

RESPIRATORY MECHANICS OF HIGH ALTITUDE WATERFOWL

by

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ABSTRACT

Birds living at high altitude (>3,000 meters) are not only able to cope with reduced oxygen availability due to hypobaria, but they are also able to achieve one of the most metabolically costly form of locomotion at these altitudes: flight. To perform such a metabolically demanding activity, in addition to energetically expensive daily tasks such as foraging, predator escape, and reproduction, all in oxygen limited (hypoxic) conditions, means that high altitude birds must enhance oxygen supply to maintain oxygen homeostasis. The primary means of increasing oxygen supply is increasing ventilation of the respiratory gas exchange surface. However, the metabolic cost of ventilation is unknown for birds at rest, as is whether this cost varies among bird species. In this thesis, the cost and work of breathing are compared in fourteen avian species to determine whether variation in the work of breathing is due to mechanical or morphological changes in the respiratory system, and if any observed changes are associated with living at high altitude. High altitude birds tended to have large and compliant respiratory systems compared with low altitude taxa, which reduces the work of breathing. However, the evidence also suggests that respiratory morphology and mechanics in birds may be more constrained by life history strategy than by evolutionary time at altitude, although species in this study that have no high altitude sister taxa (their lineages have never radiated to high altitude) struggled the most with increasing oxygen supply. Finally, birds at rest were estimated to have a lower cost of breathing than mammals, contrary to the hypothesis that cost of breathing would be high in birds due to the heavy flight muscles weighing down the sternum.

PREFACE

This thesis is the original intellectual product of the author, J. York, under the supervision of W.K. Milsom. 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 in accordance with Oregon Department of Fish and Wildlife Scientific Taking Permit 101-15 and el Ministerio del Ambiente de Perú carta numero 005-2014 y resolución directoral numero 36087-2012.

The data chapters in this thesis (chapters 2 and 3) are being prepared to submit for publication. The contributions of the listed authors are as follows:

Chapter 2:

JMY collected and analyzed the data, prepared the figures, and wrote the manuscript.
MS analyzed the CT scan data.
WKM assisted with data collection and manuscript revisions.

Chapter 3:

JMY collected and analyzed the data, prepared the figures, and wrote the manuscript.
BAC assisted in animal care during experiments.
CMI and SLL provided hypoxic ventilatory response data.
LA and RC assisted with animal capture and care.
GRS, NJD, and PBF assisted with field work.
KGM assisted with field work and provided phylogenetic data.
WKM assisted with data collection and manuscript revisions.

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LIST OF SYMBOLS AND ABBREVIATIONS

BMR- basal metabolic rate
bpm- breaths per minute
C- compliance
cm H₂O- centimeters of water
CT- computerized tomography
f_R- breathing frequency
FRC-functional residual capacity
I- inertance
IAV- inspiratory aerodynamic valving
L- liters
min- minute
mL- milliliters
P- pressure
R- resistance
TLS- trilaminar substance
V- volume
 \dot{V} - flow
 \ddot{V} - acceleration
VC- vital capacity
 \dot{V}_{eff} - effective ventilation
V_t- tidal volume

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Chapter 1

Introduction

The avian respiratory system evolved from the earliest true lung that originated in the common ancestor of sarcopterygians and tetrapods. The ancestral vertebrate lung was derived from the respiratory pharynx of basal fishes and was probably paired, tubular, and lacked cartilaginous airways (Perry and Sander, 2004). The complex extant avian lung is small in volume, but has an extremely high surface area; it does not expand or contract during normal breathing but is instead ventilated by compartmentalized air sacs. This allows unidirectional air flow across the gas exchange surface and (mostly) crosscurrent blood flow (Maina, 2005). How and why did this complex system arise? The earliest amniotes had chambered lungs with a cartilaginous bronchus, and they moved away from ancestral buccal respiration and began to use aspiration breathing, although these methods are not mutually exclusive (Perry, 1998; Brainerd and Owerkowicz, 2006). This required switching the respiratory muscles from the cranial and hypobranchial to the axial and abdominal muscles (Jenkin and Milsom, 2014). The common ancestor of saurischinan dinosaurs had postcranial pneumaticity suggesting an avian-like lung where the ventilation and gas exchange portions of the respiratory system were spatially distinct (Wedel, 2006; Wedel, 2009; Bensen et al., 2012). Saurischinan dinosaur lungs were heterogeneous and had asymmetrical branches, indicating that they may have had unidirectional airflow allowing crosscurrent gas exchange (Wedel, 2003; O'Connor, 2006). This ancestral group then branched into the sauropod dinosaurs, who have been hypothesized to have used this efficient gas exchange strategy to achieve their massive body size, and the theropod dinosaurs,

who, instead of increasing in size, became bipedal, very fast, and eventually began true, flapping flight (Sereno, 1999; Perry and Sander, 2004; Claessens et al., 2009; Sander et al., 2011). The evolutionary origin of vertebrate flight may have been enabled by the release of respiratory constraints allowed by unidirectional ventilation and crosscurrent gas exchange. This is a matter of some recent debate due to evidence suggesting unidirectional airflow may also occur in extant crocodylians and non-avian sauropsids, and may therefore be an ancestral trait for all diapsids (Cieri and Farmer, 2016).

The function of the extant avian respiratory system is complex and not yet entirely understood. Airflow begins in the trachea, which has complete cartilaginous rings, and can be very long (2.7-fold longer than in a mammal of a similar size) because the neck of most birds functions in reaching and grabbing like the forelimb of other tetrapods (Hinds and Calder, 1971). The trachea divides at the syrinx into the primary bronchi leading to the lungs. The air does not enter the first set of secondary bronchi that arise within the lungs (ventrobronchi), but instead is aerodynamically directed past them to the abdominal and posterior thoracic air sacs by variable resistance and relative angle of the primary and secondary bronchi. In some species, a constriction called the *segmentum accelerans* positioned just before the ventral secondary bronchi increases the efficiency of this aerodynamic valving (see Figure 1.1; Wang et al., 1992). This aerodynamic flow control is termed “inspiratory aerodynamic valving.” On exhalation, the air enters the lung via the dorsal secondary bronchi (dorsobronchi). The lung consists of unidirectional air passages called parabronchi (tertiary bronchi); off these passages branch the spherical, anastomosing air capillaries in very close association

with cross-hatching networks of blood capillaries (Maina, 2015). The blood capillaries are very rigid, and the shape of the air capillaries is maintained by surface tension, the avian-specific surfactant trilaminar substance (TLS), and the nearly constant flow of air through the lung (Bernhard et al., 2001). The strength of these structures, along with the extremely high surface area and lack of pulmonary dead space makes the lung as a whole quite rigid, especially compared to the lungs of mammals or non-avian sauropsids. Between inspiration and expiration, the avian lung changes only 1-10% in volume (Jones et al., 1985; Ponganis et al., 2015). This allows the blood-gas barrier to be extremely thin, directly increasing the rate of oxygen and carbon dioxide diffusion (Perry, 1990; Watson et al., 2007). On subsequent inhalation, the air in the parabronchi is pulled from the lung into the anterior air sacs and then exhaled out the trachea, although the “expiratory dynamic valving” by which this occurs is not yet understood (Maina, 2015). In mammals, the lung must act as both the ventilator and the gas exchange surface; thus the structure of the lung is a compromise constrained by both functions. In birds, the division of the ventilator and the gas exchanger into spatially separate structures relieves each from the functional constraints of the other and increases the efficiency of both ventilation and gas exchange.

No matter how efficient, breathing is dynamic work. The intensity of the work is determined by the specific morphology and mechanics of the individual respiratory system. In birds, the work of breathing is done primarily by the external oblique and the appendicocostalis muscles, which are almost exclusively respiratory muscles (Klein and Codd, 2010). This is in contrast to mammals and non-avian sauropsids, whose

abdominal and intercostal muscles have both a respiratory and a locomotor function. Mammals use their diaphragm and intercostal muscles to create negative pressure in the pleural cavity, thereby actively inflating the lung. The exhalation, particularly at rest, is accomplished passively by the elastic recoil of the lung and chest wall. In birds, expiration is powered by a combination of elastic recoil of the air sacs and chest wall and by active contraction of the external oblique muscles (Codd et al., 2005). Birds have also been reported to use their pelvic and tail muscles during expiration, although this is a matter of some debate (Baumel et al., 1990; Claessens, 2009; Klein and Codd, 2010). How can a costal muscle power air sac expansion all the way from the cervical to the caudal end of the bird? The avian sternum hinges with each sternal rib allowing motion of the posterior end of the sternum, which expands and contracts the abdominal cavity (Hillenius and Ruben, 2004). Birds also have unciniate processes on the ribs, which enhance this sternal rotation, help to lower the sternum on inspiration, and brace the dorsal sternum during expiration (Codd, 2010).

For birds, the work to move the sternum during respiration is non-trivial. The flight muscles originate on the ventral side of the sternum and average 17% of total body mass (Dial et al., 1991; Greenewalt, 1962). When weight is added to the sternum (also called “sternal loading”) barnacle geese increase metabolic rate 2.3% for every percent body mass loaded, in contrast to adding weight to the back of the bird which increases metabolic rate 0.95% for every percent body weight loaded (Tickle et al., 2010). This has implications for flight muscle hypertrophy in birds preparing for migration (Portugal et al., 2009). Due to high load on this critically important sternal movement, cost of

breathing has been hypothesized to be higher in birds relative to other tetrapods, but metabolic cost of breathing has only been estimated in one bird species: the helmeted guinea fowl (*Numida meleagris*; Ellerby et al., 2005; Markley and Carrier, 2010).

Markley and Carrier (2010) measured oxygen consumption during rest and running, and then artificially ventilated birds with a cannula inserted into the caudal air sac until spontaneous ventilation ceased. The difference in oxygen consumption between the ventilating and non-ventilating birds was assumed to be the cost of breathing. Oddly, Markley and Carrier found, at rest, oxygen consumption increased in the non-ventilating as compared to the ventilating birds. They hypothesized that increased CO₂ loss during artificial ventilation caused a pH challenge that required active compensation.

Regardless, they were unable to estimate the cost of breathing at rest, but estimated that, during running, respiration accounted for 1.4-3.6% of running metabolic rate, or 0.5-1.5 mL O₂ min⁻¹ kg⁻¹. This is within the range of the cost of breathing reported for mammals and somewhat lower than values reported for non-avian sauropsids. They concluded, “Measuring [the metabolic] cost of ventilation is confounded by the manipulation of ventilation rate... the cost of ventilation may be irresolvable.”

In this thesis, we measured the cost of breathing in fourteen bird species by measuring the work done to ventilate anesthetized birds with known volumes and frequencies, and then applying these work values to the volumes and breathing frequencies measured in the same individuals at rest, before anesthesia, in both normoxia and hypoxia. To translate these measured mechanical “work” values to metabolic “cost” values, we needed to assume a metabolic efficiency for the respiratory muscles. We also relate the

work of breathing to the mechanical and morphological properties of each respiratory system. The primary goal of this thesis was not only to investigate respiratory morphology, respiratory mechanics, and work of breathing for birds in general, but to consider whether these characteristics vary between bird species as a function of altitude. Recent studies suggest that birds that live or fly at altitude breathe more slowly and deeper than related low altitude species, a breathing pattern that is more effective for gas exchange but one that may also be more expensive given the costs of sternal loading (Scott and Milsom, 2007; Lague et al., submitted). How could a more metabolically expensive breathing pattern be maintained when oxygen is limited by reduced atmospheric pressure?

As Johan Steen (1971) put it, “Of the naturally occurring hypoxic conditions, life at high altitude is the most permanent.” We hypothesized that birds living under high altitude oxygen-limited conditions would exhibit mechanical and morphological changes to the respiratory system that reduce the cost of breathing in general, and support the differences in breathing pattern in birds living at high altitude compared to birds living at low altitude. In the first chapter, we compare three species of geese of similar body size: a low altitude resident, a high altitude transient, and a high altitude resident to investigate how the morphology of the respiratory system differs using computerized tomography (CT) scans. In the second chapter, we compare eleven species of ducks, including four pairs of sister taxa that serve as four phylogenetically independent comparisons of the effect of altitude on avian respiratory mechanics.

Definitions

The field of pulmonary mechanics has its own language; the following is a short review of commonly measured variables. The respiratory muscles do work to overcome the combination of elastic and resistive forces exerted by the rest of the respiratory system. These forces are determined by the individual mechanical and morphological properties of the chest wall, lungs, airways, and, in birds, the air sacs. The work done by the muscles must be equal and opposite to the forces applied by these other components of the respiratory system, such that the equation of motion for breathing is described by:

$$P = \left(\frac{1}{C}\right)V + R\dot{V} + I\ddot{V}$$

Where P is pressure, C is compliance, R is resistance, I is inertance, and V, \dot{V} , and \ddot{V} are volume, flow, and acceleration, respectively. The three components of this equation reflecting the mechanical properties of the respiratory system are compliance, resistance, and inertance. Inertance accounts for the kinetic energy stored by the acceleration of gases through the airway. However, unlike locomotory muscles, the work done by the respiratory muscles to overcome inertance is vanishingly small. The elastic and flow resistive forces, determined by the coefficients compliance and resistance, account for most of the work done by the respiratory muscles and thus the negligible contribution of inertance and acceleration are ignored.

Compliance

In the equation of movement for breathing, the inverse of compliance (also called elastance) is multiplied by the volume, and this is the elastic (potential) energy component of the equation. Thus, if the system is stiff, compliance is low, elastance is

high, and the elastic energy portion of the breath will be high. Compliance can be measured as either static or dynamic compliance.

Static compliance is a measurement of the stiffness of the respiratory system, calculated as the change produced in volume for a given change in pressure under conditions of no flow. Compliance has the units of $\text{mL cm H}_2\text{O}^{-1}$. Thus an animal with a higher compliance has a greater volume change for a given pressure change, or a less stiff/more compliant system. Here we report static compliance values over the linear, most physiologically relevant portion of the curve, from a pressure of 5 cm H₂O to -5 cm H₂O from functional residual capacity (FRC).

Dynamic compliance is a measurement of the stiffness and elasticity of the respiratory system as the system is dynamically changing. Dynamic compliance is different from static compliance because it is affected by elastic stiffening of the system with the addition of a flow to the volume change. As the frequency or volume of each breath increases, it requires a greater pressure change to achieve a given volume, and therefore the compliance of the system is reduced. Dynamic compliance is calculated as the slope of the line that connects the two points of zero flow on the pressure-volume loop. When flow is zero, the flow-resistive pressure is zero and therefore the elastic pressure is the only contributor to the total pressure. Thus our measurement of elasticity and stiffness is the change in volume divided by the change in pressure between the two instances of zero flow.

Resistance

Resistance is a measurement of the frictional heat energy of viscous fluid flow through the airways. The units are $\text{cm H}_2\text{O mL}^{-1} \text{min}^{-1}$. It is measured as the difference in pressure between two points of isovolume on the pressure-volume loop, divided by the flow (Mead, 1961). Resistance will be very high if the diameter of the airways is very small relative to the flow, and therefore the pressure difference between the air entering the animal and leaving to the atmosphere is very large.

Tau

The time constant (τ) is calculated as the product of compliance and resistance. It is a measurement of the time (in units of seconds) it takes for air to passively leave the system following inspiration. It combines both the elastic and flow resistive properties of the respiratory system, but does not take into account muscular work done by birds during normal expiration.

Respiratory system volumes

The various volumes that can be measured from the *in vivo* respiratory system are shown in Figure 1.2. All volumes are measured beginning at functional residual capacity (FRC), which is the volume of air in the respiratory system when the glottis is open to the atmosphere and the respiratory muscles are relaxed. The inspiratory capacity is the potential volume of air that could be added to the respiratory system from FRC, the expiratory reserve volume is the volume of air that could be removed from the system from FRC, and the vital capacity is the sum of the inspiratory capacity and expiratory

reserve volume, or the entire volume that could potentially be utilized by the animal. The reserve capacity is the volume of air that cannot be removed from the respiratory system; this can be measured with inert gas washout techniques, but was not measured in this study.

Effective ventilation, efficacy, and optimal breathing

The total ventilation (or minute ventilation; \dot{V}) is the product of breathing frequency (f_R) and tidal volume (V_t). It includes the entire volume of air that the animal expends energy to move from the environment into and out of the respiratory system. However, not all of that air actually reaches the gas exchange surface due to the presence of a dead space volume. Therefore, the effective ventilation (\dot{V}_{eff}) is the amount of air per unit time that comes into contact with the gas exchange surface. In this thesis, we use the term “effective” specifically to refer to the amount of effective ventilation. We also express the effective ventilation per unit energy, and we term this the “efficacy” of breathing. Optimal breathing (or the optimal combination) is the combination of V_t and f_R that minimizes the work of breathing (the least metabolically expensive combination of V_t/f_R for a given minute ventilation).

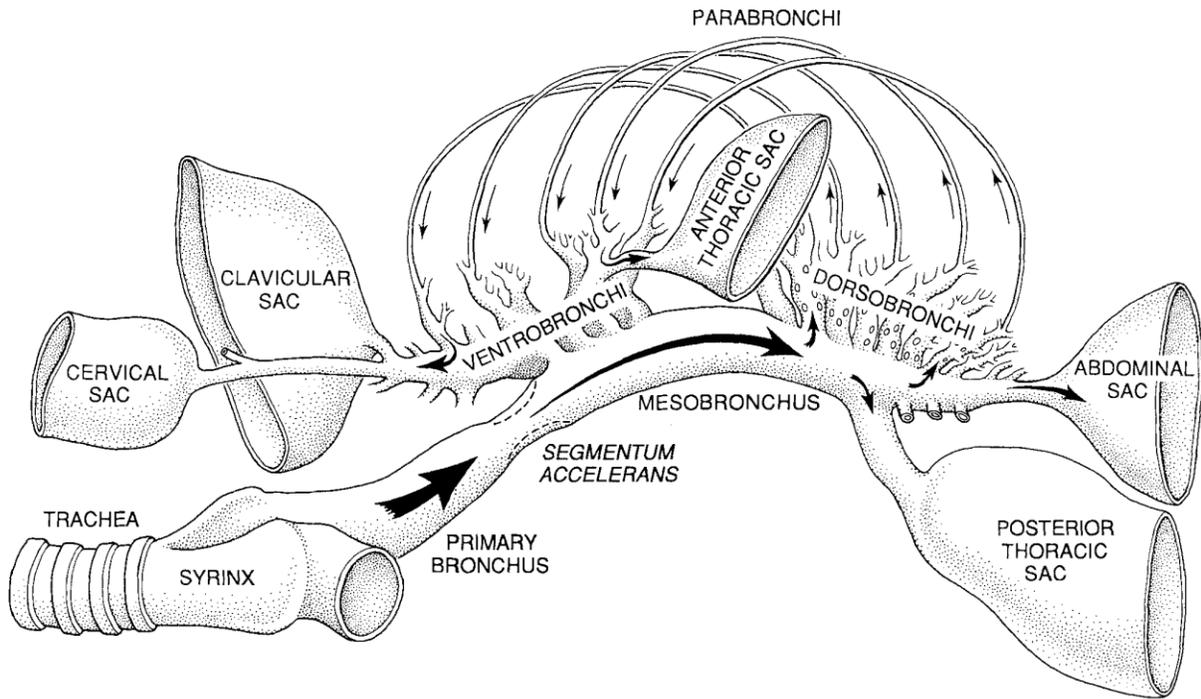


Figure 1.1: Flow of air in the avian respiratory system. Inspiratory air flow (black arrows) is controlled through inspiratory aerodynamic valving (IAV). [Figure from Wang et al., 1992].

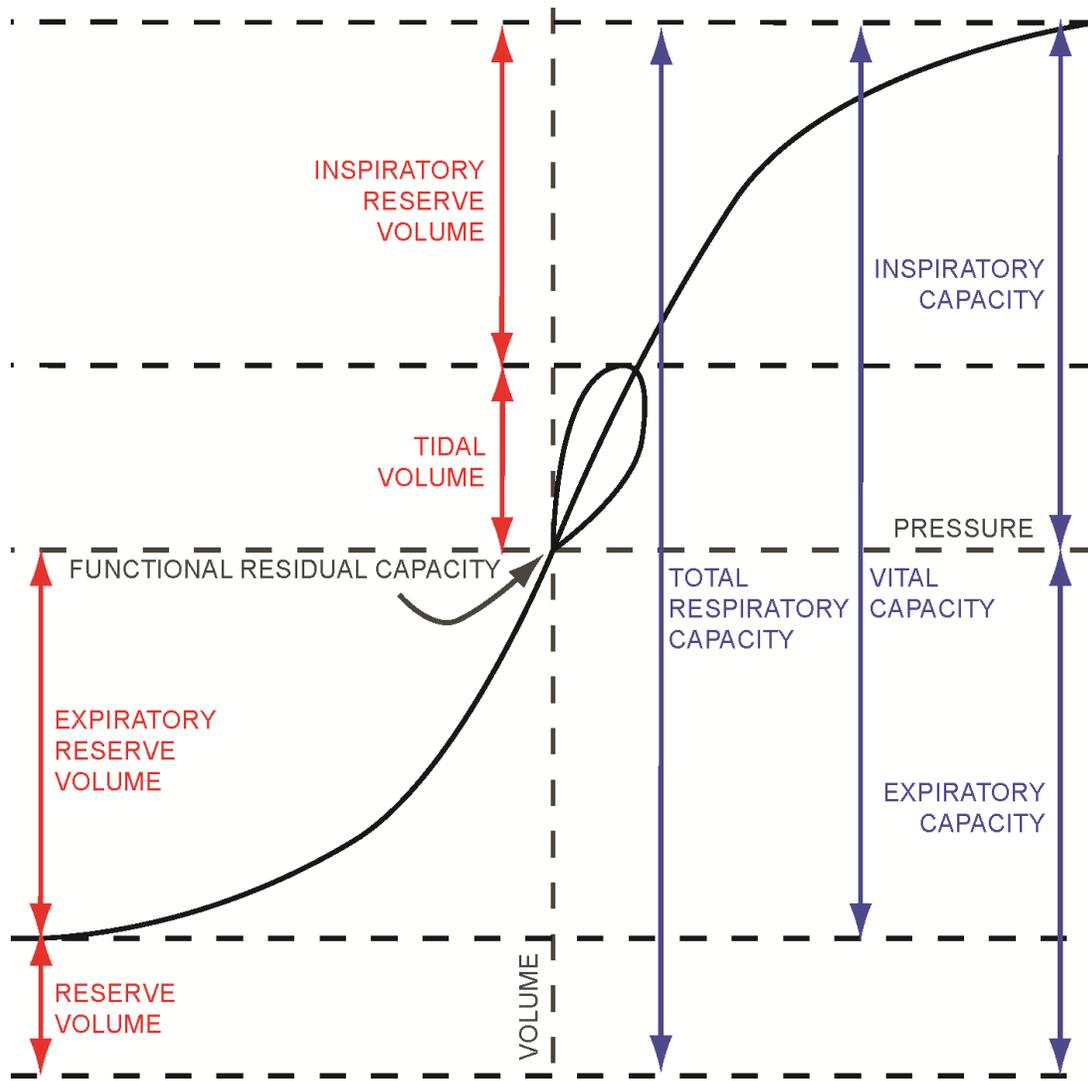


Figure 1.2: Volumes and capacities of the respiratory system. The loop at the center of the figure is the pressure-volume loop of one breath. The solid line represents the volume change over the entire vital capacity.

