute values, Andean geese had a more compliant system and required less work to breathe than barnacle geese, while bar-headed geese were intermediate in work and compliance values. However, when normalized to the size of the respiratory system (i.e. the VC), static compliance and total dynamic work of breathing were similar for all three species, indicating that it is the size of the respiratory system that lowers the work of breathing in Andean and bar-headed geese, not a difference in the mechanical properties of their respiratory systems.

We were able to estimate the cost of breathing by measuring the work required to pump ventilate the geese and applying these values to the combinations of V_t and f_R used by the resting, awake geese. We then converted work values to metabolic cost values (see Materials and methods). The metabolic cost of breathing increased 10-fold in bar-headed geese as ventilation increased 3-fold from normoxia to hypoxia (0.38% to 3.8% of BMR), whereas the cost of breathing increased 7.4-fold in barnacle geese as ventilation increased 3-fold (0.78% to 5.82% of BMR). The Andean goose did not increase ventilation significantly in hypoxia (Lague et al., 2017), and so the cost of breathing only increased 1.3-fold (0.68% to 0.90% BMR). These values are in rough agreement with those of Markley and

Carrier (2010) and Ellerby et al. (2005), who estimated the cost of breathing to be 1–2% BMR in birds at rest. However, these findings do not support the hypothesis that the metabolic cost of breathing might be higher in birds than in other tetrapods due to the weight of the pectoral flight muscles (Markley and Carrier, 2010).

Andean goose

Among our species, the size of the respiratory system was largest in Andean geese. The respiratory system accounted for 44% of total body volume at maximum capacity and 24% at functional residual capacity. Andean geese also had the highest dynamic compliance and the lowest value of τ , indicative of rapid passive emptying of the system. The former would reduce the work required to overcome elastic forces during inspiration, while the latter would reduce the amount of active force required during expiration. Our calculations suggest it would be less work for Andean geese than for the other two species to ventilate large volumes and deliver more oxygen to the gas exchange surface per breath. This is consistent with their use of a slower, deeper (more effective) breathing pattern for an equal power output. Based on reported values of V_t and f_R for this species in normoxia and hypoxia (Lague et al., 2017), Andean geese increase ventilation only slightly (1.4-fold) from normoxia (14 kPa O_2) to

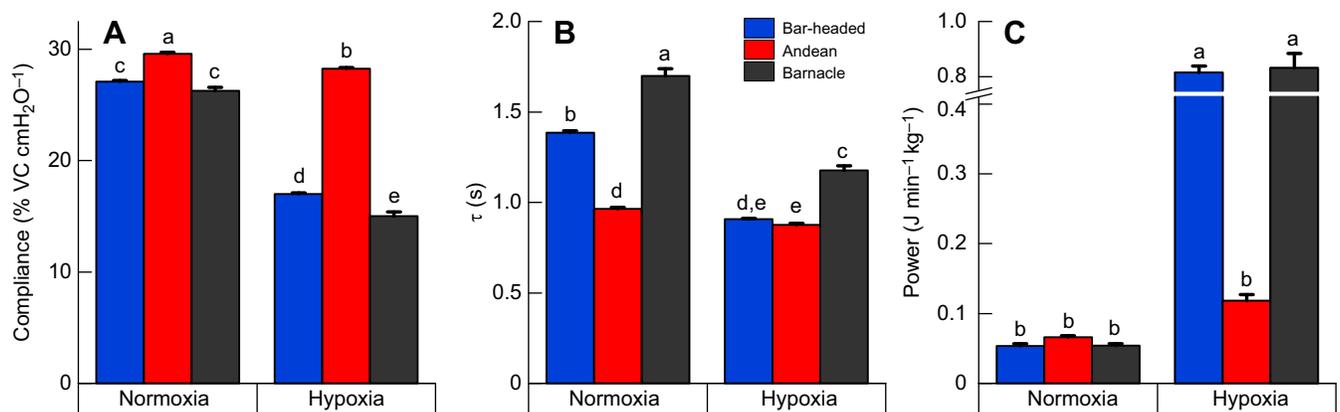


Fig. 6. Measurements and calculations of respiratory mechanics for f_R and V_t used by each species *in vivo* in normoxia and hypoxia. Normoxia is 21 kPa O_2 for bar-headed and barnacle geese and 14 kPa O_2 for Andean geese; hypoxia is 5 kPa O_2 for all three species. (A) Dynamic compliance expressed with V_t as a percentage of VC. (B) Time constant. (C) Power output. Lowercase letters indicate significant differences between groups (one-way ANOVA, $P<0.05$, *post hoc* Tukey test).

