CARDIORESPIRATORY RESPONSES TO HYPOXIA IN HIGH- AND LOW-ALTITUDE GEESE AND DUCKS

by

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ABSTRACT

High-altitude (HA) life is challenging due to the reduced partial pressure of oxygen (hypoxia). Hence, HA vertebrates have evolved increased capacities in their oxygen transport cascade enhancing oxygen transfer. The extent of interspecies variation in these responses within waterfowl, a taxon prolific at HA, remains largely unknown. This thesis investigated 17 waterfowl groups at different altitudes to address the overarching hypotheses that waterfowl use multiple cardiorespiratory strategies to maintain oxygen supply during hypoxia, and that HA exposure alters the waterfowl hypoxic ventilatory and cardiovascular responses.

A comprehensive analysis of metabolic, cardiovascular, and ventilatory responses to progressive decreases in equivalent fractional composition of inspired oxygen was made on resting low-altitude (LA) barnacle geese, LA bar-headed geese, HA bar-headed geese, Andean geese, and crested ducks. Andean geese and crested ducks, lifelong HA residents, exhibited fundamentally different mechanisms for maintaining oxygen supply during hypoxia than bar-headed geese, transient HA migrants. Bar-headed geese robustly increased ventilation and heart rate, whereas Andean species increased lung oxygen extraction and stroke volume. Also, HA-reared bar-headed geese exhibited reduced oxygen consumption during hypoxia compared to LA-reared bar-headed geese.

Similar cardiovascular studies were performed on five HA duck species (yellow-billed pintail, cinnamon teal, ruddy duck, speckled teal, and Puna teal) in Peru and six related LA duck species (northern pintail, cinnamon teal, ruddy duck, green-winged teal, gadwall, and mallard duck) in the USA. Heart rate and oxygen pulse remained generally unchanged. Instead, most HA ducks exhibited higher blood-oxygen carrying capacity and lower heart rate variability than LA
ducks. While heart rate, stroke volume, oxygen pulse, and blood-oxygen carrying capacity contributed to all 17 groups’ hypoxic cardiovascular responses, the predominant responses were increased stroke volume and, in HA taxa, blood-oxygen carrying capacity. Only bar-headed geese increased heart rate appreciably.

This thesis identifies multiple cardiovascular and respiratory strategies by which waterfowl maintain oxygen supply during hypoxia, and provides insight into how HA rearing impacts these responses. This thesis also suggests that short-term HA performance utilizes primarily functional enhancements (e.g. rapid heart rate and ventilation increases), whereas lifelong HA residency is supported predominantly by structural changes (e.g. lung and cardiac morphology enhancements).
PREFACE

Sabine Laguë was the primary contributor to the experimental design, data collection, data analysis, and manuscript preparation. W. K. Milsom and A. P. Farrell provided supervision, assistance with experimental design, and helped with thesis and manuscript preparation. Because the majority of this research was conducted abroad in the field, many collaborators were instrumental in helping data collection take place.


All procedures were approved by the University of British Columbia’s Animal Care Committee in accordance with the Canadian Council on Animal Care (A12-0013).
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<tr>
<td>$\beta \text{O}_2$</td>
<td>oxygen capacitance</td>
</tr>
<tr>
<td>$C_a \text{O}_2$</td>
<td>arterial oxygen content</td>
</tr>
<tr>
<td>$C_v \text{O}_2$</td>
<td>venous oxygen content</td>
</tr>
<tr>
<td>$E_L \text{O}_2$</td>
<td>lung oxygen extraction</td>
</tr>
<tr>
<td>$E_t \text{O}_2$</td>
<td>tissue oxygen extraction</td>
</tr>
<tr>
<td>$F_e \text{CO}_2$</td>
<td>fractional carbon dioxide composition of expired gas</td>
</tr>
<tr>
<td>$F_{\text{ECO}_2}$</td>
<td>fractional oxygen composition of expired gas of the chamber</td>
</tr>
<tr>
<td>$F_e \text{O}_2$</td>
<td>fractional oxygen composition of expired gas of the bird</td>
</tr>
<tr>
<td>$F_i \text{CO}_2$</td>
<td>fractional carbon dioxide composition of inspired gas</td>
</tr>
<tr>
<td>$f_H$</td>
<td>heart rate</td>
</tr>
<tr>
<td>$F_i \text{O}_2$</td>
<td>fractional oxygen composition of inspired gas</td>
</tr>
<tr>
<td>$f_R$</td>
<td>breathing frequency</td>
</tr>
<tr>
<td>$F_R I$</td>
<td>incumbent flow rate</td>
</tr>
<tr>
<td>Hb</td>
<td>hemoglobin</td>
</tr>
<tr>
<td>$[\text{Hb}]$</td>
<td>total blood hemoglobin concentration</td>
</tr>
<tr>
<td>$[\text{HCO}_3^-]_a$</td>
<td>arterial bicarbonate ion concentration</td>
</tr>
<tr>
<td>Hct</td>
<td>hematocrit</td>
</tr>
<tr>
<td>K</td>
<td>Krogh’s diffusion constant</td>
</tr>
<tr>
<td>$M_b$</td>
<td>body mass</td>
</tr>
<tr>
<td>$M_{H}$</td>
<td>heart mass relative to body mass</td>
</tr>
<tr>
<td>$P_{50}$</td>
<td>partial pressure of oxygen at which hemoglobin is 50% saturated</td>
</tr>
<tr>
<td>$P_a \text{CO}_2$</td>
<td>arterial partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
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<td>-------------------------------------</td>
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<tr>
<td>$P_aO_2$</td>
<td>arterial partial pressure of oxygen</td>
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<td>$P_B$</td>
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<td>$P_vO_2$</td>
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<td>$\dot{Q}$</td>
<td>cardiac output</td>
</tr>
<tr>
<td>RR interval</td>
<td>interval between successive heartbeats</td>
</tr>
<tr>
<td>$S$</td>
<td>barrier surface area</td>
</tr>
<tr>
<td>$\tau$</td>
<td>barrier thickness</td>
</tr>
<tr>
<td>$\dot{V}_C$</td>
<td>flow rate through chamber</td>
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<tr>
<td>$\dot{V}CO_2$</td>
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<tr>
<td>$\dot{V}O_2$</td>
<td>whole animal oxygen consumption</td>
</tr>
<tr>
<td>$\dot{V}_R$</td>
<td>total ventilation</td>
</tr>
<tr>
<td>$\dot{V}_R/\dot{Q}$</td>
<td>ventilation-perfusion ratio</td>
</tr>
<tr>
<td>$V_s$</td>
<td>cardiac stroke volume</td>
</tr>
<tr>
<td>$V_T$</td>
<td>tidal volume</td>
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# LIST OF ABBREVIATIONS

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACR</td>
<td>air convection requirement</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BCR</td>
<td>blood convection requirement</td>
</tr>
<tr>
<td>BTPS</td>
<td>body temperature and pressure, saturated</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
</tr>
<tr>
<td>HA</td>
<td>high altitude or high-altitude</td>
</tr>
<tr>
<td>HAR</td>
<td>high-altitude-reared</td>
</tr>
<tr>
<td>HRV</td>
<td>heart rate variability</td>
</tr>
<tr>
<td>HVR</td>
<td>hypoxic ventilatory response</td>
</tr>
<tr>
<td>LA</td>
<td>low altitude or low-altitude</td>
</tr>
<tr>
<td>LAR</td>
<td>low-altitude-reared</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>PCA</td>
<td>principle components analysis</td>
</tr>
<tr>
<td>RMSSD</td>
<td>root mean square of successive differences of the RR intervals</td>
</tr>
<tr>
<td>RQ</td>
<td>respiratory quotient; the quotient of $\dot{V}co_2$ and $\dot{V}o_2$</td>
</tr>
<tr>
<td>SDRR</td>
<td>standard deviation of RR intervals</td>
</tr>
<tr>
<td>STPD</td>
<td>standard temperature and pressure, dry</td>
</tr>
<tr>
<td>TPR</td>
<td>total peripheral resistance</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I would first and foremost like to thank my supervisors and mentors, Dr. Bill Milsom and Dr. Tony Farrell, for their guidance and support. Bill’s curiosity and love for learning is infectious, and his creativity and storytelling were great companions for fieldwork. I am grateful for his openness towards his students conducting field research, and for the opportunities he gave me to conduct science in and dialogue with scientists from a wide array of settings. It truly expanded my vision as a scientist. Tony’s mentorship in my undergraduate honours thesis played a large role in my pursuit of graduate studies, and I am grateful for his supervision in my PhD. He inspired and guided me to become a better scientist and communicator, teaching me to think outside of the box. I am also grateful for his timely encouragement. Both Bill and Tony saw potential in me and were integral in my development as a scientist. I am thankful for them both.

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Finally, I cannot begin to express the gratitude I feel towards my husband, Craig. Thank you for your loving support, patience, and encouragement. Thank you for being steady and continually reminding me to look beyond the present circumstances and be true to who I am.
DEDICATION

For my parents, who first inspired my love of the mountains and the creatures that inhabit them, and for those who walked alongside me during this season while I climbed some metaphorical ones.
CHAPTER 1: OBTAINING OXYGEN IN THIN AIR:

CARDIORESPIRATORY RESPONSES TO HYPOXIA IN WATERFOWL

1.1 Challenges of life at high altitude

Life at high altitude (HA) is physiologically challenging for animals. These physiological challenges include cold, dehydration, limited food availability, and particularly low environmental oxygen (hypoxia), which is the subject of my doctoral research (Bouverot, 1985b). There are three major HA regions in the world: the East African Plateau (average altitude: 2,400-3,700 m), the Himalayas (average altitude: 4,500 m), and the Andes (average altitude: 4,000 m) (Bouverot, 1985b). Despite the challenges posed by HA, a wide variety of vertebrate taxa live either permanently or temporarily at HA. Among these taxa are fishes (e.g. scaleless carp at 3,200 m) (Matey et al., 2008), amphibians (e.g. Andean frogs at 4,700 m) (Monge and Monge, 1968), reptiles (e.g. iguanids at 5,500 m) (Brand, 2005; Mani, 1974), mammals (e.g. deer mice at 4,300 m) (Snyder et al., 1982b), and a wide range of birds (e.g. hummingbirds, passerines, and waterfowl at 3,000-7,000 m) (Scott, 2011). In addition, the World Health Organization reported that in 1996 over ~140 million humans live above 2,500 m, including several permanent human habitations above 4,000 m located in the South American Andes, the East African Plateau, and the South-Central Asian Himalayas (Hainsworth and Drinkhill, 2007). Of the many physiological challenges posed by HA, however, the only stress ubiquitously present is hypoxia (Monge and Leon-Velarde, 1991).

All vertebrates require oxygen to sustain life. Oxidative phosphorylation is a substantially more efficient method of synthesizing ATP (28-38 mol ATP/mol oxygen) than anaerobic
glycolysis (2 mol ATP/mol oxygen) (Brand, 2005), and produces wastes (CO₂ and H₂O) that are easily excreted. The reduced barometric pressure (P₉) at HA reduces the partial pressure of oxygen (PO₂) and oxygen concentration of the air, creating a phenomenon called hypobaric hypoxia (Figure 1.1) (Bouverot, 1985b). Living in hypobaric hypoxia requires physiological adjustments and adaptations that ensure an adequate oxygen flow from the environment to the mitochondria when oxygen availability is reduced (i.e. reduced oxygen concentration) and the driving force for oxygen diffusion (i.e. PO₂) is reduced. The flow of oxygen from the atmosphere to the mitochondria in vertebrates can be conceptualised by the oxygen transport cascade.

1.2 Oxygen transport cascade in the maintenance of oxygen supply

The oxygen transport cascade is a conceptual view of the series of steps responsible for acquiring oxygen from the environment and transporting it to mitochondria. The steps of the oxygen cascade for air breathing vertebrates are as follows: (i) ventilation – convection of air across a gas-exchange surface; (ii) lung oxygen diffusion – oxygen diffusion from air into the blood; (iii) circulation – convection of oxygen in the blood throughout the body; (iv) tissue oxygen diffusion – oxygen diffusion from the blood to the cells; and (v) mitochondrial respiration – oxygen diffusion from the cellular cytosol to the mitochondria and its consumption via oxidative phosphorylation (Figure 1.2A) (Scott, 2011; Weibel, 1984a). Oxygen flows through these steps to match oxygen consumption (\(\dot{V}O₂\)) in either a diffusive or convective manner.
1.2.1 Diffusive steps in the oxygen transport cascade

Diffusion is the molecular movement of oxygen across a surface. The two diffusive steps in the oxygen transport cascade take place between the lungs and the blood and between the blood and the tissues. The rate at which oxygen diffuses is determined by the $\text{PO}_2$ gradient across the lung or tissue, the surface area of the tissue ($S$), the thickness of the tissue ($\tau$), and Krogh’s diffusion constant ($K$), which equals the product of the oxygen diffusion coefficient and the oxygen solubility coefficient for the tissue in question (Weibel, 1984b):

$$\dot{V}_{O_2}(A-B) = K \times \frac{S}{\tau} \times (\text{PO}_2(A) - \text{PO}_2(B)) \quad (1.1).$$

As such, the flow of oxygen through diffusive steps is heavily determined by morphology, imposing predetermined constraints on the potential of these steps to respond to physiological stresses such as hypoxia. Nonetheless, several vertebrate taxa have enhanced the diffusive capacity of these steps morphologically by increasing surface area or by decreasing the diffusion distance. Decreases in diffusion distance at the lungs is a result of increased capillarity or reduced barrier thickness between parabronchi and capillary blood, whereas at the tissues it can be due to increased tissue capillarity, increased mitochondrial density, or mitochondria being located closer to the capillaries. Although there is capacity for plasticity within morphology, as also seen in endurance athletic training, this capacity for plasticity is limited, and these changes occur over more prolonged timelines (e.g. months to years) than those of the convective steps (e.g. seconds to weeks) (Hainsworth and Drinkhill, 2007; Powell et al., 1998).
1.2.2 Convective steps in the oxygen transport cascade: Ventilation

Convection is the mass transport of oxygen as determined by the carrier flow rate and the oxygen content of the medium. Considered in terms of ventilation, the Fick equation for ventilation reads:

\[ \dot{V}_{O_2} = f_R \times V_T \times (F_{I}O_2-F_{E}O_2) \quad (1.2), \]

where \( F_{I}O_2 \) is the fractional oxygen composition of inspired air, \( F_{E}O_2 \) is the fractional oxygen composition of expired air, \( f_R \) is breathing frequency, and \( V_T \) is tidal volume (Weibel, 1984b). The product of the latter two factors is the carrier flow rate, total ventilation (\( \dot{V}_R \)). The percent of oxygen extracted from the inspired air ((\( F_{I}O_2-F_{E}O_2/F_{I}O_2 \))x100), lung oxygen extraction, is primarily reliant upon factors that determine diffusion (Equation 1.1) (Weibel, 1984b).

1.2.3 Convective steps in the oxygen transport cascade: Circulation

Similarly, when considered in terms of circulation, the Fick equation for circulation also takes into account the carrier flow rate (i.e. cardiac output; \( \dot{Q} \)) and the oxygen content of the medium:

\[ \dot{V}_{O_2} = f_H \times V_S \times \beta_{O_2} \times (P_{a}O_2-P_{v}O_2) \quad (1.3), \]

where \( \beta_{O_2} \) is oxygen capacitance (or blood-oxygen carrying capacity), \( P_{a}O_2 \) is the arterial partial pressure of oxygen, \( P_{v}O_2 \) is the venous partial pressure of oxygen, \( f_H \) is heart rate, and \( V_S \) is stroke volume (Weibel, 1984b). The product of the latter two factors is \( \dot{Q} \).

Blood-oxygen carrying capacity is effected by a variety of factors, including hematocrit (Hct), total blood hemoglobin concentration ([Hb]), and allosteric modulators of oxygen-hemoglobin (Hb) binding. Though Hct changes can be triggered by external factors, such as
exposure to hypoxia at HA, the manufacture of red blood cells is slow and, beyond the rapid release of stored splenic red blood cells, Hct changes are relatively slow (e.g. Hct increases occur over weeks of HA exposure in mammals) (Bouverot, 1985a). In contrast, the effect of allosteric modulators on Hb is rapid. The major allosteric modulators for Hb are organic phosphates, H⁺, NO, and CO. For example, Hb binding to H⁺ via the Bohr effect will decrease Hb-oxygen affinity, facilitating oxygen unloading at the tissues (West, 2012). The primary organic phosphates that modulate Hb function differ between fish (ATP or GTP), mammals (2,3-diphosphoglycerate), and birds (inositol pentaphosphate); however, when bound to Hb they all reduce Hb-oxygen affinity (Mairbaurl and Weber, 2012; West, 2012). Similarly, temperature also effects Hb-oxygen affinity, though in an inverse manner (i.e. increases in temperature lead to decreases Hb-oxygen affinity) (West, 2012). It is the capacity for rapid regulation of the convective steps of the oxygen transport cascade, namely ventilation and circulation, which are critical for maintaining oxygen transport through the cascade under hypoxic conditions.

The Fick equation describing convective cardiovascular oxygen transport can also be expressed using terms that already encompass βo₂. Because oxygen content is the product of PO₂ and βo₂, Equation 1.3 can also be expressed as:

\[ V_{O_2} = f_H \times V_S \times (C_{aO_2}-C_{vO_2}) \] (1.4),

where \( C_{aO_2} \) is arterial oxygen content, and \( C_{vO_2} \) is venous oxygen content. Tissue oxygen extraction is described by the percent change in oxygen content between arterial and venous systems \(( (C_{aO_2} - C_{vO_2}/C_{aO_2}) \times 100)\), and is primarily reliant upon factors that determine diffusion (Equation 1.1) (Weibel, 1984b). Finally, this equation can also be further simplified when variables such as stroke volume, \( C_{aO_2} \), and \( C_{vO_2} \) are unknown by using cardiac oxygen pulse, the product of stroke volume and the arterial-venous oxygen content difference:
\[ \dot{V}_{o_2} = f_H \times O_2 \text{ pulse} \quad (1.5). \]

1.3 Hypoxic ventilatory and cardiovascular responses

1.3.1 The oxygen transport cascade under hypoxic conditions

Without compensation, a hypoxic environment will reduce the PO\(_2\) at all levels of the oxygen transport cascade relative to their low-altitude (LA) state, regardless of whether the step is diffusive or convective (Figure 1.2B). Because the PO\(_2\) gradient is a key factor driving oxygen diffusion, diffusion rates are constrained by low PO\(_2\) levels and thus also by HA and hypobaric hypoxia. Similarly, the convective steps of ventilation and circulation are also impacted by reduced oxygen concentrations, because oxygen concentration is the product of PO\(_2\) and \(\beta_o\) (Weibel, 1984b). Thus, decreased environmental PO\(_2\) and oxygen concentrations decreases the flow of oxygen through the cascade as well as the total oxygen concentration of the blood, rendering oxygen acquisition and transport the most important physiological functions at HA.

1.3.2 Hypoxic ventilatory and cardiovascular responses

Living in a hypobaric hypoxic environment requires physiological adjustments and adaptations that ensure adequate transportation of oxygen from the environment to the mitochondria to compensate for the reduced availability and driving force of oxygen. Ventilation and circulation, the two convective steps of the oxygen transport cascade, represent the key steps that allow animals to respond rapidly and plastically to changes in oxygen supply and demand. For example, \(\dot{V}_R\) and \(\dot{Q}\) increase rapidly (within seconds to minutes) in LA animals such as humans when exposed to hypoxia (Hainsworth and Drinkhill, 2007; West, 1982). Thus, in order to compensate for the decreased availability of environmental oxygen, these two convective
steps and their contributing components play a critical role in the physiological response to hypoxia.

The hypoxic ventilatory response (HVR) describes the suite of ventilatory changes that accompany hypoxia exposure and ensure blood-oxygen loading at the lungs. As defined by Equation 1.2, there are three factors that can be altered to maintain or increase $\dot{V}_O_2$ during hypoxia: breathing frequency, tidal volume, and lung oxygen extraction. Breathing frequency and tidal volume, whose product is $V_R$, both have the potential to rapidly change in response to hypoxic exposure. Lung oxygen extraction, however, is primarily reliant upon factors that determine diffusion (Equation 1.1) (Weibel, 1984b). As such, diffusion of oxygen across the avian lung is heavily determined by morphology and, because of the importance of the oxygen gradient, can be constrained under hypoxic conditions. The HVR constitutes the combined responses of breathing frequency, tidal volume, and lung oxygen extraction that help to load the blood with oxygen during hypoxic exposure.

The hypoxic cardiovascular response describes the suite of cardiovascular changes during hypoxia exposure that regulate oxygen delivery to the tissues. There are several cardiovascular enhancements that could increase oxygen transport to the tissues during hypoxia including:

1) enhanced oxygen carrying capacity of the blood (e.g. increased Hct, increased [Hb], and decreased Hb-P_{so} (the $P_{a}O_2$ at which blood is 50% saturated));
2) increased $\dot{Q}$ (e.g. increased heart rate and/or stroke volume); and
3) increased tissue oxygen extraction from the blood (Equation 1.4). Of these three strategies, two (increased oxygen carrying capacity and increased tissue oxygen extraction) would not immediately require increased work on the part of the heart when modestly enhanced. Increasing $\dot{Q}$, however, would put further metabolic demands on the
heart, a hypoxia-sensitive organ in most endothermic vertebrates, at a time when oxygen supply is reduced. While acutely increasing metabolic demand, increases in $\dot{Q}$ (through any combination of increases in heart rate and stroke volume) can occur rapidly in response to a hypoxic stress. Conversely, changes in tissue oxygen extraction independent of changes in the $PO_2$ gradient are constrained by the same basic morphological parameters as lung oxygen diffusion (Weibel, 1984b). Similarly, aside from splenic release, large increases in either Hct and [Hb] do not occur rapidly. While increases in these two parameters will certainly increase blood-oxygen carrying capacity, too large of increases in these factors would pose a viscous threat to the circulatory system. As such, the hypoxic cardiovascular response constitutes the combined responses of heart rate, stroke volume, tissue oxygen extraction, and factors determining blood-oxygen carrying capacity, all of which help regulate oxygen delivery to the tissues during hypoxic exposure.

Thus, the HVR and hypoxic cardiovascular response are critical to the maintenance of oxygen supply during hypoxic exposure. These responses will be used throughout this thesis to describe the hypoxic responses of birds, a taxon whose specializations of the oxygen transport cascade have enhanced both diffusive and convective components, serving as “pre-adaptations” for maintaining oxygen supply during hypoxia.

1.4 The avian oxygen transport cascade: Exaptations for enhanced oxygen transport

1.4.1 Enhancements to the avian oxygen transport cascade

Among vertebrates, the avian oxygen transport cascade is characterized by its enhanced efficiency, with prominent enhancements at each diffusive and convective step that support the high oxygen transport rates required for supporting the demands of endothermy and locomotion.
These enhancements have permitted birds to inhabit some of the most varied and extreme environments on earth, extending the taxon across a wide range of temperatures (Antarctic winters endured by emperor penguins (Goldsmith and Sladen, 1961) to life in the Arabian desert sustained by hoopoe larks (Williams and Tieleman, 2005)), athletic demands (rapid bipedal sprints in the ostrich (Rubenson et al., 2011) to the longest non-stop migration in the bar-tailed godwit (Gill et al., 2005)), and hypoxic challenges (deep diving in the emperor penguin (Meir and Ponganis, 2009) to HA flying in the bar-headed goose (Hawkes et al., 2011)). Moreover, birds are remarkably hypoxia-tolerant when considering their high metabolic rates, a tolerance facilitated by several characteristics of their oxygen transport cascade and integral in facilitating HA flight.