Chapter 2

Respiratory mechanics, morphometrics, and cost of breathing of the bar-headed goose (*Anser indicus*), barnacle goose (*Branta leucopsis*), and Andean goose (*Chloephaga melanoptera*)

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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 form of locomotion, 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). A complete understanding of the respiratory adaptations that might enable cardiorespiratory performance, however, requires knowledge of the volumes of the different components of the respiratory system as well as of their mechanical properties.

Bird lungs lie flush against the dorsal thoracic ribs and back body; they are relatively small and rigid, expanding and contracting very little during normal breath cycles (Jones et al., 1985; Ponganis et al., 2015). The air sacs are extensive, branching from the bronchi in the lungs and expand to fill the body of the bird from the top of the hind limbs to the lower part of the neck and are continuous with the pneumatic spaces of the vertebrae and long bones (Duncker, 1971). They 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 volume because they are immediately ruptured by any invasive procedure to the chest or abdomen.

Previous studies have attempted to use terminal methods to study air sac morphology, such as Zeuthen (1942), who strangled chickens on inspiration, froze them, and filled the respiratory system with paraffin. Many studies used a similar method of filling the respiratory system with resin, silicone, or latex (Akester, 1960; King and Payne, 1962; Duncker, 1972; Duncker, 1977; Dubach, 1981; Jaensch et al., 2002). These fluid filling techniques are generally only able to estimate maximum respiratory volumes, are terminal, and are subject to material shrinkage. Other studies have used inert gas washout techniques to estimate volumes (Dehner, 1946; Schied 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 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; Gumpfenberger 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*), the high altitude resident Andean goose (*Chloephaga melanoptera*), and the low altitude resident barnacle goose (*Branta leucopsis*).

In the wild, bar-headed and barnacle geese migrate in the fall 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). 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.

The bar-headed goose migrates primarily at high altitudes, over the Himalayas between their breeding grounds in northern China and Mongolia (at about 2,300 meters) and their wintering grounds in India (sea level; Hawkes et al., 2012). While a previous anecdotal account (Swan, 1961) of bar-headed geese flying over Makalu (8,485 meters) is frequently cited as their ability to fly over Mount Everest (8,848 meters), more recent research using satellite transmitters suggests that bar-headed geese primarily fly through the passes (generally 5,000 meters) and reach a maximum altitude of about 7,200 meters (Hawkes et al., 2012). This is still an impressive feat, as the oxygen available at these altitudes is about half that of sea level, and the bar-headed goose

increases oxygen demand 15 to 20-fold in flight versus rest (Meir et al., unpublished). Andean geese are residents of the Andes in western South America. They do not migrate but spend their entire lives in wetlands at altitudes greater than 3,000 meters (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).

Amongst the physiological traits of both Andean and bar-headed geese that underlie their high altitude success (see Scott et al., 2015 and Dzal et al., 2015 for review) are increased lung vascularity and lung mass relatively to body size (Scott et al., 2011; Maina et al., unpublished), hemoglobin with an increased oxygen affinity (McCracken et al., 2010), and, in bar-headed geese, an ability to increase ventilation to a greater extent than other species, especially by increasing tidal volume (Black and Tenney, 1980; Scott and Milsom, 2007; Lague et al., submitted). As a ventilation strategy, increasing tidal volume is generally more metabolically expensive than increasing breathing frequency (Milsom and Vitalis, 1984; Vitalis and Milsom, 1986), but it is more effective as the effective ventilation is increased.

In the present study, we measured the lung mass, air sac volumes, and pulmonary mechanics of the three species. We hypothesized that bar-headed and Andean geese would have large respiratory systems 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 relative to barnacle geese.

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 all three 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 3,180 meters and were two years old. Mean body masses of bar-headed geese were 2.77 ± 0.14 kg, barnacle geese were 2.38 ± 1.6 kg, and Andean geese were 2.29 ± 0.15 kg (mean \pm SE). For the mechanics experiments, bar-headed and barnacle geese were anesthetized with isoflurane (1-5%), and Andean geese were anesthetized with intravenous propofol as described by Mulcahy (2007). Each bird was then intubated and attached to a constant volume ventilator that actively inflated the respiratory system (inhalation) and allowed for passive deflation (exhalation) of the birds respiratory system. 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 cm H_2O , and this pressure determined the limit of volume and frequency used for each individual. Flow was measured with a differential pressure transducer and a pneumotachograph that connected the endotracheal tube to the ventilator, and tracheal pressure was measured on the lung side of the pneumotachograph with a pressure transducer (see Figure 2.1A).

Flow curves were integrated to produce volume, and pressure-volume loops were generated using LabChart software (ADInstruments). 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 x,y-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 (see Figure 2.1B). Total work per breath is the sum of these two work components (elastic and resistive). The resistive work done to move the air through the endotracheal tube alone 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.

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 cm H₂O and deflate to a minimum of -30 cm H₂O in a step-wise fashion (25 mL per step). This was repeated 2-3 times. Animals were then either recovered or sacrificed and dissected for measurements of lung mass and volume of the trachea (details below). Static compliance was measured as the slope of the static deflation curve at its steepest point (between 5 and -5 cm H₂O).

Measurements of dynamic compliance, tau, elastic, and resistive work at each pump frequency were plotted versus tidal volume using Origin® 2016 software. 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. The selected combinations were either arbitrarily chosen or measured in the same individuals while resting and awake in normoxia and hypoxia. Levels of normoxia and hypoxia are expressed throughout this thesis as percent O₂, this is the PO₂ the birds are exposed to as a percent of sea level atmospheric pressure (101.3 kPa). Values were compared using one-way ANOVAs with post-hoc Tukey tests.

For the Andean geese, experiments were conducted in the field at the altitude where the birds were captured and raised. Thus it is possible that we overestimate the compliance and underestimate the work of breathing in the Andean geese due to low atmospheric pressure. To correct for this, comparisons were made at volumes expressed as a percent of the vital capacity that was measured at altitude as the volume change between respiratory pressures of 30 and -30 cm H₂O.

Lung masses and tracheal volumes

To measure lung mass, three barnacle, three bar-headed, and all six Andean geese were sacrificed 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, tracheal 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. Thus, this volume is more accurately the volume of the extra-pulmonary airways: the trachea, syrinx, and extra-pulmonary primary bronchi. For

simplicity we refer to this volume as the tracheal volume. We used this volume (and the tracheal volumes measured during CT scans) as an estimate of the dead space volume associated with each breath.

CT Scans

We used computerized tomography (CT) scanning to further investigate differences in morphology between the three species. We selected two bar-headed and two barnacle geese (one of each sex). For the Andean geese, at this time, we have only collected one scan on a female bird (the scan of the male is still to come). The birds were anesthetized (isoflurane 1-5%), intubated, and whole body scans were performed in the prone position at resting lung volume (glottis open to atmosphere). The birds were then hyperventilated, inflated to a tracheal pressure of 30 cm H₂O, and scanned again. Quantitative CT analysis was performed, and images were rendered using Amira 3D software. Values were compared using one-way ANOVA with post-hoc Tukey tests.

Results

Respiratory capacities

While the body mass of the birds did not differ (Figure 2.2A; $F_{2,19}=2.2$; $p=0.135$), the vital capacity of the Andean goose was significantly larger than that of the barnacle goose (Figure 2.2B; $F_{2,15}=29.9$; $p<0.001$). 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 2.1); extracted lung mass did not differ significantly between the three species

($F_{2,3}=3.7$; $p=0.81$). Tracheal volume normalized to vital capacity did not differ between species (Figure 2C; $F_{2,3}=4.3$; $p=0.13$).

CT scans

Images of CT scans for a representative of each species are shown in Figure 2.3. Body volume scaled with body mass in the birds that were CT scanned (see Table 2.1). Lung volume appeared largest in the Andean goose and smallest in the barnacle geese. The lung volumes changed between FRC and 30 cm H₂O pressure by 12% in the Andean and bar-headed geese and by 18% in the barnacle geese. Air sac volumes also appeared largest in the Andean goose and smallest in the barnacle geese and increased by 45-60% for the same pressure change between FRC and maximum inflation in all species (Table 2.1). Statistical comparisons of CT scan data have not been made due to the low sample size.

Static mechanics

The static compliance of the total respiratory system was greater in Andean and bar-headed geese compared to barnacle geese (Figure 2.4A; Table 2.2; $F_{2,15}=8.1$; $p=0.004$), but these differences were directly related to respiratory system size. When compliance was normalized to vital capacity (percent change in vital capacity for a given change in pulmonary pressure; Figure 2.4B), the static compliance of barnacle geese was actually significantly higher than that of Andean geese (Table 2.2; $F_{2,15}=4.4$; $p=0.03$).

Dynamic Mechanics

As dynamic respiratory mechanics vary as a function of the tidal volume and breathing frequency combination used, we chose to compare the three species both at common tidal volumes and frequencies (Figure 2.5 and 2.6) and at the f_R/V_t combination used by each species *in vivo* under normoxic and hypoxic conditions (Figure 2.7 and 2.8). For the common comparison, we chose a tidal volume of 7% vital capacity and a frequency of 20 breaths per minute (roughly equivalent to resting values) and then also reported how the dynamic values change if either frequency or volume were doubled.

Dynamic compliance was always highest in the Andean geese, even when normalized to vital capacity (Figure 2.5 A and B). Dynamic compliance was also higher in the bar-headed geese compared to the barnacle geese, but when normalized to vital capacity, compliance in the bar-headed and barnacle geese were equal. The time constant, tau, was longest for the barnacle geese and shortest for Andean geese (Figure 2.5 C).

These relationships were maintained when ventilation was doubled, regardless of whether it was due to a doubling of breathing frequency or of tidal volume. As expected, increasing ventilation reduced dynamic compliance; however, counter to our expectations, increasing frequency reduced compliance more than increasing tidal volume (Figure 2.5 A and B).

The minute work of breathing for all three breathing strategies is compared in Figure 2.6. Mass-specific minute work did not differ significantly between species except during high frequency breathing when it was lowest for the barnacle geese. Elastic work

accounted for the majority of the minute work during low V_t /low f_R breathing. The work required to overcome resistive forces increased when f_R or V_t doubled in bar-headed and barnacle geese but not in Andean goose (Figure 2.6 B). Effective ventilation at these combinations of V_t and f_R was highest for Andean geese and equal for bar-headed and barnacle geese (Figure 2.6 C). The efficacy of ventilation (the air brought to the gas exchange surface per unit energy spent), however, was highest for the barnacle geese and lowest for the bar-headed geese with the low V_t /low f_R breathing pattern. During high frequency breathing, efficacy dropped for all three species, to a greater extent for the bar-headed geese than the other two species. During high volume breathing, the Andean goose was the most efficacious (Figure 2.6 D).

When we compared respiratory mechanics values for the combinations of tidal volume and breathing frequency used by each species *in vivo*, we found that compliance remained highest in Andean geese, especially in hypoxia (5% O_2 ; Figure 2.7 A). Tau was longest for barnacle geese and shortest for Andean geese in normoxia (21% O_2 for barnacle and bar-headed geese, 14% O_2 for Andean geese). In hypoxia, tau was equal for Andean and bar-headed geese (Figure 2.7 B). In normoxia, all three species expended the same minute work to breathe, but in hypoxia, the minute work did not change for Andean geese (primarily because total ventilation did not change significantly), but was up to 15-fold higher in both barnacle and bar-headed geese (Figure 2.7 C). Bar-headed geese delivered the most O_2 to the gas exchange surface per minute in normoxia, and in hypoxia, all three species delivered equal amounts of O_2 to the gas exchange surfaces (Figure 2.7 D). Therefore, the efficacy of O_2 delivery was

highest in bar-headed geese in normoxia, and equal for all the species in hypoxia (Figure 2.7 E).

When minute work of breathing was plotted for a constant minute ventilation, versus the different combinations of frequency and volume that produce that minute ventilation, the curves were roughly u-shaped. Thus, for any given level of minute ventilation, there was an optimal combination of tidal volume and breathing frequency where minute work was lowest. We plotted these curves for each species for the levels of minute ventilation each used in normoxia and hypoxia and then compared these predicted optimal combinations with the actual ranges of breathing frequencies used *in vivo* (Figure 2.8). We found that barnacle geese always used the predicted optimal combination but that bar-headed geese only used the predicted optimal combination in hypoxia. The Andean geese used a slower, deeper breathing strategy than the predicted optimum in both normoxia and hypoxia. This should increase effective ventilation in both conditions but would come at a metabolic cost.

Discussion

We wanted to investigate how the mechanics and morphology of respiratory system in the bar-headed, Andean, and barnacle goose varied and compared to the overall work of breathing in each. Generally, we found that both the static mechanics and the work of breathing under the low V_T /low f_R condition were similar for all three species when normalized to the size of the respiratory system. The data also show that it is more costly to increase tidal volume than to increase breathing frequency, particularly for

barnacle and bar-headed geese. Interestingly, our calculations suggest that doubling ventilation by either strategy increased the work of breathing more than it increased the amount of oxygen delivered to the gas exchange surface. The overall efficacy of ventilation therefore decreased (Figure 2.6 D). Similar results have been reported during exercise in mammals where the relative cost of breathing can increase from less than 3% to more than 10% of total metabolic rate (Aaron et al., 1992).

The size of the respiratory system was much larger in Andean geese, even though they had the smallest body mass and body volume. The respiratory system accounted for 58% of total body volume at maximum capacity and 35% at functional residual capacity. Andean geese also had the highest dynamic compliance and the lowest value of tau, indicative of a more 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. Therefore, our calculations suggest it would be less work for Andean geese to ventilate large volumes and deliver more oxygen to the gas exchange surface per breath. This was reflected in the very high effective ventilation achieved for the same percent change in vital capacity and the increased effectiveness of ventilation during slow, deep breathing. Based on reported values of V_t and f_R for this species in normoxia and hypoxia (Lague et al., submitted), Andean geese increase ventilation only slightly (1.4-fold) from normoxia to hypoxia and, according to our calculations, would not increase the minute work of breathing significantly. Therefore, O_2 delivery must have decreased by half in hypoxia. This suggests that these geese must either increase oxygen extraction or decrease oxygen

demand in hypoxia. Lague et al. (submitted) report that this species does not suppress metabolism but increases oxygen extraction to a large extent (up to 90% extraction). Akester (1960) noted that the actively flying duck and pigeon had 4-fold more parabronchi per unit lung volume than the flightless domestic fowl. If the Andean goose has a higher density of parabronchi and a larger lung it would help explain their ability to extract more oxygen without increasing ventilation. This could also help to explain why bar-headed geese had the highest lung mass, even though their lung volume measured by CT scan was smaller than the Andean goose. It could be that the high number of parabronchi in the Andean goose lung causes the lung to have an overall lower density than the bar-headed goose lung.

The Andean goose did not use the optimal combination of breathing frequency and tidal volume predicted by the u-shaped curves but instead appear to expend energy to use a deeper, slower breathing pattern that should increase oxygen delivery. Unfortunately, our measurements do not allow us to calculate the cost-benefit balance (the O_2 delivered for the O_2 expended) of this strategy.

The barnacle goose had the smallest respiratory system, accounting for 38% of the total body volume at maximum capacity and 16.5% at functional residual capacity. Despite having the largest static compliance (when normalized to vital capacity), their respiratory system stiffened during dynamic inflation, yielding the lowest dynamic compliance. Thus while the work required to overcome resistive forces was lowest under the low V_t , low f_R

condition in this species, it was the greatest under the high V_t condition. The net result was that the barnacle goose had the highest breathing efficacy during low V_t /low f_R breathing, but this advantage disappeared under the high V_t condition. The time constant was consistently highest for the barnacle goose, meaning they would need to compensate with increased muscle work during expiration to achieve the same breathing frequency as the other two species. Therefore, the work to overcome elastic forces was the majority of the work for barnacle geese during breathing, although 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). The barnacle geese always used the optimal combination of tidal volume and breathing frequency predicted by the curves. Based on our calculations, for the barnacle goose to increase ventilation more than 3-fold in hypoxia, the minute work would increase 18-fold. The efficacy of O_2 delivery in hypoxia would therefore be extremely low.

The size of the bar-headed goose respiratory system was between that of the Andean and barnacle geese. Their respiratory system accounted for 45% of the total body volume at maximum capacity and 24% at functional residual capacity. Interestingly, 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. The CT data suggests that the FRC of bar-heads is smaller than Andean geese, supporting the latter. A greater capacity to empty the respiratory system for the bar-headed goose is also supported by an increased compliance below FRC (Figure 2.4 A). Our calculations suggest that bar-headed geese deliver more O_2 to

the gas exchange surface in normoxia than the other two species, due to an effective slow, deep breathing pattern. However, they have a very low O₂ delivery efficacy in hypoxia; our estimates indicated they increase minute work 14-fold for a 3-fold increase in ventilation, similar to the barnacle geese. This is consistent with our finding that bar-headed geese only use the optimal combination of tidal volume and breathing frequency in hypoxia when O₂ is limited, while in normoxia they expend energy to breathe with a more effective pattern.

Recent studies using mathematical modelling 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 this study, we know that compliance in the avian respiratory system is directly related to capacity. 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 8 species from 5 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). The volumes of the separate air sacs from our data set have not yet been analyzed.

While we hypothesized that the high altitude bar-headed and Andean geese would enhance gas exchange and reduce work of breathing by increasing the size and compliance of the respiratory system relative to low altitude barnacle geese, in this study we are not relating any of our findings to adaptation to altitude, nor are we attempting to correct our statistical comparisons for phylogeny. This is because we only compare three taxa in this study. The barnacle goose genus (*Branta*) is the sister taxa to the bar-headed goose genus (*Anser*), while the Andean goose is part of a genus of sheldgeese more closely related to ducks (*Chloephaga*). Therefore, we cannot determine if any differences measured here are due to phylogeny, adaptive evolution, or simply due to chance. Moreover, in comparing only three species, we are 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 mechanics of the respiratory system to reduce work of breathing, more data from appropriate species will be required to parse out the effects of altitude versus phylogeny on respiratory mechanics in birds.

Future directions

In this study, we compared wild caught, captive raised Andean geese with domesticated, captive raised bar-headed and barnacle geese. Therefore, differences due to plasticity may be confounding the data somewhat. Future studies could compare

all three species with individuals raised at sea level, or with 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 effect respiratory responses in these species (Lague et al., submitted).

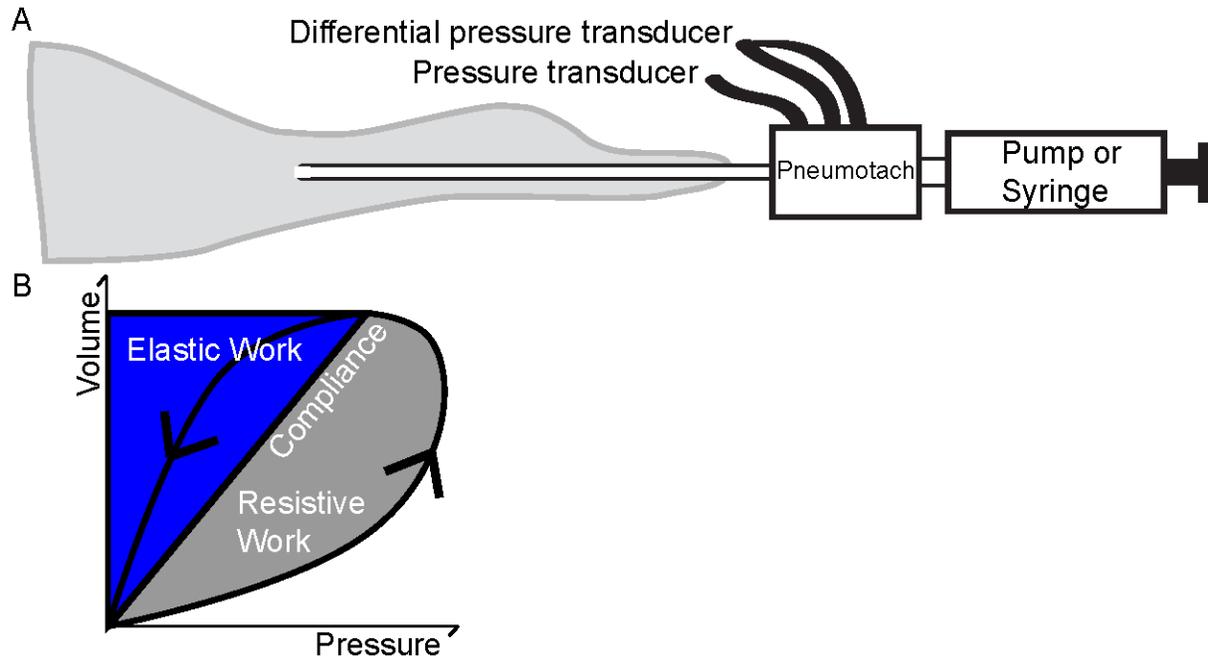


Figure 2.1: (A) Schematic of the experimental set-up to measure flow and pressure. A constant volume pump was used for dynamic measurements and a syringe was used for static measurements. Flow was measured using a calibrated differential pressure transducer connected to a pneumotachograph. Tracheal pressure was measured on the lung side of the pneumotachograph connecting the syringe or pump to the intubation tube. **(B) A hypothetical pressure-volume loop illustrated with measured variables.** Flow moves around the loop in the direction of the arrows for a full breath cycle. Dynamic compliance is the slope of the line from the origin to the point of maximum volume. Elastic work is proportional to the area in blue. Resistive work is proportional to the area in grey.