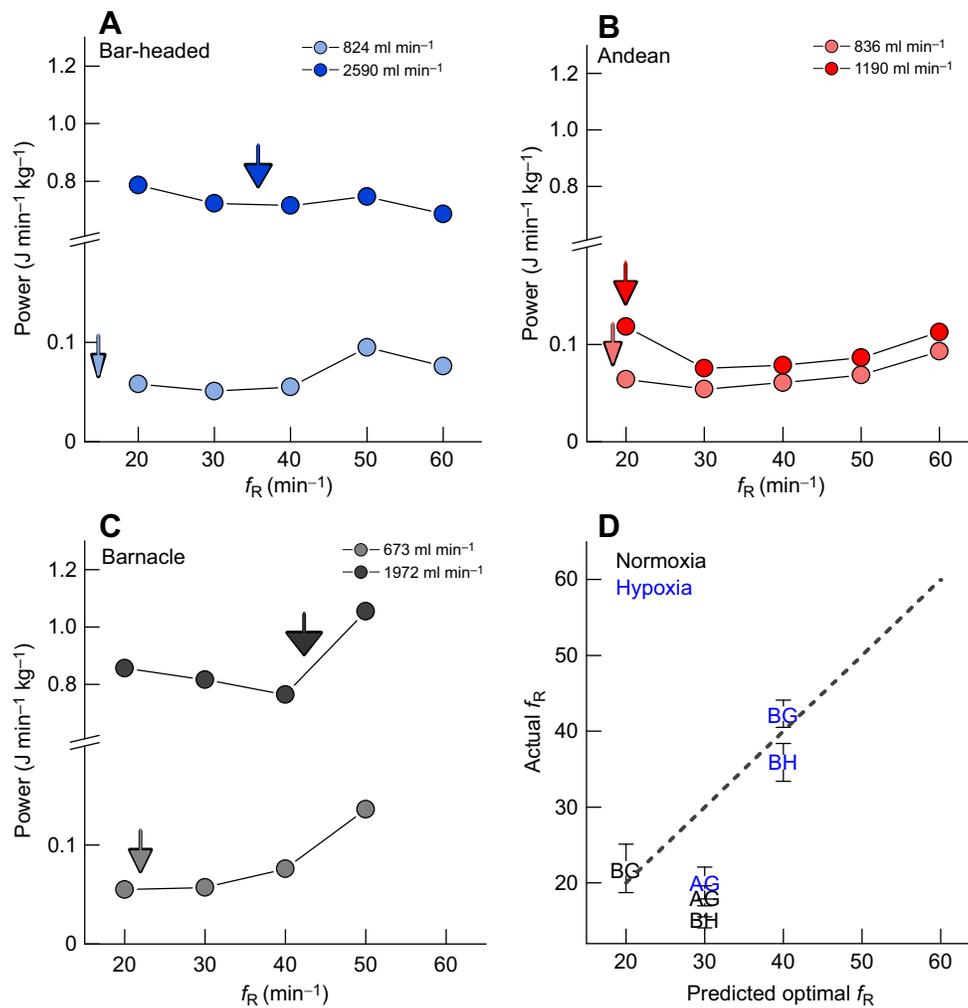


Fig. 7. Optimal combinations of V_t and f_R in vivo. (A–C) Solid lines indicate power curves for the constant levels of total ventilation each species used in normoxia (light colors; 21 kPa O_2 for bar-headed and barnacle geese, 14 kPa O_2 for Andean geese) and hypoxia (dark colors; 5 kPa O_2). The minute ventilation used by each species is indicated in the key for each plot. Arrows show the actual frequency used by each species (and hence also V_t); the width of each arrowhead is the s.e.m. (D) Comparison of actual frequency with the predicted optimal frequency in normoxia and hypoxia. The dashed line indicates where points should fall if birds are using optimal frequencies. BH, bar-headed goose; AG, Andean goose; BG, barnacle goose.

hypoxia (5 kPa O_2) and therefore, according to our calculations, do not significantly increase the power output required to breathe in hypoxia. This implies that O_2 delivery must have decreased by half in hypoxia and, to maintain resting metabolic rate, these geese must either increase oxygen extraction from the air they breathe or decrease oxygen demand in hypoxia. Lague et al. (2017) report that this species does not suppress metabolism but increases oxygen extraction to a large extent and a recent study shows that the mass-specific volume of the lung and the volume density of the gas exchange tissue in the lung of the Andean goose are particularly high. The respiratory surface area per unit body mass is the highest value so far reported in birds (Maina et al., 2017), which helps to explain their ability to extract more oxygen without increasing ventilation.