Enhancements to the avian oxygen transport cascade are well documented in the literature (Scott, 2011). In terms of ventilation, birds are thought to be more hypocapnia tolerant than mammals because they continue increasing \( \dot{V}_R \) in hypoxia despite the respiratory alkalosis that develops (lower arterial partial pressure of carbon dioxide; \( P_aCO_2 \)), thereby enhancing oxygen uptake and transport (Scheid, 1990; Scott, 2011). Morphologically, the unidirectional flow of air through the cross-current gas exchange system of the avian lungs is considered functionally more efficient than mammalian tidal ventilation (Scott, 2011). The blood-gas exchange surface area in birds is also larger and the diffusion barrier thinner (2.5-fold thinner than mammals), yet still structurally robust compared to that of mammals (Powell, 2000; Scheid, 1990; West, 2009). Compared to mammals of similar body size, birds have a disproportionally large heart, and thus are also capable of exhibiting a larger \( \dot{Q} \), due to an enhanced stroke volume (Calder, 1968; Grubb, 1983; Smith et al., 2000). In addition, birds do not undergo pulmonary vasoconstriction in hypoxia, allowing them to maintain gas exchange, and avoid pulmonary
edema, a major contributor to acute mountain sickness in mammals (Faraci et al., 1984; reviewed in Scott et al., 2015). Also, birds have an enhanced capacity for oxygen diffusion from the blood to the tissues (and thus also to the mitochondria) compared to mammals due to a higher capillary to muscle fibre ratio, as evident in the flight muscle, heart, and brain (Faraci, 1991; Mathieu-Costello et al., 1998; Scott, 2011). Avian flight muscle in particular is characterized by fast-contracting aerobic muscle, abundant mitochondria, and a high fat oxidation capacity (Scott, 2011). These enhancements help to maintain oxygen transport at rest and during metabolically-demanding activities, such as flight, even despite the decreases in $PO_2$ encountered at HA.

1.4.2 Avian locomotion at high altitude: The challenge of matching oxygen supply and demand in hypoxia

Three types of primary forms of locomotion commonly associated with HA waterfowl are flight, dabbling (primarily surface swimming), and diving (swimming underwater). With all three activities birds must overcome the forces of drag. Flight is one of the most energetically costly means of locomotion in vertebrates, requiring a 10-20-fold increase in resting $\dot{V}o_2$ (Butler et al., 1977). Flight is especially challenging at HA due to hypobaric hypoxia, which increases the work required by the bird to produce lift during flight, thereby increasing oxygen demand under conditions of decreased oxygen availability (Altshuler and Dudley, 2006). Thus, flight or any other energetically demanding activity at HA can pose a mismatch between oxygen supply and oxygen demand. During dabbling and diving, birds must overcome the forces of both drag and buoyancy under situations frequently demanding breath-holding. Diving and surface swimming in the tufted duck (*Aythya fuligula*), although similar in their energetic costs, require an increase in resting $\dot{V}o_2$ by an average of 3.5-fold to a maximum of 5-6-fold at maximum
speed (Butler, 2000). Thus, at HA the breath-holding bouts and increases in $\dot{V}O_2$ that occur during diving happen in the context of decreased oxygen availability, potentially placing physiological limits on these activities at HA. While the enhancements to the avian oxygen transport cascade mentioned above act as exaptations (or pre-adaptations) for life and flight at HA (Scott, 2011), these factors alone may not be sufficient to fuel flight at HA. Sustaining energetically demanding forms of locomotion at HA requires even further physiological enhancements to the oxygen transport cascade.

1.5 High-altitude waterfowl

The Himalayas and the Andes are home to a wide variety of endemic waterfowl species. Waterfowl (Anseriformes) and are a group comprised of geese, swans, and ducks. Despite their geographic separation, a characteristic that HA Andean and Himalayan waterfowl share is the strong environmental pressure of HA hypoxia. One Himalayan bird that has received considerable interest from the scientific community is the bar-headed goose (Anser indicus).

One of the most impressive athletic feats on Earth is the biannual HA migration of bar-headed geese across the Himalayas. They migrate from their moderately HA breeding grounds on the Mongolian and Qinghai-Tibetan plateaus (2,000-4,500 m) through the Himalayan mountains to overwinter at altitudes close to sea level in India (<1,000 m) (Bishop et al., 2015; Hawkes et al., 2011). During this migration they routinely fly at altitudes of ~4,500-6,000 m (Hawkes et al., 2011), where the PO$_2$ is less than half that which is inspired at sea level (Figure 1.1). The following spring they return to the plateaus, however, this time ascending from sea level climbing between 4,000-6,000 m in ~8 h. What is truly exceptional about this species is that they sustain continuous hypoxic flight without relying on updrafts and likely while
encountering headwinds (Hawkes et al., 2011). While by all means true HA performers, bar-headed geese are only exposed to moderate hypoxia seasonally and severe hypoxia transiently (~1 day) (Hawkes et al., 2011).

In comparison, many Andean waterfowl are exposed to appreciable levels of hypoxia lifelong. The Andean waterfowl community encompasses several species of ducks, as well as gooselike ducks called sheldgeese, which are not true geese (Livezey, 1986; McCracken et al., 2009a). Most Andean HA waterfowl inhabit areas between 2,000-5,500 m (Figure 1.1), and are uncommon at intermediate altitudes due to the lack of wetland habitat on mid-elevational slopes (McCracken et al., 2009a). Andean geese are sheldgeese that live all year at altitudes of 3,000-5,500 m (Fjeldså and Krabbe, 1990; McCracken et al., 2010) comparable to or higher than bar-headed goose breeding grounds and indeed even comparable to or higher than some bar-headed goose migration elevations (Hawkes et al., 2011) (Figure 1.1). Although evolutionarily diverged, both bar-headed geese and Andean geese have independently evolved a high oxygen-affinity Hb, but through different amino acid substitutions (bar-headed geese: Pro-α119 to Ala-α119; Andean geese: Leu-β55 to Ser-β55) (McCracken et al., 2010). However, beyond the characterization of the high oxygen-affinity Hb of Andean geese and of select species of Andean ducks (McCracken et al., 2010; Natarajan et al., 2015), very little is known about the physiology of Andean waterfowl, unlike the growing knowledge base for bar-headed geese.

### 1.5.1 Enhancements in the bar-headed goose oxygen transport cascade

Bar-headed geese facilitate HA life and performance by recognized enhancements at each step of their oxygen transport cascade (Figure 1.2A). With regards to the diffusive steps, these specializations include having larger lungs with a high surface area, enhancing the capacity for
pulmonary oxygen diffusion (Scott et al., 2010). Bar-headed geese also have a heightened blood-oxygen carrying capacity due to their aforementioned Hb mutation, favouring oxygen loading at the lungs in hypoxic environments (Weber et al., 1993). Indeed, the $P_{50}$ of bar-headed goose Hb is considerably lower (3.96 kPa) than that of its closely related lowland sister, the greylag goose (*Anser anser*) (5.27 kPa) (Weber et al., 1993). The bar-headed goose left cardiac ventricle also has increased capillary density, which decreases diffusion distances, thereby enhancing ventricular muscle oxygenation at low $PO_2$ levels (Scott et al., 2010). Likewise, cytochrome c oxidase (the enzyme catalyzing oxygen reduction during oxidative phosphorylation) in bar-headed goose cardiac myocytes has a higher affinity towards its reduced substrate, reducing the sensitivity of oxidative phosphorylation to intracellular hypoxia and minimizing reactive oxygen species production (Scott et al., 2010). Finally, the mitochondria in the flight muscle of bar-headed geese are positioned closer to the capillaries to minimize intracellular oxygen diffusion distances (Scott et al., 2009). Thus, while the diffusive steps of the bar-headed goose oxygen transport cascade increase oxygen acquisition and diffusion capacity under hypoxic conditions, it is the coupling of these changes with the enhanced convective steps that produce the increased oxygen transport demanded by HA life and performance.

1.5.2 Bar-headed goose hypoxic ventilatory and cardiovascular responses

Generally very little is known about the hypoxic ventilatory and cardiovascular responses of HA waterfowl. The most comprehensively described responses, however, are those of bar-headed geese.

The bar-headed goose HVR is characterized by a more effective breathing pattern, responding to hypoxia by initially increasing tidal volume rather than breathing frequency. This
reduces the proportion of $V_R$ attributed to ventilating dead space (Scott and Milsom, 2007). This species also demonstrates a reduced sensitivity to the hypocapnic alkalosis that accompanies increased ventilation in hypoxia, allowing them to achieve large increases in $V_R$ (Black and Tenney, 1980a; Scott and Milsom, 2007). As the convective step supporting oxygen extraction from the environment, the HVR helps to ensure blood-oxygen loading.

Studies concurrently monitoring heart rate, stroke volume, and $\dot{Q}$ in birds are few in number (especially during hypoxia), and vary markedly in their conclusions. While it is generally accepted that birds increase $\dot{Q}$ during exercise and hypoxia (Faraci, 1986; Shams and Scheid, 1989; Smith et al., 2000), it is unclear to what degree this increased perfusion is facilitated by increases in heart rate or stroke volume. During exercise, changes in $\dot{Q}$ are reportedly driven by increases in heart rate in geese (Fedde et al., 1989), ducks (Kiley et al., 1979), and turkeys (Boulianne et al., 1993a; Boulianne et al., 1993b), while increases in $\dot{Q}$ are driven by up to a 2-fold increase in stroke volume in emus (Grubb et al., 1983) and chickens (Barnas et al., 1985). During submersion hypoxia, diving ducks are reported to maintain stroke volume (Jones and Holeton, 1972). Indeed, there is also considerable variance in the literature documenting $\dot{Q}$ in bar-headed geese under hypoxic conditions (Table 1.1). For example, when bar-headed geese breathed air with a $F_{O_2}$ similar to that at the summit of Mount Everest (0.07 $F_{O_2}$), Hawkes et al. (Hawkes et al., 2014) reported that their birds decreased $\dot{Q}$ by ~20%, whereas Fedde et al. (Fedde et al., 1989) and Black and Tenney (Black and Tenney, 1980a) reported no change in $\dot{Q}$. However, when Black and Tenney (Black and Tenney, 1980a) exposed their birds to a further reduction in oxygen (~0.04 $F_{O_2}$ corresponding to a $P_{O_2}$ of 3.2 kPa), $\dot{Q}$ increased by a remarkable 7-fold. Thus, it is unclear whether and to what degree bar-headed
geese increase \( \dot{Q} \) during hypoxia, and whether changes in \( \dot{Q} \) result primarily from changes in heart rate or stroke volume.

1.5.3 The unknown effects of high-altitude rearing on the hypoxic cardiorespiratory responses of waterfowl

No studies have examined the hypoxic ventilatory and cardiovascular responses of waterfowl at HA. While providing evidence of physiological differences between bar-headed geese and closely related low-altitude species, all studies to date have been conducted on groups of bar-headed geese born and raised for generations at sea level (Black and Tenney, 1980a; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). Thus, nothing is known about the potential influence of phenotypic plasticity (i.e. acclimatization) and developmental plasticity arising from HA exposure on the physiology of this species or indeed any other species of HA waterfowl.

Hypoxic responses are known to vary depending on when during a bird’s development it is exposed to hypoxia and the duration of the exposure. For example, in chickens, while hypoxic exposure early in development had no developmental effects (Ferner and Mortola, 2009), hypoxic exposure for the entire duration of incubation, or even during the final week, yielded a blunted HVR (Ferner and Mortola, 2009; Mortola, 2011; Szdzuy and Mortola, 2007). However, despite the fact that LA avian species decrease \( \dot{V}_{O_2} \) and growth rate during sustained hypoxic exposure (Mortola, 2011), bar-headed goose embryos maintained \( \dot{V}_{O_2} \) when exposed acutely to ambient hypoxia (11.7 kPa) (Snyder et al., 1982a). In adult animals, many rapid physiological changes occur minutes to hours after acute hypoxic exposure and can be modified during chronic
acclimatization (Ivy and Scott, 2015; Powell et al., 1998). Notably, Black and Tenney acclimated LA bar-headed geese for 4 weeks to simulated altitude (5,640 m), and reported higher resting levels of $\dot{V}O_2$, $\dot{V}R$, and $\dot{Q}$, as well as greater increases in $\dot{V}R$ and $\dot{Q}$ during exposure to progressive hypoxia (Black and Tenney, 1980a). Thus, while it is evident that the HVR and hypoxic cardiovascular response of bar-headed geese can be affected by acute HA acclimation, to what degree these responses might be impacted by HA rearing is unknown.

1.6 Thesis objectives and hypotheses

The general objective of my thesis was to examine how waterfowl (geese and ducks) from different altitudes regulate their cardiovascular and respiratory systems to maintain oxygen supply during hypoxia. My comparative approach used 17 different HA and LA waterfowl populations (Figure 1.3) in order to test the overall hypothesis that waterfowl use more than one cardiovascular and ventilatory strategy to maintain oxygen supply during hypoxia based on differences in their lifetime exposure to altitude (e.g. absent versus transient versus lifetime exposure). I further hypothesized that HA exposure during a bird’s lifetime would alter their HVR and hypoxic cardiovascular response. In order to examine these hypotheses, I compared the cardiovascular (17 groups) and respiratory (6 groups) responses to progressive hypoxia in waterfowl that were reared at either HA or LA. The general form of my thesis is as follows.

Chapter 2 provides a description of methodology common to the subsequent data chapters (Chapters 3-6). Chapter-specific materials and methods are included within each data chapter.

Chapter 3 addresses the potential impact of HA rearing on the responses of bar-headed geese to hypoxia (Figure 1.3). Here I comprehensively compare changes in the convective steps
of the oxygen transport cascade, ventilation and circulation, during acute progressive hypoxic exposure in bar-headed geese born and reared at sea level to a population of bar-headed geese born and reared at 3,200 m. I predicted that, because of HA rearing and acclimatization, high-altitude-reared (HAR) bar-headed geese would show further enhancements in the overall magnitude of \( \dot{V}_{R} \) and \( \dot{Q} \) to those previously reported during short-term HA acclimation of this species (Black and Tenney, 1980a). In addition, because bar-headed geese are known to be capable of large \( \dot{V}_{R} \) increases during hypoxic exposure (Black and Tenney, 1980a; Scott and Milsom, 2007), I predicted that \( \dot{V}_{R} \) would be greater in the HAR bar-headed geese than in the low-altitude-reared (LAR) bar-headed geese.

Chapter 4 compares the ventilatory and cardiovascular strategies of HA-migrating bar-headed geese with those of non-migratory waterfowl residing lifelong in the HA Andes (Andean geese and crested ducks (Lophonetta specularioides)) to determine whether Andean geese and crested ducks met the challenge of HA life through strategies convergent with those in bar-headed geese, or whether the large differences in lifetime exposure to altitude and performance demands resulted in divergent strategies of HA adaptation (Figure 1.3). I predicted that, given the fact that all three species are endemic to HA regions, Andean geese and crested ducks would respond similarly to bar-headed geese when exposed to hypoxia. Thus, I predicted that during progressive hypoxic exposure Andean geese and crested ducks would exhibit large increases in \( \dot{Q} \) and \( \dot{V}_{R} \), and that the increases in \( \dot{V}_{R} \) that would be primarily supported by increases in tidal volume, as previously reported for bar-headed geese (Scott and Milsom, 2007).

Chapter 5 compares the cardiovascular responses to progressive hypoxia among five species of HA resident Andean ducks (yellow-billed pintail, cinnamon teal, ruddy duck, speckled
teal, and Puna teal) to six species of related LA ducks (northern pintail, cinnamon teal, ruddy duck, green-winged teal, gadwall, and mallard duck) (Figure 1.3). I predicted that the HA populations would demonstrate an enhanced blood-oxygen carrying capacity during hypoxia compared to the LA populations (e.g. decreased Hb-\textsubscript{P\textsubscript{50}} and/or increased Hct and [Hb]), and that this enhancement would be greatest for the populations that had spent the longest time at HA after diverging from their LA ancestors. Moreover, I hypothesized that any differences in the hypoxic cardiovascular responses of the HA and LA ducks would be primarily attributable to structural enhancements of the cardiovascular system (e.g. increased blood-oxygen carrying capacity and increased stroke volume) in the HA populations that enhanced their ability to match oxygen supply and demand during hypoxia.

Chapter 6 examines the heart rate variability (HRV) of the waterfowl in Chapter 5, providing the first measures of HRV for any waterfowl. It compares HRV for five species of HA Andean ducks (yellow-billed pintail, cinnamon teal, ruddy duck, speckled teal, and Puna teal) and six species of related LA ducks (northern pintail, cinnamon teal, ruddy duck, green-winged teal, gadwall, and mallard duck) during exposure to and recovery from severe hypoxia (Figure 1.3). Hypoxia exposure tends to reduce HRV (Cornolo et al., 2004; Melin et al., 2003; Sharshenova et al., 2006). I predicted that the HA Andean duck species would exhibit a greater magnitude of HRV than the LA Andean duck species during hypoxic exposure, consistent with previous reports for HA acclimatized rats and adult humans, and children born at HA (Cornolo et al., 2004; Melin et al., 2003; Sharshenova et al., 2006).

Chapter 7 concludes my thesis and compiles the observed ventilatory and cardiovascular strategies of waterfowl for maintaining routine oxygen supply during hypoxia as a synthesis,
while further discussing these findings in the context of the current literature and providing future directions for research.
Figure 1.1: Decreases in barometric pressure ($P_B$), inspired partial pressure of oxygen ($P_{I\text{O}_2}$), and the inspired fractional composition of oxygen ($F_{I\text{O}_2}$) equivalent to sea level with increases in altitude.

Relationship between x and y variables is adapted from Bouverot (Bouverot, 1985b). The tagged bar-headed goose migration data refers to actual telemetry studies conducted on bar-headed geese (Bishop et al., 2015; Hawkes et al., 2011), whereas altitude denoted by the anecdotal evidence for the bar-headed goose migration refers to a one-time sighting described by Swan (Swan, 1970). Altitude information for Andean geese, Andean ducks, and low altitude ducks are derived from the literature (Fjeldså and Krabbe, 1990; Hilty and Brown, 1986; Livezey, 1986; McCracken et al., 2009a; McCracken et al., 2009c; Munoz-Fuentes et al., 2013).
Figure 1.2: The driving partial pressure of oxygen (PO$_2$) through the vertebrate oxygen transport cascade decreases with increasing altitude.

(A) A generalized schematic of the main diffusive and convective portions of the vertebrate oxygen transport cascade, with the four chambers of the heart abbreviated (right atrium, RA; right ventricle, RV; left atrium, LA; left ventricle, LV). (B) Decreases in the PO$_2$ through the oxygen transport cascade at sea level (0 m) and at high altitude (e.g. Mt. Everest Summit: 8848 m). The solid lines are based off of data collected in Chapter 3 and the dotted lines are hypothetical. Arterial PO$_2$ values are represented by “a” and venous PO$_2$ values by “v”.
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Species</th>
<th>Scientific Name</th>
<th>Altitude</th>
<th>Study Type</th>
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<tbody>
<tr>
<td>3</td>
<td>Barnacle goose</td>
<td><em>Branta leucopsis</em></td>
<td>Low Altitude (0 m)</td>
<td>HVR and HCVR</td>
</tr>
<tr>
<td>3</td>
<td>Bar-headed goose</td>
<td><em>Anser indicus</em></td>
<td>Low Altitude (0 m)</td>
<td>HVR and HCVR</td>
</tr>
<tr>
<td>4</td>
<td>Bar-headed goose</td>
<td><em>Anser indicus</em></td>
<td>High Altitude (3,200 m)</td>
<td>HVR and HCVR</td>
</tr>
<tr>
<td>4</td>
<td>Bar-headed goose</td>
<td><em>Anser indicus</em></td>
<td>High Altitude (3,200 m)</td>
<td>HVR and HCVR</td>
</tr>
<tr>
<td>4</td>
<td>Andean goose</td>
<td><em>Chloephaga melanoptera</em></td>
<td>High Altitude (3,200 m)</td>
<td>HVR and HCVR</td>
</tr>
<tr>
<td>4</td>
<td>Crested duck</td>
<td><em>Lophonetta alticola</em></td>
<td>High Altitude (3,200 m)</td>
<td>HVR and HCVR</td>
</tr>
<tr>
<td>5 and 6</td>
<td>Yellow-billed pintail</td>
<td><em>Anas georgica</em></td>
<td>High Altitude (3,800 m)</td>
<td>HCVR</td>
</tr>
<tr>
<td>5 and 6</td>
<td>Cinnamon Teal</td>
<td><em>Anas c. orinomus</em></td>
<td>High Altitude (3,800 m)</td>
<td>HCVR</td>
</tr>
<tr>
<td>5 and 6</td>
<td>Ruddy duck</td>
<td><em>Oxyura j. ferruginea</em></td>
<td>High Altitude (3,800 m)</td>
<td>HCVR</td>
</tr>
<tr>
<td>5 and 6</td>
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<td><em>Anas oxyptera</em></td>
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<td>HCVR</td>
</tr>
<tr>
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<td><em>Anas puna</em></td>
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<td>HCVR</td>
</tr>
<tr>
<td>5 and 6</td>
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<td><em>Anas acuta</em></td>
<td>Low Altitude (1,300 m)</td>
<td>HCVR</td>
</tr>
<tr>
<td>5 and 6</td>
<td>Cinnamon Teal</td>
<td><em>Anas c. cyanosera</em></td>
<td>Low Altitude (1,300 m)</td>
<td>HCVR</td>
</tr>
<tr>
<td>5 and 6</td>
<td>Ruddy duck</td>
<td><em>Oxyura j. jamaicensis</em></td>
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<td>HCVR</td>
</tr>
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<td>5 and 6</td>
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<td><em>Anas platyrhynchos</em></td>
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<td>HCVR</td>
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</table>

Figure 1.3: A compilation of waterfowl studied in this thesis.
For each waterfowl group this compilation summarizes the chapter containing the data of a particular group, the originating altitude of the group, as well as which response was studied: the hypoxic ventilatory response (HVR) and/or the hypoxic cardiovascular response (HCVR).
Table 1.1: Ventilatory, metabolic, and cardiovascular variables during progressive hypoxic exposure in bar-headed geese.

Mean data from the literature (1: Black and Tenney, 1980a; 2: Fedde et al., 1989; 3: Scott and Milsom, 2007; 4: Hawkes et al., 2014) for a suite of variables in low-altitude bar-headed geese are compared when provided. Ventilatory data are expressed in either standard temperature and pressure, dry (STPD) or body temperature and pressure, saturated (BTPS) and are labeled accordingly in the table when this information was disclosed in the paper. Variables expressed in the table are as follows: arterial partial pressure of oxygen (P_{O_2}), arterial partial pressure of carbon dioxide (P_{CO_2}), arterial pH (pH_a), arterial oxygen content (C_{O_2}), venous partial pressure of carbon dioxide (P_{CO_2}), venous pH (pH_v), venous oxygen content (C_{O_2}), hematocrit (Hct), total blood hemoglobin concentration ([Hb]), oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory quotient (RQ; VCO_2/VO_2), total ventilation (V_T), tidal volume (V_T), breathing frequency (f_{breath}), air convection requirement (ACR), lung oxygen extraction (E_{L,O_2}) cardiac output (Q), stroke volume (V_S), heart rate (f_{HR}), blood convection requirement (BCR), tissue oxygen extraction (E_{T,O_2}), difference in arterial and venous oxygen content (C_{O_2}-C_{O_2}), total peripheral resistance (TPR), and the ventilation-perfusion ratio (V_{R}/Q).