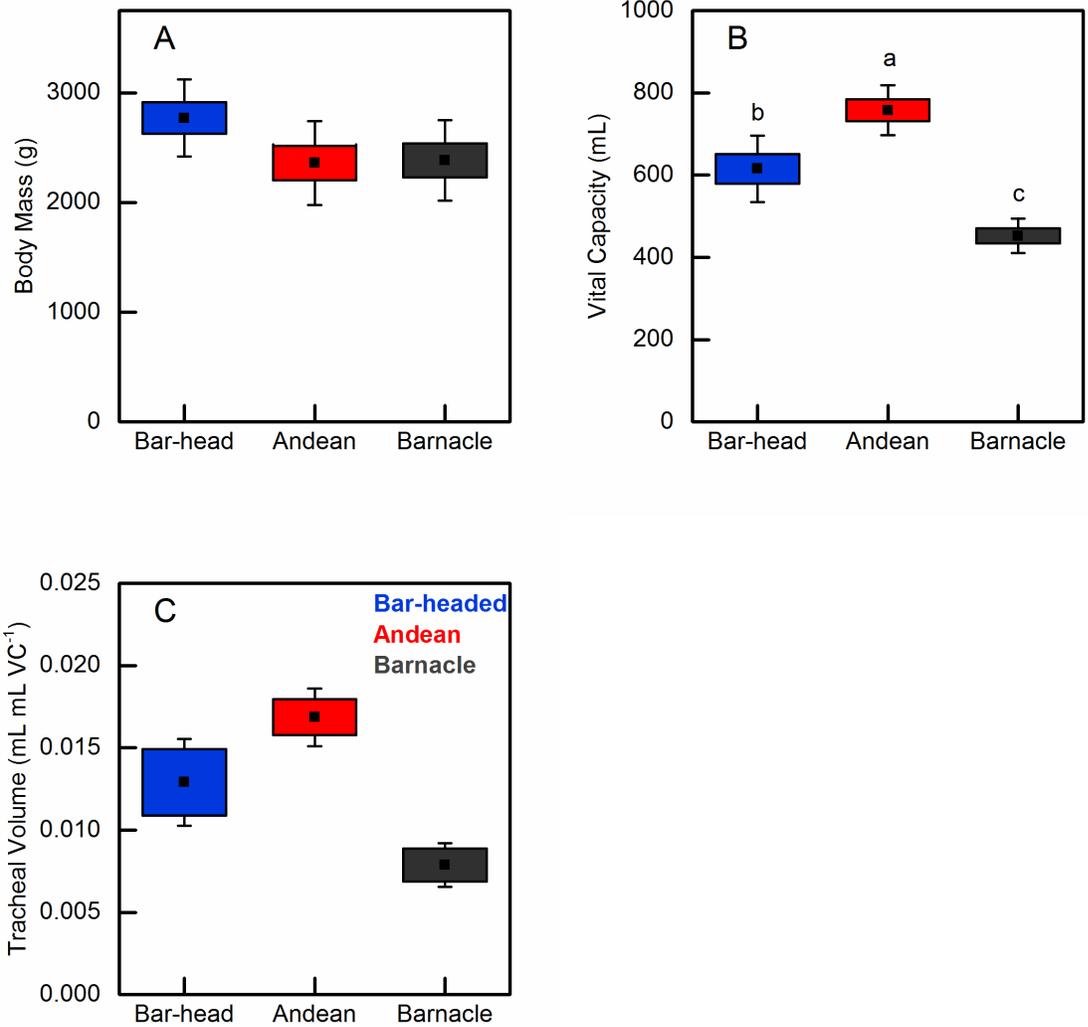


Figure 2.2: Body size and size of the respiratory system of the three species compared. (A) Body mass (B) Vital capacity (C) Tracheal volume normalized to vital capacity. Letters indicate significant differences between species (one-way ANOVA; $p < 0.05$). Black squares indicate means, colored boxes standard error of the mean, and whiskers 95% confidence interval.

Table 2.1: Volume and mechanics measurements for three species of geese.

Values reported as mean \pm standard error. One-way ANOVA was used for statistical comparison where possible. Mechanics values were obtained from the static inflation/deflation curves. *In vivo* values for tidal volume and breathing frequency were taken from Lague et al. (submitted). Dissection values were obtained from birds post-mortem. CT values were obtained from the CT scans. N.S. indicates non-significant difference, * indicates all three groups differ, BH-BG indicates bar-headed differ from barnacle geese, BH indicates bar-headed goose differs from the other two groups, BG indicates barnacle goose differs from the other two groups.

<i>Mechanics values</i>	Bar-head	Andean	Barnacle	Significance
n	6	6	6	-
Body mass (kg)	2.77 ± 0.1	2.29 ± 0.2	2.38 ± 0.2	N.S. (p=0.135; F=2.2)
Inspiratory capacity (mL)	372 ± 22	552 ± 21	293 ± 19	* (p<0.001; F=40.2)
Expiratory capacity (mL)	243 ± 18	206 ± 12	159 ± 16	BH-BG (p=0.005; F=7.6)
Vital capacity (mL)	615 ± 36	758 ± 27	452 ± 19	* (p<0.001; F=30)
<i>In vivo values</i>				
n	6	7	6	-
Tidal volume (mL BTPS)	56 ± 3	35 ± 3	32 ± 3	BH (p<0.001; F=18.5)
Tidal volume (mL STPD)	45 ± 2	33 ± 2	25 ± 3	BH (p<0.001; F=14.2)
Breathing frequency (min ⁻¹)	15 ± 1	18 ± 1	22 ± 3	N.S. (p=0.075; F=3.1)
<i>Dissection values</i>				
n	3	5	3	-
Lung mass (g)	27 ± 0.7	24 ± 1	21 ± 2	N.S. (p=0.81; F=3.7)
Heart mass (g)		21.7 ± 0.9		
Tracheal volume (mL)		11.4 ± 1		
<i>CT values</i>				
n	2	1	2	-
Body volume (cm ³)	1756 ± 112	1422	1430 ± 36	
Inflated lung volume (mL)	83 ± 7	103	51 ± 1	
Resting lung volume (mL)	74 ± 5	92	46 ± 0.2	
Inflated air sac volume (mL)	707 ± 1	722	491 ± 19	
Resting air sac volume (mL)	348 ± 28	409	190 ± 4	
Tracheal volume (mL)	8 ± 0.9	5.6	4.7 ± 0.02	
Pneumatic volume long bones (mL)	11 ± 0.3	11	7.5 ± 2	
Pneumatic volume spine (mL)	5 ± 0.3	6.4	3.2 ± 0.2	
Bone (cm ³)	166 ± 0.3	171	126 ± 0.8	
Inflated pulmonary vessel vol (mL)	0.9 ± 0.2		0.6 ± 0.1	
Resting pulmonary vessel vol (mL)	1.4 ± 0.3	0.07	0.8 ± 0.1	
Fat (cm ³)	89 ± 20	153	242 ± 5	

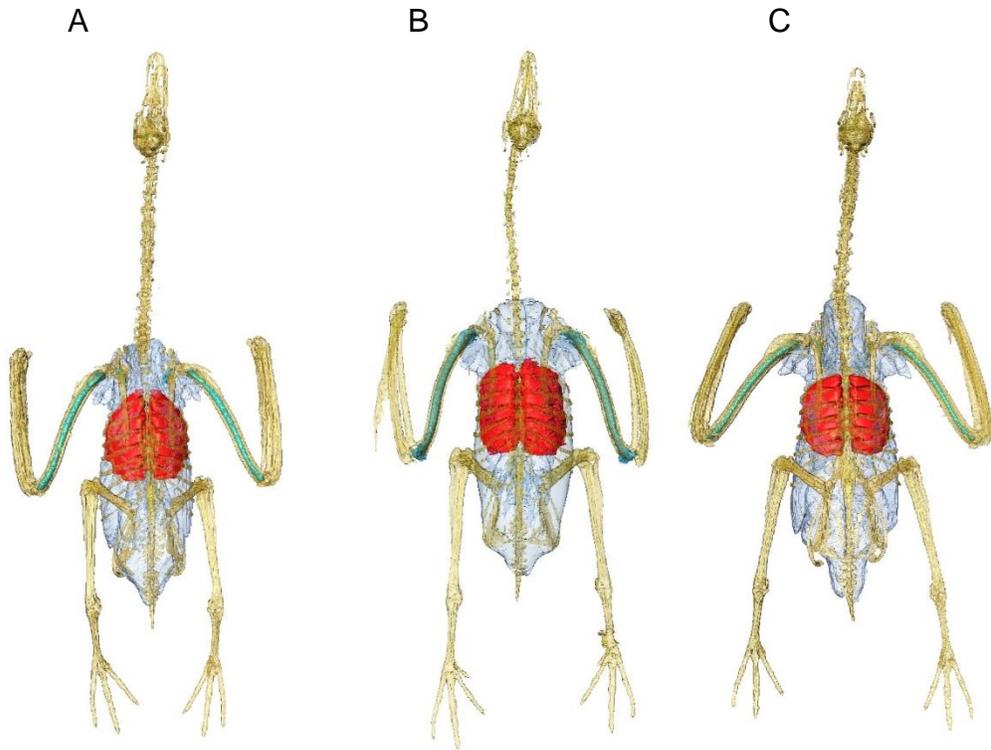


Figure 2.3: CT scans the respiratory system of each species, inflated to 30 cm H₂O. (A) Bar-headed goose (B) Andean goose and (C) barnacle goose. Lungs (red), air sacs (light blue), air spaces in long bones (cyan), and bones (yellow) are shown. Images are taken from the dorsal side of prone birds.

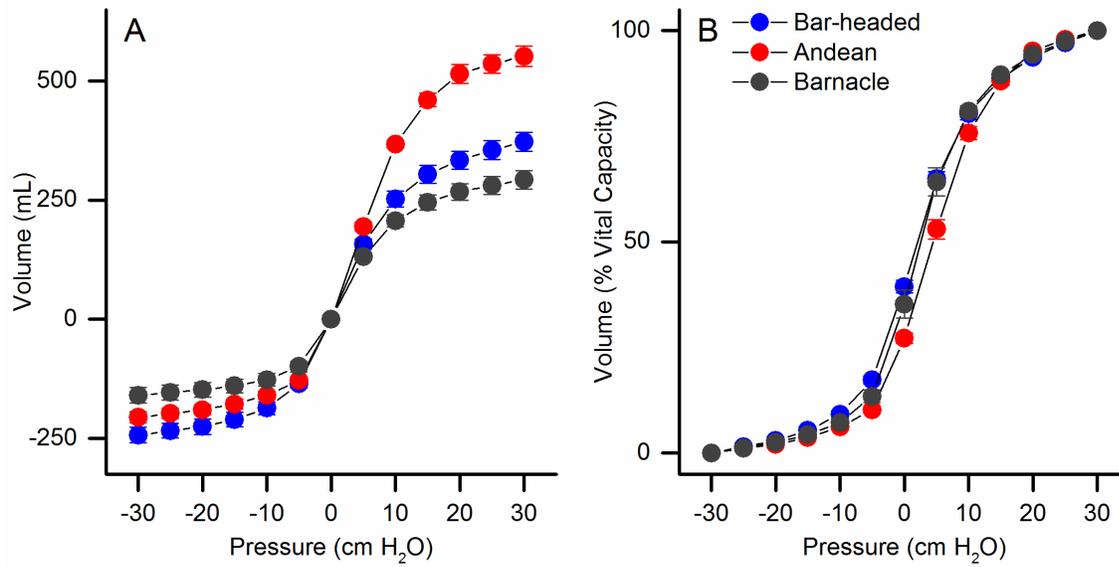


Figure 2.4: Static compliance curves (A) expressed as volume change (mL) for a given pressure change and (B) expressed as percent of vital capacity change (%) for a given pressure change. Curves shown are means ± standard errors.

Table 2.2: Static compliance values measured as the slope of the static compliance curve at its steepest point (from curves in Figure 2.5 A and B). Values are expressed as means \pm SE. Barnacle goose has significantly lower static compliance than the other two species (indicated by letters; one-way ANOVA; $p=0.004$; $F=8.1$). When volume is normalized to vital capacity, barnacle geese have significantly higher compliance than Andean geese, bar-headed geese do not differ from the other two species ($p=0.03$; $F=4.4$)

Species	Static compliance $mL\ cm\ H_2O^{-1}$	Static compliance % of Vital Capacity $cm\ H_2O^{-1}$
Bar-headed	29.4 ± 1.9^A	4.77 ± 0.2^{AB}
Andean	32.3 ± 1.5^A	4.28 ± 0.2^B
Barnacle	23.0 ± 1.5^B	5.07 ± 0.2^A

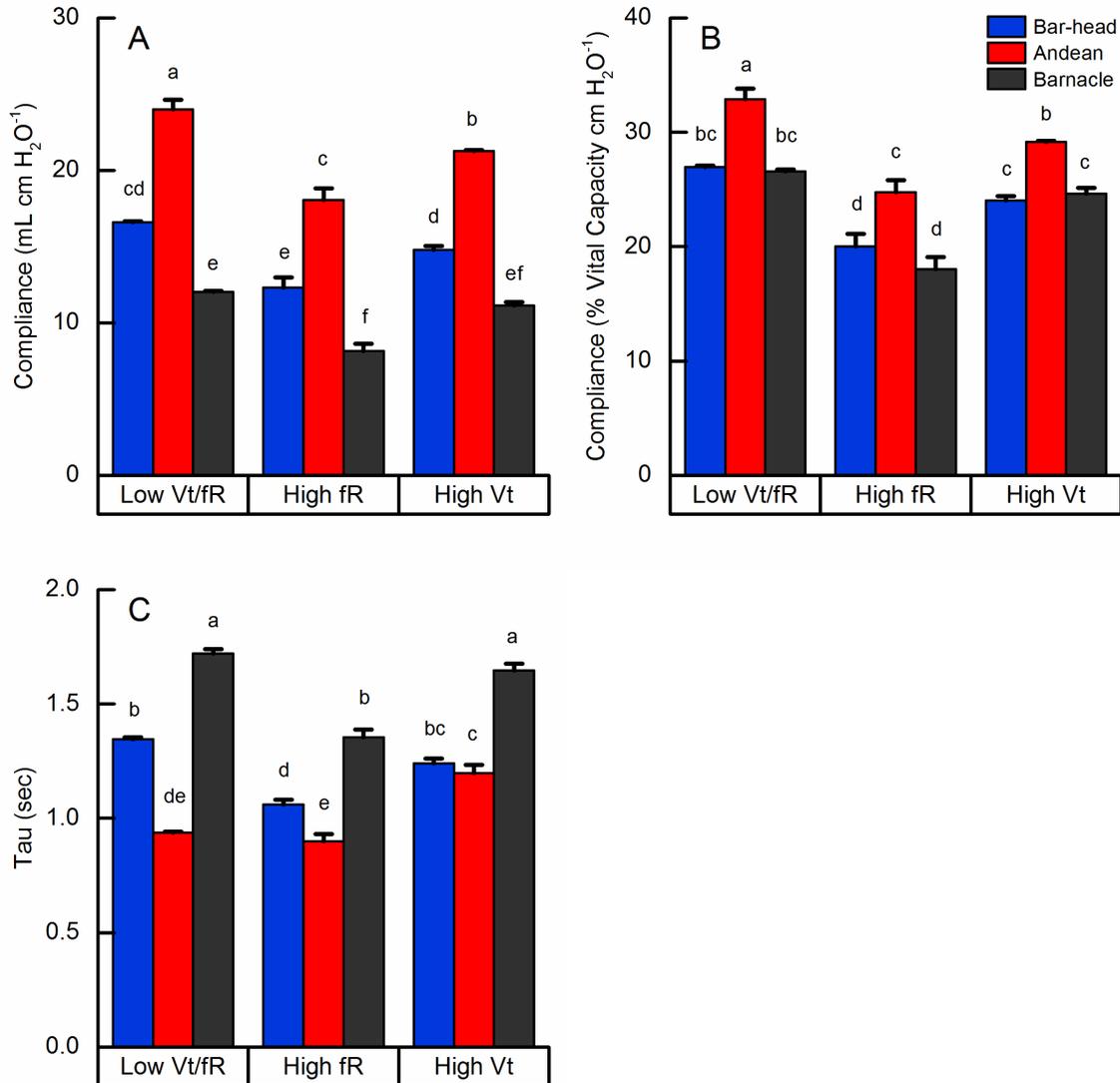


Figure 2.5: Dynamic compliance and tau compared for three different breathing strategies. (A) Dynamic compliance expressed with volume in mL. (B) Dynamic compliance expressed with volume as percent of vital capacity. (C) The time constant (tau). Low Vt/fR is 20 breaths per minute, 7% vital capacity tidal volume, High fR is a 40 breaths per minute, 7% vital capacity tidal volume, and High Vt is 20 breaths per minute, 14% vital capacity tidal volume. Letters indicate significant differences between total work values (one-way ANOVA; $p < 0.05$; post-hoc Tukey test).

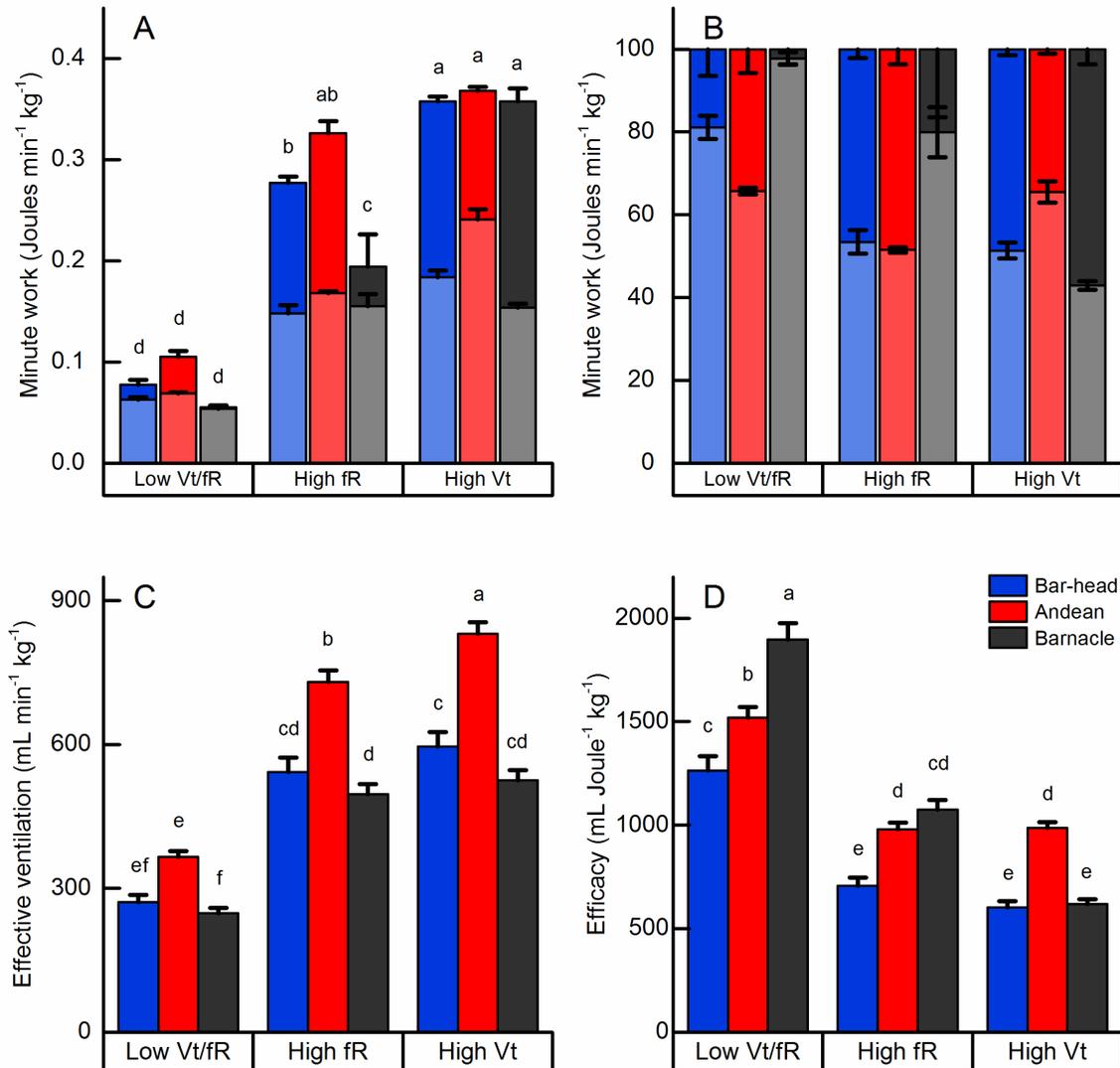


Figure 2.6: Work required and effectiveness of three the different breathing strategies compared. (A) Minute work of breathing. Light part of each column is the elastic component of work, dark part of each column is the resistive component, and the whole column is total minute work required. (B) Proportion of elastic work (light) and resistive work (dark) associated with each pattern (C) Effective ventilation produced with each breathing pattern. (D) Efficacy of air delivery. Low Vt/fR is 20 breaths per minute, 7% vital capacity tidal volume, High fR is a 40 breaths per minute, 7% vital capacity tidal volume, and High Vt is 20 breaths per minute, 14% vital capacity tidal volume. Letters indicate significant differences between total work values (one-way ANOVA; $p < 0.05$; post-hoc Tukey test).

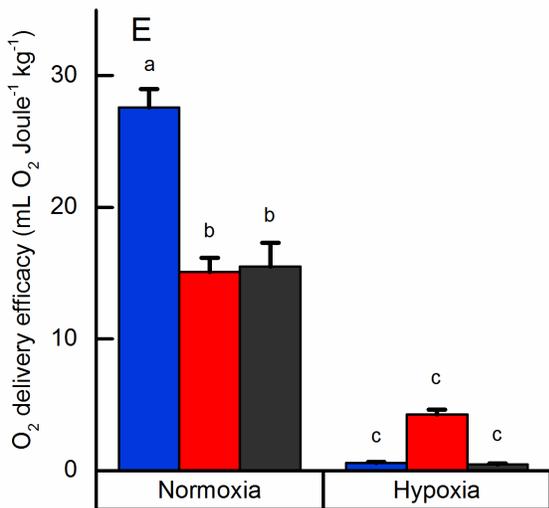
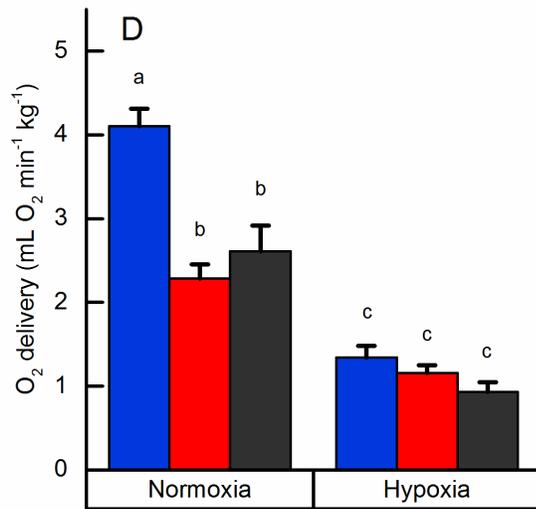
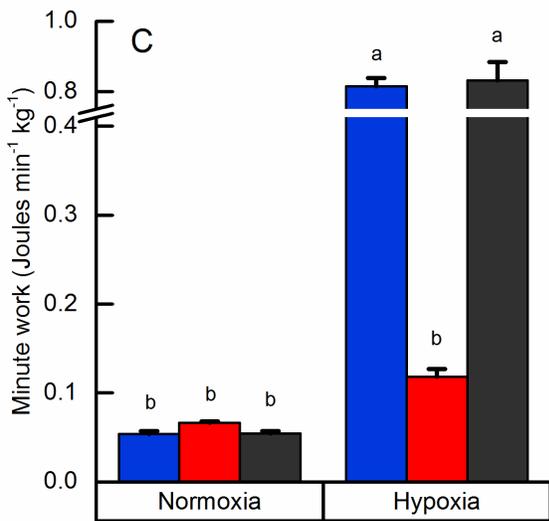
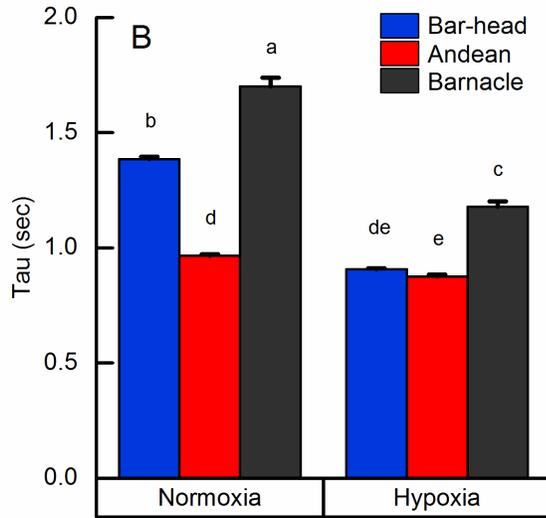
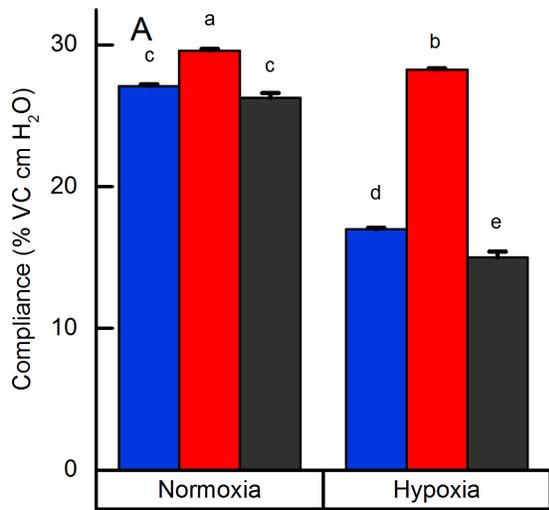


Figure 2.7: Respiratory mechanics measurements and calculations for the breathing frequency and tidal volume used by each species *in vivo* in normoxia and hypoxia. Normoxia is 21% O₂ for bar-headed and barnacle and 14% O₂ for Andean geese, hypoxia is 5% O₂ for all three species. (A) Dynamic compliance, (B) Tau, (C) Minute work, (D) O₂ delivered to the gas exchange surface, (E) Efficacy of O₂ delivery. Letters indicate significant differences (one-way ANOVA, p<0.05, post-hoc Tukey test).