The Andean goose did not use the energetically optimal combination of f_R and V_t predicted by the U-shaped curves but instead appeared to use a slightly more expensive, deeper and slower breathing pattern that should increase oxygen delivery. Unfortunately, our measurements do not allow us to calculate the cost-benefit balance (the increase in O_2 delivered for the extra O_2 expended) of this strategy.

Barnacle goose

The barnacle goose had the smallest respiratory system, accounting for 39% of the total body volume at maximum capacity and 17% at functional residual capacity. Despite having a larger static compliance when normalized to VC, their respiratory system

Table 2. Cost of breathing estimates

Species	Normoxia						Hypoxia		
	21% O_2			14% O_2			5% O_2		
	BMR (J min ⁻¹ kg ⁻¹)	Work (J min ⁻¹ kg ⁻¹)	Cost (% BMR)	BMR (J min ⁻¹ kg ⁻¹)	Work (J min ⁻¹ kg ⁻¹)	Cost (% BMR)	BMR (J min ⁻¹ kg ⁻¹)	Work (J min ⁻¹ kg ⁻¹)	Cost (% BMR)
Bar-headed goose	276	0.054	0.38%				430	0.815	3.80%
Andean goose				195	0.066	0.68%	262	0.118	0.90%
Barnacle goose	183	0.071	0.78%				286	0.832	5.82%

Measured work of breathing ('Work') was multiplied by 20 to correct for active expiration and muscle efficiency. BMR (basal metabolic rate, in J) was calculated from the equation given by Romijn and Lokhorst (1966).

stiffened during dynamic inflation, yielding the lowest dynamic compliance. Thus, while the work required to overcome resistive forces was lowest under the normalized low V_t , low f_R condition in this species, it was the greatest under the high V_t condition. The time constant τ was consistently highest for the barnacle goose, suggesting they might require increased muscle work during expiration to achieve the same f_R as the other two species. The work to overcome elastic forces constituted the majority of the work required to breathe for barnacle geese, although, as noted in Materials and methods, we may be underestimating total resistive work due to the subtraction of the resistive work to move the air through the endotracheal tube (and thus the trachea).

Barnacle geese always used the optimal combination of V_t and f_R predicted by the curves. Based on our calculations, for the barnacle goose to increase ventilation 3-fold in hypoxia, the power output required would increase 18-fold.

Bar-headed goose

The size of the bar-headed goose respiratory system accounted for 46% of the total body volume at maximum capacity and 25% at functional residual capacity. They had the largest expiratory reserve volume (capacity to empty the lungs and air sacs from FRC during active expiration). This could be the result of a larger overall FRC or a very low residual volume. A greater capacity to empty the respiratory system for the bar-headed goose is consistent with the increased static compliance below FRC, a finding also seen in several other high-altitude waterfowl species (York et al., 2017; Fig. 3B). Our calculations suggest that bar-headed geese use an effective slow, deep breathing pattern in normoxia (Fig. 7A). However, our estimates indicated they increase power output 14-fold for a 3-fold increase in ventilation, only slightly less than the barnacle geese. This is consistent with our finding that bar-headed geese only use the optimal combination of V_t and f_R in hypoxia when O_2 is limited, while in normoxia they breathe with a more effective but more expensive pattern.