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<th>Factor</th>
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<td></td>
<td></td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
</tr>
<tr>
<td>P_{O_2}</td>
<td>Torr</td>
<td>93 91 88 103</td>
<td>28.5 32 39 39</td>
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<tr>
<td>P_{CO_2}</td>
<td>Torr</td>
<td>32 34 25 41</td>
<td>11 17 13 24</td>
</tr>
<tr>
<td>pH_a</td>
<td>pH units</td>
<td>7.47 7.44 7.40 --</td>
<td>7.66 7.59 7.49 --</td>
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<tr>
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<td>ml dl(^{-1})</td>
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<td>12.1 9.2 -- 11.2</td>
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<tr>
<td>P_{CO_2}</td>
<td>Torr</td>
<td>35 38 40.9 52</td>
<td>18 22 22.3 28</td>
</tr>
<tr>
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<td>4.2 3.6 -- 6.1</td>
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<tr>
<td>Hct</td>
<td>%</td>
<td>47.8 -- -- 40.1</td>
<td>43.9 -- -- 38.5</td>
</tr>
<tr>
<td>[Hb]</td>
<td>g dl(^{-1})</td>
<td>13.9 -- -- 16.5</td>
<td>12.9 -- -- 16.9</td>
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<tr>
<td>VO_2</td>
<td>ml O_2 min(^{-1}) kg(^{-1})</td>
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<td>12.7 15.7 58.96 14.1</td>
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<tr>
<td>VCO_2</td>
<td>ml O_2 min(^{-1}) kg(^{-1})</td>
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<td>-- 15.7 -- 11.4</td>
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<tr>
<td>RQ</td>
<td></td>
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<td>-- 1.0 -- 0.81</td>
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<tr>
<td>VR</td>
<td>ml min(^{-1}) kg(^{-1})</td>
<td>286 BTPS 335 359 --</td>
<td>591 BTPS 713 1117 --</td>
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<td>-- 40.5 63 --</td>
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<tr>
<td>f_{breath}</td>
<td>min(^{-1})</td>
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<td>-- 17.6 17.7 19.7</td>
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<td>ACR</td>
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<td>30.7 31.5 -- --</td>
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<tr>
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<td>ml min(^{-1}) kg(^{-1})</td>
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<td>151 220 -- 261</td>
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<tr>
<td>V_S</td>
<td>ml kg(^{-1})</td>
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<td>-- 0.81 -- 2.75</td>
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<tr>
<td>f_{HR}</td>
<td>min(^{-1})</td>
<td>-- 220 -- 96</td>
<td>-- 230 -- 95</td>
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<tr>
<td>BCR</td>
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<td>11.9 14.0 -- --</td>
</tr>
<tr>
<td>E_{T,O_2}</td>
<td>%</td>
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<td>54.0 60.9 -- --</td>
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<tr>
<td>C_{O_2}-C_{O_2}</td>
<td>ml dl(^{-1})</td>
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<td>7.9 5.6 -- 5.1</td>
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<td>-- 0.65 -- --</td>
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<tr>
<td>VR/Q</td>
<td>ml air ml blood(^{-1})</td>
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<td>3.9 STPD 3.2 -- --</td>
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</table>
CHAPTER 2: MATERIALS AND METHODS

2.1 Animals

All experimental procedures were conducted according to guidelines approved by the Animal Care Committee at the University of British Columbia under the guidelines of the Canadian Council on Animal Care. Specific details on the species used, their holding conditions, and where the experiments were conducted is contained in the data chapters. The following are general procedures used in more than one data chapter.

2.2 Surgical procedures

For the cardiovascular measurements without an electrocardiogram (ECG) (Chapters 3 and 4), surgery was conducted under general and local anesthesia. All birds were first weighed, gently restrained and induced with isoflurane (4%) supplemented with oxygen (100%) by facemask prior to intubation. General anesthesia was maintained with isoflurane and oxygen. The right brachial artery and vein were accessed via a small incision and blunt dissection and cannulated with polyurethane cannulae (PU-90; 0.102 cm internal diameter x 0.410 cm outer diameter) filled with 1000 IU ml$^{-1}$ heparinized saline (Black and Tenney, 1980a; Scott and Milsom, 2007). Arterial cannulae were advanced ~4.0 cm and venous cannulae were advanced to rest outside the right atrium opening to obtain mixed venous blood. Birds were recovered for at least 24 h prior to the hypoxic exposure experiments.

2.3 Experimental protocol for hypoxic exposure

For Chapters 3-6, each bird was placed in a flexible cradle that permitted unrestricted
breathing. Its head was placed in an opaque plexiglass chamber large enough to accommodate free movement of the neck and head, sealed around the neck with a flexible latex collar, and supported by the cradle (Figure 2.1). Birds in the experimental apparatus were allowed 60-90 min to adjust to their surroundings. Then to simulate progressive hypoxia, air with varying levels of sea level-equivalent $F_iO_2$ was delivered at a flow rate ($\dot{V}_C$) through the box ranging between 5-10 l min$^{-1}$. $F_iO_2$ was altered by mixing $N_2$ and air through a series of calibrated rotameters. Birds were exposed to 20-25-min step reductions in equivalent $F_iO_2$ and a 25-min recovery period at ambient $F_iO_2$ followed the hypoxic exposures.

2.4 Cardiorespiratory measurements and data acquisition

Whole animal $\dot{V}o_2$ (Chapters 3 and 4) was calculated from $\dot{V}_C$, $F_iO_2$, and the fractional oxygen composition of expired gas from the chamber ($F_{EC}O_2$), which were directly measured by a gas analyzer (Sable Systems, Las Vegas, NV, USA). Water vapour was removed from the gas prior to analysis (Withers, 1977). Tidal volume and breathing frequency were measured from the head mask outflow using a pneumotachograph connected to a differential pressure transducer (Validyne, Northridge, CA, USA). Mean arterial pressure and all respiratory variables were recorded to a computer using PowerLab data acquisition software (ADInstruments, Colorado Springs, CO, USA).

Arterial blood pressure (Chapters 3 and 4) was continuously monitored throughout the experiment using a pressure transducer (Deltran, Utah Medical Products Inc., Midvale, USA) connected to the brachial artery cannula. Strategic sampling of arterial and venous blood (0.4 ml per sample) occurred 15 min after exposure to each $F_iO_2$, as well as after 5 and 25 min into ambient recovery. Any blood remaining after analysis was returned to the bird. Blood samples
were immediately analyzed for partial pressures of oxygen and carbon dioxide, oxygen content, [Hb], Hct, arterial pH (pHₐ) and plasma ions including HCO₃⁻, CₐO₂ (mmol l⁻¹) and CᵥO₂ (mmol l⁻¹) were determined at 41°C using the Tucker method (Tucker, 1967) with a FireSting oxygen probe (PyroScience, Aachen, Germany). The oxygen probe was calibrated with 0% oxygen (3 g l⁻¹ Na₂SO₃; Sigma-Aldrich, Oakville, ON, Canada) and water saturated with ambient air (21% oxygen at sea level and 12% oxygen at 3,200 m) prior to each experiment. [Hb] (g dl⁻¹), Hct (%), arterial bicarbonate ion concentration ([HCO₃⁻]ₐ; mmol l⁻¹), PₐO₂ (kPa), PₐCO₂ (kPa), and pHₐ were analyzed from arterial blood at 41°C using CG8+ cartridges with the i-STAT VetScan Analyzer (Abaxis, Union City, CA, USA). All i-STAT values were corrected for bird blood according to Harter et al. (Harter et al., 2015). [HCO₃⁻]ₐ was calculated using the Henderson-Hasselbach equation, assuming a pK of 6.090 and a CO₂ solubility coefficient of 0.2117 mmol l⁻¹ kPa⁻¹ in plasma (Helbecka et al., 1964; Scott and Milsom, 2007). With the exception of the blood variables, all data were acquired and analyzed using the PowerLab data acquisition and analysis software (ADInstruments, Colorado Springs, CO, USA) at a sampling frequency of 1000 Hz per channel. Mean values were derived for each variable for a 1-2 min period before each blood sample (i.e. after 12-15 min of each hypoxic FIO₂ exposure, and after 3-5 min and 22-25 min of the normoxic recovery). CₐO₂ and CᵥO₂ were acquired using software designed for the FireSting oxygen probes (PyroScience, Aachen, Germany).

For Chapters 5 and 6, heart rate was continuously monitored via three subcutaneously inserted ECG leads using a PowerLab Bio Amp (ADInstruments, Colorado Springs, CO, USA) and analyzed using PowerLab analysis software (ADInstruments, Colorado Springs, CO, USA) at a sampling frequency of 1000 Hz per channel. Arterial oxygen saturation was measured continuously using a MouseOx oxygen sensor (Starr Life Sciences, Oakmont, PA, USA) and
recorded by the accompanying data acquisition software provided by Starr Life Sciences (Starr Life Sciences, Oakmont, PA, USA). Hematocrit was determined at the end of the experiment in duplicate by a Zipocrit hematocrit centrifuge (LW Scientific, Lawrenceville, GA, USA). Hemoglobin concentration was acquired at the end of the experiment using a Hemocue Hb 201+ System (Ängelholm, Sweeden).

2.5 Data analysis

The following respiratory variables were measured directly. Tidal volume (ml kg \(^{-1}\)) and breathing frequency (min \(^{-1}\)) were derived from the integrated differential pressure signal and \(\dot{V}_R\) (ml \(\text{min}^{-1} \text{kg}^{-1}\)) was calculated as their product. \(\dot{V}_O_2\) (ml \(\text{min}^{-1} \text{kg}^{-1}\)) was calculated as:

\[
\dot{V}_O_2 = \frac{\dot{V}_C \times (F_{I}O_2 - F_{E}CO_2)}{M_b} \quad (2.1),
\]

where \(M_b\) is body mass (kg). Water vapour had been removed prior to gas analysis (Withers, 1977). The respiratory quotient (RQ) was assumed to be 1.0. It has been shown that RQ does not change with acute hypoxic exposure in bar-headed geese (Hawkes et al., 2014), and is unaffected by prolonged hypoxic exposure in deer mice (Cheviron et al., 2012; McClelland et al., 1998). Thus, while RQ in birds can range from <0.7-1.0 and my calculations ignore this variation, they use the same value throughout, thus allowing for comparison without affecting my overall conclusions (Scott and Milsom, 2007). Tidal volume was reported in terms of body temperature and pressure, saturated (BTPS), assuming a constant body temperature of 41°C and taking into account changes in barometric pressure and air density at altitude (Dejours, 1975). \(\dot{V}_O_2\) was reported in standard temperature and pressure, dry (STPD).
The following respiratory variables were calculated. Air convection requirement (ACR) was calculated as the quotient of $\dot{V}_R$ and $\dot{V}_O_2$. Lung oxygen extraction (%) was calculated as:

$$\text{Lung O}_2\text{ Extraction} = \left( \frac{F_I O_2 - F_E O_2}{F_I O_2} \right) \times 100 \quad (2.2),$$

where the calculated fractional expired level of oxygen of the bird ($F_E O_2$) was calculated as:

$$F_E O_2 = \left( \frac{\dot{V}_R \times F_I O_2 - \dot{V}_O_2}{\dot{V}_R} \right) \quad (2.3).$$

Air convection requirement, and lung oxygen extraction were reported in terms of STPD.

The following cardiovascular variables were measured directly. Heart rate (min$^{-1}$) was calculated from the peaks in the pulsatile arterial blood pressure trace (Chapters 3 and 4) or from the peaks of the ECG trace (Chapters 5 and 6). Mean arterial pressure (kPa) was calculated as the sum of diastolic pressure plus 1/3 pulse pressure.

The following cardiovascular variables were calculated. $\dot{Q}$ (ml min$^{-1}$ kg$^{-1}$) was calculated from the Fick equation given known values of $\dot{V}_O_2$ and $C_a O_2 - C_v O_2$. Stroke volume (ml kg$^{-1}$) was calculated as the quotient of $\dot{Q}$ and heart rate. Oxygen pulse was calculated as the quotient of $\dot{V}_O_2$ and heart rate (ml oxygen kg$^{-1}$). Total peripheral resistance (kPa min kg ml$^{-1}$) was calculated as the quotient of mean arterial pressure and $\dot{Q}$ (Bech and Nomoto, 1982). Blood convection requirement (BCR) was calculated as the quotient of $\dot{Q}$ and $\dot{V}_O_2$. Tissue oxygen delivery was calculated as the product of $\dot{Q}$ and $C_a O_2$ (ml$^{-1}$ min$^{-1}$ kg$^{-1}$). Tissue oxygen extraction (%) was calculated as:

$$\text{Tissue O}_2\text{ Extraction} = \left( \frac{C_a O_2 - C_v O_2}{C_a O_2} \right) \times 100 \quad (2.4).$$
2.6 Statistical analyses

Refer to the abbreviated methods section in each chapter for information on what statistical analyses were employed.

2.7 Methodological considerations

With any methodology, it is important to consider the possible consequences inherent in the assumptions. I assumed that my sample size for each waterfowl group was sufficiently large to avoid Type II errors (“false negatives”). My replication was similar to those in the literature for equivalent experiments (Black and Tenney, 1980a; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). To avoid Type I errors (“false positives”), conservative statistical analyses (i.e. repeated measures analysis of variance (ANOVA) rather than a series of t-tests) were selected for comparing multiple exposures within a group and comparing between groups.

The majority of my birds were caught haphazardly from a much larger wild populations and it was not possible to control for potential differences in individual life history or other factors that might impact their physiology. However, because the specific aim of this study was to compare the physiological responses of wild birds at altitude, the importance of using these particular populations to test my hypotheses outweighed the need to control these factors.

A number of assumptions made in the calculation of certain variables could also impact my results. For example, there are implications of assuming an RQ of 1.0 to calculate $\dot{V}O_2$, as described in Section 2.5. In brief, while RQ in birds can range from <0.7-1.0 and my calculations ignore this variation, they use the same value throughout, thus allowing for comparison without affecting my overall conclusions. There is also evidence that RQ is unchanged by either acute (Hawkes et al., 2014) or prolonged hypoxic exposure (Cheviron et al., 2012; McClelland et al., 2012).
In addition, the following primary variables were calculated rather than measured directly: $V_R$, $F_{E,O_2}$, air convection requirement, lung oxygen extraction, cardiac output, stroke volume, blood convection requirement, tissue oxygen extraction, and total peripheral resistance. The primary ramification of calculating a variable rather than directly measuring it is that the error terms for the measured variables are combined for the calculated variable. In the context of random sampling this could lead to greater variability and a greater chance of a Type II error. Ultimately there are constraints on what variables can and cannot be measured with some ease in remote field locations. As such, while I acknowledge that the above factors may contribute to my data analysis and interpretation, I considered the importance of making measurements on wild birds in the field to far outweigh the consequences of potentially increasing variability in my derived variables.
Figure 2.1: Experimental setup.
A schematic of the general experimental setup used in Chapters 3-6.
CHAPTER 3: ALTITUDE MATTERS: DIFFERENCES IN CARDIOVASCULAR AND RESPIRATORY RESPONSES TO HYPOXIA IN BAR-HEADED GEESE REARED AT HIGH AND LOW ALTITUDES

3.1 Introduction

Birds exhibit enhancements at each step of their oxygen transport cascade that help to support the high flux of oxygen required for flight and endothermy (Scott, 2011). This cascade describes the flow of oxygen from the atmosphere to the mitochondria in vertebrates and the steps include ventilation, pulmonary oxygen diffusion, perfusion, and tissue oxygen diffusion. Bar-headed geese, which migrate biannually over the Himalayan mountain range (Bishop et al., 2015; Hawkes et al., 2011; Scott et al., 2015), exhibit further enhancements at each level of this oxygen transport cascade (Black and Tenney, 1980a; Meir and Milsom, 2013; Scott, 2011; Scott and Milsom, 2006; Scott and Milsom, 2007; Scott et al., 2015; Weber et al., 1993). While providing evidence of physiological differences between bar-headed geese and closely related LA species, all studies to date have been conducted on groups of bar-headed geese born and raised for generations at sea level (Black and Tenney, 1980a; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). Thus, relatively little is known about the influence of phenotypic plasticity (i.e. acclimatization) and developmental plasticity on the physiology of this species.

Hypoxic responses vary depending on when during an animal’s development it is exposed to hypoxia and the duration of the exposure. In chickens, hypoxic exposure had no reported developmental effects when it occurred early in development or for an acute duration (Ferner and Mortola, 2009). When exposure to hypoxia occurred during the entire duration of
incubation, or even during the final week, however, the HVR of the chicks was blunted. This was a result of reduced ventilatory chemosensitivity (Ferner and Mortola, 2009; Mortola, 2011; Szdzuy and Mortola, 2007). Sustained hypoxic exposure in LA birds also decreased $\dot{V}O_2$ and growth rate (Mortola, 2011). However, embryos of some birds successfully hatch with normal growth rates and $\dot{V}O_2$ at altitudes of 4,000-6,500 m (Carey et al., 1982; Leon-Velarde and Monge, 2004). For example, bar-headed geese embryos maintained $\dot{V}O_2$ when exposed acutely to ambient hypoxia (11.7 kPa) (Snyder et al., 1982a). Thus, though hypoxia exposure during development can alter physiological responses in birds, some HA bird species have adapted to mitigate these effects.

In adult animals, many rapid physiological changes occur minutes to hours after acute hypoxic exposure and can be modified by chronic acclimatization (Ivy and Scott, 2015; Powell et al., 1998). Black and Tenney measured changes in $\dot{V}O_2$, $\dot{V}R$, and $\dot{Q}$ during progressive hypoxic exposure in bar-headed geese following short-term (4 week) acclimation to simulated altitude (5,640 m) (Black and Tenney, 1980a). The acclimated bar-headed geese did not become polycythemic, a trait characteristic of other species endemic to HA regions. They also displayed higher resting $\dot{V}O_2$, $\dot{V}R$, and $\dot{Q}$ under ambient conditions, as well as greater increases in $\dot{V}R$ and $\dot{Q}$ during exposure to progressive hypoxia (Black and Tenney, 1980a). Thus, the HVR and hypoxic cardiovascular response of bar-headed geese can be affected by short-term HA acclimation.

In the present study I extended this work by examining the effects of HA rearing and development on the physiological responses to hypoxia in bar-headed geese. This is significant, considering that the effects of pre- or postnatal hypoxic exposure can differ significantly, and persist throughout adult life (Bavis, 2005; Ivy and Scott, 2015). My primary objective, therefore,
was to compare the changes in the convective steps in the oxygen transport cascade, ventilation and circulation, of LAR bar-headed geese born and reared at 0 m to a group of wild HAR bar-headed geese born and reared at 3,200 m during short-term progressive hypoxic exposure. These responses were compared with those of LAR barnacle geese (*Branta leucopsis*), a member of a closely related genus that also migrates, but only at LA. I predicted that, because of HA rearing and acclimatization, HAR bar-headed geese would show further enhancements in the overall magnitude of $\dot{V}_R$ and $\dot{Q}$ to those previously reported during short-term HA acclimation of this species (Black and Tenney, 1980a). In addition, because bar-headed geese are known to be capable of large $\dot{V}_R$ increases during hypoxic exposure (Black and Tenney, 1980a; Scott and Milsom, 2007), I predicted that $\dot{V}_R$ would be greatest in the HAR bar-headed geese and lowest in the barnacle geese.

### 3.2 Materials and methods

#### 3.2.1 Animals

The experiments on the LAR geese were performed at the University of British Columbia, where the geese were housed at the Centre for Comparative Medicine. Cardiovascular measurements were made on 6 cannulated bar-headed geese (*Anser indicus*), (2.5 ± 0.2 kg) and respiratory measurements were made on 5 non-cannulated bar-headed geese (2.4 ± 0.1 kg). All cardiorespiratory measurements were made on 7 cannulated barnacle geese (*Branta leucopsis*) (2.5 ± 0.2 kg). Cardiorespiratory measurements were also obtained from 5 cannulated HAR bar-headed geese (2.1 ± 0.1 kg) that were born in the wild at 3,200 m (reduced oxygen partial pressure and barometric pressure) at Lake Qinghai, China and reared in captivity for at least one year at the lake. All experimental animals were fed similar diets, housed in outdoor pens under
natural conditions, and experienced similar levels of (in)activity. The HAR bar-headed geese, however, were born and reared in hypobaric hypoxia. All experimental procedures were conducted according to guidelines approved by the Animal Care Committee at the University of British Columbia under the guidelines of the Canadian Council on Animal Care.

3.2.2 Surgical procedures

See Chapter 2 for a detailed explanation of the surgical procedures used for the brachial artery and vein cannulations.

3.2.3 Experimental protocol

Birds were exposed to 25-min step reductions in sea level-equivalent F\textsubscript{I}\textsubscript{O\textsubscript{2}} (ambient [0.21 at 0 m or 0.134 at 3,200 m], 0.12, 0.09, and 0.07), and a 25-min recovery period at ambient F\textsubscript{I}\textsubscript{O\textsubscript{2}} followed the hypoxic exposures. For the respiratory trials, birds were exposed further to 0.05 F\textsubscript{I}\textsubscript{O\textsubscript{2}}. See Chapter 2 for a detailed experimental protocol.

3.2.4 Data measurement and analysis

See Chapter 2 for details regarding all measurements, calculations, and data analysis.

3.2.5 Statistical analysis

Data are presented as means $\pm$ s.e.m unless stated otherwise. Within each species, all data were analyzed using one-way repeated measures ANOVA and Holm-Sidak post hoc tests. Comparisons between each species were made using two-way (species and F\textsubscript{I}\textsubscript{O\textsubscript{2}}) repeated
measures ANOVA and Holm-Sidak post-hoc tests within each $F_O_2$. For statistical comparisons, $P<0.05$ was used to determine statistical significance. Variables analyzed with a one-way repeated measures ANOVA that did not meet assumptions for either normality or equal variance in barnacle geese were transformed with $x' = \ln(x)$ for $Q$, lung oxygen extraction, and blood convection requirement and with $x' = x^2$ for tidal volume. Similarly, variables were transformed when they did not meet assumptions for either normality or equal variance analyzed for a two-way repeated measures ANOVA (i.e. $x' = \ln(x)$ and $x' = 1/(1-x)$ for $\dot{V}_R$, tidal volume, and $P_aO_2$). Student t-tests were used to compare Hct and [Hb] prior to and following the experiment to ensure that no blood dilution had been incurred throughout the experiment. Statistical analyses were carried out using SigmaStat (version 3.0; Systat Software).

### 3.3 Results

#### 3.3.1 Metabolic response

Both bar-headed and barnacle geese maintained $\dot{V}O_2$ during progressive hypoxia (Figure 3.1A), with $\dot{V}O_2$ increasing significantly during hypoxic exposure in LAR bar-headed geese ($P<0.001$), almost significantly in HAR bar-headed geese ($P=0.051$), and remaining unchanged in barnacle geese ($P=0.720$). The $\dot{V}O_2$ of the HAR bar-headed geese was significantly lower than that of the LAR bar-headed geese at every level except 0.07 $F_O_2$ ($P=0.004$).