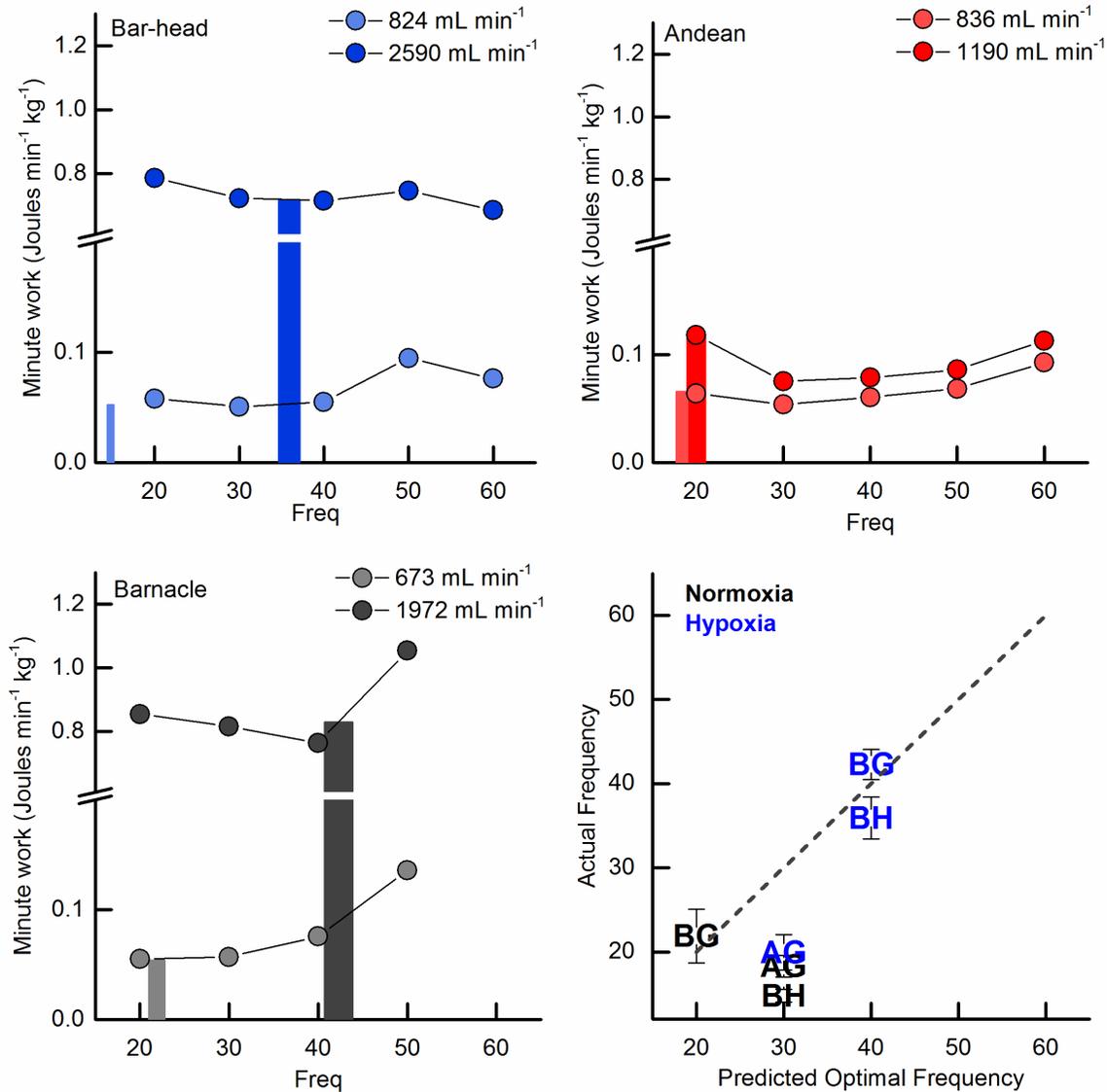


Figure 2.8: Optimal combinations of tidal volume and breathing frequency *in vivo*.

Lines indicate minute work curves for levels of total ventilation each species used in normoxia (light shade; 21% O₂ for bar-headed and barnacle geese, 14% O₂ for Andean geese) and hypoxia (dark shade; 5% O₂). The minute ventilations used by each species are indicated in the key for each plot. Bars show actual frequency used by each species (and hence also tidal volume). Width of each bar is the standard error of the mean. Lower right plot compares actual frequency with the predicted optimal frequency in normoxia (black) and hypoxia (blue). Dashed line indicates where points should fall if birds are using optimal frequency. BH is bar-headed goose, AG is Andean goose, and BG is barnacle goose.

Chapter 3

Respiratory mechanics of eleven avian species resident at different altitudes

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Introduction

In the previous chapter, we investigated the respiratory mechanics and morphometry of a high altitude exercise champion, the bar-headed goose, as well as a species which has resided at high altitudes for more than a million years, the Andean goose (K.G. McCracken, personal communication). Our findings were consistent with our predictions, but we were not able to speculate on whether the differences we measured were due to the effects of altitude or simply due to phylogeny. In this chapter, we expand our study group to include eleven additional species of waterfowl, five that reside at high altitudes in the Andes and six species found at low altitudes, to directly investigate the effect of altitude on avian respiratory mechanics.

For animals living at high altitude, when oxygen is limited, it is not always possible to decrease oxygen demand as metabolically expensive but vital tasks such as predator evasion, hunting or foraging, migration, and reproduction must still be accomplished.

Animals must therefore cope by increasing oxygen supply (Hochachka, 1985).

Increasing supply usually occurs at multiple levels of the oxygen transport cascade, for example by increasing ventilation, increasing cardiac output, left- or right-shifting the O₂-hemoglobin equilibrium curve at the lung or tissue, respectively, or increasing myoglobin content or vascularity at the muscles (see Monge and Leon-Velarde, 1991 for review).

The first oxygen transport step, ventilation, is often the first response when oxygen demand outstrips supply (Powell et al., 1998). Increasing ventilation can be accomplished either by increasing the volume of the breath (tidal volume, V_t) or the frequency of respiration (f_R) such that:

$$\textit{Total ventilation} = f_R * V_t$$

In vertebrates such as fish, which can bring nearly all the “inhaled” respiratory medium into direct contact with the gas exchange surface, increasing f_R is an equally effective strategy as increasing V_t . However, for tetrapods, the dead space volume must be taken into account, such that:

$$\textit{Effective ventilation} = f_R * (V_t - \textit{dead space volume})$$

The dead space volume is the volume of respiratory medium that the animal expends energy to move from the environment into the respiratory system, but which never actually contacts the gas exchange surface and so does not increase oxygen loading. In the case of sauropsids, who use active expiration, this volume is not only actively moved into the animal but must also be actively pushed out to prepare for the next

breath, thus doubling the cost per unit volume compared to animals using exclusively passive expiration (e.g., mammals at rest). The volume of respiratory medium that is actually brought to the respiratory surface per minute is called the effective ventilation (\dot{V}_{eff}). Because dead space volume is a constant, increasing V_t increases \dot{V}_{eff} more than an equal increase of f_R (see Figure 3.1).

However, because the work to overcome elastic forces of the chest wall and expansion of the lung increases exponentially with increasing V_t but linearly with increasing f_R , the more effective strategy of increasing V_t is generally more metabolically expensive than increasing f_R (Vitalis and Milsom, 1986). We wondered if the deeper, slower breathing pattern measured in the bar-headed goose (Scott and Milsom, 2007) was found in other high altitude species, and whether those species could have altered the mechanical design of their respiratory systems to reduce the metabolic cost of potentially employing this higher tidal volume strategy. We also wondered if the differences in respiratory system size and compliance measured in Chapter 2 would be seen consistently in the high altitude group studied here. Therefore, we compared the mechanics of the respiratory system and the work of breathing in eleven species of waterfowl from two sites (see Table 3.1 for a list). Eight of these were members of sister taxa in which one species or subspecies was found primarily at high altitude and the other at low altitude. This allowed us to make four phylogenetically independent comparisons of the effects of altitude on respiratory mechanics.

Among the five highland species, there is variation in the evolutionary time since they split with their lowland sister taxa and radiated to altitude. The highland puna teal and speckled teal are considered to be separate species from their closest relatives, the lowland silver teal (*Anas versicolor*, not studied here) and the lowland green-winged teal. The highland cinnamon teal and ruddy duck are separate subspecies from their lowland sister taxa, the cinnamon teal being the most recently diverged species (K.G. McCracken, personal communication). The highland yellow-billed pintail is a separate species from its closest relative, the northern pintail, but they diverged more recently than the ruddy ducks. Some gene flow still exists between the highland yellow-billed pintails and yellow-billed pintails found at low altitude sites in South America (McCracken et al., 2009b). In this study, we also include the mallard duck and gadwall, lowland species who have no high altitude sister taxa. All of these species are considered to be dabbling ducks (genus *Anas*), meaning that they forage primarily while on the surface of the water, except the ruddy ducks, which are stiff-tailed diving ducks and the only representatives in this study of the genus *Oxyura* (see Table 3.1 and Figure 3.2).

With these four independent comparisons on the effects of altitude on respiratory mechanics and our observations from the previous chapter we wanted to answer four primary questions: (1) Are there morphological or mechanical changes in the respiratory system associated with radiation to altitude? (2) Are there relative benefits of increasing tidal volume over breathing frequency? (3) What is the actual cost of breathing for these

birds *in vivo*? (4) Do these birds use an energetically “optimal” combination of tidal volume and breathing frequency?

Methods

Highland species were collected from Lake Titicaca, Puno, Perú, at 3,812 meters altitude. Lowland species were collected from either Summer Lake Wildlife area at 1,260 meters or Malheur National Wildlife Refuge at 1,256 meters in Oregon, USA. Species collected at the high altitude site were the puna teal (*Anas puna*; n=6), speckled teal (*Anas flavirostris*; n=6), Andean ruddy duck (*Oxyura jamaicensis ferruginea*; n=11), yellow-billed pintail (*Anas georgica*; n=6), and cinnamon teal (*Anas cyanoptera orinomus*; n=8). Lowland species collected were the American green-winged teal (*Anas crecca*; n=8), ruddy duck (*Oxyura jamaicensis jamaicensis*; n=6), northern pintail (*Anas acuta*; n=6), northern cinnamon teal (*Anas cyanoptera septentrionalium*; n=8), mallard duck (*Anas platyrhynchos*; n=6), and gadwall (*Anas strepera*; n=6). This group contains eight sister taxa: the highland speckled teal and the lowland green-winged teal are sister species as are the highland yellow-billed pintail and the lowland northern pintail. The highland and lowland cinnamon teals and ruddy ducks are subspecies found at different altitudes. See Figure 3.2 for phylogeny (Gonzalez et al., 2009).

Birds were captured at night and held in kennels with access to food and water until the morning. They were then anesthetized with intravenous propofol, intubated, and connected to a constant volume respirator. Birds were hyperventilated until the

spontaneous drive to breathe was lost. They were then ventilated sequentially with at least three volumes (11.55, 17.33, 23.11, 28.9, or 34.68 mL) and up to five frequencies at each volume (20, 30, 40, 50, and 60 min⁻¹). Tracheal pressure was not permitted to go above 30 cm H₂O. Pressure at the trachea was measured with a pressure transducer calibrated with a glass manometer, and flow was measured with a differential pressure transducer connected to either side of a pneumotachograph positioned between the intubation tube and the respirator. Once dynamic data were collected, the birds were again hyperventilated, disconnected from the respirator, and a 100 mL glass syringe was used to inflate and then deflate the respiratory system in a step-wise manner (10-20 mL per step) from functional residual capacity (FRC; lungs open to atmospheric pressure) to 30 cm H₂O, back to FRC, to -30 cm H₂O, and then back to FRC again. This was done 2-3 times. At the end of experiments, the endotracheal tubes were ventilated with the same volumes and frequencies. Data were recorded with a WinDaq Data Acquisition system.

Birds were then either allowed to recover and released or sacrificed with an overdose of intravenous propofol (all birds in Peru were sacrificed; three individuals of each species in Oregon were sacrificed in accordance with our collection permits). Lungs, hearts, and tracheas were dissected from sacrificed birds. Lung and heart mass as well as extra-pulmonary airway volumes were measured. The extra-pulmonary airway (hereafter referred to as tracheal) volume was used as a proxy for dead space volume.

All experiments were conducted in the field at the altitude where the birds were collected. Thus it is possible that we overestimate the compliance and underestimate the work values in the highland species due to hypobaric conditions at the highland site. To correct for this, comparisons were made at volumes expressed as a percent of the vital capacities measured at each site as the volume change between respiratory pressures of 30 and -30 cm H₂O. We also normalized values to vital capacity because we were interested in the differences in the mechanics of the respiratory systems independent of differences in body size.

Static compliance was measured as the steepest slope of the relationship between volume and pressure under conditions of zero flow. We also calculated static compliance as the change in volume expressed as a percent of vital capacity for a given change in pressure.

To analyze dynamic data, flow measurements were integrated to produce volume curves, and volume was plotted against pressure as pressure-volume loops. Dynamic compliance was measured as in Chapter 2. The resistive work to move the air through the endotracheal tube alone was subtracted from total resistive work. Flow resistive and elastic work sum to total work per breath. Tau, the time constant, is calculated by estimating resistance as the difference in pressure between two points of isovolume and dividing by the flow, and the product of resistance and compliance is tau.

For measurements of dynamic mechanics, we make comparisons at the same combination of f_R/V_t for all species (approximately the average value derived for all species breathing ambient O_2), with values of V_t expressed as % vital capacity to directly compare the mechanical properties of individual respiratory systems. *In vivo* each species will use a different combination of f_R/V_t when breathing ambient air and will also increase ventilation with different combinations in hypoxia. Therefore, to compare the mechanics between species *in vivo*, we estimated compliance and work values at the f_R/V_t combination used by each species while breathing 13% and 6% O_2 . We chose 13% O_2 because it was the level of ambient oxygen availability at our high altitude site, and the low altitude species used a very similar combination of f_R/V_t when breathing 13% and the ambient level of O_2 at the low altitude site: 18% O_2 (see Figure 3.11). We chose 6% O_2 for hypoxia as it was the lowest common level of oxygen for which data had been collected (Ivy et al., unpublished).

Data were analyzed using LabChart software from ADInstruments and graphs and statistics were generated using Origin® 2016. Compliance, tau, elastic work, and resistive work were plotted versus tidal volume and frequency isopleths were produced as linear fits to this data. The equations of these lines were then used to estimate values of the variables at desired combinations of tidal volume and breathing frequency. T-tests were used to compare sister taxa.

Results

Respiratory system morphology and static mechanics

The species compared have a wide range of body masses (from roughly 0.3 to 1 kg), which correlated with their vital capacities (Figure 3.3 A, $R^2 = 0.83$). The highland ruddy ducks (RD) had a somewhat smaller vital capacity than would be predicted for their body mass, while the vital capacities of yellow-billed pintails (YBP), gadwalls (GW), and mallard ducks (MD) were slightly larger than predicted. There were no significant differences between lung mass of any species when normalized to body mass (Figure 3.3 B; two-way ANOVA; $p=0.68$). The highland birds all tended to have lower dead space volumes, but there was no significant effect of altitude (two-way ANOVA; $p=0.42$). Among sister taxa, only the highland yellow-billed pintails had a significantly smaller tracheal volume for their vital capacity than the lowland northern pintails (t-test; $p=0.008$). It was common for one lung to be larger than the other in any individual bird, but whether it was the right or left lung was inconsistent between individuals, and in some birds both lungs were of a similar size.

Static compliance was expressed in $\text{mL cm H}_2\text{O}^{-1}$ (Figure 3.4 A) and as the change in volume expressed as a percent of vital capacity (Figure 3.4 B). The slopes of the curves in Figure 3.4 are listed in Table 3.2 and Figure 3.5. Before normalization to vital capacity, three of the highland species were significantly more compliant than their lowland sister taxa (t-tests: speckled teal/green winged teal, $p<0.001$; ruddy duck/lowland ruddy duck, $p=0.04$; cinnamon teal/lowland cinnamon teal, $p<0.001$). This was because they had larger vital capacities. When expressed as change in volume per

unit change in pressure (Figure 3.4 A), the slopes of the curves for all species were directly proportional to the size of the respiratory system (Figure 3.6; $R^2=0.68$). When normalized to the size of the respiratory system, there was a non-significant trend for the highland species to be more compliant (Figure 3.5 B), although between sister taxa, only the speckled teal and yellow-billed pintail were still significantly more compliant than the lowland green-winged teal and northern pintail (t-tests: ST/GT $p=0.036$; YBP/NP $p=0.021$).

Cost and benefit of increasing tidal volume

We compared the work of breathing in each species when breathing at equal tidal volumes (as a percent of vital capacity) and frequencies because the variables of dynamic mechanics are specific to each combination of V_t/f_R . Dynamic compliance was higher for all highland sister taxa, except the ruddy ducks who had lower or equal compliance, and this remained true when frequency or volume was doubled (Table 3.3). We found that in general the time constant was reduced when f_R or V_t were increased but saw no consistent differences in the time constant between sister taxa. Our calculations suggest that doubling V_t would be more expensive than doubling f_R for all species except the highland speckled and cinnamon teals (Figure 3.7). In general, the highland sister taxa did either equal or more work than the lowland groups per minute (Table 3.3). When we compared effective ventilation, only the yellow-billed pintail delivered significantly more air than their lowland sister taxon, the northern pintail, and this was directly related to the relative size of the tracheal volume (Figure 3.3C). We then calculated the level of effective ventilation per unit energy spent (efficacy) and

found that the three smallest species (green-winged teal, speckled teal, and lowland cinnamon teal) were the most efficacious. However, the highland species, except the ruddy duck, were generally more efficacious than their lowland sister taxa. The highland ruddy duck, lowland northern pintail, mallard duck, and gadwall were the least efficacious.

Dynamic mechanics and work of breathing in vivo

In the previous plots, we compared the species at common combinations of V_t and f_R . Here we calculate the dynamic compliance and the time constant for each species from our data using their *in vivo* breathing pattern (Figure 3.8). Based on these calculations, dynamic compliance (Figure 3.8 A and B) would be reduced in the ruddy duck and cinnamon teal compared to their lowland relatives while breathing 13% O_2 but not 6% O_2 (t-tests; RD/LRD $p < 0.001$; CT/LCT $p = 0.003$). While breathing 6% O_2 , only the dynamic compliance of the speckled teal would be significantly less than its lowland sister taxon, the green-winged teal (t-test; $p = 0.01$). In Figure 3.8 C and D, we report the time constant (τ) for the combination of breathing frequency and tidal volume used by each species *in vivo* based on our measurements. We found no significant differences among sister taxa for τ , except the highland cinnamon teal, which was estimated to have a slightly faster time constant than the lowland cinnamon teal in both 13 and 6% O_2 (t-test; $p = 0.015$ and 0.024).

We calculated the minute work (power output) and the volume of oxygen brought to the gas exchange surface per unit minute work using the *in vivo* measurements of tidal

volume and breathing frequency for each species breathing 13 and 6% O₂ (Figure 3.9). Not surprisingly, the minute work of breathing was significantly higher and the amount of O₂ delivered to the respiratory exchange surfaces tended to be lower when the birds were breathing 6% O₂ compared to 13% O₂ (Figure 3.9; paired t-test; minute work $p < 0.001$; O₂ delivery $p = 0.28$). In general, the highland birds had reduced minute work compared to the lowland taxa but ventilated approximately equal volumes of oxygen per unit work. When breathing 13% O₂ (Figure 3.9 A), however, the highland ruddy ducks were estimated to do significantly more work per minute than the lowland ruddy ducks (t-test; $p < 0.001$) yet brought less oxygen to the gas exchange surface for that work (Figure 3.9 C; $p < 0.001$). When breathing 6% O₂ (Figure 3.9 B), this relationship switched ($p < 0.001$). The speckled teal and green-winged teal had the opposite pattern to the ruddy ducks (Figure 3.9 D; $p = 0.008$), and when breathing both 13 and 6% O₂, the yellow-billed pintail was estimated to be more efficacious than the northern pintail (t-test; $p < 0.001$). In 6% O₂, the mallard duck was estimated to have the highest minute work of breathing—approximately 3-fold more than any other species. Per unit energy, the mallard duck was also estimated to bring less oxygen to the gas exchange surface than any other bird.

Do birds use an energetically optimal combination of tidal volume and breathing frequency?