Sternal recumbency

In this study, we measured both respiratory mechanics and CT scan volumes while the birds rested in sternal recumbency (prone position). We chose sternal recumbency as it was found to be the position that least restricted lung and air sac volumes during CT scans in red-tailed hawks (Malka et al., 2009) and also because the respiratory and metabolic measurements taken from Lague et al. (2016) were done while the birds rested in sternal recumbency. However, during normal breathing, birds use a rocking motion of the sternum to expand the pleural cavity, and this may be restricted when the birds rest on their sternums. Tickle and colleagues (2010) observed that barnacle geese sat more often when carrying a sternal load as compared with a back or leg load. Tickle et al. (2012) found that standing was 25% more metabolically expensive than sitting for the barnacle goose, and they hypothesized that sitting allows for an energetically cheaper breathing strategy of costal expansion rather than sternal rocking. Therefore, our cost and work of breathing measurements may be more representative of sitting birds, and during standing, running or flight, the cost and work of breathing would be expected to be higher.

Air sac and lung volumes

Recent studies using mathematical modeling have suggested that efficient unidirectional airflow through the parabronchi requires in-phase air sac pressure changes, and that this timing is dependent on the relative compliance of the caudal and cranial air sacs

(Urushikubo et al., 2013; Harvey and Ben-Tal, 2016). From the current study, we know that compliance in the avian respiratory system is directly related to VC. Therefore, knowing the relative sizes of the caudal versus cranial air sacs informs our understanding of aerodynamic flow control through the avian respiratory system. If the caudal air sacs are more compliant than the cranial air sacs, the majority of flow through the lung occurs on expiration. Duncker (1972) compiled relative volume data for the air sacs of eight species from five avian families and found that the caudal air sacs (abdominal and posterior thoracic) had a greater combined volume than the combined cranial air sacs (anterior thoracic, clavicular and cervical). Our data also fit this pattern: in all three species, the caudal air sacs have a combined volume that is at least 19% larger (barnacle goose at rest) and up to 77% larger (bar-headed goose inflated) than the combined volume of the cranial air sacs. Indeed, the difference between species in overall air sac size is primarily due to the caudal air sacs, the migratory bar-headed goose having significantly larger inflated caudal air sacs than either the resident Andean goose or the barnacle goose (see CT scan images in Movie 1).

With the CT data, we also confirmed what was previously known from dissection studies: that Andean geese have very large lungs (Maina et al., 2017), more than twice the volume of the barnacle geese lungs. The lungs also change between 8% and 19% in volume between resting and maximum inflation (30 cm H_2O). This supports the idea of a fairly 'rigid' avian lung that changes relatively little in volume during normal breathing, although perhaps in some species it changes more than the previously measured maximal change of around 10% in volume (Jones et al., 1985; Ponganis et al., 2015).

Altitude and phylogeny

Our data are consistent with the hypothesis that the high-altitude bar-headed and Andean geese enhance gas exchange and reduce the work of breathing by increasing the size and compliance of the respiratory system relative to that of low-altitude species such as barnacle geese. However, we are not relating any of our findings to adaptation to altitude per se, nor are we attempting to correct our statistical comparisons for phylogeny. This is because we only compared three taxa in this study. The barnacle goose genus (*Branta*) is the sister taxon to the bar-headed goose genus (*Anser*), whereas the Andean goose is part of a genus of sheldgeese (*Chloephaga* spp.) more closely related to ducks (e.g. *Anas* spp.). Therefore, we cannot determine whether any differences measured here are due to phylogeny, adaptive evolution or simply chance. Moreover, in comparing only three species, we were not able to determine the effects of a bimodal condition such as altitude. We present the data here and merely speculate on how morphological and mechanical changes might be beneficial when birds are required by exercise or lack of oxygen to increase oxygen delivery. While our data are consistent with our hypothesis that high-altitude species have changed the morphology and mechanics of the respiratory system to reduce the work of breathing with a slow, deep breathing pattern, data from a previous study (York et al., 2017) indicate that while high-altitude ducks are larger than their low-altitude sister taxa, the respiratory system is not disproportionately larger and the total work of breathing is not consistently lower when normalized to the size of the respiratory system.