#### 3.3.2 Hypoxic ventilatory response

The hypoxic ventilatory responses of each study group and the differences present in the relative contributions of breathing frequency and tidal volume to $\dot{V}_R$ are depicted in a Hey plot
(Fig. 3.1B), a graphical depiction of breathing patterns (tidal volume and breathing frequency) at different levels of $\dot{V}_R$ (Guz and Widdicombe, 1970). At a given level of $\dot{V}_R$, bar-headed geese breathed at a slower rate with significantly larger tidal volumes (Figure 3.1B, Figure 3.2A,B) than the barnacle geese, and this difference in pattern was sustained in hypoxia. The increase in $\dot{V}_R$ was greatest in the HAR bar-headed geese and was lowest for the barnacle geese (Figure 3.1C). $\dot{V}_R$ of HAR bar-headed geese was higher than that of barnacle geese during every exposure (P<0.001). LAR bar-headed geese trended towards having a larger $\dot{V}_R$ than barnacle geese at 0.05 $F_iO_2$ (P=0.057). The air convection requirement, the ratio of $\dot{V}_R$ and $\dot{V}O_2$, increased in hypoxia in all groups, significantly so in both LAR groups (Figure 3.3A). Lung oxygen extraction, the percentage of the inspired oxygen extracted from inspired gas, increased initially in both LAR groups between 0.21 and 0.12 $F_iO_2$ (Figure 3.3B), and then remained constant between 30-50% beyond 0.12 $F_iO_2$. All differences in both the resting levels of $\dot{V}_R$ between HAR and LAR bar-headed geese disappeared when my data were expressed as STPD rather than BTPS (Figure 3.1D).

3.3.3 Hypoxic cardiovascular response

3.3.3.1 Blood-oxygen carrying capacity and acid-base status

Hct and [Hb] were not significantly different among HAR bar-headed geese (Hct: 38.8 ± 2.8%, [Hb]: 117.3 ± 7.0 g l$^{-1}$) or LAR bar-headed geese (Hct: 43.9 ± 4.3%, [Hb]: 125.1 ± 7.5 g l$^{-1}$), and LAR barnacle geese (Hct: 43.1 ± 1.9%, [Hb]: 112.6 ± 4.0 g l$^{-1}$). In all three groups of geese, Hct and [Hb] were unchanged during progressive hypobaric hypoxia.
$P_aO_2$ decreased with progressive decreases in $F_I O_2$, and was lower (P<0.001) in the bar-headed geese than in barnacle geese at or below an $F_I O_2$ of 0.07 (Figure 3.4A). $C_aO_2$ was similar between the groups of geese and decreased with hypobaric hypoxia (Figure 3.4B). Plotting $C_aO_2$ as a function of $P_aO_2$ generated in vivo oxygen equilibrium curves (Figure 3.5) that are representative of the arterial saturation given the prevailing acid-base conditions that accompanied hypobaric hypoxia (see Table 3.1). Differences in these oxygen equilibrium curves reflect the higher oxygen-affinity of the bar-headed goose blood (Black and Tenney, 1980a; Weber et al., 1993) compared to that of barnacle geese and differences in the pH$_a$ at each $F_I O_2$ (Figure 3.6).

The starting pH$_a$ of the HAR bar-headed geese was higher than that of the LAR bar-headed geese (P<0.001) and was accompanied by higher starting [HCO$_3^-$] (P=0.009) (Figure 3.6A,B). $P_aCO_2$ decreased (P<0.001) and pH$_a$ increased (P<0.001) in all three groups of geese during progressive hypoxia (Figure 3.6, Table 3.1). Both bar-headed goose study groups experienced a respiratory alkalosis during hypoxic exposure down to 0.07 $F_I O_2$ (Figure 3.6A,B). At that point, LAR bar-headed geese were recovered to normoxia (Figure 3.6A), whereas HAR bar-headed geese were further exposed to 0.05 $F_I O_2$ (Figure 3.6B). Between 0.07 to 0.05 $F_I O_2$ (Figure 3.6B), the pH$_a$ of HAR bar-headed geese remained unchanged, but [HCO$_3^-$] decreased significantly (Figure 3.6B), indicative of a metabolic acidosis. This also occurred in the barnacle geese, but the metabolic acidosis was triggered at a less extreme level of hypoxia (0.07 $F_I O_2$) (Figure 3.6C). Intriguingly, only during ambient recovery did pH$_a$ fall significantly in any group. LAR bar-headed geese recovered their pH$_a$ within 5-min of normoxia after being exposed to 0.07 $F_I O_2$. The HAR bar-headed geese and barnacle geese that were exposed to 0.05 $F_I O_2$ both had a persistent acidosis and low [HCO$_3^-$]$_a$ even after 25-min recovery (Figure 3.6B,C).
3.3.2 Cardiac output

All three groups of geese increased $\dot{Q}$ by 2-3 fold, yielding similar maximum values during progressive hypoxia (Figure 3.7A). The increase in $\dot{Q}$ became significant at a different $F_1O_2$ between the groups: 0.09 in HAR bar-headed geese, 0.07 in LAR bar-headed geese, and 0.05 in barnacle geese (Figure 3.7A). The relative contributions of increases in heart rate and stroke volume to $\dot{Q}$ are depicted in a cardiac equivalent of a Hey plot for individuals at all exposures (Figure 3.7B). Increases in stroke volume accounted for most of the increase in $\dot{Q}$ in barnacle geese and LAR bar-headed geese (Figure 3.7B,C) - their heart rates increased only modestly (Figure 3.7D) while stroke volume roughly doubled (Figure 3.7C). This was also the case initially in the HAR bar-headed geese, however at 0.07 $F_1O_2$ heart rate increased substantially, with an associated decrease in stroke volume. The net overall result, however, was a trend for $\dot{Q}$ to increase earlier and more rapidly in bar-headed geese than barnacle geese, and more so in the HAR bar-headed geese than in the LAR bar-headed geese (Figure 3.7A).

While the increase in $\dot{Q}$ of HAR bar-headed geese was triggered at a higher $F_1O_2$, it is evident from Figure 3.8A, which plots the changes in cardiac variables as a function of $P_aO_2$, that this response was associated with this group of geese having a lower $P_aO_2$ at any given $F_1O_2$ during hypoxia (Figure 3.4A). Significant increases in $\dot{Q}$ occurred at the same $P_aO_2$ in all three groups of geese ($\sim$6 kPa). That this reflects the differences in the Hb-oxygen equilibrium curves is clear from the extent of the overlap when $\dot{Q}$ is plotted as a function of $C_aO_2$ (Figure 3.8B). Similarly, significant changes in the contributions of stroke volume and heart rate to $\dot{Q}$ occurred $\leq$6 kPa (Figure 3.8C,D).
3.3.3.3  *Tissue oxygen delivery and extraction*

Neither blood convection requirement (the quotient of $\dot{Q}$ and $\dot{V}O_2$) nor tissue oxygen delivery (the product of $\dot{Q}$ and $C_ao_2$) changed significantly in any of the three groups of geese during progressive hypoxia (Table 3.1). The percentage of the oxygen extracted from arterial blood fluctuated between 30 and 50% and also did not change significantly either during progressive hypoxia or between any of the three groups of geese (Table 3.1).

3.3.3.4  *Blood pressure and total peripheral resistance*

While $\dot{Q}$ increased during hypoxic exposure, mean arterial pressure was generally maintained, decreasing minimally at 0.07 $F_iO_2$ in the two LAR groups of geese (Table 3.1).

3.4  *Discussion*

In this study I comprehensively compared the metabolic, ventilatory, and cardiovascular responses of HAR bar-headed geese to those of LAR bar-headed geese. In addition, I compared these responses to those of barnacle geese, a member of a closely related genus, to provide further insight into responses unique to bar-headed geese. I found that HAR bar-headed geese exhibited a reduced $\dot{V}O_2$ compared to LAR bar-headed geese. When exposed to progressive hypoxia, HAR bar-headed geese exhibited a larger HVR and initiated cardiac responses earlier than LAR bar-headed geese, supporting my initial hypothesis. Explanations for these differences are discussed below.
While it cannot be determined with absolute certainty the extent to which the differences present between the HAR and LAR bar-headed geese can be attributed exclusively to differences in barometric pressure during rearing, many potentially confounding variables were controlled across study groups. All groups of birds were held in outdoor pens with access to indoor shelter during the winter. All groups were healthy, fed similar diets and had been housed for at least a year without flying. While body mass and body composition have been shown to vary seasonally in barnacle geese (Portugal et al., 2007), the relationship between heart rate and \( \dot{V}o_2 \), when normalized for body weight, was unaffected in five of the six seasonal sampling periods and was also unaltered by molt (Portugal et al., 2009). Furthermore, many of the variables measured in this study have been previously measured on LAR bar-headed geese and barnacle geese, allowing us to compare results. Resting values for \( \dot{V}o_2 \) and \( \dot{V}_R \) in my LAR bar-headed geese in normoxia were comparable to those previously described in the literature (Black and Tenney, 1980a; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). My values for \( C_{a}O_2 \), \( C_vO_2 \), Hct and \([Hb]\) also fell within the range of values previously reported in the literature (Black and Tenney, 1980a; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). The literature values for \( Q \), stroke volume and heart rate of LAR bar-headed geese in normoxia vary widely. Fedde et al. (Fedde et al., 1989) reported a high heart rate and low stroke volume, while Hawkes et al. (Hawkes et al., 2014) reported a low heart rate and high stroke volume. My values fall midway between the two.

\( \dot{V}o_2 \) was significantly lower in the HAR bar-headed geese compared to LAR bar-headed geese, suggesting that rearing at altitude leads to a reduction in metabolism and in the demand for oxygen. All groups maintained or increased \( \dot{V}o_2 \) when exposed to hypoxia (Figure 3.1A).
The small increases seen in $\dot{V}O_2$ in all groups may reflect an increased cost of ventilation and associated events. The net response suggests that the cardiorespiratory adjustments were sufficient to match oxygen supply to oxygen demand at all but the most severe levels of hypoxia.

There was evidence of a metabolic acidosis, indicative of recruitment of anaerobic metabolism, in barnacle geese at 0.07 F$_{I}O_2$ and in HAR bar-headed geese at 0.05 F$_{I}O_2$. On return to control conditions, the HAR bar-headed geese recovered to control acid-base status faster (within 25-min) than the barnacle geese. Both groups exposed to 0.05 F$_{I}O_2$ experienced a significant decrease in pH$_a$ upon recovery to normoxia (Figure 3.6B,C). This may reflect the sequestering of lactate and H$^+$ during hypoxia that was rapidly released into the blood on return to resting conditions. Because LAR bar-headed geese were recovered to ambient conditions after breathing 0.07 F$_{I}O_2$ rather than 0.05 F$_{I}O_2$, I cannot ascertain whether the ability of bar-headed geese to avoid metabolic acidosis until more severe levels of hypobaric hypoxia is an adaptation or a consequence of HA rearing. Nevertheless, the ability to recover quickly from severe hypoxia would be an asset during the HA migration of bar-headed geese.

The magnitude by which $\dot{V}R$ increased during progressive hypoxia in the LAR bar-headed geese and barnacle geese of my study was within the range reported in previous studies (Black and Tenney, 1980a; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). In addition, as previously reported, bar-headed geese exhibited a higher overall tidal volume and lower breathing frequency at any given $\dot{V}R$ than barnacle geese, a pattern hypothesized to be a more effective breathing pattern that reduces effective dead space ventilation (Scott and Milsom, 2007). The demonstration that this breathing pattern was common to LAR and HAR bar-headed geese supports the suggestion that this is an adaptation specific to bar-headed geese.

Furthermore, I found that the increase in $\dot{V}R$ in hypoxia was greatest in magnitude in the HAR
bar-headed geese and lowest in the barnacle geese (P<0.001; Figure 3.1C). These findings support my hypothesis and are consistent with previous findings of a greater increase in $\bar{V}_R$ in bar-headed geese following short-term acclimation to simulated altitude (Black and Tenney, 1980a).

Differences in both the resting levels of ventilation and the HVR disappeared when my data were expressed as STPD rather than BTPS (Figure 3.1, Figure 3.9B). Expressing volume as a function of STPD reveals the molar amount of air (and thus oxygen) moved. In this instance, STPD values are not significantly different for either LAR or HAR bar-headed geese, indicating that the differences in ventilation reported in BTPS were due to the thinner air.

Expressing volumes as a function of BTPS on the other hand is standard for showing how much gas an animal ventilates. Black and Tenney found that the resting ventilation in bar-headed geese acclimated under hypobaric conditions (equivalent to 5,640 m) for four weeks was approximately double that of sea level-acclimated birds when measured at similar levels of $P_{O_2}$ under normobaric conditions (Black and Tenney, 1980a). In the present study, while the level of $\bar{V}_R$ at an inspired partial pressure of oxygen of 12 kPa ($F_{O_2} = 0.21$ at 3,200 m; sea level equivalent $F_{O_2} = 0.12$) was roughly 30% higher in the HAR bar-headed geese relative to the LAR bar-headed geese, this difference was not significant. This suggests, that despite apparent similarities, the changes seen following short-term acclimation (Black and Tenney, 1980a) are more akin to ventilatory acclimatization to hypoxia, while those seen in the HAR bar-headed geese appear to reflect hypoxic desensitization (Powell et al., 1998).

Ventilatory acclimatization to hypoxia is defined as the further increase in ventilation, compared with the rapid initial response, which occurs over hours to days of acclimatization (Powell et al., 1998). This secondary increase has been ascribed to plasticity in oxygen sensing
by the carotid body chemoreceptors and in central integration of chemoreceptor input (Powell, 2007). Over many months at HA, however, this hypoxic ventilatory response can be gradually attenuated by hypoxic desensitization (Brutsaert, 2007; Powell et al., 1998). While the increases in breathing during ventilatory acclimatization to hypoxia improve oxygen uptake, hypoxic desensitization could be representative of longer-term HA exposure and the ability to effectively transport oxygen without magnified convective transport. This would help reduce respiratory water loss, and reduce the metabolic cost of breathing (Powell, 2007; Storz et al., 2010). Despite the apparent hypoxic desensitization, $V_R$ remained elevated relative to $V_o_2$ in the HAR bar-headed geese, however, indicative of a reduction in lung oxygen extraction (Figure 3.3B).

Differences between species in blood-oxygen carrying capacity were driven primarily by differences in intrinsic oxygen affinity (i.e. Hb-P$_{50}$) and in vivo blood-oxygen affinity (i.e. blood-oxygen affinity subject to in vivo changes in temperature, pH, and allosteric modulators). There were no inter- or intraspecies differences in [Hb] or Hct either in normoxia or with progressive hypoxia. The greater oxygen-affinity of the HbA isoform of bar-headed geese is well documented (Weber et al., 1993), although the properties of the HbD isoform have yet to be studied. Neither Hct nor [Hb] in bar-headed geese were affected by rearing altitude, a finding also reported after short-term (4 weeks) acclimation of bar-headed geese to simulated altitude (5,640 m) (Black and Tenney, 1980a). This is also similar to patterns described in HA-acclimatized Tibetan humans (Simonson et al., 2015) and deer mice (Lui et al., 2015). As a result of the differences in intrinsic and in vivo blood-oxygen affinity, however, at any given P$_a$O$_2$ bar-headed goose blood will be more saturated than that of barnacle geese (Figure 3.5). Furthermore, the data suggest that at any given P$_a$O$_2$, the blood of the HAR bar-headed geese would be slightly more saturated than that of the LAR bar-headed geese (Figure 3.5). These small differences are
most likely explained by the higher levels of \([\text{HCO}_3^-]\) (\(P=0.009\)) at 0.12 \(\text{F}_2\text{O}_2\) and pH (\(P<0.001\)) at all levels of hypoxia in the HA study group. Such an alkalosis would left-shift the oxygen equilibrium curve and enhance oxygen loading at the lung, possibly another feature of HA rearing. A respiratory alkalosis occurred in all groups during progressive hypoxic exposure due to heavy ventilation, aiding to further enhance blood-oxygen carrying capacity (Figure 3.6).

Based on the \(in vivo\) oxygen equilibrium curves, the blood \(P_{50}\) of the two groups of bar-headed geese are unlikely to be appreciably different. While birds have organic phosphates (inositol pentophosphate) for altering Hb-oxygen affinity, there is little evidence of an IPP-induced change in \(P_{50}\) with HA exposure (Weber, 2007). This suggests that isoHb switching did not occur in response to environmental hypoxia. While large, reversible changes in blood \(P_{50}\) could be achieved by altering the expression levels of HbA and HbD, my data suggest that bar-headed geese do not do this. Similar results have been reported for HA versus LA hummingbirds (Projecto-Garcia et al., 2013), sparrows (Cheviron et al., 2014), house wrens (Galen et al., 2015), and waterfowl (Natarajan et al., 2015).

All groups in the present study increased \(\dot{Q}\) by 2.0-2.5-fold during severe hypoxia. Previous reports of the magnitude and direction of change in \(\dot{Q}\) during severe hypoxia in bar-headed geese vary widely. Hawkes et al. (Hawkes et al., 2014) reported that, in bar-headed geese breathing 0.07 \(\text{F}_2\text{O}_2\), \(\dot{Q}\) decreased by ~20\%, whereas Fedde et al. (Fedde et al., 1989) and Black and Tenney (Black and Tenney, 1980a) reported no change in \(\dot{Q}\) at this level of hypoxia. However, when Black and Tenney exposed their birds to a further reduction in oxygen to ~0.05 \(\text{F}_2\text{O}_2\), corresponding to a \(P_{50}\text{O}_2\) of ~3.5 kPa, \(\dot{Q}\) increased by a remarkable 7-fold (Black and Tenney, 1980a). These differences most likely reflect the steepness of the exponential
cardiovascular response curve beyond the inflection point and small differences in $P_aO_2$.

Analysis of the data based on $F_iO_2$ suggests that increases in $\dot{Q}$ in the present study were initiated first in the HAR bar-headed geese (0.09 $F_iO_2$), next in the LAR bar-headed geese (0.07 $F_iO_2$), and last in the barnacle geese (0.05 $F_iO_2$) (Figure 3.7A). When expressed as a function of $P_aO_2$ (or $C_aO_2$), however, all groups produced significant increases in $\dot{Q}$ at a similar $P_aO_2$ of ~6 kPa (Figure 3.8A, Figure 3.9C), indicating that the differences in which $\dot{Q}$ increases are initiated when plotted as a function of $F_iO_2$ reflect differences in the blood-oxygen affinity. Black and Tenney made a similar observation. They too noted that the differences they saw in the changes in $\dot{Q}$ during progressive hypoxia following short-term acclimation in bar-headed geese could be accounted for by differences in $C_aO_2$ (Black and Tenney, 1980a). Unlike Black and Tenney, however, I did not see an increase in the overall magnitude of $\dot{Q}$ at a given $P_aO_2$ with lifelong exposure to HA. Under resting conditions, $\dot{Q}$ does not differ in HA versus LA native or domestic mammals either. Total blood flow has been found unaltered or slightly reduced in humans, alpaca, llama, rats and wild mice living at altitude (Banchero et al., 1971; Klausen, 1966; Monge et al., 1955; Sillau et al., 1976; Turek et al., 1973). Thus, the differences present between the study of short-term acclimatization by Black and Tenney (Black and Tenney, 1980a) and of HA rearing in my study not only suggest that both acclimatization to hypoxia and hypoxic desensitization occur with ventilatory responses, but also that similar acclimatization and desensitization to hypoxia occur with regard to blood flow in bar-headed geese, and may act to reduce the costs of convective transport of blood as they do for respiratory gases.

Previous studies on geese reported changes in heart rate as the primary contributor to changes in $\dot{Q}$ with stroke volume remaining largely unchanged (Faraci, 1986; Fedde et al., 1989;
Smith et al., 2000). In the present study, this was true of the LA groups down only to an F\textsubscript{I}O\textsubscript{2} of 0.12. Below this, all groups increased \(\dot{Q}\) during progressive hypoxia down to a P\textsubscript{a}O\textsubscript{2} \approx 6 kPa (Figure 3.9C) more by increasing stroke volume than heart rate (Figure 3.7C, Figure 3.8C). HAR bar-headed geese were the only group in which P\textsubscript{a}O\textsubscript{2} fell <6 kPa, at which point heart rate increased substantially (Figure 3.8D) associated with a decrease in stroke volume (Figure 3.8C). The large increases in stroke volume seen in the present study could have been mediated either by extrinsic factors (circulating hormones or neurotransmitters) or intrinsic factors (cardiac muscle fiber contractile properties associated with the Frank-Starling response – the relationship between cardiac contractility and venous return) (Smith et al., 2000). As outlined in Shiels and White (Shiels and White, 2008), limited information exists on the Frank-Starling response in avian cardiomyocytes, though it is known to facilitate large increases in stroke volume during hypoxia in fish (Farrell, 1991a; Shiels and White, 2008). Further studies are required to determine the underlying mechanisms of this response.

3.4.1 Conclusions

The primary differences present between the HAR and LAR bar-headed geese were ventilatory and metabolic in nature. But, at this point, I cannot discern the differential effects of phenotypic plasticity (i.e. acclimatization) from developmental plasticity on the physiology of this species. The reduction in resting \(\dot{V}\textsubscript{O}2\) was one of the most significant differences observed in the HAR bar-headed geese. I also observed an increase in resting \(\dot{V}\textsubscript{R}\) and in the HVR that could be explained by the differences in barometric pressure at which the measurements were made. Even taking this into account, however, HAR bar-headed geese still exhibited a large air convection requirement (ratio of \(\dot{V}\textsubscript{R}\) to \(\dot{V}\textsubscript{O}2\)), compensating for of a reduction in lung oxygen
extraction (Figure 3.9A). This may help maintain blood acid-base balance at the expense of oxygen uptake.

All geese increased \( \dot{Q} \) by \( \sim 2 \)-fold to a similar overall magnitude, but \( \dot{Q} \) increased earlier and more rapidly in bar-headed geese than barnacle geese as environmental oxygen fell, and more so in the HAR bar-headed geese than in LAR bar-headed geese. However, this could be explained by the differences in \textit{in vivo} blood-oxygen affinity. All groups increased perfusion at a similar \( P_{a\,O_2} \) during hypoxic exposure. An unexpected finding was the prominent role of increases in stroke volume in increasing \( \dot{Q} \) in all groups, including the barnacle geese.

Further studies are required to determine the underlying mechanisms of the differences reported here between HAR bar-headed geese and LAR bar-headed geese, and the extent to which these differences may also facilitate HA flight.

Given that differences were observed within a single species reared at different altitudes, I was interested in whether the strategies employed by bar-headed geese to maintain oxygen supply during hypoxia would be shared by other waterfowl adapted to HA life.
Figure 3.1: Oxygen consumption was maintained throughout hypoxia exposure by increases in total ventilation.