As described above, the total work of breathing has two components: the work required to overcome elastic forces (elastic work) and the work required to overcome flow resistive forces (resistive work). For a constant level of minute ventilation, frequency

increases and tidal volume decreases, resistive work increases, and elastic work decreases. When summed, the total work curve tends to be u-shaped with the different components (elastic and resistive work) contributing proportionately different amounts at each combination. Usually there is an “optimal” combination of tidal volume and breathing frequency where minute work is minimized. We compared how the resistive and elastic components of work change at a common constant level of minute ventilation (500% vital capacity per minute) in Figure 3.10. While there was high variability, we found no significant differences in the proportion of total work required to overcome elastic forces at 40 bpm (and 12.5% vital capacity) between sister taxa. Nonetheless, the shape of the power curve was greatly affected by the extent to which resistive work increased with increasing frequency; the greater the increase the more u-shaped the curve.

We plotted the change in minute work for the level of minute ventilation used by each species while breathing ambient levels of O_2 (18% O_2 for lowland species and 13% O_2 for highland species), and 6% O_2 (Figure 3.11). We found that, in general, birds expend energy to use a higher V_t /slower f_R pattern than the predicted optimal combination (Figure 3.12) when breathing ambient levels of O_2 , but that when oxygen was more limited (in 6% O_2) birds generally moved toward a more optimal combination of V_t/f_R (Figure 3.12).

Discussion

Morphological and mechanical variation associated with altitude

Our data revealed increases in static and dynamic compliance associated with altitude (Table 3.2 and 3.3). For any given pressure change, there was a greater volume change in the respiratory system of highland species, and this difference was primarily due to reduced stiffness at low respiratory volumes (removing air below FRC). This was consistent with our measurements in bar-headed geese. In a passively expiring mammal, this would have little physiological consequence, but because birds expire actively, increased expiratory compliance should reduce the cost of every breath. We found little difference in tracheal volume or lung mass between the highland and lowland sister taxa. Of the three species for which no sister taxa were studied, the highland puna teal had a small tracheal volume for its body mass, while the lowland mallard duck and gadwall had small lungs and large tracheal volumes for their body mass. For the latter two species, this may have limited their potential to radiate to altitude and explain the lack of highland sister taxa. Overall vital capacity scaled directly with body mass in our species, and we found no effect of evolutionary time at altitude on either vital capacity or lung mass.

Costs and benefits of deep versus rapid breathing

Consistent with the previous chapter, we found increasing tidal volume to be a more expensive strategy than increasing breathing frequency in most species. The exceptions were the Andean goose and two highland species in this study, the speckled and cinnamon teals. Interestingly, these two species have spent the most and least

evolutionary time at altitude, respectively (the Andean goose has been at altitude even longer than the speckled teal), suggesting this is not associated with high altitude adaptation, although it would certainly be beneficial when increasing ventilation at altitude. This change could be due to the increase in compliance, decrease in resistance, or a combination of the two that was estimated in these two species.

The benefit of increasing tidal volume over breathing frequency in terms of greater oxygen delivery to the gas exchange surface is directly related to the proportion of dead space volume in the respiratory system. The greater the dead space volume, the more advantageous using a higher tidal volume strategy would potentially be. If the dead space volume is relatively small, then the advantage of increasing V_t versus f_R will be reduced. In this study, we used tracheal (extra-pulmonary airway) volume as an estimate of dead space volume. Only the highland yellow-billed pintails had a reduced tracheal volume compared to their lowland sister taxon, the northern pintail. This gave rise to greater effective ventilation and an increase in the O_2 delivery efficacy compared to the northern pintails *in vivo*. For the other sister taxa, relative tracheal volume was not different, and therefore neither was the effective ventilation.

The species with the highest efficacy were the smallest birds, regardless of altitude. This size effect may be related to homeostatic constraints such as the need to thermoregulate with high surface area to volume ratios or maintain higher mass specific metabolic rates with high heart rates and levels of ventilation at rest. The least efficacious species, the mallard duck, highland ruddy duck, gadwall, and northern

pintail, had stiff respiratory systems and either a high airway resistance (ruddy duck), or fairly large tracheal volumes (mallard duck, gadwall, and northern pintail).

Cost of breathing in vivo

Our data indicate that when breathing 6% O₂, the highland taxa were generally estimated to have expended less or equal energy to breathe per unit time and delivered an approximately equal amount of oxygen to the gas exchange surface per unit energy spent. This implies that some highland taxa may be delivering less oxygen to the system overall and may be employing other strategies to cope with hypoxia—such as decreasing O₂ demand or increasing O₂ extraction. It is important to note that the least efficacious species were those that have no highland sister taxa—the gadwall and mallard duck. The mallard duck in particular was estimated to have brought very little oxygen to the gas exchange surface per unit of energy spent, a finding further supporting the suggestion that respiratory limitations may explain why these lineages have not radiated to altitude.

When do birds breathe optimally?

As already described, for a constant level of minute ventilation, as the breathing frequency and work to overcome resistive forces increase, the tidal volume and work to overcome elastic forces will decrease. When summed, the total minute work curve tends to be u-shaped. Thus there is an “optimal” combination of tidal volume and breathing frequency where total minute work is minimized. We expected that birds would always use this optimal combination. We found that only two species, the

speckled teal and lowland ruddy duck, were estimated to always (in normoxia and hypoxia) use the optimal combination. In normoxia, all other species expended energy to use a slower, deeper breathing strategy than the optimum. In hypoxia, when oxygen was limited, birds shifted closer to the optimal combination of f_R/V_t . If they didn't use the optimum, they were estimated to always use a slower, deeper strategy, which would increase effective ventilation per breath; birds never expended energy to use a more rapid, shallow breathing strategy than the optimum. These findings are consistent with our observation that barnacle geese always use the optimal combination, but that bar-headed and Andean geese expend energy to use a more effective breathing pattern. Future studies could calculate the increased cost versus the increased O_2 delivery of the 'less than optimal' strategy

It is important to note that our predictions of the optimal combination of f_R/V_t are somewhat skewed due to our subtraction of the resistive work created by the intubation tube. By subtracting this value, we do not take into account the resistive work of moving the air through the trachea, and thus we underestimate the contribution of resistive work to total work. This will reduce the estimated total work of breathing, especially at high frequencies, and may explain why some of the curves in Figure 3.11 are not u-shaped at all, but continue to decrease with increasing breathing frequency. Given this, our predicted optimal combinations may, in reality, be somewhat closer to the deeper, slower combinations used by the birds.

Those ruddy ducks!

The highland ruddy duck did not fit with the high compliance, low resistance trend seen in the other highland species. This could be for several reasons. The ruddy ducks were our only diving species in this study, and their respiratory mechanics and morphology may be under different constraints than the dabbling ducks. Birds dive upon inhalation, and avian divers may require a stiff chest wall to prevent barotrauma due to excess compression. Anecdotally, the highland ruddy ducks were simple to catch, as they were not able to dive more than once. This is in contrast to the lowland ruddy ducks that were very difficult to catch, as they were able to dive repeatedly. This is likely due to an inability of the highland ruddy ducks to replenish O₂ stores rapidly, but this has not been investigated. The lack of consistency with the pattern seen in other birds may also be due to phylogeny; all other species in this study are in the genus *Anas*, while ruddy ducks are members of the genus *Oxyura*, the stiff-tailed diving ducks. They are more closely related to geese than to other duck species (see Figure 3.2; Gonzalez et al., 2009). Also, evolutionarily, the common ancestor of the high and lowland ruddy duck subspecies originated at high altitudes and the low altitude subspecies radiated down, the opposite pattern from that seen in the other sister taxa studied here (McCracken and Sorenson, 2005). The non-conforming pattern in respiratory mechanics seen in the ruddy ducks is likely due to a combination of these factors.

Chapter conclusions

We set out to address whether there are morphological or mechanical changes in the avian respiratory system associated with high altitude and if any metabolic benefits to

these changes reduce the cost of breathing *in vivo*. We found little difference in tracheal volume or lung mass between the highland and lowland sister taxa but estimated that highland species had more compliant respiratory systems. Despite this, the work and efficacy of ventilation were similar between highland and lowland taxa. This could reflect exaptation (preadaptation) of the dabbling lowland sister taxa to highland hypoxia. This would be consistent with the hypothesis that allopatric speciation occurred between these high and low altitude taxa due to the lack of wetland habitat at intermediate altitudes (McCracken et al., 2009a). Additionally, the low breathing efficacy of mallard ducks and gadwalls may help explain their inability to expand their altitudinal niche.

Consistent with Chapter 2, we estimated that the ducks were more likely to use an optimal combination of tidal volume and frequency when oxygen is limited. If they did not use the optimal predicted combination, they always breathed slower and deeper rather than more rapidly and shallowly. In theory, this would increase the effective ventilation for a given level of minute ventilation. According to our calculations, for the highland speckled and cinnamon teals, increasing V_t over f_R would be also be energetically favorable.

We describe these species as being either highland or lowland, and, while some of them are indeed restricted to a certain altitudinal range, birds are highly mobile and using these dichotomous labels may be somewhat misleading. As previously mentioned, while northern pintails are mainly restricted to low altitude wetlands in North America (below 2,500 m), yellow-billed pintails are found at both high and low altitude in

South America, and while the individuals we collected for this study were at high altitude, there is still high gene flow among the population as a whole (McCracken et al., 2009b). The Eurasian subspecies of the American green-winged teal (*Anas crecca crecca*) has been reported to fly over the Himalayas at 5,600 meters (Groebbels, 1932). Mallard ducks, as another example, are considered a lowland species that never radiated to altitude as they are usually not seen above 2,000 meters and have no highland sister taxa, but there are anecdotal reports of mallards nesting at 3,300 meters and one incidence of a mallard (identified by one primary feather) colliding with an airplane at 6,400 meters (A. Rush, personal communication; Manville, 1963). The avian respiratory system is already highly specialized for flight, the most energetically costly form of locomotion, and this may allow birds to move between altitudes with minimal further modification. This may also help explain the lack of consistent differences such as those seen among the geese. However, given more evolutionary time, perhaps further modification would be seen, similar to that of the Andean goose.

Future directions

Although we investigated the effect of altitude here, the data presented for the ruddy duck may suggest that waterfowl respiratory mechanics are more constrained by life history strategy (i.e. dabbling, flying, or diving) than by habitat. The highly specialized avian respiratory system may lend itself to maintaining oxygen delivery at altitude, so it would be interesting to investigate how altitude affects the respiratory mechanics of mammalian taxa, such as deer mice or bats that inhabit a wide altitudinal range.

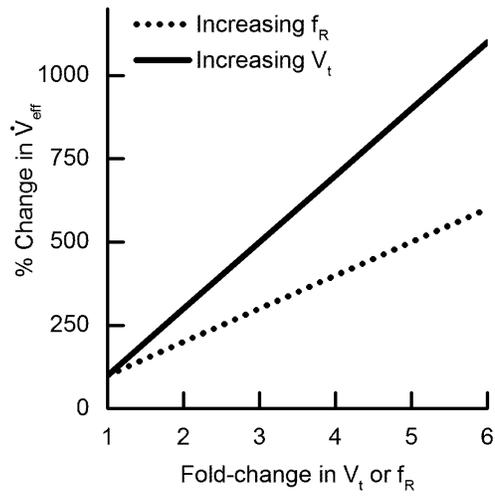


Figure 3.1: Change in effective ventilation for an equal-fold change in tidal volume (solid line) or breathing frequency (dotted line).

Table 3.1: Species compared in this study and respiratory mechanics values. We found no significant effect of altitude on any of the variables presented (two-way ANOVA, $p > 0.05$).

	Species	Abbrev.	Body mass <i>g</i>	Vital capacity <i>mL</i>	Vital capacity <i>mL kg⁻¹</i>	Inspiratory capacity <i>mL kg⁻¹</i>	Expiratory capacity <i>mL kg⁻¹</i>	Whole heart <i>g kg⁻¹</i>	Right ventricle <i>g kg⁻¹</i>	Left ventricle <i>g kg⁻¹</i>	Total lung <i>g kg⁻¹</i>	Trachea <i>mL</i>
Highland	Puna teal <i>Anas puna</i>	PT	396 ± 15	157 ± 4	399 ± 14	246 ± 12	154 ± 10	9.4 ± 0.6	1.5 ± 0.2	6.1 ± 0.4	16.7 ± 0.9	1.9 ± 0.3
	Speckled teal <i>Anas flavirostris</i>	ST	378 ± 9	132 ± 3	350 ± 8	224 ± 9	126 ± 10	12.9 ± 0.6	2.3 ± 0.2	7.7 ± 0.3	16.3 ± 1.6	1.3 ± 0.3
	Ruddy duck <i>Oxyura jamaicensis ferruginea</i>	RD	773 ± 51	193 ± 6	260 ± 19	194 ± 12	67 ± 10	9.5 ± 0.5	1.3 ± 0.1	5.8 ± 0.4	16.1 ± 1.3	1.6 ± 0.1
	Yellow-billed pintail <i>Anas georgica</i>	YBP	618 ± 20	234 ± 6	373 ± 7	236 ± 8	137 ± 4	10.4 ± 0.6	2.0 ± 0.1	6.2 ± 0.5	14.6 ± 1.3	2.8 ± 0.4
	Cinnamon teal <i>Anas cyanoptera orinomus</i>	CT	440 ± 13	159 ± 4	360 ± 13	240 ± 13	121 ± 8	11 ± 0.6	1.9 ± 0.2	6.6 ± 0.4	14.4 ± 1.2	2.6 ± 0.3
Lowland	Green-winged teal <i>Anas crecca</i>	GT	283 ± 10	94 ± 3	331 ± 18	206 ± 8	125 ± 14	12.3 ± 2	2.9 ± 0.4	9.0 ± 2	17.1 ± 1.5	1.3 ± 0.3
	Lowland ruddy duck <i>Oxyura jamaicensis jamaicensis</i>	LRD	476 ± 32	153 ± 7	329 ± 28	217 ± 16	112 ± 17	9.7 ± 0.9	2.1 ± 0.5	7.3 ± 0.5	18.1 ± 1.3	1.4 ± 0.2
	Northern pintail <i>Anas acuta</i>	NP	857 ± 12	249 ± 10	291 ± 12	183 ± 9	108 ± 7	10.7 ± 0.1	2.2 ± 0.3	8.0 ± 0.3	13.8 ± 1.9	4.9 ± 0.2
	Lowland cinnamon teal <i>Anas cyanoptera septentrionalium</i>	LCT	300 ± 13	104 ± 4	350 ± 21	218 ± 14	132 ± 16	9.2 ± 0.4	1.9 ± 0.3	6.8 ± 0.2	16.7 ± 2.4	1.8 ± 0.2
	Mallard duck <i>Anas platyrhynchos</i>	MD	932 ± 21	330 ± 19	359 ± 23	248 ± 20	111 ± 5	7.4 ± 0.4	1.3 ± 0.3	5.9 ± 0.2	13.5 ± 1.7	6.0 ± 0.1
	Gadwall <i>Anas strepera</i>	GW	766 ± 40	284 ± 10	372 ± 16	226 ± 16	147 ± 11	10.5 ± 3	2.1 ± 0.6	8.1 ± 2	13.8 ± 0.6	5.4 ± 0.4

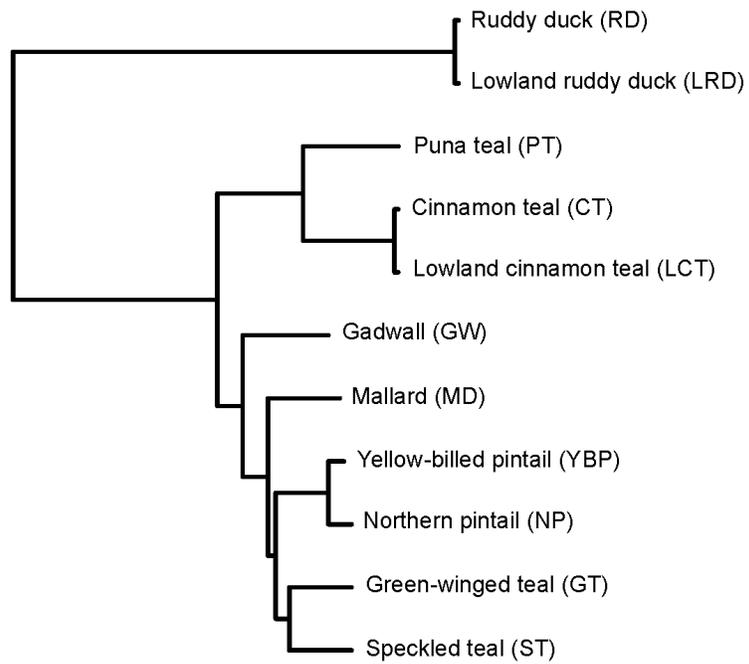


Figure 3.2: Phylogeny of the species compared in this study. [Adapted from Gonzalez et al., 2009].

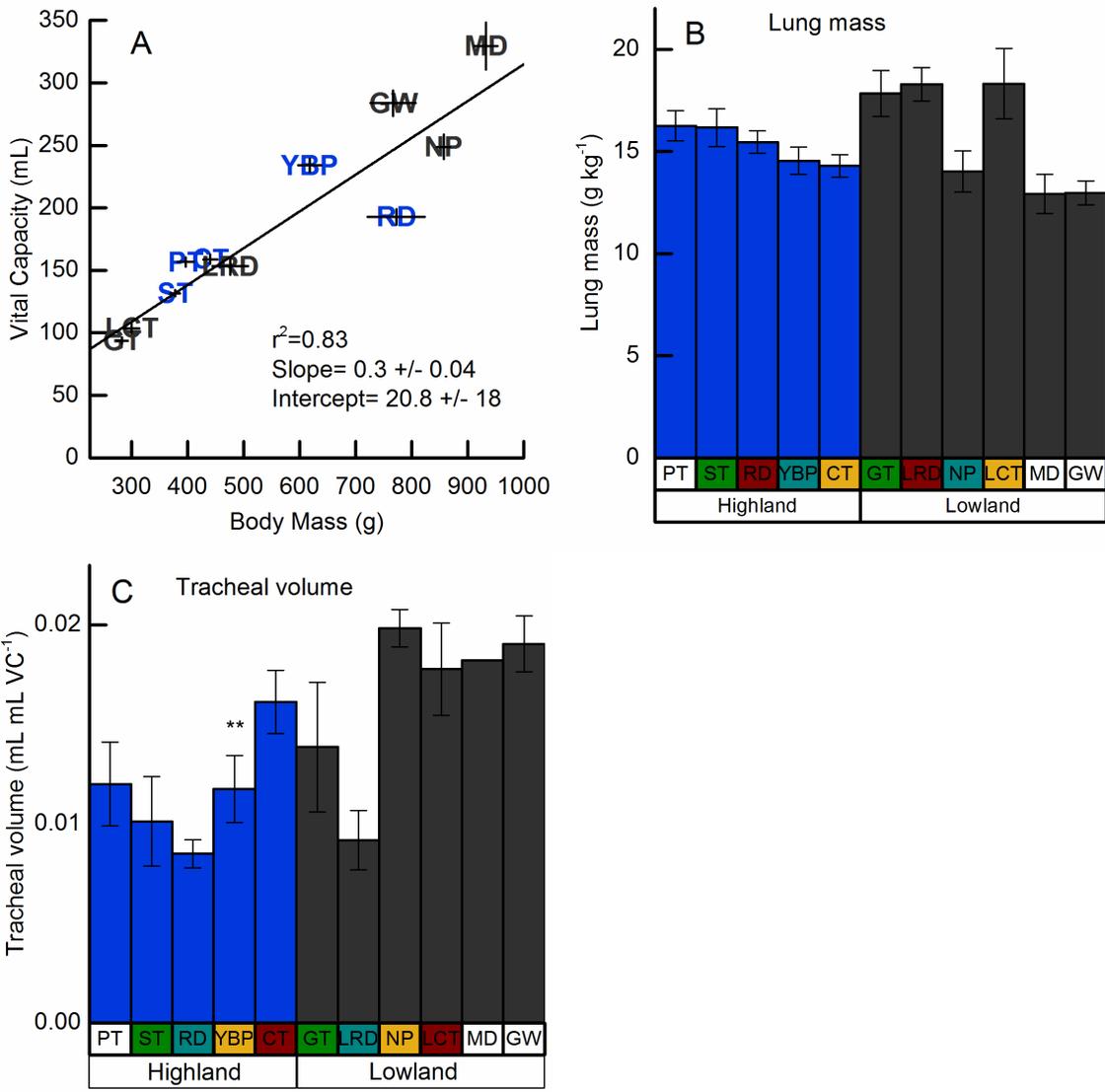


Figure 3.3: (A) Vital capacity versus body mass (B) Lung mass normalized by body mass (C) Tracheal volume normalized to vital capacity. Blue bars are highland species, grey bars are lowland species. Colors below bars identify sister taxa. ** indicates significant difference between sister taxa (t-test; $p < 0.01$). Highland species are in blue, lowland species are in grey.

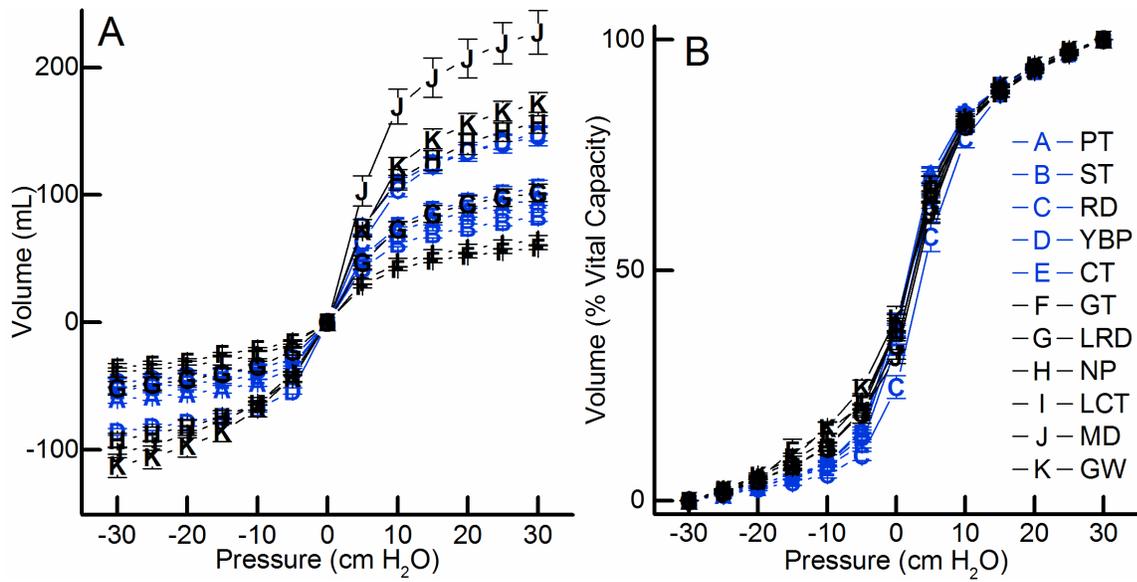


Figure 3.4: Static compliance curves of the intact respiratory system. (A) Static compliance measured as the change in volume for a given change in pressure, along the line of zero flow. High altitude species are in blue, low altitude species are in black. (B) Static compliance measured as the change in percent of vital capacity with for a given change in pressure

Table 3.2: Static compliance measured as the slope of the static compliance curves in Figure 3.2. Values are means +/- standard error. * indicates significant difference between sister taxa (*= $p < 0.05$; ***= $p < 0.001$).