Finally, in this study, we compared wild-caught, captive-raised Andean geese with domesticated, captive-raised bar-headed and barnacle geese. Therefore, differences due to environmental and developmental plasticity may confound the data somewhat. Future studies should compare individuals of all three species raised at sea

level, or individuals that were all captured from the wild. This would allow investigation of the role of plasticity versus genotype, which has already been shown to affect respiratory responses in these species (Lague et al., 2016).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: W.K.M.; Methodology: J.M.Y., M.S., W.K.M.; Software: M.S.; Formal analysis: J.M.Y., M.S.; Investigation: J.M.Y., K.G.M., W.K.M.; Resources: K.G.M.; Data curation: J.M.Y.; Writing - original draft: J.M.Y.; Writing - review & editing: J.M.Y., M.S., K.G.M., W.K.M.; Visualization: J.M.Y., M.S.; Supervision: W.K.M.; Project administration: K.G.M., W.K.M.; Funding acquisition: J.M.Y., K.G.M., W.K.M.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.170738.supplemental>

References

- Akester, A. R.** (1960). The comparative anatomy of the respiratory pathways in the domestic fowl (*Gallus domesticus*), pigeon (*Columba livia*), and domestic duck (*Anas platyrhynchos*). *J. Anat.* **94**, 487-505.
- Black, C. P. and Tenney, S. M.** (1980). Oxygen-transport during progressive hypoxia in high-altitude and sea-level waterfowl. *Respir. Physiol.* **39**, 217-239.
- Butler, P. J., Woakes, A. J. and Bishop, C. M.** (1998). Behavior and physiology of Svalbard barnacle geese *Branta leucopsis* during their autumn migration. *J. Avian Biol.* **29**, 536-545.
- Dehner, E.** (1946). An apparatus for determining the respiratory volume of large aquatic birds. *Science* **103**, 171-172.
- Dubach, M.** (1981). Quantitative analysis of the respiratory system of the house sparrow, budgerigar and violet-eared hummingbird. *Respir. Physiol.* **46**, 43-60.
- Duncker, H. R.** (1971). *The Lung Air Sac System of Birds: A Contribution to the Functional Anatomy of the Respiratory Apparatus*. Berlin: Springer-Verlag.
- Duncker, H.-R.** (1972). Structure of avian lungs. *Respir. Physiol.* **14**, 44-63.
- Duncker, H. R.** (1977). Development of the avian respiratory and circulatory systems. In *Respiratory Function in Birds, Adult and Embryonic* (ed. J. Piiper), pp. 260-273. Berlin: Springer-Verlag.
- Dzal, Y. A., Jenkins, S. E. M., Lague, S. L., Reichert, M. N., York, J. M. and Pamerter, M. E.** (2015). Oxygen in demand: how oxygen has shaped vertebrate physiology. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **186**, 4-26.
- Ellerby, D. J., Henry, H. T., Carr, J. A., Buchanan, C. I. and Marsh, R. L.** (2005). Blood flow in guinea fowl *Numida meleagris* as an indicator of energy expenditure by individual muscles during walking and running. *J. Physiol.* **564**, 631-648.
- Gumpenberger, M. and Henninger, W.** (2001). The use of computed tomography in avian and reptile medicine. *Semin. Avian Exotic Pet Med.* **10**, 174-180.
- Harvey, E. P. and Ben-Tal, A.** (2016). Robust unidirectional airflow through avian lungs: new insights from a piecewise linear mathematical model. *PLoS Comput. Biol.* **12**, e1004637.
- Hawkes, L. A., Balachandran, S., Batbayar, N., Butler, P. J., Chua, B., Douglas, D. C., Frappell, P. B., Hou, Y., Milsom, W. K., Newman, S. H. et al.** (2012). The paradox of extreme high-altitude migration in bar-headed geese *Anser indicus*. *Proc. Biol. Sci.* **280**, 20122114.
- Jaensch, S. M., Cullen, L. and Raidal, S. R.** (2002). Air sac functional anatomy of the sulphur-crested cockatoo (*Cacatua galerita*) during isoflurane anesthesia. *J. Avian Med. Surg.* **16**, 2-9.
- Jones, J. H., Effmann, E. L. and Schmidt-Nielsen, K.** (1985). Lung volume changes during respiration in ducks. *Respir. Physiol.* **59**, 15-25.
- Jonker, R. M., Kraus, R. H. S., Zhang, Q., van Hooft, P., Larsson, K., van der Jeugd, H. P., Kurvers, R. H. J. M., van Wieren, S. E., Loonen, M. J. J. E., Crooijmans, R. P. M. A. et al.** (2013). Genetic consequences of breaking migratory traditions in barnacle geese *Branta leucopsis*. *Mol. Ecol.* **22**, 5835-5847.
- King, A. S. and Payne, D. C.** (1962). The maximum capacities of the lungs and air sacs of *Gallus domesticus*. *J. Anat.* **96**, 495-508.
- Krautwald-Junghanns, M.-E., Schumacher, F. and Tellhelm, B.** (1993). Evaluation of the lower respiratory tract in psittacines using radiology and computer tomography. *Vet. Radiol. Ultrasound* **34**, 382-390.
- Krautwald-Junghanns, M.-E., Valerius, K.-P., Duncker, H.-R. and Sohn, H.-G.** (1998). CT-assisted versus silicone rubber cast morphometry of the lower respiratory tract in healthy amazons (genus *Amazona*) and grey parrots (genus *Psittacus*). *Res. Vet. Sci.* **65**, 17-22.