(A) Oxygen consumption (STPD) plotted as a function of the fractional oxygen composition of inspired gas ($F_{O_2}$) in all groups. (B) Hey plot depicting the breathing patterns of all groups. For any level of total ventilation, bar-headed geese had a higher tidal volume and lower breathing frequency (right-shifted curve). Dashed lines represent breathing frequency ($f_R$) isopleths. (C) Total ventilation as a function of the fractional oxygen composition of inspired gas ($F_{O_2}$) in all groups in BTPS. (D) Total ventilation as a function of the fractional oxygen composition of inspired gas ($F_{O_2}$) in all groups in STPD. Values represent means ± s.e.m (blue triangle: high-altitude-reared bar-headed geese; green diamond: low-altitude-reared bar-headed geese; purple square: barnacle geese). Significant differences ($P<0.05$) in the y-axis variable from values during exposure to ambient air within a species are indicated by open symbols. Significant differences ($P<0.05$) in the y-axis variable between species are indicated by different symbols (Figures 1A, 1C, and 1D): *high-altitude-reared bar-headed geese versus barnacle geese, †high-altitude-reared bar-headed geese versus low-altitude-reared bar-headed geese, and ‡low-altitude-reared bar-headed geese versus barnacle geese.
Figure 3.2: Changes in the components of total ventilation during progressive hypoxic exposure.

(A) Breathing frequency was initially higher in barnacle geese, but increased in all groups throughout decreases in fractional oxygen composition of inspired gas ($F_{1}O_{2}$). (B) Tidal volume (in BTPS) was greater in magnitude in bar-headed geese than barnacle geese, though it increased in all groups. Values represent means ± s.e.m (blue triangle: high-altitude-reared bar-headed geese; green diamond: low-altitude-reared bar-headed geese; purple square: barnacle geese). Significant differences (P<0.05) between species are indicated by different symbols: *high-altitude-reared bar-headed geese versus barnacle geese, **high-altitude-reared bar-headed geese versus low-altitude-reared bar-headed geese, and ♂low-altitude-reared bar-headed geese versus barnacle geese.
Figure 3.3: Air convection requirement and lung oxygen extraction during progressive hypoxia. (A) Air convection requirement plotted as a function of the fractional oxygen composition of inspired gas ($F_{O_2}$) in all groups. (B) Changes in lung oxygen extraction in all groups exposed to progressive hypoxia. Values are reported in STPD and represent means ± s.e.m (blue triangle: high-altitude-reared bar-headed geese; green diamond: low-altitude-reared bar-headed geese; purple square: barnacle geese). Significant differences (P<0.05) in values from those during ambient exposure within a species are indicated by open symbols.
Figure 3.4: The relationship of arterial partial pressure of oxygen (P_\text{aO}_2) and arterial oxygen content (C_\text{aO}_2) with inspired oxygen level throughout hypoxic exposure.

(A) P_\text{aO}_2 decreased with decreasing fractional oxygen composition of inspired gas (F_\text{IO}_2) in all groups, though high-altitude-reared bar-headed geese had a lower P_\text{aO}_2 at any given F_\text{IO}_2 than either low-altitude-reared group. Significant differences (P<0.05) in P_\text{aO}_2 between species are indicated by different symbols: *high-altitude-reared bar-headed geese versus barnacle geese, αhigh-altitude-reared bar-headed geese versus low-altitude-reared bar-headed geese, and βlow-altitude-reared bar-headed geese versus barnacle geese. (B) C_\text{aO}_2 decreased with decreasing F_\text{IO}_2. Values are means ± s.e.m (blue triangle: high-altitude-reared bar-headed geese; green diamond: low-altitude-reared bar-headed geese; purple square: barnacle geese). Significant differences (P<0.05) from ambient exposure performance within a species are indicated by open symbols.
Figure 3.5: Arterial oxygen content (C\textsubscript{a}O\textsubscript{2}) at a given level of arterial partial pressure (P\textsubscript{a}O\textsubscript{2}) differed between bar-headed geese and barnacle geese. The relationship between C\textsubscript{a}O\textsubscript{2} and P\textsubscript{a}O\textsubscript{2} was such that at any given P\textsubscript{a}O\textsubscript{2} bar-headed geese carried a greater content of oxygen in their arterial blood. Values are means ± s.e.m (blue triangle: high-altitude-reared bar-headed geese; green diamond: low-altitude-reared bar-headed geese; purple square: barnacle geese). Significant differences (P<0.05) in C\textsubscript{a}O\textsubscript{2} from values obtained at ambient exposures within a species are indicated by open symbols.
Figure 3.6: Arterial acid-base status was altered during hypoxia exposure due to respiratory alkalosis and metabolic acidosis.

These plots represent changes in arterial acid-base status (arterial pH, pHₐ; arterial bicarbonate ion concentration, [HCO₃⁻]) throughout the experimental exposures in (A) low-altitude-reared bar-headed geese, (B) high-altitude-reared bar-headed geese, and (C) barnacle geese. The first shaded point represents the starting ambient exposure and is marked with the corresponding F̄O₂ (0.21 for low-altitude groups and 0.12 for the high-altitude group). Each subsequent point denotes further decreases in F̄O₂ as described in the methods. Recovery in ambient F̄O₂ after 5- and 25-min is represented by the black symbols. The dotted isopleths represent arterial partial pressure of CO₂ (PₐCO₂; kPa). Significant differences (P<0.05) in values from starting levels within a species are indicated by ϕ for pHₐ and λ for [HCO₃⁻].
Figure 3.7: Changes in cardiac output and its contributing components as a function of equivalent fractional oxygen composition of inspired gas ($F_{IO_2}$).

(A) Cardiac output plotted as a function of $F_{IO_2}$. (B) The hypoxic cardiovascular response of all groups was characterized generally by large increases in stroke volume with modest increases in heart rate. All points represent individual data. Dashed lines represent heart rate ($f_H$) isopleths. (C) Stroke volume and (D) heart rate plotted as a function of $F_{IO_2}$. Values are means ± s.e.m (blue triangle: high-altitude-reared bar-headed geese; green diamond: low-altitude-reared bar-headed geese; purple square: barnacle geese). Significant differences (P<0.05) from values obtained with ambient exposure within a species are indicated by open symbols.
Figure 3.8: Changes in cardiac output and its contributing components as a function of arterial partial pressure of oxygen (P$_{\text{a}}$O$_2$) or arterial oxygen content (C$_{\text{a}}$O$_2$).
(A) Cardiac output plotted as a function of P$_{\text{a}}$O$_2$. (B) Cardiac output plotted as a function of C$_{\text{a}}$O$_2$. (C) Stroke volume and (D) heart rate plotted as a function of P$_{\text{a}}$O$_2$. All variables increased initially with decreasing at a P$_{\text{a}}$O$_2$ ~6 kPa. Values are means ± s.e.m (blue triangle: high-altitude-reared bar-headed geese; green diamond: low-altitude-reared bar-headed geese; purple square: barnacle geese). Significant differences (P<0.05) from values obtained with ambient exposure within a species are indicated by open symbols.
Figure 3.9: A summary of the trends in the hypoxic ventilatory response and hypoxic cardiovascular response of bar-headed geese and barnacle geese. Graphs summarizing the hypoxic ventilatory responses and cardiovascular responses of barnacle geese, low-altitude-reared bar-headed geese, and high-altitude-reared bar-headed geese. The Y axis of the graphs in column A is the fractional composition of oxygen (FIO₂). The top of each box is the inspired FIO₂, while the bottom of each box is the expired FIO₂. The difference between these two represents the amount of oxygen extracted from the respired gas (ELO₂; %), while the number inside the box represents the area of box, which is equal to the oxygen consumption in ml oxygen min⁻¹ kg⁻¹ (VR x FIO₂ x ELO₂). The shading of the boxes depicts different FIO₂ levels: dark grey = 0.12, light grey = 0.07, and striped = 0.05 FIO₂. The graphs in column B illustrate how total ventilation (in STPD) increased in each species with decreasing FIO₂. The graphs in column C depict the relationship between cardiac output (Q; solid symbols, left axis) and arterial oxygen content (CaO₂; hatched symbols, right axis of column D) with arterial partial pressure of oxygen (PaO₂) in each species. The Y axis of the graphs in column D is the CaO₂. The top of each box is the CaO₂, while the bottom of each box is the venous oxygen content (CvO₂). The difference between these two represents the amount of oxygen extracted from the blood by the tissues (ETO₂; %), while the number inside the box represents the area of box, which is equal to the oxygen consumption in ml oxygen min⁻¹ kg⁻¹ (Q x CaO₂ x ET O₂). The shading of the boxes depicts different FIO₂ levels: dark grey = 0.12, light grey = 0.07, and striped = 0.05 FIO₂. Significant differences (P<0.05) in values from those obtained at ambient exposure for VR and Q within a species are indicated by open symbols in the graphs in columns B and C.
Table 3.1: Changes in cardiovascular variables during hypoxic exposure in bar-headed geese and barnacle geese.
Changes in blood gases and cardiovascular variables with decreasing fractional inspired oxygen \( (F_\text{O}_2) \): \( P_\text{O}_2 \), arterial partial pressure of oxygen (kPa); \( P_\text{CO}_2 \), venous partial pressure of oxygen (kPa); \( C_\text{aO}_2 \), arterial content of oxygen (mmol l\(^{-1}\)); \( C_\text{vO}_2 \), venous content of oxygen (mmol l\(^{-1}\)); \( pH_a \), arterial pH; \( pH_v \), venous pH; MAP, mean arterial pressure (kPa); TPR, total peripheral resistance (kPa); blood convection requirement; tissue oxygen delivery (ml min\(^{-1}\) kg\(^{-1}\)); and tissue oxygen extraction (%). Venous values were provided where available. \(^{a,b,c,d}\)Significant differences (P<0.05) of a variable within species from values at ambient \( F_\text{O}_2 \) are indicated by different letters.

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CHAPTER 4: DIVERGENT STRATEGIES IN HIGH-ALTITUDE GEESE
FOR OBTAINING OXYGEN AT HIGH ALTITUDE

4.1 Introduction

Life at HA is challenging; it is cold, dry and hypoxic. An adequate supply of oxygen is essential for vertebrate life, often demanding high rates of oxygen uptake from thin air that has little oxygen to give, especially for high-energy-consuming tissues, such as the heart and brain. Hypoxia in particular is believed to have driven novel adaptations to improve oxygen transport in several HA species.

Two high-altitude populations that have received decades of scientific scrutiny are those of Tibetan and Andean humans that exhibit differing adaptations for coping with hypoxia. Tibetans have high resting levels of ventilation and a robust ventilatory response to hypoxia (Beall, 2002), but a normal [Hb] and Hct (Beall, 2007). Conversely, Andeans have a substantially lower ventilatory response than Tibetans and even than lowlanders (Beall, 2007; Beall et al., 1997), instead exhibiting an enhanced blood-oxygen carrying capacity (higher [Hb] and higher C\textsubscript{a}O\textsubscript{2} (Beall, 2007), as well as greater lung volumes and pulmonary diffusion capacities than lowlanders (Brutsaert et al., 2000). The extent to which convergent physiological strategies have evolved across other high-altitude populations is poorly understood.

Many species of birds are endemic to HA, and as a taxon birds have generally evolved several unique respiratory and cardiovascular specializations that permit greater hypoxia-tolerance than mammals. These features are distributed throughout the oxygen transport cascade, the steps involved in oxygen transfer from atmosphere to mitochondria (ventilation, lung oxygen
diffusion, circulation, and tissue oxygen diffusion). Morphologically, bird lungs have a larger surface area and thinner diffusion barrier than mammalian lungs, with a unidirectional ventilation pattern that is more effective for gas exchange than the tidal ventilation pattern of mammals (Scott, 2011). Birds tolerate hypocapnia (reduced $P_{a}CO_2$) better than mammals (Scheid, 1990), permitting greater $\dot{V}_{R}$ increases during hypoxia when $P_{a}CO_2$ levels fall. The larger hearts of birds produce a larger $Q$ than mammals (Calder, 1968; Grubb, 1983; Smith et al., 2000) and the capillary exchange capacity in tissues of birds is greater (Scott et al., 2015).

The bar-headed goose is an elite HA performer, migrating across the Himalayan mountains (at >4,500 m) between summer breeding grounds on the Mongolian and Tibetan plateaus (2,000-4,000 m) and overwintering sites at LA in India (<1,000 m) (Bishop et al., 2015; Hawkes et al., 2011). This species appears to have evolved an enhanced oxygen transport capacity at several steps in the oxygen cascade by having large lungs (Scott, 2011), a pronounced $\dot{V}_{R}$ increase during hypoxia using a more effective breathing pattern (Black and Tenney, 1980a; Scott and Milsom, 2007) (Chapter 3), Hb with an enhanced blood-oxygen affinity (Weber et al., 1993), increased capillarity of flight and cardiac muscles (Scott, 2011; Scott et al., 2009), and a redistribution of mitochondria towards the cell membrane to reduce intracellular oxygen diffusion distances (Scott et al., 2009).

Although many other avian species live their entire lives at HA, it is not clear if they have evolved similar mechanisms for improving oxygen transport. Andean geese and crested ducks are lifelong residents at HA in the Andes (3,500-5,500 m), and they routinely fly but do not migrate vast distances (Fjeldså and Krabbe, 1990; McCracken et al., 2010). Andean geese have evolved a Hb with an even greater oxygen-affinity than that of bar-headed geese ($P_{50}$ of stripped HbA values: pH 7.4 and 37°C are 0.38 kPa and 0.82 kPa, respectively) (Black and Tenney,
1980a; McCracken et al., 2010; Natarajan et al., 2015; Weber et al., 1993), but little else is known about their physiology.

I designed this study to determine whether Andean geese and crested ducks meet the challenge of HA life through strategies convergent with those in bar-headed geese (Chapter 3), or whether the large differences in lifetime exposure to altitude and performance demands have resulted in divergent strategies of HA adaptation. I anticipated the former, given the fact that all three species are endemic to HA regions, I predicted that Andean geese and crested ducks would respond similarly to bar-headed geese when exposed to hypoxia. To test this I compared the response of metabolic, ventilatory, and cardiovascular variables to progressive stepwise reductions in inspired levels of oxygen in bar-headed geese to those of Andean geese and crested ducks, all individuals wild-caught at HA. I predicted that during progressive hypoxic exposure Andean geese and crested ducks would exhibit large increases in $\dot{Q}$ and $\dot{V}_R$, and that the $\dot{V}_R$ increases would be primarily supported by tidal volume increases, as previously reported for bar-headed geese (Scott and Milsom, 2007).

4.2 Materials and methods

4.2.1 Animals

Measurements were made on 7 wild bar-headed geese ($Anser indicus$; 2.1 ± 0.1 kg) captured at Lake Qinghai, China (3,200 m), and 7 wild Andean geese ($Chloephaga melanoptera$; 2.1 ± 0.1 kg) and 6 wild crested ducks ($Lophonetta specularioides$; 0.98 ± 0.04 kg), captured in the Andes (>4,000 m) and held for 6 months at 3,200 m in San Pedro de Casta, Peru. All experiments were conducted in the field at 3,200 m at the respective sites. All experimental procedures were conducted according to guidelines approved by the Animal Care Committee at
the University of British Columbia under the guidelines of the Canadian Council on Animal Care.

4.2.2 Surgical procedures

See Chapter 2 for a detailed explanation of the surgical procedures used for the brachial artery and vein cannulations.

4.2.3 Experimental protocol

Birds were exposed to 25-min step reductions in sea level-equivalent F\textsubscript{1}O\textsubscript{2} (0.133, 0.12, 0.09, 0.07, and 0.05 for bar-headed geese and Andean geese) and a 25-min recovery period at ambient F\textsubscript{1}O\textsubscript{2} (0.133) followed the hypoxic exposures. See Chapter 2 for a detailed experimental protocol.

4.2.4 Measurements

See Chapter 2 for details regarding all measurements, calculations, and data analysis.

4.2.5 Statistical analyses

Data are presented as means ± s.e.m unless stated otherwise. Within each species, all data were analyzed using one-way repeated measures ANOVA and Holm-Sidak post hoc tests. Comparisons between species were made using two-way (species and F\textsubscript{1}O\textsubscript{2}) repeated measures ANOVA and Holm-Sidak post-hoc tests within each F\textsubscript{1}O\textsubscript{2}. P<0.05 determined statistical significance. Variables analyzed with a one-way repeated measures ANOVA that failed
normality or equal variance assumptions were transformed with $x' = \ln(x)$ for Andean geese ($\dot{V}O_2$, $\dot{V}R$, blood convection requirement, tissue oxygen extraction) and bar-headed geese ($\dot{V}R$, $Q$, $[HCO_3^-]_a$). $x = x^2$ transformed $[HCO_3^-]_a$ for crested ducks and $x' = -1/(x^2)$ transformed mean arterial pressure in Andean geese. Similarly, two-way repeated measures ANOVA transformations included $x' = \ln(x)$ ($C_aO_2$, $Q$, heart rate, stroke volume), $x' = \sqrt{x}$ ($C_aO_2$), and a box cox transformation with $\lambda = -1.5$ ($pH_a$). Student t-tests compared Hct and [Hb] prior to and following each experiment, as well as lung oxygen extraction between groups. Statistical analyses were carried out using SigmaStat (version 3.0; Systat Software).

4.3 Results

4.3.1 Metabolic and ventilatory responses

Bar-headed geese, Andean geese, and crested ducks maintained starting levels of $\dot{V}O_2$ even during the most extreme hypoxic exposure that simulated equivalent $FIO_2$ levels lower than the top of Mt. Everest (Figure 4.1A) - a powerful reflection of the HA adaptations of these birds. Remarkably, neither Andean geese nor crested ducks increased $\dot{V}R$ despite the severity of hypoxia (Figure 4.1B). As a result, the air convection requirement, the ratio of $\dot{V}R$ to $\dot{V}O_2$, remained unchanged in Andean geese ($P=0.098$) and crested ducks ($P=0.703$) (Figure 4.1C). In contrast, bar-headed geese maintained their routine $\dot{V}O_2$ through >2-fold increases in the air convection requirement ($P<0.001$) (Figure 4.1C), following 2.5-fold increases in $\dot{V}R$ ($P<0.001$) (Figure 4.1B), mediated by increases in both tidal volume ($P<0.001$) and breathing frequency ($P<0.001$) (Figure 4.2A-C).
With each decrease in $F_{\text{I}}O_2$ and associated decrease in $P_{\text{a}}O_2$, lung oxygen extraction, the amount of oxygen extracted by the animal from the inspired air, progressively increased in Andean geese ($P<0.001$) and crested ducks ($P=0.024$), achieving maximum levels approaching 90% (Figure 4.1D). Conversely, lung oxygen extraction was unchanged in bar-headed geese ($P=0.241$) and remained at a much lower level (~40%) (Figure 4.1D). Lung oxygen extraction was similar in both Andean geese and crested ducks ($P=0.748$), and significantly greater than that of bar-headed geese ($P<0.001$). Thus, the non-migratory, high-altitude resident species (Andean geese and crested ducks) have evolved markedly different strategies for maintaining oxygen supply during severe hypoxia compared to the transiently high-altitude, long-distance migrating bar-headed geese (increased lung oxygen extraction versus ventilation).

### 4.3.2 Blood gases and acid-base status

$P_{\text{a}}O_2$ decreased in all species at each $F_{\text{I}}O_2$ (Figure 4.3A), and the *in vivo* blood-oxygen equilibrium curves were similar among three species (Figure 4.3B), indicating that the two different ventilatory strategies for maintaining oxygen uptake were equally effective at blood-oxygen loading. Although Andean geese have an intrinsically higher Hb-oxygen affinity than the bar-headed goose (Black and Tenney, 1980a; Weber et al., 1993), the latter experienced a left-shift of its *in vivo* oxygen equilibrium curve to render it similar to the *in vivo* oxygen equilibrium curves of the Andean species (Figure 4.3B). This likely arose from a Bohr shift resulting from the respiratory alkalosis incurred by bar-headed geese (Figure 4.4A), who were significantly more alkalotic than Andean geese ($P<0.001$) (Figure 4.4B) and crested ducks ($P=0.014$) (Figure 4.4C), and experienced a larger overall pH$_a$ change (~0.45 pH$_a$ units in bar-headed geese versus
0.2 pH units in the Andean species). As birds recovered in ambient conditions following hypoxia exposure, all species became acidotic (Figure 4.4A-C) and $[\text{HCO}_3^-]_a$ was restored to initial levels within 25-min.

None of the birds exhibited high [Hb] or Hct levels, which typically occur in lowlanders due to hypoxia-induced erythropoiesis. Hct and [Hb] were similar between bar-headed geese (Hct: 31.0 ± 2.3%; [Hb]: 97.5 ± 5.8 g l$^{-1}$), Andean geese (Hct: 35.4 ± 1.6%; [Hb]: 108 ± 4.1 g l$^{-1}$), and crested ducks (Hct: 33.3 ± 2.3%; [Hb]: 103 ± 5.9 g l$^{-1}$). Stepwise hypoxia did not affect either Hct or [Hb], suggesting that splenic release of red blood cells was not a response to severe hypoxia (or to experimental stress) and that hemodilution did not occur with repetitive blood sampling.

### Cardiovascular responses

$\dot{Q}$, the volume of blood pumped by the heart per minute, was similar between species under starting conditions, although heart rate was higher and stroke volume lower in the crested ducks (Figure 4.5). $\dot{Q}$ increased by ~2-fold to similar maximal levels between species during severe hypoxia exposure (Figure 4.5A). However, the increase in $\dot{Q}$ was achieved primarily by an increase in heart rate in bar-headed geese ($P=0.018$) (Figure 4.5B), but predominantly by increases in stroke volume in the Andean species (Andean geese ($P=0.028$) and crested ducks ($P=0.045$)) (Figure 4.5C, Figure 4.6). The large increases in $\dot{Q}$ during severe hypoxia were associated with maintained mean arterial pressure (Figure 4.7A) and decreased total peripheral resistance (Figure 4.7B), which reached statistical significance in both Andean species and almost reached significance ($P=0.055$) in bar-headed geese. The blood convection requirement,
the ratio of $Q$ to $\dot{V}_{O_2}$, increased significantly in Andean geese ($P=0.013$) and crested ducks ($P=0.012$) at the most severe level of hypoxia (Figure 4.5D). All three species similarly maintained tissue oxygen extraction (Figure 4.8), the amount of oxygen extracted from arterial blood, despite the fall in $P_{\text{a}O_2}$ and $C_aO_2$ during hypoxia. Nonetheless, whereas bar-headed geese matched their increases in $\dot{V}_R$ and $Q$ and maintained a ventilation-perfusion ratio ($\dot{V}_R/Q$) of $\geq 1.0$, Andean geese significantly decreased ($P=0.042$) the $\dot{V}_R/Q$ ratio during hypoxia to minimum levels of 0.40 (Figure 4.9).