Species	Altitude	Static Compliance <i>mL cm H₂O⁻¹</i>	Static Compliance <i>% Vital Capacity cm H₂O⁻¹</i>
Puna teal	Highland	8.70 ± 0.23	5.56 ± 0.13
Speckled teal	Highland	7.09 ± 0.24 ***	5.47 ± 0.22 *
Ruddy duck	Highland	9.71 ± 0.74 *	4.75 ± 0.25
Yellow-billed pintail	Highland	12.9 ± 0.60	5.51 ± 0.26 *
Cinnamon teal	Highland	8.48 ± 0.30 ***	5.36 ± 0.22 *
Green-winged teal	Lowland	4.41 ± 0.31	4.68 ± 0.24
Lowland ruddy duck	Lowland	6.97 ± 0.69	4.49 ± 0.24
Northern pintail	Lowland	11.8 ± 0.83	4.72 ± 0.15
Lowland cinnamon teal	Lowland	4.87 ± 0.32	4.69 ± 0.25
Mallard	Lowland	14.3 ± 1.27	4.34 ± 0.23
Gadwall	Lowland	11.5 ± 0.68	4.02 ± 0.12

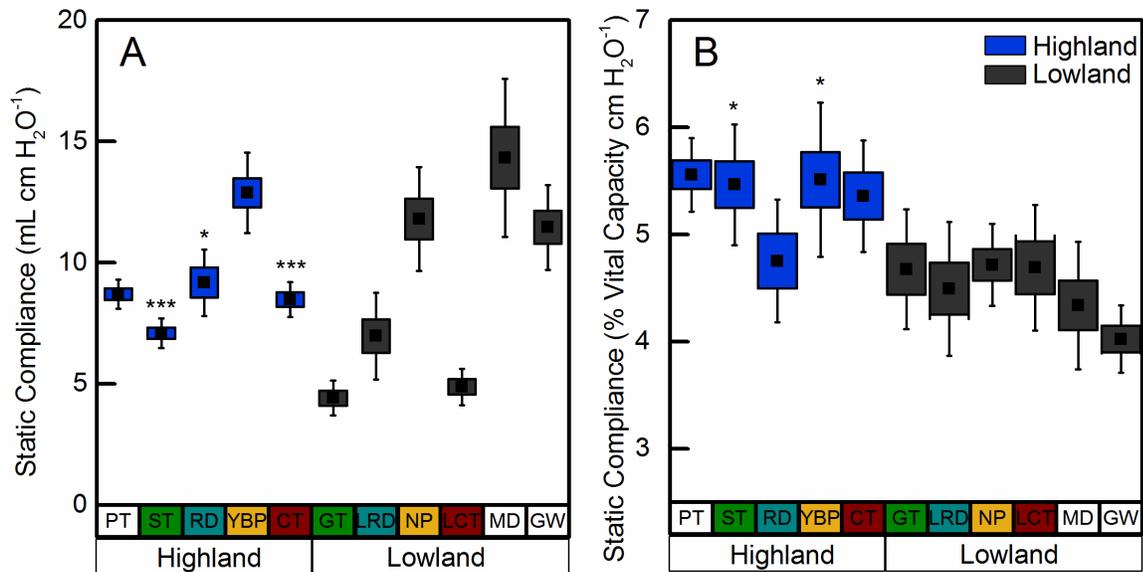


Figure 3.5: Static compliance measured as the slope of the static compliance curves for highland species (blue) and lowland species (grey). Sister taxa are identified by colors along the x-axis. (A) Static compliance measured as change in volume for a given change in pressure, or the slope of the curve in Figure 4 A. (B) Static compliance as change in volume expressed in percent vital capacity for a given change in pressure, or the slope of the curve in Figure 4 B. Mean (black squares), standard error (colored boxes), and 95% confidence interval (whiskers) are plotted. * indicates significant difference between sister taxa (*= $p < 0.05$; **= $p < 0.001$).

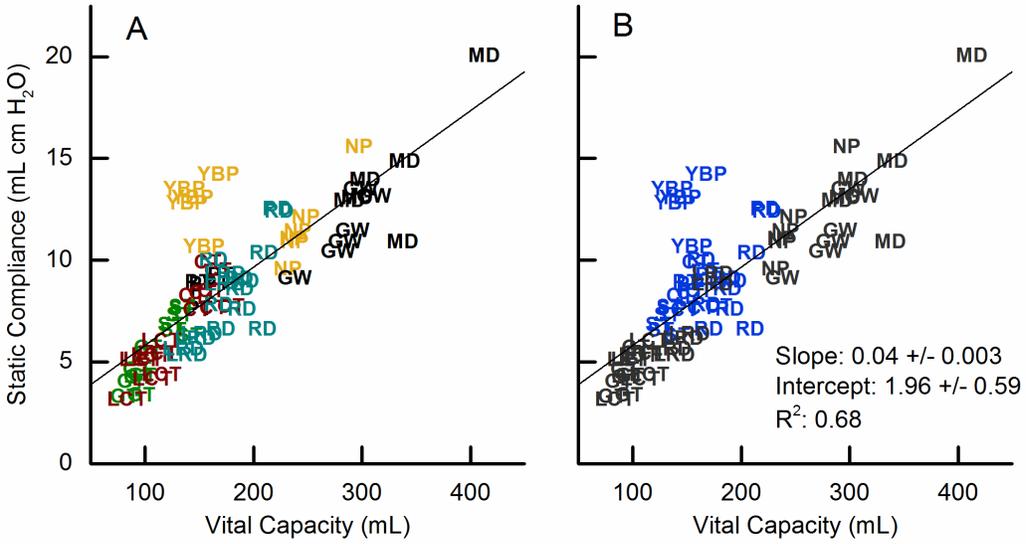


Figure 3.6: Static compliance (expressed as mL per cm H₂O) versus vital capacity (mL). (A) Species are colored by sister taxa. (B) Species are colored by altitude (blue high altitude; grey low altitude). Slope and intercepts expressed as mean +/- standard error.

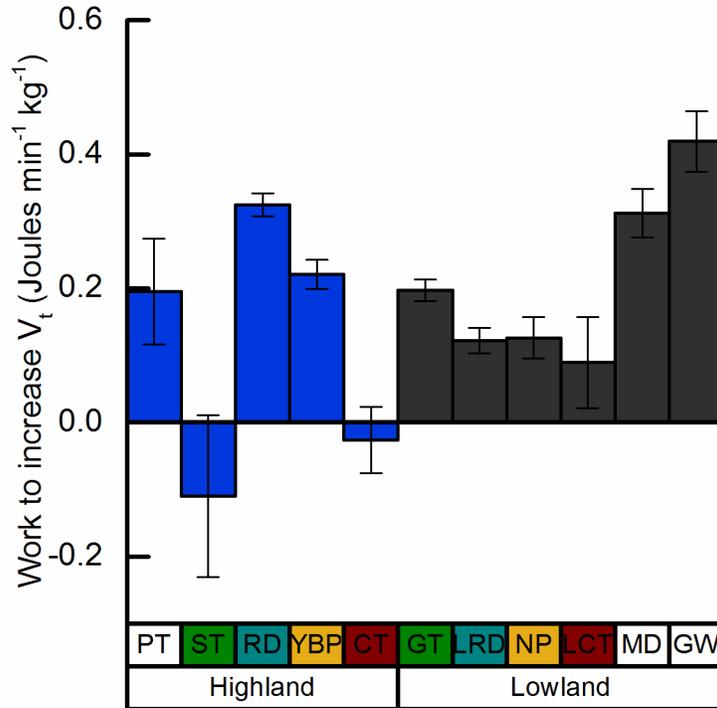


Figure 3.7: Difference between cost to increase tidal volume and cost to increase breathing frequency. Increasing tidal volume is a more expensive strategy for all birds except speckled teal and highland cinnamon teal (ST and CT). Blue bars are highland species, grey bars are lowland species.

Table 3.3: Respiratory mechanics measurements for three breathing strategies compared. Comparing the costs and benefits of doubling tidal volume or breathing frequency. The first column for every variable is representative of the average V_t/f_R for all the species at rest (7.3% VC/20 bpm). In the second column f_R is doubled, and in the third column V_t is doubled. Values are mean \pm SE. * beneath the values indicate significant differences between sister taxa (*= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$).

		Compliance (% VC cm H ₂ O ⁻¹)			Tau (seconds)			Minute work (Joules min ⁻¹ kg ⁻¹)		
V _I (% VC)		7.3	7.3	14.6	7.3	7.3	14.6	7.3	7.3	14.6
f _R (min ⁻¹)		20	40	20	20	40	20	20	40	20
Highland	Puna teal	4.39 ± 0.04	2.92 ± 0.04	3.36 ± 0.04	1.15 ± 0.01	0.90 ± 0.01	1.05 ± 0.01	0.14 ± 0.02	0.44 ± 0.02	0.63 ± 0.14
	Speckled teal	5.16 ± 0.15 ***	2.61 ± 0.09 ***	3.29 ± 0.12 ***	1.99 ± 0.18 *	0.97 ± 0.02 *	1.29 ± 0.03 **	0.10 ± 0.07	0.61 ± 0.08 ***	0.50 ± 0.16
	Ruddy duck	2.90 ± 0.04 ***	2.31 ± 0.11	2.64 ± 0.13	1.54 ± 0.04	0.98 ± 0.07	1.51 ± 0.11 *	0.17 ± 0.01 ***	0.56 ± 0.01	0.88 ± 0.02 ***
	Yellow-billed pintail	5.93 ± 0.16 ***	3.29 ± 0.06	4.52 ± 0.08 ***	1.25 ± 0.12	0.81 ± 0.10	1.08 ± 0.14	0.13 ± 0.01	0.57 ± 0.01 **	0.79 ± 0.03 **
	Cinnamon teal	4.79 ± 0.14 ***	2.80 ± 0.04 ***	3.50 ± 0.05 ***	1.29 ± 0.16	0.71 ± 0.05 ***	1.19 ± 0.08	0.12 ± 0.01	0.36 ± 0.02	0.33 ± 0.07
Lowland	Green-winged teal	3.03 ± 0.03	2.31 ± 0.01	2.40 ± 0.01	1.53 ± 0.03	1.02 ± 0.01	1.20 ± 0.01	0.20 ± 0.01	0.26 ± 0.01	0.45 ± 0.02
	Ruddy duck	3.57 ± 0.08	2.23 ± 0.01	2.59 ± 0.01	1.44 ± 0.04	1.14 ± 0.01	1.18 ± 0.01	0.11 ± 0.01	0.55 ± 0.01	0.67 ± 0.03
	Northern pintail	3.41 ± 0.01	3.20 ± 0.16	2.63 ± 0.13	1.14 ± 0.02	0.83 ± 0.03	1.03 ± 0.04	0.14 ± 0.01	0.49 ± 0.02	0.62 ± 0.05
	Cinnamon teal	3.51 ± 0.06	2.06 ± 0.09	2.62 ± 0.11	1.55 ± 0.04	0.94 ± 0.01	1.20 ± 0.01	0.14 ± 0.01	0.35 ± 0.02	0.44 ± 0.11
	Mallard	3.90 ± 0.09	3.67 ± 0.17	3.57 ± 0.17	1.06 ± 0.03	0.73 ± 0.08	1.10 ± 0.12	0.17 ± 0.01	0.64 ± 0.02	0.95 ± 0.05
Gadwall	4.48 ± 0.08	3.51 ± 0.19	3.60 ± 0.19	1.14 ± 0.04	0.78 ± 0.03	1.19 ± 0.04	0.16 ± 0.06	0.65 ± 0.02	1.07 ± 0.07	

		\dot{V}_{eff} (mL min ⁻¹ kg ⁻¹)			Efficacy (L Joule ⁻¹ kg ⁻¹)		
V_t (% VC)		7.3	7.3	14.6	7.3	7.3	14.6
f_R (min ⁻¹)		20	40	20	20	40	20
Highland	Puna teal	486 ± 21	972 ± 42	1068 ± 37	8.87 ± 0.39	5.64 ± 0.25	4.27 ± 0.15
	Speckled teal	439 ± 14	878 ± 28	948 ± 18	11.9 ± 0.38 ***	3.82 ± 0.12	5.04 ± 0.09 **
	Ruddy duck	327 ± 30	653 ± 60	695 ± 67	2.50 ± 0.23 ***	1.51 ± 0.14 ***	1.02 ± 0.10 ***
	Yellow-billed pintail	451 ± 13 ***	902 ± 27 ***	994 ± 21 ***	5.67 ± 0.17 ***	2.57 ± 0.08 ***	2.04 ± 0.04 ***
	Cinnamon teal	421 ± 22	842 ± 44	951 ± 37	8.02 ± 0.42	5.39 ± 0.28 **	6.58 ± 0.26
Lowland	Green-winged teal	385 ± 25	771 ± 50	863 ± 51	6.79 ± 0.44	10.7 ± 0.68	6.75 ± 0.40
	Ruddy duck	412 ± 38	824 ± 75	880 ± 79	7.97 ± 0.73	3.15 ± 0.29	2.76 ± 0.25
	Northern pintail	309 ± 17	618 ± 35	733 ± 36	2.63 ± 0.15	1.48 ± 0.08	1.39 ± 0.07
	Cinnamon teal	382 ± 28	764 ± 55	887 ± 58	9.20 ± 0.66	7.27 ± 0.53	6.73 ± 0.44
	Mallard	392 ± 32	784 ± 64	914 ± 65	2.53 ± 0.21	1.31 ± 0.11	1.03 ± 0.07
Gadwall	399 ± 19	799 ± 38	937 ± 42	3.26 ± 0.16	1.61 ± 0.08	1.15 ± 0.05	

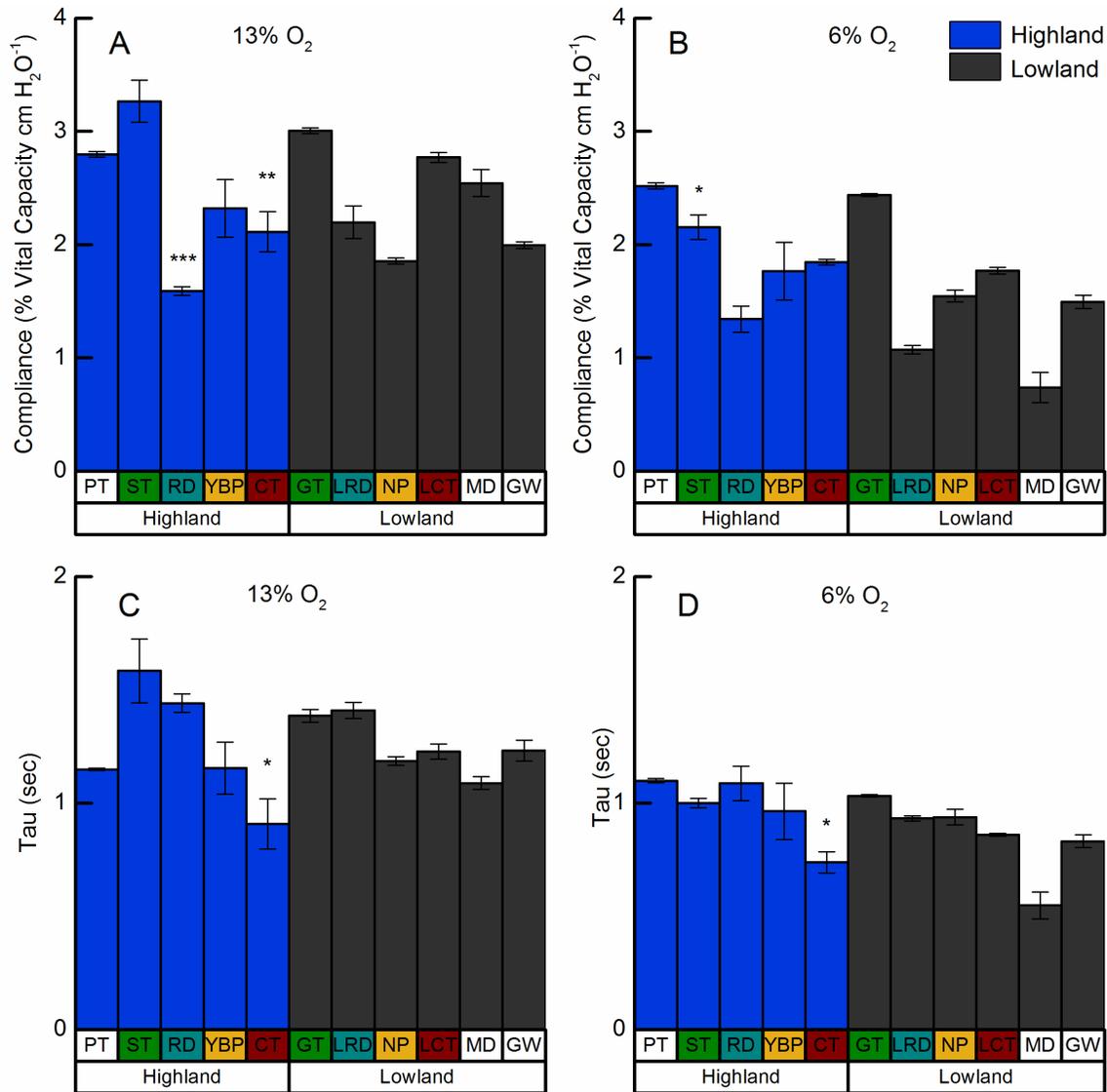


Figure 3.8: Dynamic compliance (A and B) and time constant (Tau; C and D) measurements for the actual combination of tidal volume and breathing frequency used by each species breathing 13% O₂ (A and C) and 6% O₂ (B and D). Blue bars are highland taxa, and grey bars are lowland taxa. Sister taxa are identified by colors below bars. * show significant differences (*= $p < 0.05$; **= $p < 0.01$; *= $p < 0.001$) between sister taxa.**

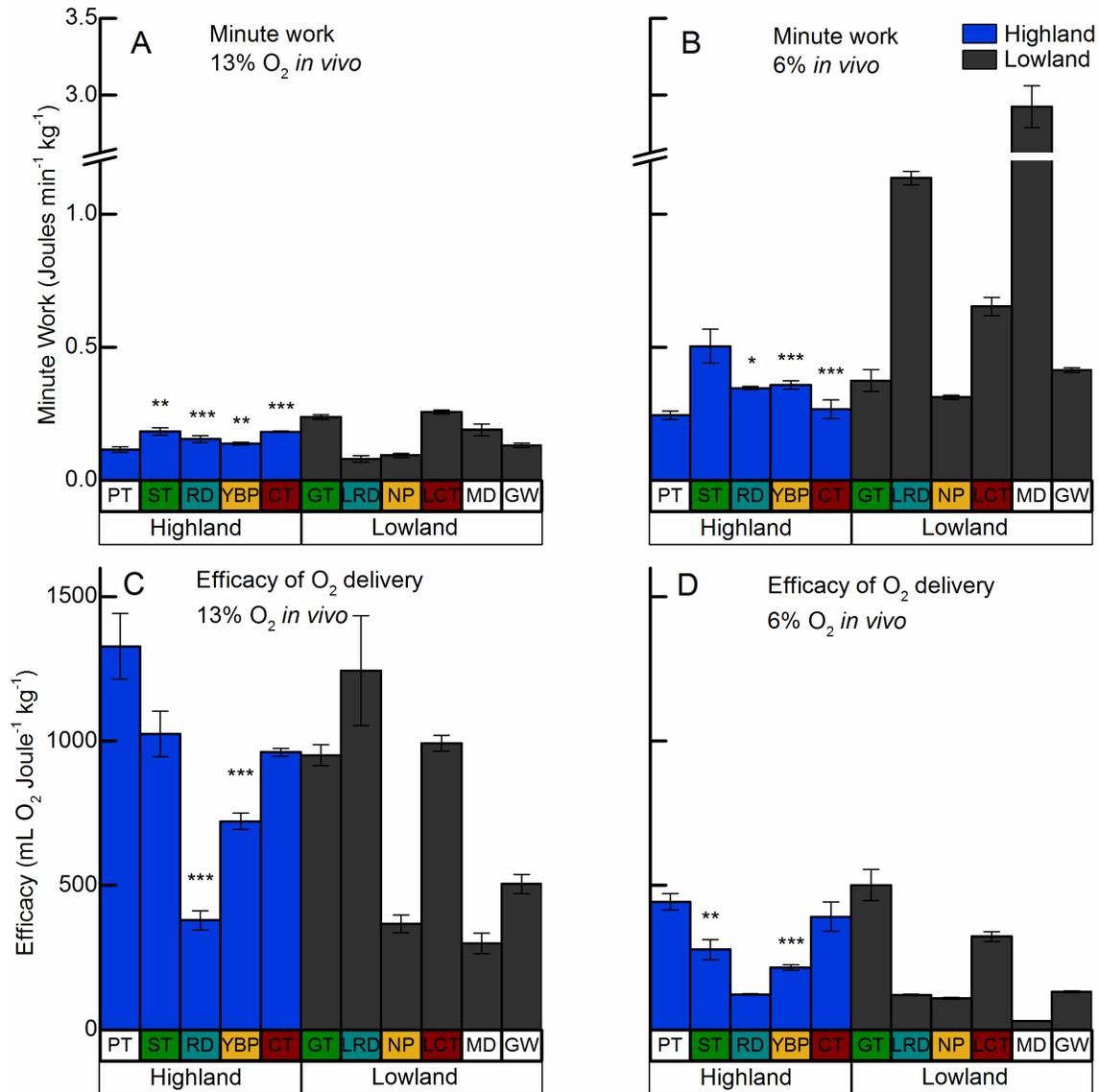


Figure 3.9: Minute work of breathing per kg (A and B) and volume of oxygen brought to the gas exchange surface per joule (C and D) while breathing 13% O₂ (A and C) and 6% O₂ (B and D). Blue bars are highland taxa, and grey bars are lowland taxa. Sister taxa are identified by colors below bars. * indicate significant differences between sister taxa (t-test; **= $p < 0.01$; *= $p < 0.001$).**