- Lague, S. L., Chua, B., Farrell, A. P., Wang, Y. and Milsom, W. K.** (2016). Altitude matters: differences in cardiovascular and respiratory responses to hypoxia in bar-headed geese reared at high and low altitudes. *J. Exp. Biol.* **219**, 1974-1984.
- Lague, S. L., Chua, B., Alza, L., Scott, G. R., Frappell, P. B., Zhong, Y., Farrell, A. P., McCracken, K. G., Wang, Y. and Milsom, W. K.** (2017). Divergent respiratory and cardiovascular responses to hypoxia in bar-headed geese and Andean birds. *J. Exp. Biol.* **220**, 4186-4194.
- Lee, S. Y. and Milsom, W. K.** (2016). The metabolic cost of breathing in red-eared sliders: an attempt to resolve an old controversy. *Respir. Physiol. Neurobiol.* **224**, 114-124.
- Lumb, A. B.** (2017). *Nunn's Applied Respiratory Physiology*. Toronto: Elsevier.
- Maina, J. N.** (2005). *The Lung-Air Sac System of Birds: Development, Structure, and Function*. Berlin: Springer-Verlag.
- Maina, J. N., McCracken, K. G., Chua, B., York, J. M. and Milsom, W. K.** (2017). Morphological and morphometric specialization of the lung of the Andean goose, *Chloephaga melanoptera*: a lifelong high-altitude resident. *PLoS ONE* **12**, e0174395.
- Malka, S., Hawkins, M. G., Jones, J. H., Pascoe, P. J., Kass, P. H. and Wisner, E. R.** (2009). Effect of body position on respiratory system volumes in anesthetized red-tailed hawks (*Buteo jamaicensis*) as measured via computed tomography. *Am. J. Vet. Res.* **70**, 1155-1160.
- Markley, J. S. and Carrier, D. R.** (2010). The cost of ventilation in birds measured via unidirectional artificial ventilation. *Comp. Biochem. Physiol. A* **155**, 146-153.
- McCracken, K. G., Barger, C. P. and Sorenson, M. D.** (2010). Phylogenetic and structural analysis of the HbA ($\alpha^2\beta^2$) and HbD ($\alpha^2\beta^1\delta^1$) hemoglobin genes in two high-altitude waterfowl from the Himalayas and the Andes: bar-headed goose (*Anser indicus*) and Andean goose (*Chloephaga melanoptera*). *Mol. Phylogenet. Evol.* **56**, 649-658.
- Mulcahy, D. M.** (2007). Free-living waterfowl and shorebirds. In *Zoo Animal and Wildlife Immobilization and Anesthesia* (ed. G. West, D. Heard and N. Caulkett), Vol. 2, pp. 299-324. Chichester: Wiley.
- Natarajan, C., Projecto-Garcia, J., Moriyama, H., Weber, R. E., Muñoz-Fuentes, V., Green, A. J., Kopuchian, C., Tubaro, P. L., Alza, L., Bulgarella, M. et al.** (2015). Convergent evolution of hemoglobin function in high-altitude Andean waterfowl involves limited parallelism at the molecular sequence level. *PLoS Genet.* **11**, e1005681.
- Newell, S. M., Roberts, G. D. and Bennett, R. A.** (1997). Imaging techniques for avian lower respiratory diseases. *Semin. Avian Exot. Pet Med.* **6**, 180-186.
- Orosz, S. E. and Toal, R. L.** (1992). Tomographic anatomy of the golden eagle (*Aquila chrysaetos*). *J. Zoo Wildl. Med.* **23**, 39-46.
- Otis, A. B.** (1954). The work of breathing. *Physiol. Rev.* **34**, 449-458.
- Otis, A. B., Fenn, W. O. and Hermann, R.** (1950). Mechanics of breathing in man. *J. Appl. Physiol.* **2**, 592-607.
- Petnehazy, O., Benczik, J., Takacs, I., Petras, Z., Sütö, Z., Horn, P. and Repa, I.** (2012). Computed tomographical (CT) anatomy of the thoracoabdominal cavity of the male turkey (*Meleagris gallopavo*). *Anat. Histol. Embryol.* **41**, 12-20.
- Ponganis, P. J., St Leger, J. and Scadeng, M.** (2015). Penguin lungs and air sacs: implications for baroprotection, oxygen stores and buoyancy. *J. Exp. Biol.* **218**: 720-730.
- Romijn, C. and Lokhorst, W.** (1966). Heat regulation and energy metabolism in the domestic fowl. In *Physiology of the Domestic Fowl* (ed. C. Horton-Smith and E.C. Amoroso), pp. 211-227. Edinburgh: Oliver & Boyd Ltd.
- Scheid, P. and Piiper, J.** (1969). Volume, ventilation, and compliance of the respiratory system in the domestic fowl. *Respir. Physiol.* **6**, 298-308.
- Scott, G. R. and Milsom, W. K.** (2007). Control of breathing and adaptation to high altitude in the bar-headed goose. *Am. J. Regul. Integr. Comp. Physiol.* **293**, R379-R391.
- Scott, G. R., Schulte, P. M., Egginton, S., Scott, A. L. M., Richards, J. G. and Milsom, W. K.** (2011). Molecular evolution of cytochrome c oxidase underlies high-altitude adaptation in the bar-headed goose. *Mol. Biol. Evol.* **28**, 351-363.
- Scott, G. R., Hawkes, L. A., Frappell, P. B., Butler, P. J., Bishop, C. M. and Milsom, W. K.** (2015). How bar-headed geese fly over the Himalayas. *Physiology* **30**, 107-115.
- Storz, J. F. and Moriyama, H.** (2008). Mechanisms of hemoglobin adaptation to high altitude hypoxia. *High Alt. Med. Biol.* **9**, 148-157.
- Swan, L. W.** (1961). The ecology of the high Himalayas. *Sci. Am.* **205**, 68-78.
- Thomas, S. P.** (1975). Metabolism during flight in two species of bats, *Phyllostomus hastatus* and *Pteropus gouldii*. *J. Exp. Biol.* **63**, 273-293.