4.4 Discussion

Here I provide compelling evidence for the existence of two strikingly different ventilatory and cardiovascular strategies for maintaining oxygen supply during hypoxic conditions simulating altitudes greater than the summit of Mt. Everest. Andean geese and crested ducks, non-migratory species that reside year-round at HA in the Andes, match oxygen supply to oxygen demand under resting conditions during hypoxic exposures in a remarkably different way than the bar-headed goose, a migratory species that flies across the Himalayan mountain range. The increase in lung oxygen extraction by the Andean species precludes the need for a robust ventilatory response to hypoxia (summarized in Figure 4.10). In contrast, bar-headed geese ventilate heavily to deliver more oxygen to the gas exchange surfaces, as consistent with previous studies (Black and Tenney, 1980a; Scott and Milsom, 2007) (Chapter 3). This produces a respiratory alkalosis that enhances Hb-oxygen loading via a Bohr shift, such that bar-headed geese maintain similar $C_aO_2$ in vivo despite having an intrinsically lower Hb-oxygen affinity compared to Andean geese (Black and Tenney, 1980a; McCracken et al., 2010; Weber et al.,
1993). All species increased $\dot{Q}$ in hypoxia, ensuring sufficient oxygen delivery to the tissues (summarized in Figure 4.10), but the Andean species did so primarily by increasing stroke volume whereas bar-headed geese did so by increasing heart rate. I suggest that while increased breathing frequency, tidal volume, and heart rate are functional responses that can be rapidly recruited to support the transient performance demands of long-distance HA flight in bar-headed geese, they are associated with acute metabolic costs (Klabunde, 2012; Otis, 1954). In contrast, the presumed structural (morphological) changes to the lungs and heart that support the divergent responses to hypoxia in Andean species are supported by metabolic costs incurred throughout development and lifelong maintenance.

The divergent strategies of hypoxia tolerance employed between bird species have intriguing similarities with those reported for high-altitude Tibetan and Andean humans. High-altitude Tibetan humans have high levels of resting ventilation and respond to hypoxia with a robust ventilatory response (Beall, 2002; Beall et al., 1998; Wu and Kayser, 2006), much like the Himalayan bar-headed geese. In contrast, Andean high-altitude humans have a blunted ventilatory response to hypoxia and rely predominantly on enhanced pulmonary oxygen diffusing capacity to maintain oxygen uptake (Beall, 2007; Beall et al., 1998; Winslow et al., 1989), like the Andean geese, in addition to their enhanced blood-oxygen carrying capacity (Beall, 2007). It has been argued that the response of Andean high-altitude human populations is maladaptive because increasing Hct increases blood viscosity, which exacerbates the pulmonary hypertension resulting from pulmonary artery hypoxic vasoconstriction (Dempsey and Morgan, 2015). The high blood-oxygen carrying capacity of the avian Andean species, however, reflects the intrinsic increased Hb-oxygen affinity that enhances blood-oxygen carrying capacity without increasing blood viscosity (or requiring respiratory alkalosis). In addition, there is no indication
that the pulmonary vasculature constricts in response to hypoxia in birds (Black and Tenney, 1980b; Faraci et al., 1984; West et al., 2007). This allows lifelong high-altitude avian residents to adopt a strategy primarily based on structural enhancements (e.g. morphological changes to the lungs and heart increasing lung oxygen diffusion and cardiac stroke volume) for coping with hypoxia, rather than a strategy predominantly based on functional enhancements (e.g. large increases in heart rate and ventilation) as seen in the bar-headed geese. These radically different strategies for maintaining oxygen supply in a hypoxic environment appear to reflect the demands of lifelong high-altitude residency versus transient high-altitude performance.

4.4.1 Conclusion

Thus, to my surprise, HA waterfowl used different ventilatory and cardiovascular strategies for maintaining oxygen supply during hypoxic exposure, with the responses being supported primarily by structural enhancements in lifelong HA residents (e.g. Andean geese and crested ducks) as opposed to functional enhancements in transient HA performers (e.g. bar-headed geese). I propose that these differences are primarily due the duration of HA exposure, i.e. whether the animals is a transient HA performer (e.g. bar-headed goose) or a lifelong HA resident (e.g. Andean goose). If this hypothesis is correct, then other species of HA resident waterfowl that do not migrate long distances should also express these traits.
Figure 4.1: Oxygen consumption, total ventilation, air convection requirement, and lung oxygen extraction during exposure to progressive hypoxia.
The relationship between arterial partial pressure of oxygen (P$_{a}$O$_{2}$) and (A) oxygen consumption rate (in STPD), (B) total ventilation (C) air convection requirement, and (D) lung oxygen extraction (B-D in BTPS). All values are means ± s.e.m (blue diamond: bar-headed goose; orange circle: Andean goose; red square: crested duck). Significant differences (P<0.05) from starting values within a species are indicated by open symbols.
Figure 4.2: Hypoxic ventilatory response patterns.
(A) Tidal volume (in BTPS) and (B) breathing frequency are depicted in relation to arterial partial pressure of oxygen ($P_aO_2$). (C) Changes in total ventilation (in BTPS) and its components: tidal volume (x axis) and breathing frequency ($f_R$; dotted isopleths) during exposure to progressive hypoxia. All values represent means ± s.e.m (blue diamond: bar-headed goose; orange circle: Andean goose; red square: crested duck). Significant differences ($P<0.05$) in total ventilation from starting values within a species are indicated by open symbols.
Arterial partial pressure of oxygen and oxygen content during progressive hypoxia.

(A) Arterial partial pressure of oxygen ($P_{a}O_2$) decreased with decreasing fractional composition of oxygen in inspired gas ($F_{I}O_2$) in all groups. Significant differences ($P<0.05$) in $P_{a}O_2$ between species are indicated by different symbols: $\alpha$ Andean geese versus crested duck and $\beta$ bar-headed geese versus crested duck. There were no significant differences present between Andean geese and bar-headed geese. (B) Arterial oxygen content ($C_{a}O_2$) decreased as a function of decreasing $P_{a}O_2$, producing *in vivo* oxygen equilibrium curves. All values represent means ± s.e.m (blue diamond: bar-headed goose; orange circle: Andean goose; red square: crested duck). Significant differences ($P<0.05$) from starting values within a species are indicated by open symbols.
**Figure 4.4: Arterial acid-base status during progressive hypoxia exposure and recovery.**

Changes in arterial acid-base status (arterial pH, $pH_a$; arterial bicarbonate ion concentration, $[HCO_3^-]$) throughout experimental exposures in (A) bar-headed geese, (B) Andean geese, and (C) crested ducks. The point labeled 0.12 $F_I O_2$ represents the starting exposure. Each subsequent point denotes further decreases in $F_I O_2$: 0.09, 0.07 and for the geese 0.05. Recovery to ambient $F_I O_2$ after 5- and 25-min is represented by black symbols. Grey dashed isopleths represent arterial partial pressure of CO$_2$ ($P_a$CO$_2$; kPa) and the black dotted line is the blood buffer line (Helbecka et al., 1964). Significant differences ($P<0.05$) from starting values within a species are indicated by $\phi$ for $pH_a$ and $\lambda$ for $[HCO_3^-]$. 


Figure 4.5: Cardiovascular responses to progressive hypoxia.
Changes in (A) cardiac output, its components, (B) heart rate and (C) stroke volume, and (D) the blood convection requirement, in relation to arterial partial pressure of oxygen (P$_{a}$O$_2$) during exposure to progressive hypoxia. All values are means ± s.e.m (blue diamond: bar-headed goose; orange circle: Andean goose; red square: crested duck). Significant differences (P<0.05) from starting values within a species are indicated by open symbols.
Figure 4.6: Cardiovascular response patterns during progressive hypoxia.
Changes in cardiac output and its components: stroke volume (x axis) and heart rate ($f_H$; dotted isopleths) in response to progressive hypoxia. All values represent means ± s.e.m (blue diamond: bar-headed goose; orange circle: Andean goose; red square: crested duck). Significant differences (P<0.05) in cardiac output from starting values within a species are indicated by open symbols.
Figure 4.7: Mean arterial pressure and total peripheral resistance responses during progressive hypoxia. (A) Mean arterial pressure and (B) total peripheral resistance are depicted as a function of arterial partial pressure of oxygen ($P_{a}O_2$). All values represent means ± s.e.m (blue diamond: bar-headed goose; orange circle: Andean goose; red square: crested duck). Significant differences (P<0.05) from starting values within a species are indicated by open symbols.
Figure 4.8: Tissue oxygen extraction during progressive hypoxia.
Tissue oxygen extraction is depicted as a function of arterial partial pressure of oxygen ($P_aO_2$). All values represent means $\pm$ s.e.m (blue diamond: bar-headed goose; orange circle: Andean goose; red square: crested duck). Significant differences ($P<0.05$) from starting values within a species are indicated by open symbols.
Figure 4.9: Ratio between ventilation and perfusion during hypoxia.
The quotient of total ventilation and cardiac output is depicted as the ventilation perfusion ratio and graphed as a function of arterial partial pressure of oxygen ($P_aO_2$). All values represent means ± s.e.m (blue diamond: bar-headed goose; orange circle: Andean goose; red square: crested duck). Significant differences (P<0.05) from starting values within a species are indicated by open symbols.
Figure 4.10: Summary of hypoxic responses.

(A) Ventilation is plotted against the fractional composition of inspired (I) and expired (E) oxygen. Oxygen consumption (presented numerically within each box in ml min^{-1} kg^{-1}) is equal to the area of each box, while the oxygen extracted from each breath (%) is presented numerically above each box. (B) Ventilation (STPD) is plotted versus F_{I}O_{2}. (C) Cardiac output (\dot{Q}, left axis, solid symbols) and arterial oxygen content (C_{a}O_{2}; right axis, hatched symbols) are plotted versus arterial partial pressure of oxygen (P_{a}O_{2}). Significant differences (P<0.05) from starting values within a species are indicated by open symbols in graphs B and C. (D) Cardiac output is plotted against the oxygen content of arterial (a) or venous (v) blood. The oxygen consumed from blood (presented numerically within each box in ml min^{-1} kg^{-1}) is equal to the area of each box while the oxygen extracted by the tissues (%) is presented numerically beside each box. Shaded boxes represent the least severe hypoxia exposure (0.12 F_{I}O_{2}) and hatched boxes represent the most severe hypoxia exposure (0.07 F_{I}O_{2} for crested ducks, 0.05 F_{I}O_{2} for the geese).
CHAPTER 5: DIFFERENCES IN CARDIOVASCULAR RESPONSES TO PROGRESSIVE HYPOXIC EXPOSURE IN DUCKS: HIGH-ALTITUDE DUCKS HAVE GREATER OXYGEN CARRYING CAPACITY THAN LOW-ALTITUDE DUCKS

5.1 Introduction

A major role of the cardiovascular system is to maintain adequate delivery of oxygen to tissues to support their rates of $\dot{V}O_2$ both at rest and during periods of increased activity. This becomes challenging when environmental PO$_2$ levels fall, such as at HA (hypobaric hypoxia). Under these conditions, either $Q$, the oxygen carrying capacity of arterial blood, or some combination of the former likely need to increase if $\dot{V}O_2$ is to be maintained. Absolute levels of tissue oxygen extraction from the blood must be maintained, and if oxygen delivery is not maintained then the relative percent extraction of oxygen from the blood must increase. $Q$ is the product of heart rate and stroke volume. $C_aO_2$ is a function of the oxygen carrying capacity of the blood, which in turn is determined by Hct, the mean corpuscular Hb concentration, and the Hb-oxygen binding affinity. Tissue oxygen extraction from the blood is the difference between $C_aO_2$ and $C_vO_2$, representing a function of perfusion and tissue diffusion capacity. In species adapted to live under hypoxic conditions, these enhancements may occur to both “structural” components (e.g. larger hearts and thus larger stroke volumes, increased Hct, increased Hb-oxygen binding affinity and/or an enhanced tissue diffusion capacity) or “functional” components (e.g. increased heart rate). Birds in general are more tolerant of hypoxia than mammals. They have a disproportionally large heart for their body size relative to mammals, and thus exhibit a relatively
larger $\dot{Q}$, and cardiac stroke volume (Calder, 1968; Grubb, 1983; Smith et al., 2000). Birds also have a larger capillary exchange capacity in their tissues (Scott et al., 2015).

It has recently been shown that some species of waterfowl (Anseriformes) that are endemic to or living transiently at HA have further cardiovascular specializations that make them exceptionally tolerant to hypoxia (Chapters 3 and 4). Bar-headed geese, which perform impressive biannual HA migrations over the Himalayas (routine migration altitude: ~4,500-6,000 m) (Hawkes et al., 2011), have been reported to increase their $\dot{Q}$ 2.5-fold (Chapters 3 and 4) to 7-fold (Black and Tenney, 1980a) when exposed to severe hypoxia ($<0.07 F_{\text{IO}_2}$) at rest. They do this by a combination of increases in heart rate and stroke volume, with heart rate as the primary contributor (Chapters 3 and 4). Andean geese, life-long HA residents of the Andes, similarly increase $\dot{Q}$ by 2.5-fold when exposed to severe hypoxia at rest, but achieve this predominantly through increases in stroke volume (Chapter 4). Neither species has a significantly elevated Hct or total [Hb] (Black and Tenney, 1980a) (Chapters 3 and 4). In contrast, short-term acclimation of LA Pekin ducks (Anas platyrhynchos, forma domestica) to simulated HA (5,640 m) significantly increased Hct (Black and Tenney, 1980a). HA waterfowl species such as bar-headed geese and Andean geese can apparently avert the need to increase either Hct or [Hb] by exhibiting mutations in their Hb that decrease their Hb-$P_{50}$, thereby increasing Hb-oxygen binding affinity (Black and Tenney, 1980a; Weber et al., 1993) and allowing them to maintain blood-oxygen carrying capacity in hypoxia. Despite these similarities, bar-headed geese (transient HA performers) generally responded to hypoxia using primarily functional enhancements, while the hypoxic responses of Andean geese and crested ducks (lifelong HA residents) were facilitated predominantly by structural enhancements (Chapter 4). The extent to
which the hypoxic responses of HA resident Andean geese are common to other HA resident bird species is unknown.

Resident to the HA Andean plateau (up to ~5,500 m) are several species of ducks that differ in their activity level and the evolutionary time spent at HA (McCracken et al., 2009a). These waterfowl include the Puna teal (*Anas puna*), speckled teal (*Anas oxyptera*), cinnamon teal (*Anas orinomus*), yellow-billed pintail (*Anas georgica*), and Andean ruddy duck (*Oxyura jamaicensis ferruginea*). Their predominant forms of locomotion broadly range from dabbling (Puna teal, cinnamon teal), through diving (Andean ruddy duck) and occasional long-distance flying (speckled teal, yellow-billed pintail). Nevertheless, all these species are derived from LA waterfowl populations (Johnson and Sorenson, 1999), but exhibit differences in genetic divergence that are suggestive of varying degrees of genetic isolation at HA (McCracken et al., 2009c; McCracken et al., 2009a). For example, the yellow-billed pintail is the most recent species to diverge from their LA ancestor in arriving at HA (McCracken et al., 2009b), followed by the cinnamon teal. Conversely, the Puna teal and speckled teal are the most diverged from their respective LA ancestors since arriving at HA. This leaves the ruddy duck as intermediate in evolutionary time since divergence from their LA ancestors (Fjeldså and Krabbe, 1990; Hilty and Brown, 1986; Livezey, 1986; McCracken et al., 2009c; McCracken et al., 2009b; McCracken et al., 2009a; Munoz-Fuentes et al., 2013; Natarajan et al., 2015). Duration of HA exposure, among other factors, may have already exerted evolutionary pressure on the blood-oxygen affinity of these HA waterfowl.

The present study investigated the divergence in the cardiovascular responses to hypoxia among these five species of HA ducks (yellow-billed pintail, cinnamon teal, ruddy duck, speckled teal, and Puna teal) and six species of related LA ducks (northern pintail, cinnamon...
teal, ruddy duck, green-winged teal, gadwall, and mallard duck). Within this broader species comparison was embedded four closer population comparisons at either the subspecies level (HA and LA cinnamon teal populations, and HA and LA ruddy duck populations), or the species level (HA yellow-billed pintail versus LA northern pintail and HA speckled teal versus LA green-winged teal) (Figure 5.1). I hypothesized that the HA populations would demonstrate an enhanced oxygen transport capacity during hypoxic hypoxia compared to the LA populations (e.g. decreased Hb-P$_{50}$ and/or increased Hct and [Hb]), and that this enhancement would be greatest for the populations that had spent the longest time at HA after diverging from their LA ancestors. I also hypothesized that any differences in the hypoxic cardiovascular responses of the HA and LA ducks would be primarily attributable to structural enhancements of the cardiovascular system (e.g. increased blood-oxygen carrying capacity and increased stroke volume) in the HA populations, similar to the findings for HA resident Andean geese and crested ducks (Chapter 4).

5.2 Materials and methods

All experimental procedures were conducted according to guidelines approved by the Animal Care Committee at the University of British Columbia under the guidelines of the Canadian Council on Animal Care.

5.2.1 Animals

The cardiovascular and metabolic measurements made on six LA populations were performed at Summer Lake in Oregon, USA (1,300 m). They were caught from the wild on the lake and studied within a maximum of two days post-capture. They comprised: mallard ducks
(Anas platyrhynchos; 0.96 ± 0.02 kg, N=8), gadwalls (Anas strepera; 0.75 ± 0.04 kg, N=8),
northern pintails (Anas acuta; 0.84 ± 0.01 kg, N=10), cinnamon teals (Anas c. cyanoptera; 0.31 ±
0.01 kg, N=11), ruddy ducks (Oxyura j. jamicensis; 0.42 ± 0.04 kg, N=8), and green-winged
teals (Anas carolinensis; 0.29 ± 0.01 kg, N=10).

The cardiovascular and metabolic measurements made on five HA populations were
performed at Lake Titicaca, Peru (3,800 m). They were caught from the wild on the lake and
studied within a maximum of two days post-capture. They comprised: yellow-billed pintails
(Anas georgica; 0.61 ± 0.02 kg, N=12 cinnamon teals (Anas c. orinomus; 0.44 ± 0.01 kg, N=12),
ruddy ducks (Oxyura j. ferruginea; 0.73 ± 0.07 kg, N=6), speckled teals (Anas oxyptera; 0.37 ±
0.01, N=10), and Puna teals (Anas puna; 0.40 ± 0.01 kg, N=8).

All variables were measured for each individual with two exceptions. Arterial oxygen
saturation was measured on fewer HA ducks: 6 yellow-billed pintails, 5 HA cinnamon teals, 7
HA ruddy ducks, 6 speckled teals, and 5 Puna teals. Heart mass was limited to representative
individuals due to permit regulations, namely: 3 northern pintails, 3 LA cinnamon teals, 5 LA
ruddy ducks, 2 green-winged teals, 3 mallard ducks, 6 yellow-billed pintails, 10 HA cinnamon
teals, 8 HA ruddy ducks, 6 speckled teals, and 5 Puna teals. Hearts could not be sampled from
gadwalls.

5.2.2 Duck preparation

All ducks were outfitted with a 3-lead ECG. For the animals upon which arterial oxygen
saturation was also measured, a 3-cm tall band was plucked on their necks to accommodate the
MouseOx oxygen sensor and collar. No birds were cannulated. See Chapter 2 for more detailed
procedures.
5.2.3 Hypoxic exposure protocol

Ducks were exposed to 20-min step reductions in sea level-equivalent \( F_iO_2 \): 0.18 (LA ducks only; ambient exposure for LA ducks at 1,300 m), 0.133 (all ducks; ambient exposure for HA ducks at 3,800 m), 0.12, 0.09, 0.07, and 0.06. A 20-min recovery at ambient \( F_iO_2 \) followed the hypoxic exposures. See Chapter 2 for a detailed experimental protocol.

5.2.4 Data measurement and analysis

Whole animal \( \bar{V}o_2 \) was calculated from the incurrent flow rate (\( FR_i \)), \( F_iO_2 \), fractional inspired carbon dioxide composition (\( F_iCO_2 \)), fractional expired oxygen composition (\( F_EO_2 \)), and fractional expired carbon dioxide composition (\( F_ECO_2 \)), which were directly measured by a gas analyzer (Sable Systems, Las Vegas, NV, USA). Water vapour was removed from the gas prior to analysis. \( \bar{V}o_2 \) (ml min\(^{-1}\) kg\(^{-1}\)) was calculated as:

\[
\bar{V}o_2 = FR_i \left( F_iO_2 - \frac{(1-F_iO_2-F_iCO_2)}{(1-F_EO_2-F_ECO_2)} \cdot F_EO_2 \right)
\]

(Lighton, 2008), standardized to body mass (kg), and reported in terms of STPD.

Heart rate was continuously monitored via 3-lead ECG using a PowerLab Bio Amp (ADInstruments, Colorado Springs, CO, USA) and analyzed using PowerLab analysis software (ADInstruments, Colorado Springs, CO, USA) at a sampling frequency of 1000 Hz per channel. Heart rate (min\(^{-1}\)) was calculated from the peaks in the pulsatile arterial blood pressure trace. Cardiac oxygen pulse (ml oxygen kg\(^{-1}\)) was calculated as the quotient of \( \bar{V}o_2 \) and heart rate. Arterial oxygen saturation (%) was measured continuously using a MouseOx oxygen sensor (Starr Life Sciences, Oakmont, PA, USA) and recorded by the accompanying data acquisition.
software provided by Starr Life Sciences (Starr Life Sciences, Oakmont, PA, USA). Hct (%) was determined at the end of the experiment in duplicate by a Zipocrit hematocrit centrifuge (LW Scientific, Lawrenceville, GA, USA). [Hb] (g dl\(^{-1}\)) was acquired at the end of the experiment using a Hemocue Hb 201\(^+\) System (Ängelholm, Sweeden), and incorporates both Hct and mean corpuscular Hb concentration. Mean values were derived for heart rate, \(\bar{V}O_2\), arterial oxygen saturation, and oxygen pulse during the last 10 min of each exposure, including recovery, and the first 5-min of recovery (heart rate only). Total heart mass (g kg\(^{-1}\)) was standardized by body mass.

5.2.5 **Statistical analysis**

Data are presented as means ± s.e.m unless stated otherwise. Within each species, all data were analyzed using one-way repeated measures ANOVA and Holm-Sidak post hoc tests. Comparisons between groups were made using two-way (species and F\(_1\)O\(_2\)) repeated measures ANOVA and Holm-Sidak post-hoc tests within each F\(_1\)O\(_2\). For statistical comparisons, P<0.05 was used to determine statistical significance. Variables analyzed with a one-way repeated measures ANOVA that did not meet assumptions for either normality or equal variance were transformed with: \(x' = x^2\) (arterial oxygen saturation and \(\bar{V}O_2\) for yellow-billed pintail), \(x' = \ln(x)\) (\(\bar{V}O_2\) for gadwall), \(x' = \sqrt{x}\) (\(\bar{V}O_2\) for mallard duck), \(x' = \log(x)\) (heart rate for northern pintail and mallard duck), \(x' = 1/\sqrt{x}\) (heart rate and oxygen pulse for green-winged teal), and \(x' = 1/\ln(x)\) (heart rate for speckled teal). Similarly, variables were transformed when they did not meet assumptions for either normality or equal variance analyzed for a two-way repeated measures ANOVA with: \(x' = x^2\) (select comparisons of arterial oxygen saturation), \(x' = \ln(x)\)
(select comparisons of \( \dot{V} \text{O}_2 \), heart rate, and oxygen pulse). Student t-tests were used to compare Hct, [Hb], and heart mass relative to body mass between HA-LA pairs. Tests that failed normality were ran with a Mann-Whitney Rank Sum Test. Statistical analyses were carried out using SigmaStat (version 3.0; Systat Software).

A principle components analysis (PCA) was run at 0.133 and 0.06 F\textsubscript{I}O\textsubscript{2} with the contributing variables of \( \dot{V} \text{O}_2 \), heart rate, Hct, and [Hb] using R. The PCA was run on the entire data set of all HA and LA pairs (i.e. 8 groups: LA northern pintails, HA yellow-billed pintails, LA and HA cinnamon teals, LA and HA ruddy ducks, LA green-winged teals, and HA speckled teals). Average PCA scores were derived from this whole group analysis and are denoted in the relevant axis of the corresponding figure regardless of whether the figure depicts data from all four HA-LA paired groupings or just one HA-LA pair.