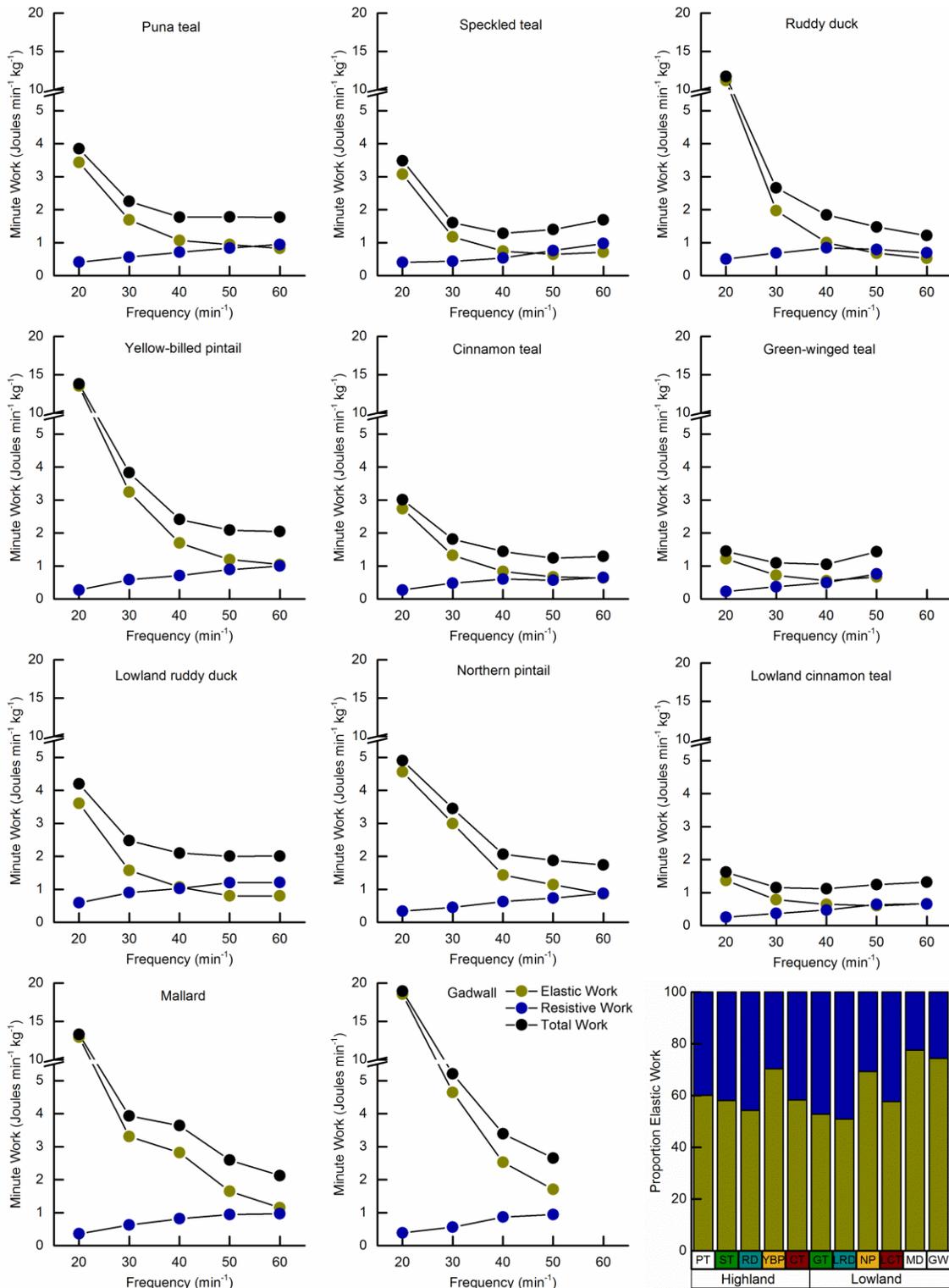


Figure 3.10: Relationship of total minute work, minute elastic work, and minute resistive work for one level of minute ventilation (500% VC per minute). Change in elastic (gold), resistive (blue), and total (black) work at a constant minute ventilation of 500% vital capacity per minute. The final panel plots the proportion of elastic (gold) and resistive (blue) work at a frequency of 40 breaths per minute and 12.5% vital capacity.

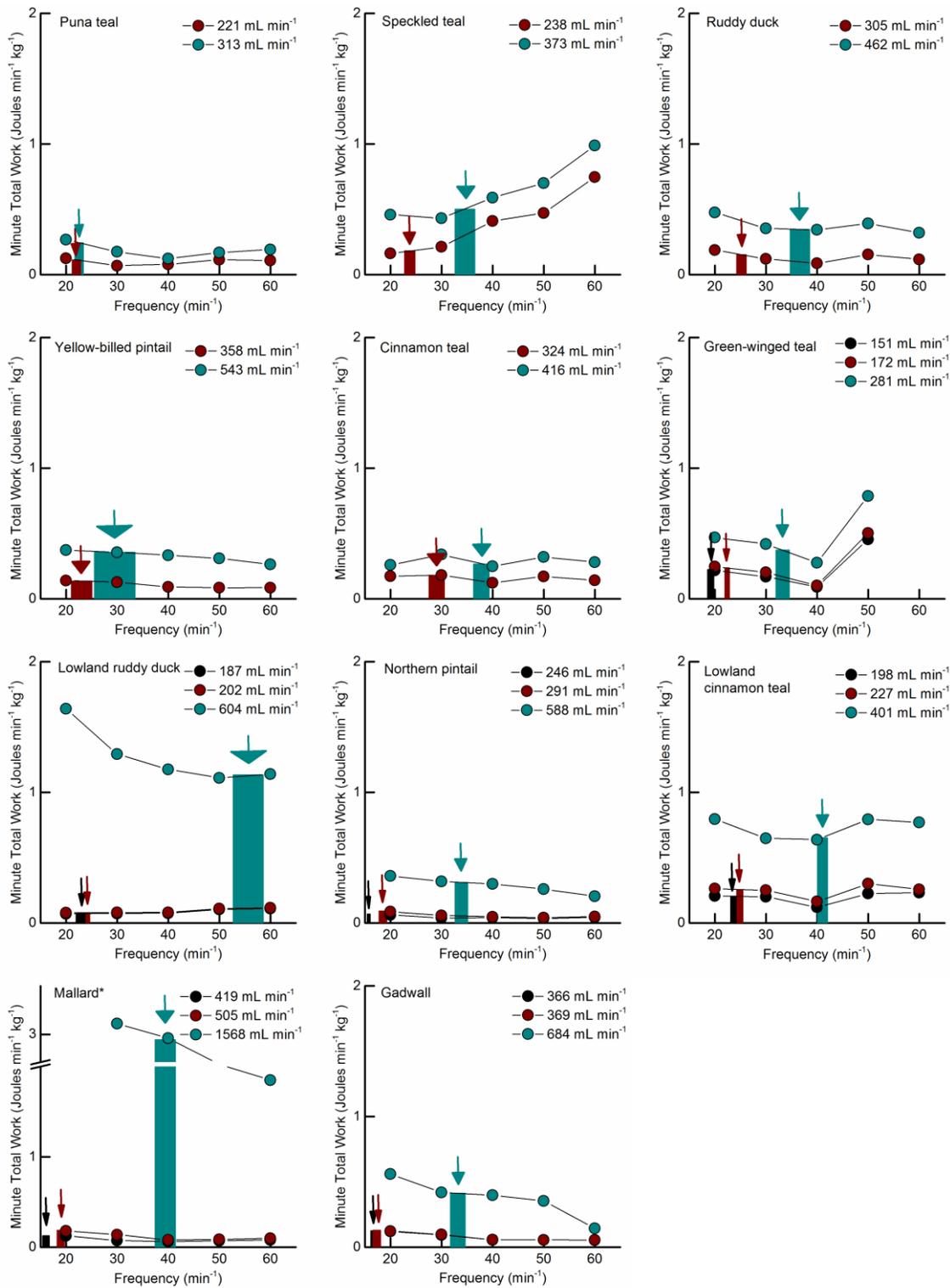


Figure 3.11: Work of breathing (power output) at combinations of breathing frequency and tidal volume for minute ventilations measured *in vivo* in 18% O₂ (black), 13% O₂ (red), and 6% O₂ (teal). Lines indicate work for the minute ventilation use by each species at that O₂ level (see individual plots for values). Bars with arrows above indicate mean \pm standard error of breathing frequencies measured in awake, unanesthetized, resting birds. *Note that the mallard duck graph uses a different scale.

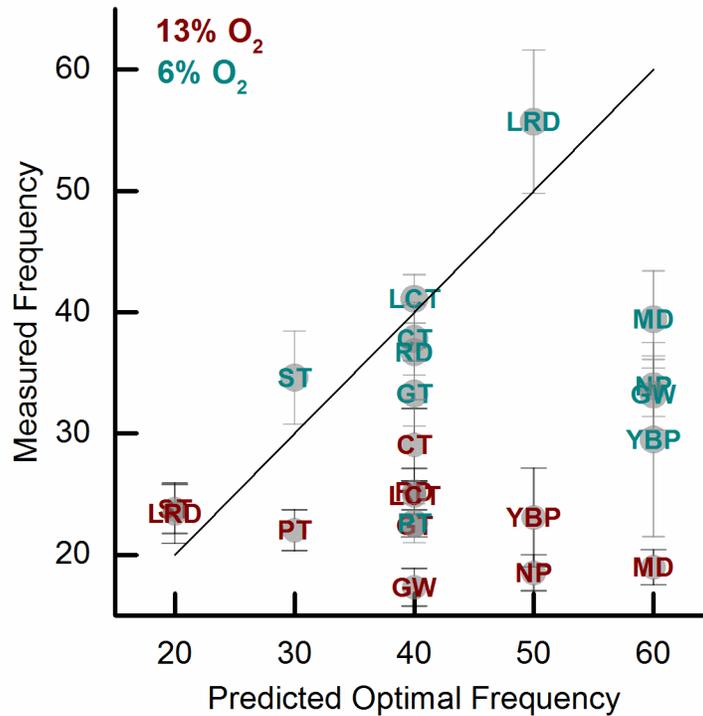


Figure 3.12: Measured breathing frequency versus predicted optimal breathing frequency. The optimal frequency is predicted by the curves in Figure 11. Black line ($x=y$) indicates where points should fall if birds were using the optimal combination of breathing frequency and tidal volume. Points that fall below this line indicate that birds are expending energy to use a higher tidal volume, points above the line show birds that expend energy to use a higher breathing frequency.

Chapter 4

Discussion

Sternal recumbency

During the measurements of respiratory mechanics and CT scans, birds were placed in sternal recumbency (prone position). Sternal recumbency may restrict the rocking movement of the sternum that is used to expand the pleural cavity during normal breathing. However, Malka and colleagues (2009) found that sternal recumbency was the position that resulted in the greatest air sac and lung volumes measured by CT scan in red-tailed hawks. This was probably due to pleural cavity compression by the flight muscles and intestinal displacement when the birds were placed in dorsal recumbency or on their side (Petnehazy et al., 2012). King and Payne (1964) found a reduction in tidal volume when both conscious and anesthetized chickens were placed in dorsal recumbency as compared to erect posture (sternal recumbency was not measured). The *in vivo* respiratory and metabolic values we use here were measured in birds resting in sternal recumbency. We therefore chose to complete experiments while the birds rested on their sternums; however, this may have caused an underestimation of work of breathing due to the lack of gravitational pull on the mass of the flight muscle attached to the sternum. Indeed, Tickle and colleagues (2010) noted that barnacle geese often sat down when carrying a sternal load, which is unusual for a bird in a stressful environment, and was not seen to the same extent in birds carrying loads on their backs or legs. They hypothesized that birds sat down to change their breathing strategy from sternal rotation to a more energetically favorable costal expansion. In a later study they found that standing was 25% more metabolically expensive than sitting

in sternal recumbency (Tickle et al., 2012). If this is the case, in this study we primarily measure the work of the costal expansion breathing strategy, and the cost of breathing during standing, running, or flight would be expected to be higher.

Subtracting endotracheal tube resistance

In these studies, birds were intubated in order to be ventilated without interference from spontaneous respiration. At the end of the studies, all endotracheal tubes were ventilated with the same volumes and frequencies as the birds, resistive work was calculated from pressure-volume loops, and the resistive work to move the air through the endotracheal tube alone was subtracted from the total resistive work. We chose to subtract this value because it artificially inflates the resistive work of breathing as the endotracheal tube is by necessity narrower than the trachea of the bird. Obviously, we are therefore underestimating the resistive work of breathing because we have not taken into account the resistive work of the trachea. Typically, the resistive work to move air through the endotracheal tube was approximately 3% of total resistive work, and it ranged from 1% at lower frequencies to 10% at higher frequencies. For the low frequencies used by the birds at rest, therefore, the error due to the subtraction of the endotracheal tube resistive work can be expected to be less than 1%. However, at high frequencies, such as those used by the mallard duck in hypoxia, the resistive work (and consequently the total work) could be underestimated by up to 10%, and this should be taken into account in the interpretation of these data.

Lung volume estimates

In these studies we estimated lung volume by dissection but chose not to present the data. Our lung volume estimates in this study were approximately half of similar values in the literature (see Maina, 2002 for a review), due to a difference in methods.

Commonly in the 1980's and early 1990's, morphometrics of the lungs (including lung volume) were measured by first fixing the lung *in situ* with glutaraldehyde under a pressure head of 25 cm H₂O (Maina and King, 1982; Powell and Mazzone, 1983; Maina, 1984; Maina, 1987; Timmwood, et al., 1987; Vidyadaran et al., 1988; Maina and King, 1989; Vidyadaran et al., 1990). The lungs were then dissected from the bird and the volume measured by the fluid immersion method described by Scherle (1970). The lungs were therefore fixed at or near maximum capacity, which could increase their volume by up to 18%. In this study, lungs were dissected without first being fixed, and volume was estimated by the same method. The large discrepancy between our values and the values measured in the fixed lungs may seem counterintuitive as avian lungs are generally thought of as "rigid" and expand and contract very little during breathing. However, *in situ* the lungs are attached to the surrounding tissues by fascia, and the process of extraction removes the attachments and pushes air and blood out of the tissue. King (1966) noted, "The avian lung contracts appreciably on being removed from the coelom." This contraction of unfixed lungs, as well as the high pressure protocol for fixed lungs, explains the significant drop in mass specific lung volume we measured. We discuss it here simply to note that future investigations should be made aware of the possibility of tissue contraction and the inability to control for the amount of blood and air that leaves the lung upon dissection. Lung volumes should be measured for fixed

lungs or lungs *in situ* using non-invasive methods such as CT scans. Our values for lung mass are in rough agreement with those of Scott and colleagues (2011). Lung masses of birds seem to scale with body mass and are consistently 1% of total body mass (see Table 4.1; Lasiewski and Calder, 1971).

Avian respiratory morphometry

Although it has been the focus of much study and attention, major questions in our understanding of the avian respiratory system remain unsettled. Primarily, how exactly is airflow directed past the lungs to the caudal air sacs on inspiration and then from the caudal air sacs to the lungs on expiration? As Krogh (1941) said, “A study of the distribution of inspired air between lungs and the separate air sacs presents very formidable experimental difficulties, and from the literature anything but a clear and consistent picture can be obtained.” Since 1941, significant progress has been made but the mechanisms of airflow control are not yet fully understood.

The current hypothesis is that airflow is directed primarily by aerodynamic means through differential resistance of the airways, the relative angles of the airways, and the relative compliance of the air sacs (Cieri and Farmer, 2016; Harvey and Ben-Tal, 2016). Unidirectional airflow through the lung can be accomplished passively simply due to the anatomy of the avian respiratory system. This has been supported by studies showing that airflow through the lung is unidirectional even when birds are mechanically ventilated after death (Scheid and Piiper, 1971) as well as studies modelling passive airflow (Maina et al., 2009; Harvey and Ben-Tal, 2016). However, anatomical

constrictions thought to achieve this passive control, such as the segmentum accelerans, are not found in all avian taxa (e.g. Maina and Nathaniel, 2001). It appears that to achieve the high efficiency of aerodynamic valving actually measured in birds, some active control from the smooth muscle surrounding the airways may be required (Urushikubo et al., 2013). The smooth muscle could help to control relative resistance of the various bronchi and ostia. The smooth muscle found around the bronchi is known to contract in response to CO₂ changes in some areas and to spontaneous vagal input in other areas (Barnas et al., 1978; Wang et al., 1992). Clearly, more investigation is needed into the role of the smooth muscle and how it might change under certain conditions. For example, in panting ostriches, air sac ventilation is very vigorous, but no respiratory alkalosis develops (Schmidt-Nielsen et al., 1969). This could be due to a shunting of blood away from the lung, but Jones (1982) found no significant redistribution of blood in the lung during panting. Therefore, ostriches must be shunting air away from gas exchange surfaces during panting, a finding that has yet to be explained (Maina et al., 2009).

The primary factor in determining the relative compliances of the air sacs is the relative size. We compare air sac volumes collected from the literature in Table 4.1. Obviously, volume varies depending on the pressure in the coelom, so we have compiled values for both functional residual capacity (FRC) and maximum inflation. Caudal air sacs are consistently larger than cranial air sacs, although Ponganis and colleagues (2015) found that caudal air sacs are smaller than cranial air sacs at functional residual capacity in Adelie and King penguins. This indicates that the compliance of the caudal

air sacs is much higher than that of the cranial air sacs, as there is a greater volume change for a given pressure change. According to Harvey and Ben-Tal (2016), this would cause the majority of air to pass through the parabronchi on expiration and increase the efficiency of unidirectional airflow. More studies investigating relative air sac size at functional residual capacity and maximum inflation are needed to understand whether this is consistently the case in the avian respiratory system.

Dubach (1981) hypothesized that active birds have a smaller air sac to lung ratio than less active birds. In avian taxa, air sac to lung volume ratio ranges from 3.71 to 21.2 at FRC, and from 2.62-15.7 at maximum capacity. In the species for which we have both FRC and maximal inflation data, the ratio approximately doubles from FRC to maximum capacity. We do find some support for the air sac to lung volume hypothesis. The smallest values are seen in active birds such as the ostrich, pigeon, and hummingbird, while species such as the domestic chicken have values approximately 3 to 5-fold larger.

Tracheal volumes

Hinds and Calder (1971) compiled tracheal volumes from 52 avian species across 24 families and found they scaled allometrically according to the equation:

$$\text{Tracheal volume} = 3.724 \times \text{Body mass}^{1.090}$$

Where tracheal volume is expressed in milliliters and body mass is expressed in kilograms. The tracheal volumes reported here are nearly all larger than predicted by the allometric equation—an average of 53% larger than predicted. This is primarily due

to the presence of large bullae on the syrinx of the males of several species studied here. The size of the trachea is a trade-off between minimizing ventilatory dead space and minimizing resistance to airflow. As mentioned previously, birds have longer tracheas than mammals of similar body masses (2.7-fold longer), but they have compensated for the increased resistance of long tracheas by increasing the radius 1.29-fold as compared to mammals (Hinds and Calder, 1971). The resistance in a tube is proportional to the radius raised to the fourth power, so any increase in tracheal radius greatly reduces resistance to airflow. Indeed, mammals and birds are hypothesized to have approximately equal tracheal resistance due to this compensation. However, because of the wider, longer trachea, birds are estimated to have 4.53-fold larger tracheal dead space volumes than similarly sized mammals, and, because they exhale actively, the dead space volume “costs” twice as much for a bird. Therefore, it is interesting to note that the majority of ducks studied here have increased this dead space volume even further, presumably due to sexual selection. A notable exception is the highland ruddy duck, whose tracheal dead space was 43% smaller than predicted by the allometric equation. The males of this species do not have syringeal bullae, and perhaps the constraints of a rapid recovery from diving have favored a small dead space volume at the cost of a high resistance, although as previously described these birds are not generally able to recover rapidly from dives. Another important observation from our data was that the tracheal volumes estimated by the CT scans were smaller than those predicted by the allometric equation, and, for Andean geese, the tracheal volume estimated by CT scan was approximately half that of the tracheal volumes measured during dissection. Unfortunately, we do not have

dissected tracheal volumes for the other two species, but future studies should verify the accuracy of the small volumes estimated with CT scans.

Avian respiratory mechanics

We predicted that highland species would have higher compliance than lowland species, and we generally found this to be true, especially when compliance was normalized to body mass or to the size of the respiratory system. As expected, the compliance values we measured were higher than those reported in the literature for mammals and lower than the compliance values reported for non-avian sauropsids and turtles (see Table 4.2). The work of breathing we estimated was similar to those reported for mammals, but 2-3 orders of magnitude higher than those of non-avian sauropsids. Non-avian sauropsids have simple, sac-like lungs, which explain their high compliance and low work of breathing estimates. Mammals have complex lungs that are difficult to inflate, and therefore their compliance is generally low, and the work of breathing high. Birds seem to have fairly high compliance, as well as high work of breathing, and this is probably due to the high resistance to airflow in the avian respiratory system from the numerous small airways as compared to the mammalian respiratory system.

Cost of breathing

In these studies, we were able to estimate the work of breathing associated with the actual breathing strategies used by the individual birds in normoxia and hypoxia. Failure to do this has caused problems during previous attempts to measure cost of breathing

in birds (Markley and Carrier, 2010). This is because manipulation of the breathing strategy can cause homeostatic imbalance, such as blood pH change, that requires active compensation by the bird making metabolic rate estimates unreliable. By temporally separating the measurements of ventilatory and metabolic responses in the awake bird and the work done to achieve those responses in the anesthetized bird we eliminate error from artificial manipulation. In addition, our work measurements can be applied to any past or future values of breathing frequency and tidal volume that might be measured in these birds.

This method also has disadvantages. The “work” of breathing we measure is only the work that must be done to actively inflate the relaxed respiratory system. When awake, birds both inspire and expire actively, therefore our measured work values should be doubled to estimate the work of a complete breath. Additionally, skeletal muscle in general is not 100% efficient, in fact, respiratory skeletal muscle is closer to 10% efficient: to apply one joule of work to the system, the muscle must do 10 joules of work (Otis et al., 1950). We therefore multiplied our work estimates by 20 to account for inspiration, expiration, and muscle efficiency. This was the same correction used by Lee and Milsom (2016) to estimate theoretical cost of breathing in red-eared sliders (*Trachemys scripta elegans*). To convert these corrected “work” values to metabolic “cost” values we calculated basal metabolic rate (BMR) by plugging the $\dot{V}O_2$ and $\dot{V}CO_2$ measured in each individual bird into the equation given by Romijn and Lokhorst (1966) for the metabolic catabolism of birds:

$$kJ = 16.19 \times (L O_2) + 5.00 \times (L CO_2)$$

Our estimated cost of breathing with these corrections can be compared for all fourteen species in Table 4.3. In normoxia, our estimates of cost of breathing range from 0.38% BMR in the bar-headed goose and lowland ruddy duck to 1.14% BMR in the mallard, and average 0.72% BMR. In hypoxia, the mallard is still highest using 15.7% of BMR simply to breathe. The Andean goose, which changed ventilation very little in hypoxia, has the lowest cost of breathing at 0.90%. The average cost of breathing in hypoxia is 3.5% BMR. These values are low, but within the general range reported in the literature of 1-10% BMR in vertebrates (Otis, 1950; Steffensen and Lomholt, 1983; Aaron et al., 1992; Skovgaard et al., 2016; Lee and Milsom, 2016). They are in agreement with the other two studies that estimate cost of breathing in birds: a rough 2% BMR by Ellerby and colleagues (2005) based on blood flow redistribution, and minimum of 1.43% running metabolism estimated by unidirectional artificial ventilation by Markley and Carrier (2010). Both of these studies were done on the guinea fowl (*Numida meleagris*). Thus, this thesis presents the first cost of breathing data measured from the avian order Anseriformes and the first estimate of cost of breathing in birds using this method.