- Tickle, P. G., Richardson, M. F. and Codd, J. R.** (2010). Load carrying during locomotion in the barnacle goose (*Branta leucopsis*): the effect of load placement and size. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **156**, 309-317.
- Tickle, P. G., Nudds, R. L. and Codd, J. R.** (2012). Barnacle geese achieve significant energy savings by changing posture. *PLoS One* **7**, e46950.
- Urushikubo, A., Nakamura, M. and Hirahara, H.** (2013). Effects of air sac compliances on flow in the parabronchi: computational fluid dynamics using an anatomically simplified model of an avian respiratory system. *J. Biomed. Sci. Eng.* **6**, 483-492.
- Ward, S., Bishop, C. M., Woakes, A. J. and Butler, P. J.** (2002). Heart rate and the rate of oxygen consumption of flying and walking barnacle geese (*Branta leucopsis*) and bar-headed geese (*Anser indicus*). *J. Exp. Biol.* **205**, 3347-3356.
- Withers, P. C., Cooper, C. E., Maloney, S. K., Bozinovic, F. and Cruz-Neto, A. P.** (2016). Physiological adaptations to extreme environments. In: *Ecological and Environmental Physiology of Mammals*. Oxford: Oxford University Press.
- York, J. M., Chua, B. A., Ivy, C. A., Alza, L., Cheek, R., Scott, G. R., McCracken, K. G., Frappell, P. B., Dawson, N. J., Laguë, S. L. et al.** (2017). Respiratory mechanics of eleven avian species resident at high and low altitude. *J. Exp. Biol.* **220**, 1079-1089.
- Zeuthen, E.** (1942). The ventilation of the respiratory tract in birds. *Det Kongelige Danske Videnskabernes Selskab.* **17**, 1-50.