5.3 Results

5.3.1 Progressive hypoxic exposure

Progressive hypoxia significantly decreased arterial oxygen saturation in all groups of ducks (Figure 5.2A-G). Arterial oxygen saturation started at ~90% in the LA ducks (0.18 F\textsubscript{I}O\textsubscript{2}) and ~85% in the HA ducks at a lower F\textsubscript{I}O\textsubscript{2} (0.133) and decreased to 40-50% in all groups at the most severe level of hypoxia (0.06 F\textsubscript{I}O\textsubscript{2}). At any given level of F\textsubscript{I}O\textsubscript{2}, HA yellow-billed pintails (\( P<0.001 \)) (Figure 5.2A), LA ruddy ducks (\( P=0.031 \)) (Figure 5.2C), and HA speckled teals (\( P=0.017 \)) (Figure 5.2D) all exhibited higher arterial oxygen saturation than their LA counterparts. In contrast, there was no difference (\( P=0.624 \)) in the arterial oxygen saturation of HA and LA populations of cinnamon teal throughout progressive hypoxia (Figure 5.2B).
Despite the ~50% decrease in arterial oxygen saturation, ten duck species maintained their \( \bar{V}o_2 \) throughout the progressive hypoxic exposure (Figure 5.3A-G). The one exception was the LA ruddy ducks, where \( \bar{V}o_2 \) increased significantly as \( F_iO_2 \) was decreased (\( P=0.007 \)) (Figure 5.3C). \( \bar{V}o_2 \) in the LA cinnamon teals was significantly greater than that of the HA cinnamon teals (\( P=0.024 \)) except at the most severe level of hypoxia even though it did not increase significantly during hypoxia exposure. Therefore, the species response of \( \bar{V}o_2 \) for cinnamon teal (Figure 5.3B) and the ruddy duck (Figure 5.3C) differed between both HA and LA populations.

Nine of the duck populations maintained heart rate during progressive hypoxic exposure (Figure 5.4A-G). The two exceptions modestly increased their heart rates during hypoxia, significantly elevating heart rate at both 0.07 and 0.06 \( F_iO_2 \) in Puna teals (\( P<0.001 \)) (Figure 5.4E) and at 0.06 \( F_iO_2 \) in mallard ducks (\( P<0.001 \)) (Figure 5.4G). The heart rate of LA green-winged teals was significantly greater than that of HA speckled teals at any given level of \( F_iO_2 \) (\( P<0.003 \)) (Fig. 5.4D). Also, LA cinnamon teals exhibited a higher overall heart rate than their HA counterparts during the three most severe levels of hypoxia (\( P=0.019 \)) (Figure 5.4B), even though there was no significant effect of hypoxia within either population.

Cardiac oxygen pulse was calculated as the quotient of \( \bar{V}o_2 \) and heart rate (Figure 5.5A-G), which from the Fick equation equals the product of stroke volume and \( C_2O_2-C_vO_2 \). Seven of the duck populations maintained oxygen pulse during progressive hypoxic exposure. Oxygen pulse increased transiently during hypoxic exposure in three groups: HA yellow-billed pintails (\( P<0.001 \)) (Figure 5.5A), LA cinnamon teals (\( P<0.001 \)) (Figure 5.5B), and LA ruddy ducks (\( P=0.003 \)) (Figure 5.5C). As a result, LA ruddy ducks had a higher oxygen pulse than their HA counterparts at 0.09 and 0.06 \( F_iO_2 \) (\( P=0.043 \)), and yellow-billed pintails had a higher oxygen
pulse than their HA counterparts at 0.07 F\textsubscript{1}O\textsubscript{2} (P=0.028). In contrast, HA Puna teals significantly decreased their oxygen pulse at 0.06 F\textsubscript{1}O\textsubscript{2} (P=0.007) (Figure 5.5E).

Measures of Hct and [Hb] provided information on the arterial oxygen carrying capacity. In three of the four HA-LA pairs, the HA population exhibited significantly higher Hct and total [Hb] than its LA counterparts (Table 5.1). The exception was the HA and LA ruddy duck populations, which did not exhibit any significant differences in Hct (P=0.891) or [Hb] (P=0.401).

Heart mass relative to body mass was generally similar between HA-LA pairs. An exception to this was that heart mass relative to body mass was significantly greater in HA speckled teals compared to LA green-winged teals (P=0.028), and almost significantly greater in HA yellow-billed pintails than in LA northern pintails (P=0.052) (Table 5.1).

5.3.2 Post-hypoxic recovery

In all populations arterial oxygen saturation returned to starting values within 5 min of ambient conditions (data not shown), as did \( \dot{V}\text{O}_2 \) in the case of the LA ruddy duck where it significantly increased (data not shown). Heart rate was significantly higher at 5- and 20-min ambient recovery in all LA groups compared to their related HA pair with the exception of LA and HA ruddy ducks, whose heart rates did not significantly differ during ambient recovery (data not shown). Heart rate tended to increase during recovery in all groups (Figure 5.6A), reaching statistical significance in five of the eleven populations. The LA northern pintails elevated their heart rates significantly (P<0.001) higher than their HA counterparts (P=0.008). Both LA and HA cinnamon teal populations also exhibited a significant tachycardia upon ambient recovery (P<0.001), with the LA cinnamon teals exhibiting a higher heart rate at 5-min ambient recovery.
(515 ± 46 min⁻¹) than the HA cinnamon teals (286 ± 26 min⁻¹). Both LA green-winged teals and HA Puna teals significantly increased their heart rate throughout 20 min of recovery in ambient air (P<0.001) (Figure 5.6A). There also was a tendency for the oxygen pulse to decrease significantly during the post-hypoxic recovery in half of the groups (Figure 5.6B). This decrease was significant in LA northern pintails (P=0.004), HA yellow-billed pintails (P<0.001), LA cinnamon teals (P<0.001), HA speckled teals (P=0.002) and HA Puna teals (P=0.007) (Figure 5.6B).

A principle components analysis using Hct, [Hb], heart rate, and \(\dot{V}O_2\) indicated that the hematological variables (Hct and [Hb]) varied in opposition to heart rate and \(\dot{V}O_2\), such that variance between populations appeared to be best described by Hct and [Hb] in the HA populations and by heart rate and \(\dot{V}O_2\) in the LA populations (Figure 5.7A-F). Interestingly, this held true at both 0.133 F\(_{O_2}\) (Figure 5.7A) and 0.06 F\(_{O_2}\) (Figure 5.7B), with PCA 1 explaining over 50% of the variability at both 0.133 F\(_{O_2}\) (54.2%) and 0.06 F\(_{O_2}\) (52.0%). Indeed, LA cinnamon teals and LA ruddy ducks exhibited higher \(\dot{V}O_2\) during hypoxia (Figure 5.3), and LA cinnamon teals, and LA green-winged teals exhibited higher heart rates at some point during hypoxic exposure (Figure 5.4).

### 5.4 Discussion

The present study compared the cardiovascular responses to progressive hypoxia exposure in five species of HA ducks to those of six species of LA ducks. While a number of previous studies have examined heart rate in LA ducks, they primarily studied a single species under conditions of exercise (Berger et al., 1970; Hawkins et al., 2000; Kiley et al., 1979) or
diving (Butler and Woakes, 1979; Stephenson et al., 1986). With regards to the hypoxic cardiovascular response, diving ducks are reported to maintain stroke volume during submersion hypoxia (Jones and Holeton, 1972). In addition, Black and Tenney exposed Pekin ducks to a similar protocol to the present study and reported increases in \( \dot{Q} \) (though they did not report changes in either of its contributing components) (Black and Tenney, 1980a). Indeed, none have investigated the role of cardiovascular components in the hypoxic response of HA resident ducks. Thus, I am not aware of any broad or phylogenetically-matched comparisons of the hypoxic cardiovascular response in ducks across an altitudinal range.

The most overarching difference between HA and LA populations was in their oxygen carrying capacity. A larger oxygen carrying capacity reduces the need to increase convective flow to sustain oxygen delivery to the tissues under hypoxic conditions. Hct and [Hb] were significantly higher in all HA groups except in the ruddy ducks (Table 5.1), and this was also clear in the PCA (Figure 5.7). Also, the literature \( P_{50} \) values are lower in HA groups compared to the LA groups for which there were data available in the literature (Natarajan et al., 2015) (Table 5.1). The increased Hb-oxygen binding affinity in the HA birds is consistent with data for many animals endemic to HA (Natarajan et al., 2015; Storz, 2010). An important exception were the ruddy duck populations, where the HA and LA ducks did not exhibit differences in Hct, [Hb] (Table 5.1), and their PCA ellipses overlapped to a greater degree than the other pairs (Figure 5.7E). Ruddy ducks were the only diving species in this study, however, and many diving mammals and birds exhibit enhanced [Hb] and lower \( P_{50} \) values commensurate with the use of blood as an oxygen store for diving (Butler, 2001; Kooymen and Ponganis, 1998; Meir and Ponganis, 2009). This may account for the elevated values seen in the LA ruddy ducks.
The higher Hct and [Hb] present in the HA populations was unexpected when compared with bar-headed geese, whose Hct or [Hb] is unchanged when either acclimated short-term to simulated HA (5,640 m) (Black and Tenney, 1980a) or when reared at HA (3,200 m) (Chapters 3 and 4). Despite the benefit to oxygen carrying capacity, increases in Hct and [Hb] (polycythemia) increase blood viscosity, and when large enough are thought to be maladaptive because the increased blood viscosity increases resistance to blood flow and can place greater demands on the heart (Smith et al., 2000). Whether the differences seen here are genotypic or represent phenotypic plasticity is unclear. LA Pekin ducks acclimated to short-term simulated HA (5,640 m) increased Hct from 45.4% to 55.9% (Black and Tenney, 1980a). Though this value is similar to the levels present in my HA ducks, drawing parallels between these studies would require studying the affects of short-term HA acclimatization on these LA duck populations. For example, though HA acclimation increased Hct and [Hb] in both HA and LA deer mice populations, the HA populations increased Hct and [Hb] less than LA deer mice (Lui et al., 2015). Regardless, most of the HA waterfowl populations present in this study manage to carry more oxygen per ml of blood than their LA counterparts; however, it is unknown whether this characteristic, which increases blood viscosity, results in consequential increases in blood flow resistance and increased cardiac demand. Given the combination of an enhanced blood-oxygen carrying capacity and generally lower resting $\dot{V}O_2$ seen in the HA groups, the HA populations likely have a larger reserve of oxygen for coping with hypoxia.

Considering the altitudes at which these studies were performed, ambient $F_iO_2$ was equivalent to a sea level $F_iO_2$ of 0.18 $F_iO_2$ for the LA birds and 0.133 $F_iO_2$ for the HA birds. As a result all pairwise statistical comparisons began at an $F_iO_2$ equivalent to 0.133. During progressive hypoxic exposure all birds either maintained or modestly increased $\dot{V}O_2$ (Figure 5.3).
Because heart rate and oxygen pulse generally did not increase when arterial oxygen saturation (and hence $C_aO_2$) fell by 50%, in order to maintain $\dot{V}O_2$ either stroke volume or the percent of oxygen extracted from the blood at the tissues must have doubled, or some combination of these two. Though neither stroke volume nor tissue oxygen extraction were measured in this study, recent studies on Andean geese, crested ducks, bar-headed geese, and barnacle geese suggest that stroke volume is likely to have increased (Chapters 3 and 4). Both Andean geese and crested ducks increased stroke volume by 1.5-3.0-fold during progressive hypoxia while tissue oxygen extraction remained relatively constant at ~40-60% (Chapter 4). Similarly, both HA bar-headed geese and LA barnacle geese increased stroke volume 1.5-2.0-fold during progressive hypoxic exposure while tissue oxygen extraction remained constant at ~30-50% (Chapters 3 and 4). In contrast to the present study and the previous chapters, several older studies on geese either identified tachycardia as the primary response to hypoxia in waterfowl (Faraci, 1991; Fedde et al., 1989; Smith et al., 2000), or did not measure heart rate or stroke volume (Black and Tenney, 1980a; Faraci, 1991).

Recovery from severe hypoxia was associated with a general tachycardia and reduced oxygen pulse. Since $\dot{V}O_2$ did not change, the two offset each other. These changes were present 20 min after the animals had returned to breathing ambient levels of oxygen. This suggests that the changes were not in response to either the progressive hypoxia or an oxygen debt, raising the possibility that they were a response to the metabolic acidosis that likely developed during exposure to the most severe levels of hypoxia, a suggestion that remains to be explored.
5.4.1 Conclusions

The progressive hypoxic exposure used in the present study reduced arterial oxygen saturation by at least 50%, yet all eleven populations of ducks (subspecies and species) maintained \( \text{\(\dot{V}\)o}_2 \) with little, if any, change in either heart rate or oxygen pulse. Instead, the primary response to hypoxic hypoxia in this group of ducks appears to be either a doubling of stroke volume, a halving of \( C_v\text{O}_2 \) to maintain tissue oxygen extraction, or some combination of the two to maintain oxygen pulse. Previous studies (Chapters 3 and 4) suggest that increases in stroke volume are most likely. Contrary to my hypothesis, consistent differences in the physiological responses did not exist between either the LA-HA pairings or between groups as a function of time since evolutionary divergence at HA. Instead, the HA groups generally benefitted from higher Hct and [Hb], higher Hb-oxygen affinities (Natarajan et al., 2015) and, in the case of the HA speckled teal, a larger heart mass relative to body mass their LA counterparts. These structural enhancements all benefit arterial blood-oxygen carrying capacity, suggesting that the HA birds have a greater reserve for hypoxia tolerance. As such, and in support of my hypothesis, the hypoxic cardiovascular responses of the HA resident ducks in this study were predominantly supported by structural enhancements (e.g. increased blood-oxygen carrying capacity and likely increased stroke volume), similar to the enhancements documents in the hypoxic cardiovascular responses of HA resident Andean geese and crested ducks (Chapter 4).

The effect of HA residence on the cardiovascular system is not exclusive simply to changes in the magnitude of cardiovascular responses such as heart rate, however; it is also known to impact the magnitude of HRV (Cornolo et al., 2004; Melin et al., 2003; Sharshenova et al., 2006). As such, I was interested in examining whether lifelong exposure to HA impacted the magnitude of HRV in HA and LA waterfowl during progressive hypoxic exposure.
### Figure 5.1: Comparison of high-altitude and low-altitude waterfowl.

Groups are compared with respect to their relative time at altitude and the relationship between a high-altitude group and a closely related low-altitude pair. Subspecies and altitude information provided from the literature (Fjeldså and Krabbe, 1990; Hilty and Brown, 1986; Livezey, 1986; McCracken et al., 2009c; McCracken et al., 2009a; Munoz-Fuentes et al., 2013).

<table>
<thead>
<tr>
<th>Low Altitude</th>
<th>High Altitude</th>
<th>Relationship</th>
<th>Relative Time at Altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern pintail <em>Anas acuta</em></td>
<td>Yellow-billed pintail <em>Anas georgica</em></td>
<td>sibling species</td>
<td>Very recent</td>
</tr>
<tr>
<td>Cinnamon teal <em>Anas c. cyanoptera</em></td>
<td>Cinnamon teal <em>Anas c. orinomus</em></td>
<td>subspecies</td>
<td>Recent</td>
</tr>
<tr>
<td>Ruddy duck <em>Oxyura j. jamicensis</em></td>
<td>Andean ruddy duck <em>Oxyura j. ferruginea</em></td>
<td>subspecies</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Green-winged teal <em>Anas carolinensis</em></td>
<td>Speckled teal <em>Anas f. oxyptera</em></td>
<td>sibling species</td>
<td>Old</td>
</tr>
<tr>
<td>Gadwall <em>Anas strepera</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mallard duck <em>Anas platyrhynchos</em></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Figure 5.2: Arterial oxygen saturation during progressive hypoxic exposure.

(A-G) Arterial oxygen (O₂) saturation decreased in all groups throughout progressive decreases in fractional inspired oxygen composition (FIO₂). Values represent means ± s.e.m with low-altitude groups indicated by solid lines and circle symbols - (A) orange: northern pintail, (B) red: cinnamon teal, (C) light blue: ruddy duck, (D) green: green-winged teal, (F) grey: gadwall, (G) black: mallard duck. High-altitude groups indicated by a dashed lines and triangle symbols – (A) orange: yellow-billed pintail, (B) red: cinnamon teal, (C) light blue: ruddy duck, (D) green: speckled teal, (E) dark blue: Puna teal. Significant differences (P<0.05) in the y-axis variable from values during exposure to ambient air within a species are indicated by open symbols and determined by one-way repeated measures ANOVA. Significant differences (P<0.05) in the y variable between phylogenetic pairs (A-D) are indicated by an asterisk and determined by two-way repeated measures ANOVA.
Oxygen consumption was maintained or increased in all groups throughout progressive decreases in fractional inspired oxygen composition (F_iO_2). Values represent means ± s.e.m with low-altitude groups indicated by solid lines and circle symbols - (A) orange: northern pintail, (B) red: cinnamon teal, (C) light blue: ruddy duck, (D) green: green-winged teal, (F) grey: gadwall, (G) black: mallard duck. High-altitude groups indicated by a dashed lines and triangle symbols – (A) orange: yellow-billed pintail, (B) red: cinnamon teal, (C) light blue: ruddy duck, (D) green: speckled teal, (E) dark blue: Puna teal. Significant differences (P<0.05) in the y-axis variable from values during exposure to ambient air within a species are indicated by open symbols and determined by one-way repeated measures ANOVA. Significant differences (P<0.05) in the y variable between phylogenetic pairs (A-D) are indicated by an asterisk and determined by two-way repeated measures ANOVA.
Figure 5.4: Heart rate during progressive hypoxic exposure.

Heart rate was maintained or increased in all groups throughout progressive decreases in fractional inspired oxygen composition ($F_{O_2}$). Values represent means ± s.e.m with low-altitude groups indicated by solid lines and circle symbols - (A) orange: northern pintail, (B) red: cinnamon teal, (C) light blue: ruddy duck, (D) green: green-winged teal, (F) grey: gadwall, (G) black: mallard duck. High-altitude groups indicated by a dashed lines and triangle symbols – (A) orange: yellow-billed pintail, (B) red: cinnamon teal, (C) light blue: ruddy duck, (D) green: speckled teal, (E) dark blue: Puna teal. Significant differences (P<0.05) in the y-axis variable from values during exposure to ambient air within a species are indicated by open symbols and determined by one-way repeated measures ANOVA. Significant differences (P<0.05) in the y variable between phylogenetic pairs (A-D) are indicated by an asterisk and determined by two-way repeated measures ANOVA.
Figure 5.5: Oxygen pulse during progressive hypoxic exposure.

Oxygen (O$_2$ pulse) was maintained or increased throughout progressive decreases in fractional inspired oxygen composition (F$\text{I}_\text{O}_2$). Values represent means ± s.e.m with low-altitude groups indicated by solid lines and circle symbols – (A) orange: northern pintail, (B) red: cinnamon teal, (C) light blue: ruddy duck, (D) green: green-winged teal, (E) grey: gadwall, (G) black: mallard duck. High-altitude groups indicated by a dashed lines and triangle symbols – (A) orange: yellow-billed pintail, (B) red: cinnamon teal, (C) light blue: ruddy duck, (D) green: speckled teal, (E) dark blue: Puna teal. Significant differences (P<0.05) in the y-axis variable from values during exposure to ambient air within a species are indicated by open symbols and determined by one-way repeated measures ANOVA. Significant differences (P<0.05) in the y variable between phylogenetic pairs (A-D) are indicated by an asterisk and determined by two-way repeated measures ANOVA.
Figure 5.6: Percent change in heart rate and oxygen pulse during post-hypoxic recovery.
Percent change from starting levels of (A) heart rate during 5-min and 20-min post-hypoxic recovery and (B) Oxygen (O₂) pulse during 20-min post-hypoxic recovery. Values represent means ± s.e.m with low-altitude (LA) groups indicated by solid bars: orange (northern pintail; NP), red (cinnamon teal; CT), light blue (ruddy duck; RD), green (green-winged teal; GT), grey (gadwall; GW), and black (mallard duck; MD). High-altitude (HA) groups indicated by dashed bars: orange (yellow-billed pintail; YBP), red (cinnamon teal; CT), light blue (ruddy duck; RD), green (speckled teal; ST), and dark blue (Puna teal; PT). Significant differences (P<0.05) in the y-axis variable from values during exposure to ambient air within a species are indicated by asterisks and determined by one-way repeated measures ANOVA.
Figure 7
Figure 5.7: Principle components analysis of differences between high- and low-altitude pairs under ambient conditions.

Contributing components involved in the analysis are hematocrit (Hct), hemoglobin concentration ([Hb]), heart rate, and whole animal oxygen consumption (\(\dot{V}_{O_2}\)). Low-altitude (LA) groups are represented by solid lines and high-altitude (HA) groups are represented by dashed lines as follows: LA northern pintail, orange solid line; HA yellow-billed pintail, orange dashed line; LA cinnamon teal, red solid line; HA cinnamon teal, red dashed line; LA ruddy duck, blue solid line; HA ruddy duck, blue dashed line; LA green-winged teal, green solid line; HA speckled teal, green dashed line. (A) Principle components analysis (PCA) under 0.133 fractional oxygen composition (\(F_{O_2}\)), (B) 0.06 \(F_{O_2}\), (C) LA northern pintail and HA yellow-billed pintail, (D) LA and HA cinnamon teal, (E) LA and HA ruddy duck, and (F) LA green-winged teal and HA speckled teal. Percent values indicate the percent of variance explained by either PCA 1 or PCA 2 for the whole group PCA.
Table 5.1: Differences in baseline cardiovascular variables in high- and low-altitude ducks.
Baseline cardiovascular variables in high- and low-altitude duck groups include: hematocrit, Hct (%); total blood hemoglobin concentration, [Hb] (g dl⁻¹); heart mass relative to body mass, M₉₀ (g kg⁻¹); and hemoglobin P₅₀, Hb P₅₀, the value at which hemoglobin is half-saturated with oxygen. Values for Hb P₅₀ (kPa) were obtained from Natarajan et al. (Natarajan et al., 2015). Significant differences (P<0.05) of a variable between high- and low-altitude species pairs for all measured values were determined by using a student t-test (northern pintail and yellow-billed pintail, cinnamon teal, ruddy duck, and green-winged teal and speckled teal).