Table 4.1: Air sac and lung volume measurements from the literature and this study. Values are expressed as mean \pm SE. Cranial air sacs include cervical, clavicular, and craniothoracic air sacs. Caudal air sacs include caudothoracic air sacs and abdominal air sacs. Pneumatic spaces are for the vertebrae only.

^AThis study

^BKrautwald-Junghanns et al, 1998

^CDubach, 1981

^DVictorow, 1909

^ECampana, 1875

^FZeuthen, 1942

^GKing and Payne, 1958

^HKing and Payne, 1962

^ISchmidt-Nielsen et al, 1969

^JPonganis et al., 2015

* Volumes expressed as % of trunk volume, not total body volume

Species	M_b	V_b	Lung mass	Lung vol	Lung mass	Lung vol	Total air sac vol	Air sac vol	Air sac to lung Vol ratio	Cranial air sacs vol	Caudal air sac vol	Caudal to cranial Vol ratio	Tracheal vol	Pneumatic spaces	Method ^{Citation}
	g	mL	g	mL	% M_b	% V_b	mL	% V_b		mL	mL	Vol ratio	mL	mL	
Amazons and grey parrots <i>Amazona and Psittacus</i>	356.6 ± 50			10.23 ± 1.74		4.62%*	37 ± 10.4	16.7%*	3.7						CT ^B
Adelie penguins <i>Pygoscelis adeliae</i>	4,630 ± 456	4,400 ± 1000		115 ± 8		2.61%	306 ± 100	6.95%	2.7	194.5	111.3	0.57	12 ± 3.2		CT ^J
King penguins <i>Aptenodytes patagonicus</i>	13,430 ± 425	13,250 ± 650		252 ± 20		1.90%	536 ± 50	4.05%	2.1	271.8	263.8	0.97	50 ± 13.3		CT ^J
Emperor penguins <i>Aptenodytes forsteri</i>	21,460 ± 845	21,000 ± 2000		353 ± 24		1.68%	1,528 ± 200	7.28%	4.3	607.3	921	1.52	72 ± 7.1		CT ^J
Torrent duck <i>Merganetta armatta</i>	425 ± 11.6		4.97 ± 0.37	5.74 ± 0.76	1.35%								1.23 ± 0.11		Fluid immersion ^{unp} _{ub}
Bar-headed goose <i>Anser indicus</i>	2,770 ± 100	1,756 ± 112	27 ± 2	74 ± 5	0.98%	4.21%	348 ± 28	#####	4.7				8 ± 0.9	5 ± 0.3	CT ^A
Andean goose <i>Chloephaga melanoptera</i>	2,290 ± 200	1,422	24 ± 1.1	92	1.07%	6.47%	409	#####	4.4				5.6	6.4	CT ^A
Barnacle goose <i>Branta leucopsis</i>	2,380 ± 200	1,430 ± 36	21 ± 3	46 ± 0.2	0.88%	3.22%	190 ± 4	#####	4.1				4.7 ± 0.02	3.2 ± 0.2	CT ^A

FRC Estimates

Species	M _b	V _b	Lung mass	Lung vol	Lung mass	Lung vol	Total air sac vol	Air sac vol	Air sac to lung Vol ratio	Cranial air sacs vol	Caudal air sac vol	Caudal to cranial	Tracheal vol	Pneumatic spaces	Method ^{Citation}
	g	mL	g	mL	% M _b	% V _b	mL	% V _b		mL	mL	Vol ratio	mL	mL	
House sparrow <i>Passer domesticus</i>	23.56 ± 1.82	34.05 ± 1.81	0.27 ± 0.08	0.8 ± 0.1	1.14%	2.35%	5.76 ± 0.9	16.90 %	7.2	2.15 ± 0.29	3.61 ± 0.72	1.68	0.08 ± 0.01	0.08 ± 0.02	Silicone cast ^C
Budgerigar <i>Melopsittacus undulatus</i>	38.16 ± 5	46.08 ± 5.66	0.31 ± 0.04	1.11 ± 0.16	0.85%	2.40%	5.0 ± 1.31	10.85 %	4.5	2.45 ± 0.66	2.55 ± 0.74	1.04	0.10 ± 0.02	0.17 ± 0.09	Silicone cast ^C
Violet-eared hummingbird <i>Colibri coruscans</i>	7.28 ± 1.16	9.29 ± 0.88	0.1	0.27 ± 0.05	1.37%	2.91%	1.59 ± 0.43	17.12 %	5.9	0.53 ± 0.15	1.06 ± 0.29	2	0.05 ± 0.01	0.08 ± 0.03	Silicone cast ^C
Rock pigeon <i>Columba livia</i>				15			49-60		3.2	16	33-44	2-2.75			Wax cast ^D
Domestic chicken <i>Gallus gallus domesticus</i>	1,600			21.9			287.8		13.1	83.4	204.4	2.45			Cocoa butter injection ^E
Domestic chicken <i>Gallus gallus domesticus</i>				6			94		15.7	26	68	2.62			Wax cast ^F
Domestic chicken <i>Gallus gallus domesticus</i>				24			358		14.9	86	272	3.16			Latex cast ^G
Domestic chicken <i>Gallus gallus domesticus</i>	3,300			52.5			342		6.5	170	172	1.01			Resin cast ^H
Ostrich <i>Struthio camelus</i>	1 x10 ⁵			3,000			7,870		2.6	1,450	3,420	2.36			Gelatin casts ^I
Adelie penguins <i>Pygoscelis adeliae</i>	4,630 ± 456	5,750 ± 1000		120 ± 7		2.09%	1,243 ± 136	21.62 %	10.4	441	802	1.82			CT ^J
King penguins <i>Aptenodytes patagonicus</i>	13,430 ± 425	18,800 ± 1000		310 ± 15		1.65%	4,950 ± 304	26.33 %	16.0	1,666	3,283	1.97			CT ^J
Emperor penguins <i>Aptenodytes forsteri</i>	21,460 ± 845	28,000 ± 2500		425 ± 40		1.52%	7,462 ± 88	26.65 %	17.6	3,005	4,457	1.48			CT ^J
Bar-headed goose <i>Anser indicus</i>	2,770 ± 100	1,777 ± 114	27 ± 2	83 ± 7	0.98%	4.67%	707 ± 1	39.79 %	8.5				8 ± 0.9	5 ± 0.3	CT ^A
Andean goose <i>Chloephaga melanoptera</i>	2,290 ± 200	1,422	24.5 ± 1	103	1.07%	7.24%	722	50.77 %	7.0				5.6	6.4	CT ^A
Barnacle goose <i>Branta leucopsis</i>	2,380 ± 200	1,448 ± 32	21 ± 3	51 ± 1	0.88%	3.52%	491 ± 19	33.91 %	9.6				4.7 ± 0.02	3.2 ± 0.2	CT ^A

Maximal inflation estimates

Table 4.2: Respiratory mechanics comparison table. *Work values are estimates of breathing at rest in normoxia.

Species	M _b g	Total respiratory capacity mL	Vital capacity mL	Tracheal vol mL	Static Compliance mL cm H ₂ O ⁻¹	Static Compliance mL cm H ₂ O ⁻¹ kg ⁻¹	Minute elastic work* J min ⁻¹ kg ⁻¹	Minute total work* J min ⁻¹ kg ⁻¹	Citation
<i>Birds</i>									
Mallard <i>Anas platyrhynchos</i>		249.4							Dehner, 1946
Mallard <i>Anas platyrhynchos</i>	932 ± 21		330 ± 19	6 ± 0.1	14.3 ± 1.27	15.3 ± 1.4		0.189 ± 0.022	This study
Black duck <i>Anas rubripes</i>		236.7							Dehner, 1946
Greater scaup <i>Nyroca marila</i>		198.3							Dehner, 1946
Red-head <i>Nyroca americana</i>		201.1							Dehner, 1946 Perry and Duncker, 1980
Rock pigeon <i>Columba livia</i>					76.52		6.76 x10 ⁻⁴		
Bar-headed goose <i>Anser indicus</i>	2770 ± 100	798 ± 9	615 ± 36		29.4 ± 1.9	10.6 ± 0.7		0.0537 ± 0.0033	This study
Andean goose <i>Chloephaga melanoptera</i>	2290 ± 200	831	758 ± 27		32.3 ± 1.5	14.1 ± 0.7		0.0662 ± 0.002	This study
Barnacle goose <i>Branta leucopsis</i>	2380 ± 200	547 ± 20	452 ± 19		23.0 ± 1.5	9.66 ± 0.6		0.0708 ± 0.0064	This study
Puna teal <i>Anas puna</i>	396 ± 15		157 ± 4	1.9 ± 0.3	8.70 ± 0.23	22 ± 0.6		0.1149 ± 0.01	This study
Speckled teal <i>Anas flavirostris</i>	278 ± 9		132 ± 3	1.3 ± 0.3	7.09 ± 0.24	28 ± 0.9		0.182 ± 0.01	This study
Ruddy duck (highland) <i>Oxyura jamaicensis ferruginea</i>	772 ± 51		193 ± 6	1.6 ± 0.1	9.71 ± 0.74	12.6 ± 1		0.155 ± 0.01	This study
Yellow-billed pintail <i>Anas georgica</i>	628 ± 20		234 ± 6	2.8 ± 0.4	12.9 ± 0.60	20.5 ± 1		0.137 ± 0.01	This study
Cinnamon teal (highland) <i>Anas cyanoptera orinomus</i>	440 ± 13		159 ± 4	2.6 ± 0.3	8.48 ± 0.30	19.3 ± 0.7		0.182 ± 0.003	This study
Green-winged teal <i>Anas crecca</i>	283 ± 10		94 ± 3	1.3 ± 0.3	4.41 ± 0.31	15.6 ± 1.1		0.237 ± 0.01	This study
Ruddy duck (lowland) <i>Oxyura jamaicensis jamaicensis</i>	476 ± 32		153 ± 7	1.4 ± 0.2	6.97 ± 0.69	14.6 ± 1.5		0.0803 ± 0.01	This study
Northern pintail <i>Anas acuta</i>	867 ± 12		249 ± 10	4.9 ± 0.2	11.8 ± 0.83	13.6 ± 1		0.0936 ± 0.01	This study
Cinnamon teal (lowland) <i>Anas cyanoptera septentrionalium</i>	300 ± 13		104 ± 4	1.8 ± 0.2	4.87 ± 0.32	16.2 ± 1.1		0.257 ± 0.01	This study
Gadwall <i>Anas strepera</i>	766 ± 40		284 ± 10	5.4 ± 0.4	11.5 ± 0.68	15 ± 0.9		0.131 ± 0.01	This study

Species	M _b	Total respiratory capacity	Vital capacity	Tracheal vol	Static Compliance	Static Compliance	Minute elastic work*	Minute total work*	Citation
	g	mL	mL	mL	mL cm H ₂ O ⁻¹	mL cm H ₂ O ⁻¹ kg ⁻¹	J min ⁻¹ kg ⁻¹	J min ⁻¹ kg ⁻¹	
<i>Non-avian sauropsids and turtles</i>									
Green lizard <i>Lacerta viridis</i>	29				1.74	60	5.15 x10 ⁻⁵		Perry and Duncker, 1978
Monitor lizard <i>Varanus exanthematicus</i>	249				88.76	356.5	1.456 x10 ⁻⁴		Perry and Duncker, 1978
Monitor lizard <i>Varanus gouldii</i>	249						4.036 x10 ⁻⁵		Perry and Duncker, 1978
Turtle <i>Pseudemys scripta</i>	275				72.25	262.7	2.344 x10 ⁻⁴		Jackson, 1971
<i>Mammals</i>									
Rat <i>Rattus norvegicus</i>	403				1.29	3.2	0.0403		Perry and Duncker, 1980
Rat <i>Rattus norvegicus</i>	423 ± 16				1.56 ± 0.2	3.69			Bennett and Tenney, 1982
Rat <i>Rattus norvegicus</i>	250				0.3	1.2	0.118		Crosfill and Widdicombe, 1961
Dog <i>Canis lupus familiaris</i>	26000				156.2 ± 60	6.01			Bennett and Tenney, 1982
Rabbit <i>Oryctolagus cuniculus</i>	2520 ± 46				8.58 ± 1	3.4			Bennett and Tenney, 1982
Mouse <i>Mus musculus</i>	26 ± 0.6				0.097 ± 0.02	3.73			Bennett and Tenney, 1982
Cat <i>Felis catus</i>	2980 ± 398				28.9 ± 2.62	9.7			Bennett and Tenney, 1982
Cat <i>Felis catus</i>	4700 ± 500				20.4 ± 5	4.34 ± 1			Kochi et al., 1988

Table 4.3: Cost of breathing estimates for all fourteen species studied. Measured work of breathing was multiplied by 20 to correct for active expiration and muscle efficiency (see text). BMR (basal metabolic rate) was calculated from the equation given by Romijn and Lokhorst, 1966.

Species	Normoxia						Hypoxia		
	18% O ₂			13% O ₂			6% O ₂		
	BMR	Work of breathing	Cost of breathing	BMR	Work of breathing	Cost of breathing	BMR	Work of breathing	Cost of breathing
	<i>J min⁻¹ kg⁻¹</i>	<i>J min⁻¹ kg⁻¹</i>	% BMR	<i>J min⁻¹ kg⁻¹</i>	<i>J min⁻¹ kg⁻¹</i>	% BMR	<i>J min⁻¹ kg⁻¹</i>	<i>J min⁻¹ kg⁻¹</i>	% BMR
Puna teal				361	0.11	0.64%	307	0.245	1.60%
Speckled teal				373	0.18	0.98%	395	0.503	2.56%
Ruddy duck				304	0.15	1.02%	284	0.346	2.44%
Yellow-billed pintail				334	0.14	0.82%	336	0.357	2.12%
Cinnamon teal				352	0.18	1.04%	350	0.267	1.52%
Green-winged teal	466	0.23	0.96%	534	0.24	0.88%	467	0.374	1.60%
Lowland ruddy duck	323	0.07	0.42%	431	0.08	0.38%	525	1.135	4.32%
Northern pintail	279	0.07	0.52%	346	0.09	0.54%	300	0.312	2.08%
Lowland cinnamon teal	452	0.20	0.88%	531	0.26	0.96%	480	0.653	2.72%
Mallard	282	0.13	0.90%	334	0.19	1.14%	373	2.925	15.68%
Gadwall	432	0.13	0.58%	477	0.13	0.54%	414	0.414	2.00%
	21% O ₂			14% O ₂			5% O ₂		
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%

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Appendix

Table 5.1: Equations for frequency isopleth lines used to generate data. Note that elastic work is expressed as $\log_{10}(\text{elastic work})$.

Species	Freq	Compliance mL cm H ₂ O ⁻¹		Tau seconds		Log (Elastic work) Joules		Resistive work Joules	
		Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Puna teal	20	-0.14088	5.4186	-0.0132	1.24145	0.08567	-3.30864	0.000422	-0.00241
	30	-0.22314	5.25868	-0.0431	1.3483	0.11532	-3.57393	0.000671	-0.00372
	40	-0.21137	4.46678	-0.0413	1.19125	0.12534	-3.5419	0.000974	-0.00516
	50	-0.16059	3.34279	-0.0516	1.21639	0.12716	-3.39819	0.0011	-0.00435
	60	-0.15882	2.89905	-0.0487	1.03967	0.14594	-3.48033	0.0012	-0.00374
Speckled teal	20	-0.25558	7.02079	-0.0962	2.69017	0.09567	-3.62765	0.000392	-0.00219
	30	-0.18189	4.70644	-0.0488	1.47918	0.10301	-3.54716	0.000372	-0.00074
	40	-0.13748	3.61482	-0.046	1.3011	0.09528	-3.34426	0.000301	0.0013
	50	-0.09241	2.67448	-0.0398	1.13024	0.0991	-3.30296	0.000516	0.000563
	60	-0.08918	2.29918	-0.0427	1.04757	0.10001	-3.18192	0.000519	0.00182
Ruddy duck (highland)	20	-0.03659	3.17204	-0.0449	1.8595	0.1205	-3.37661	0.000911	-0.0033
	30	-0.10101	3.74843	-0.0354	1.39383	0.12516	-3.37969	0.00142	-0.00604
	40	-0.06624	2.78955	-0.0359	1.27898	0.1227	-3.2485	0.0019	-0.00751
	50	-0.08222	2.62436	-0.0323	1.09125	0.10994	-3.07804	0.0017	-0.0047
	60	-0.09479	2.56817	-0.0264	0.9179	0.12659	-3.22544	0.0015	-0.00361
Yellow-billed pintail	20	-0.19367	7.34621	-0.0134	1.38518	0.1269	-3.55208	0.000379	-0.00115
	30	-0.19391	5.41647	-0.0008	0.869	0.13943	-3.4998	0.000961	-0.00386
	40	-0.25811	5.17671	0.00322	0.68819	0.15206	-3.48219	0.00125	-0.0046
	50	-0.28031	4.813	-0.037	1.03048	0.16519	-3.48311	0.0017	-0.00598
	60	-0.27066	4.06368	-0.0298	0.85126	0.18746	-3.53038	0.00204	-0.00673
Cinnamon teal (highland)	20	-0.176	6.07228	-0.0483	1.9004	0.11891	-4.193	0.000217	0.000564
	30	-0.09335	3.85885	-0.0328	1.3173	0.10282	-3.42399	0.00064	-0.00358
	40	-0.13518	3.78623	-0.0359	1.20556	0.11261	-3.44285	0.000978	-0.00563
	50	-0.1561	3.55338	-0.0406	1.18712	0.12806	-3.50829	0.000764	-0.00264
	60	-0.1614	3.20883	-0.0323	0.99597	0.13215	-3.4319	0.00104	-0.00387
Green-winged teal	20	-0.08636	3.66463	-0.0046	1.57524	0.05718	-3.19342	0.000116	0.000315
	30	-0.08618	3.15194	-0.0493	1.596	0.07419	-3.40704	0.000257	-0.00076
	40	-0.10426	3.07119	-0.0278	1.18682	0.0925	-3.56108	0.000578	-0.00372
	50	-0.05999	2.02437	-0.0505	1.26139	0.07917	-3.21392	0.000417	0.000142
	60	-0.03155	1.45774	-0.0484	1.11779	0.06701	-2.98753		
Lowland ruddy duck	20	-0.13407	4.54491	-0.0356	1.69509	0.09015	-3.32055	0.000775	-0.00525
	30	-0.10823	3.30096	-0.0402	1.48895	0.10544	-3.35968	0.00128	-0.00707
	40	-0.13934	3.24693	-0.0594	1.57322	0.12127	-3.41213	0.00167	-0.00863
	50	-0.10842	2.55283	-0.0493	1.31386	0.11656	-3.28398	0.00249	-0.01345
	60	-0.14215	2.54226	-0.0735	1.42141	0.13594	-3.32945	0.00241	-0.01052
Northern pintail	20	0.02813	4.19927	-0.0227	1.41165	0.09708	-3.13595	0.000699	-0.00298
	30	-0.10759	4.88717	-0.0123	1.02636	0.14131	-3.42291	0.000989	-0.00352
	40	-0.2347	4.91717	-0.0284	1.01913	0.15354	-3.43217	0.00153	-0.00558
	50	-0.29027	4.66899	-0.0464	1.09919	0.18765	-3.58578	0.00191	-0.00653
	60	-0.34139	4.68514	-0.0448	0.97013	0.19389	-3.52639	0.00257	-0.00889
Lowland cinnamon teal	20	-0.12245	4.40648	-0.0153	1.25255	0.06029	-3.19468	0.000195	-0.00111
	30	-0.10462	3.43797	-0.0043	1.02203	0.08116	-3.45881	0.000272	-0.00083
	40	-0.09118	2.73044	-0.0165	0.95115	0.09384	-3.48706	0.000483	-0.00248
	50	-0.09029	2.4177	-0.029	0.95148	0.09736	-3.41752	0.000549	-0.00162
	60	-0.04088	1.58265	-0.0565	1.08345	0.08798	-3.21149	0.000879	-0.00406
Mallard	20	0.15811	4.23218	0.00471	1.02858	0.11795	-3.16781	0.000791	-0.00296
	30	-0.04533	4.72927	-0.0089	0.87202	0.13158	-3.18147	0.00157	-0.00672
	40	-0.32329	6.0298	-0.0457	1.06034	0.17792	-3.40605	0.00229	-0.0096
	50	-0.2822	4.92293	-0.0631	1.12095	0.18339	-3.34631	0.00289	-0.01129
	60	-0.27826	4.41005	-0.0637	1.01917	0.18465	-3.28613	0.00319	-0.0115
Gadwall	20	0.02246	5.38243	0.00682	1.09305	0.12766	-3.33979	0.000719	-0.00304
	30	-0.12242	5.3849	0.00684	0.82912	0.15209	-3.46048	0.00109	-0.00383
	40	-0.28042	5.55778	0.0016	0.77051	0.17757	-3.53474	0.00192	-0.00738
	50	-0.42774	6.064	-0.0468	1.03025	0.19966	-3.5783	0.00221	-0.00765
	60	-0.41336	5.27063	-0.0546	0.99916	0.22288	-3.63347		

Species	Freq	Compliance mL cm H ₂ O ⁻¹		Tau seconds		Log(Elastic work) Joules		Resistive work Joules	
		Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Bar-headed goose	20	-0.04068	18.42	-0.0025	1.461	0.0104	-2.53	0.00049	-0.02003
	30	-0.10184	19.86	-0.0063	1.472	0.01427	-2.72	0.00065	-0.02182
	40	-0.12022	17.8	-0.0086	1.449	0.01706	-2.77	0.00097	-0.03481
	50	-0.08987	13.34	-0.0081	1.272	0.01582	-2.54	0.00108	-0.0336
Andean goose	60	-0.15023	16.12	-0.0098	1.274	0.01943	-2.72	0.00104	-0.02637
	20	0.04974	18.5	0.00467	0.67971	0.00983	-2.64671	0.000188	-0.00635
	30	-0.01783	21.5	0.00111	0.88418	0.01144	-2.70401	0.00038	-0.01494
	40	-0.06157	21.45	-0.0017	1	0.01276	-2.72614	0.000594	-0.0237
Barnacle goose	50	-0.0604	18.51	-0.0013	0.8559	0.01418	-2.74101	0.000708	-0.02558
	60	-0.08523	18.9	-0.0037	1.00205	0.01433	-2.65043	0.000878	-0.03045
	20	-0.02664	12.9	-0.0023	1.79892	0.01374	-2.64603	0.000806	-0.02879
	30	-0.07809	13.2	-0.0078	1.77684	0.01748	-2.73557	0.0012	-0.03999
Barnacle goose	40	-0.06671	10.3	-0.0096	1.67794	0.01873	-2.66092	0.00171	-0.05712
	50	-0.06701	8.7	-0.0102	1.48377	0.01765	-2.42713	0.0015	-0.02755
	60								