<table>
<thead>
<tr>
<th>Group</th>
<th>Hct (%)</th>
<th>[Hb] (g dl⁻¹)</th>
<th>Hb P₅₀ (kPa)</th>
<th>M₉₀ (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
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<td><strong>Low Altitude</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern pintail</td>
<td>43.7 ± 1.3*</td>
<td>15.1 ± 0.5*</td>
<td>----</td>
<td>10.7 ± 0.1</td>
</tr>
<tr>
<td>Cinnamon teal</td>
<td>43.1 ± 1.5*</td>
<td>14.0 ± 0.5*</td>
<td>4.95</td>
<td>9.2 ± 0.4</td>
</tr>
<tr>
<td>Ruddy duck</td>
<td>55.6 ± 4.0</td>
<td>19.6 ± 0.3</td>
<td>3.81</td>
<td>11.4 ± 0.4</td>
</tr>
<tr>
<td>Green-winged teal</td>
<td>45.9 ± 1.7*</td>
<td>15.2 ± 0.5*</td>
<td>----</td>
<td>10.3 ± 0.5*</td>
</tr>
<tr>
<td>Gadwall</td>
<td>44.6 ± 1.9</td>
<td>15.3 ± 0.4</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Mallard duck</td>
<td>46.5 ± 2.4</td>
<td>15.1 ± 0.6</td>
<td>----</td>
<td>7.4 ± 0.4</td>
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<tr>
<td><strong>High Altitude</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>Yellow-billed pintail</td>
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<tr>
<td>Puna teal</td>
<td>49.8 ± 1.3</td>
<td>17.0 ± 0.3</td>
<td>3.61</td>
<td>9.8 ± 0.6</td>
</tr>
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</table>
6.1 Introduction

In Chapter 4, I proposed that acute HA performance in birds, as seen in HA transient migrators such as bar-headed geese, necessitates adaptations in primarily the responses of convective components (rapid physiological changes in breathing and heart rate), while permanent HA residency, such as that seen in Andean geese and crested ducks, has resulted in a different strategy entailing enhancements to predominantly structural rather than functional components (i.e. increased lung oxygen extraction likely facilitated primarily by changes to lung morphology and a greater reliance on cardiac stroke volume to facilitate \( Q \) increases). Consistent with this, I reported in Chapter 5 that duck species endemic to HA in the Andes (Puna teal, speckled teal, cinnamon teal, yellow-billed pintail, and Andean ruddy duck) sustained resting levels of \( \overline{V}o_2 \) in response to progressive hypoxic exposure without increasing heart rate to any great extent, but rather by maintaining oxygen pulse through increases in stroke volume and/or tissue oxygen extraction. Moreover, this was also true of the same or closely related species native to LA (northern pintail, cinnamon teal, ruddy duck, green-winged teal, gadwall, and mallard duck) (Chapter 5). Since these results suggest a different pattern of cardiac regulation to hypoxic hypoxia than mammals, I was intrigued by whether or not the HRV response to acute hypoxia also varied.

All vertebrates hearts have an intrinsic, myogenic beat that is continuously modulated primarily by the interactions of extrinsic factors (sympathetic and parasympathetic controls, and
humoral influences) (Brennan et al., 2002; Larsen and Galletly, 2001). Even under steady state conditions, there may be beat-to-beat modulation, which can be considerable over short time periods (Axelsson, 2005; Smith et al., 2000). These beat-to-beat fluctuations in heart rate are termed HRV and analysis of HRV is a technique growing in physiological and clinical importance in part because HRV can be derived simply and noninvasively from the time between heartbeats, usually taken as the RR interval from an ECG. Individual variability in RR interval can be visualized in plots such as tachograms, which plot the RR interval length per each successive beat, and can reveal oscillations of a slower time-course (minutes) that are not revealed with other methods of analysis. The mean magnitude of HRV can be depicted in a number of different forms, such as the total modulation in RR intervals (SDRR; the standard deviation of the RR intervals) and short-term variability (RMSSD; the root mean square of successive differences of the RR intervals). A Pointcaré plot integrates SDRR and RMSSD within a scatter plot of a RR interval (n) plotted against its subsequent RR interval (n+1) (Figure 6.1). In humans, the length of the Pointcaré plot scatter generally is associated with SDRR, while the width of the Pointcaré plot scatter is associated with RMSSD (Brennan et al., 2002). Also, the shape of the points within the Pointcaré plot can be informative in assessing pathologies and has been well described in humans (Khandoker et al., 2013; Woo et al., 1992). Inferences on sympathovagal balance are possible from HRV analysis especially in conjunction with effects sympathetic or vagal blockers (Brennan et al., 2002; Valance et al., 2008). For example, increased heart rate in mammals, including humans, is typically associated with reduced HRV (Task Force of the European Society of Cardiology, 1996) resulting from increased sympathetic activity and decreased vagal tone (Hughson et al., 1994; Perini et al., 1996). For the reasons discussed above, HRV is being used more frequently not only to describe pathology, but also to
assess responses to external stressors (Axelsson, 2005; Brennan et al., 2002; Valance et al., 2008).

Two external stressors that can change HRV are dynamic exercise and exposure to hypoxia, because both typically alter heart rate partly through autonomic effects (Tulppo et al., 1996; Wolfel and Levine, 2001). During exercise the initial increase in heart rate is in part due to the withdrawal of vagal tone, while subsequent heart rate increases are in part a result of increased sympathetic activity. Exercise intensity determines the balance of the two autonomic modulators (Tulppo et al., 1996). Reduced HRV in exercising humans (Tulppo et al., 1996) is indicative of increased sympathetic activity. Exposure to HA similarly increases \( \dot{Q} \) in vertebrates, primarily mediated by increases in heart rate in mammals (Dzal et al., 2015; Shiels and White, 2008; Wolfel and Levine, 2001). In rats, acute or chronic exposure to simulated HA decreases HRV, but more so in acutely-exposed rats (Melin et al., 2003). Similarly, acute HA exposure in humans progressively increases sympathetic activity, while HA acclimatization is characterized by a shift towards increased HRV and a greater parasympathetic tone (Cornolo et al., 2004). Correspondingly, children born and raised at HA display a higher HRV and a higher parasympathetic modulation of their sinoatrial node than LA children (Sharshenova et al., 2006). Thus, in both humans and rats acclimatization to HA tempers the loss of HRV normally associated with acute HA exposure.

Very little HRV analysis has been completed on birds in general, and none on HA birds. HRV has been measured in embryonic chickens (Aubert et al., 2004), adult chickens (Kuo et al., 2001), quail (Valance et al., 2008), and European starlings (Cyr et al., 2009), but not on waterfowl. Furthermore, no study on adult birds has analyzed the effects of acute or chronic hypoxic exposure on HRV. My aim was to examine the effect of acute hypoxia on the HRV of
the eleven duck study groups discussed in Chapter 5 and to test the hypothesis that HA and LA species of ducks differ in the magnitude and pattern of their HRV. Therefore, I studied the cardiovascular responses to progressive hypoxia in five species of HA ducks (yellow-billed pintail, cinnamon teal, ruddy duck, speckled teal, and Puna teal) and six species of related LA ducks (northern pintail, cinnamon teal, ruddy duck, green-winged teal, gadwall, and mallard duck) (Figure 6.2). My hypotheses, based on the responses of rats and humans (Cornolo et al., 2004; Melin et al., 2003; Sharshenova et al., 2006), were that because neither HA nor LA ducks responded to hypoxia with large increases in heart rate (Chapter 5), HRV would not be altered by acute hypoxic exposure in either group, and additionally that the HA resident ducks would exhibit greater HRV during hypoxic exposure than LA ducks given their lifelong HA exposure.

6.2 Materials and methods

All experimental procedures were conducted according to guidelines approved by the Animal Care Committee at the University of British Columbia under the guidelines of the Canadian Council on Animal Care.

6.2.1 Animals

Cardiovascular measurements were made on eleven different groups of ducks. The six LA groups were studied at Summer Lake in Oregon, USA (1,300 m). These ducks were caught from the wild and studied within a maximum of 2 days post-capture. These groups include: 8 mallard ducks (*Anas platyrhynchos*; 0.96 ± 0.02 kg), 8 gadwall (*Anas strepera*; 0.75 ± 0.04 kg), 10 northern pintail (*Anas acuta*; 0.84 ± 0.01 kg), 11 cinnamon teal (*Anas c. cyanoptera*; 0.31 ± 0.01 kg), 8 ruddy duck (*Oxyura j. jamicensis*; 0.42 ± 0.04 kg), and 10 green-winged teal (*Anas
carolinensis; 0.29 ± 0.01 kg). The five HA groups were studied at Lake Titicaca in Peru (3,800 m). These birds were caught from the wild and studied within a maximum of 2 days post-capture. These groups include: 12 yellow-billed pintail (Anas georgica; 0.61 ± 0.02 kg), 12 cinnamon teal (Anas c. orinomus; 0.44 ± 0.01 kg), 6 ruddy duck (Oxyura j. ferruginea; 0.73 ± 0.07 kg), 10 speckled teal (Anas oxyptera; 0.37 ± 0.01), and 8 Puna teal (Anas puna; 0.40 ± 0.01 kg).

The comparison between the HA and LA duck species was strengthened by four HA-LA phylogenetic pairs: HA yellow-billed pintail and LA northern pintail, HA and LA ruddy duck, HA and LA cinnamon teal, and HA speckled teal and LA green-winged teal (Figure 6.2).

6.2.2 Duck preparation

All ducks were outfitted with a 3-lead ECG. See Chapter 2 for more detailed procedures.

6.2.3 Hypoxic exposure protocol

Ducks were exposed to 20-min step reductions in sea level-equivalent F\textsubscript{I}O\textsubscript{2}: 0.18 (LA ducks only; ambient exposure for LA ducks at 1,300 m), 0.133 (all ducks; ambient exposure for HA ducks at 3,800 m), 0.12, 0.09, 0.07, and 0.06. A 20-min recovery at ambient F\textsubscript{I}O\textsubscript{2} followed the hypoxic exposures. See Chapter 2 for a detailed experimental protocol.

6.2.4 Data measurement and analysis

Heart rate was continuously monitored via 3-lead ECG using a PowerLab Bio Amp (ADInstruments, Colorado Springs, CO, USA) at a sampling frequency of 1000 Hz per channel and a 5-min interval 15 min into the exposure was analyzed using PowerLab HRV analysis.
software (ADIStuments, Colorado Springs, CO, USA). Heart rate (min⁻¹) data were derived from this 5-min interval and described in full in Chapter 5. RMSSD (ms), the most common time domain measure for HRV, and SDRR (ms), a measure of total variability, were derived from this program from the same 5-min interval (Khandoker et al., 2013) (Figure 6.1).

The RR interval lengths derived from this program for 500 heartbeats were also used to generate tachograms and Pointcaré plots from a representative individual exhibiting HRV responses closest to those of the mean SDRR and RMSSD for each study group. The choice of 500 heartbeats was based on other studies using <500 heartbeats (Cyr et al., 2009), and because longer segments of data did not alter overall patterns in the Pointcaré plots and tachograms. I used Pointcaré plots and tachograms for this analysis as they provide an excellent visual representation of changes in RR interval between subsequent beats and over time (Brennan et al., 2002; Khandoker et al., 2013). Pointcaré plots are a reliable method for characterizing individual HRV because the shape of scatter, degree of scatter, and orientation of scatter on the plot can all be used to inform HRV analysis. Individual Pointcaré plots within a duck species at a given altitude were compared to make generalizations about the shape of the Pointcaré plot. The tachograms provide a visualization of temporal patterns in the HRV and are shown in the Supplementary material.

6.2.5 Statistical analysis

RMSSD, and SDRR data are presented as means ± s.e.m, while all other data are presented as individual data points. Within each species, all mean data were analyzed using one-way repeated measures ANOVA and Holm-Sidak post hoc tests. Comparisons of mean data
between groups were made using two-way (species and \(F_{IO_2}\)) repeated measures ANOVA and Holm-Sidak post-hoc tests within each \(F_{IO_2}\). For statistical comparisons, \(P<0.05\) was used to determine statistical significance. Variables analyzed with a one-way repeated measures ANOVA that did not meet assumptions for normality were transformed with: \(x' = \ln(x)\) (SDRR for Punta teal and mallard duck), \(x' = \log(x)\) (heart rate for northern pintail and mallard duck), \(x' = 1/\sqrt{x}\) (heart rate for green-winged teal), and \(x' = 1/\ln(x)\) (heart rate for speckled teal). Those that could not be transformed to meet normality (SDRR and RMSSD: yellow-billed pintail and HA ruddy duck) were analyzed using a Friedman repeated measures ANOVA on ranks. Similarly, variables were transformed when they did not meet assumptions for either normality or equal variance analyzed for a two-way repeated measures ANOVA with: \(x' = \ln(x)\) for select comparisons of heart rate, and \(x' = \sqrt{x}\) for SDRR in HA and LA ruddy ducks.

Statistical analyses were carried out using SigmaStat (version 3.0; Systat Software).

6.3 Results

The mean SDRR did not change significantly during either hypoxic exposure or ambient recovery in any group (Figure 6.3A-G), except for a modest increase in SDRR in HA ruddy ducks during 5-min ambient recovery (\(P=0.035\)) (Figure 6.3C). Nevertheless, SDRR was significantly higher in the LA counterparts of all HA-LA pairs, except the HA yellow-billed pintails and LA northern pintails. LA ruddy ducks (Figure 6.3C) and LA green-winged teals (Figure 6.3D) exhibited a higher SDRR than their HA counterparts at all exposure levels (\(P<0.001\), and LA cinnamon teals exhibited a higher SDRR than HA cinnamon teals at all
exposure levels except ambient \((P=0.046)\) (Figure 6.3B). Thus, the LA groups generally exhibited greater SDRR than the HA groups.

Similarly, the mean RMSSD did not change significantly throughout hypoxic exposure and ambient recovery in any group (Figure 6.4A-G), except the modest increase in RMSSD in HA ruddy ducks \((P=0.020)\) (Figure 6.4C). Yet, just as with SDRR, RMSSD was significantly higher in the LA counterparts of all HA-LA pairs except the HA yellow-billed pintails and LA northern pintails Figure 6.4A). LA ruddy ducks \((P=0.002)\) (Figure 6.4C) and LA green-winged teals \((P=0.003)\) (Figure 6.4D) exhibited a higher RMSSD than their HA counterparts at all exposure levels, and LA cinnamon teals exhibited a higher RMSSD than HA cinnamon teals at all exposure levels except ambient \((P=0.004)\) (Figure 6.4B). Thus, the LA groups also generally exhibited greater RMSSD than the HA groups.

The Pointcaré plots in Figure 6.5 through Figure 6.11 illustrate the spatial patterns of HRV for one individual that exhibited the same general HRV trends as the group means from each of the eleven duck groups. While the patterns seen here are generally consistent with trends in the group mean data (Figure 6.3, Figure 6.4), there was inter-individual variability; the HRV for individuals within each group did not always exhibit changes identical with their group means. Thus, while the plots in general show the lack of change in HRV upon hypoxic exposure (e.g. mallard duck in Figure 6.5, gadwall in Figure 6.6), examples of individuals where HRV appeared reduced (e.g. HA Puna teal in Figure 6.7, and LA and HA cinnamon teal in Figure 6.9) or increased (e.g. LA northern pintail in Figure 6.8 and LA ruddy duck in Figure 6.10) upon hypoxic exposure were evident. One pattern that was clearly evident, however, was the reduction in HRV in the HA ducks relative to the LA ducks in each of the four HA and LA pairs in almost all exposures in the representative Pointcaré plots (Figures 6.8-6.11). The only major exception
to this was the greater HRV present in the representative HA cinnamon teal at 5-min recovery (Figure 6.9).

The tachograms in Figure 6.12 through Figure 6.18 illustrate the temporal patterns of HRV for the same individuals for which the Poincare plots were generated in Figure 6.5 to Figure 6.11. Tachograms are better for illustrating short-term oscillations present in heart rate, while still reflecting general overall magnitude of variability. For example, the decreased HRV of the HA ducks relative to the LA ducks were still evident in the tachograms (Figure 6.15, Figure 6.16, Figure 6.17, and Figure 6.18), but interesting temporal oscillations in RR interval length otherwise masked by the Pointcaré plots were also present.

6.4 Discussion

In this study I show the first reported HRV data for waterfowl in eleven study groups of ducks. There were two primary findings of this study. First, and in support of my hypothesis, there were no significant changes in the mean HRV of any group upon exposure to severe hypoxia (0.06 F\textsubscript{1}O\textsubscript{2}). Second, HA resident waterfowl generally exhibited less HRV (as quantified by SDRR and RMSSD) than the same or closely related LA resident waterfowl species. The only exception was the lack of significant difference between the HRV of LA northern pintails and HA yellow-billed pintails. This was contrary to my hypothesis and to findings in the literature for rats and humans acclimated to HA (Cornolo et al., 2004; Melin et al., 2003; Sharshenova et al., 2006). Second, while a number of studies have examined heart rate in ducks, none report metrics for HRV or raw RR interval data (Berger et al., 1970; Butler and Woakes, 1979; Hawkins et al., 2000; Stephenson et al., 1986). Remarkably few HRV studies have been conducted on birds, and none on waterfowl. In the few studies that have measured HRV in birds (adult chickens (Kuo et
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al., 2001), embryonic chickens (Aubert et al., 2004), quail (Valance et al., 2008), and European starlings (Cyr et al., 2009)), there is little overlap in reported HRV variables given the variety of ways that HRV can be expressed and discussed (Khandoker et al., 2013). Of the aforementioned studies, none included Pointcaré plots or reported SDRR. RMSSD was reported only for quail and embryonic chickens (Aubert et al., 2004; Valance et al., 2008), and none of the studies examined the effects of acute or chronic hypoxia on HRV. One comprehensive study by Tazawa et al. examined the changes in HRV in embryos during incubation in a representative species of goose, duck, quail, peafowl, chicken, and turkey, and used a “coefficient of variability” to represent average heart rate changes during incubation (Tazawa et al., 1991); however, the sampling time course (~10% increments of incubation) provides nowhere near the level of definition of individual RR interval lengths and is too broad to allow for comparison. Thus, there are no other data with which I can compare my results highlighting the novelty of my study.

The relationship between heart rate and HRV is complex and generally understudied even in mammals (Coumel et al., 1994). However, it has been shown in human studies that increases in heart rate are typically associated with decreases in HRV (and vice versa) (Coumel et al., 1994; Mangin et al., 1998; Melin et al., 2003), and this decreased HRV is generally believed to reflect increased sympathetic activity and decreased vagal tone (Bernardi et al., 2001; Hughson et al., 1994). Studies on mammals suggest that acute hypoxia results in an increase in heart rate due to increased sympathetic activity and reduced vagal tone. This shift in autonomic balance leads to a reduction in beat-to-beat HRV that reduces chronotropic responses. Chronic exposure to hypoxia leads to a reduction in β-adrenoreceptor expression in the left cardiac ventricles of rats (Kacimi et al., 1992), as well as to a decrease in α-adrenoreceptor density in the right ventricle (Morel et al., 1999). The net result is to reduce the impact of the chronic hypoxia on
HRV and restore chronotropic sensitivity, thereby restoring HRV during acute hypoxic stress. The reduced HRV with normal or reduced heart rates in my HA ducks suggests that both vagal tone and sympathetic tone could be significantly reduced. The reduced vagal tone would reduce the HRV while a concomitant reduction in sympathetic tone would be required to prevent heart rate from rising. This is an intriguing possibility that remains to be explored, though there is some evidence for HA pikas also exhibiting blunted parasympathetic and sympathetic modulation of heart rate due to reduced β-adrenoreceptor expression in the cardiac ventricle (Pichon et al., 2013). Experiments involving the use of pharmacological agonists and antagonists are required to understand the role and significance of changes in vagal and sympathetic tone to the reduced HRV that develops over time at altitude in these waterfowl.

The analysis of individuals in addition to group mean data revealed interesting changes in HRV masked by analysis of group mean data for SDRR, RMSSD and heart rate alone. The HRV of these individuals did not always mirror the mean HRV of the group; i.e. mean trends in HRV were not necessarily representative of individual trends in all exposures. Clearly, the balance of intrinsic and extrinsic factors producing short-term changes in vagal tone varied between individuals and conditions in each of the groups.

Oscillation in heart rate and HRV were observed in several of the individuals from different groups in the present study. These oscillations could be very rapid, as seen in the LA green-winged teal (Figure 6.18A) in normoxia and hypoxia, moderate, as seen in the HA Puna teal (Figure 6.14), or slow, as seen in the gadwall (Figure 6.13), speckled teal (Figure 6.18B), and LA northern pintail and HA yellow-billed pintail (Figure 6.15) upon ambient recovery. The basis of these oscillations remains to be determined but these observations suggest that the re-
establishment of the normal balance between vagal and sympathetic tone is not always straightforward.

6.4.1 Conclusions

Here I present the first HRV data for waterfowl. In this study I found that hypoxic exposure did not significantly change HRV (as quantified by RMSSD and SDRR), which is consistent with results in Chapter 5 indicating that progressive hypoxia had little effect on heart rate in both LA and HA birds. I also found that HRV was generally lower in HA study groups than in LA study groups, a finding contrary to that reported in mammals (Cornolo et al., 2004; Melin et al., 2003; Sharshenova et al., 2006). The reduced HRV with normal or reduced heart rates in my HA birds suggests that both vagal tone and sympathetic tone must be significantly reduced. The reduced vagal tone would reduce the HVR while a concomitant reduction in sympathetic tone would be required to prevent heart rate from rising. The large inter-individual variability, however, indicates that vagal tone is easily and rapidly modulated around baseline levels under all conditions in this study. Experiments involving the use of pharmacological agonists and antagonists will be required to derive baseline or resting variables for the species studied, as well as inform the contribution of vagal and sympathetic tone in each species during different hypoxic exposures.
Figure 6.1: Representative example of a Pointcaré plot.
As a scatter plot of RR intervals against subsequent RR intervals, Pointcaré plots contain useful qualitative information with regards to the shape of the scatter, the width of the scatter (RMSSD; the root mean square of successive differences in RR intervals), and the length of the scatter (SDRR; the standard deviation of RR intervals).
Figure 6.2: Comparison of high-altitude and low-altitude waterfowl used in the present study.
Groups are compared with respect to their relative time at altitude and the relationship between a high-altitude group and a closely related low-altitude pair. Subspecies and altitude information provided from the literature (Fjeldså and Krabbe, 1990; Hilty and Brown, 1986; Livezey, 1986; McCracken et al., 2009c; McCracken et al., 2009a; Munoz-Fuentes et al., 2013).

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<thead>
<tr>
<th>Low Altitude</th>
<th>High Altitude</th>
<th>Relationship</th>
<th>Relative Time at Altitude</th>
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<td>Northern pintail <em>(Anas acuta)</em></td>
<td>Yellow-billed pintail <em>(Anas georgica)</em></td>
<td>sibling species</td>
<td>Very recent</td>
</tr>
<tr>
<td>Cinnamon teal <em>(Anas c. cyanoptera)</em></td>
<td>Cinnamon teal <em>(Anas c. orinomus)</em></td>
<td>subspecies</td>
<td>Recent</td>
</tr>
<tr>
<td>Ruddy duck <em>(Oxyura j. jamicensis)</em></td>
<td>Andean ruddy duck <em>(Oxyura j. ferruginea)</em></td>
<td>subspecie</td>
<td>Intermediate</td>
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<tr>
<td>Green-winged teal <em>(Anas carolinensis)</em></td>
<td>Speckled teal <em>(Anas f. oxyptera)</em></td>
<td>sibling species</td>
<td>Old</td>
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<tr>
<td>Puna teal <em>(Anas puna)</em></td>
<td></td>
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<td>Gadwall <em>(Anas strepera)</em></td>
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<td>Mallard duck <em>(Anas platyrhynchos)</em></td>
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Figure 6.3: Standard deviation of RR intervals (SDRR) in normoxia, severe hypoxia and ambient recovery. (A-G) SDRR is represented for all study groups as a red bar for ambient conditions (0.18 equivalent fraction of inspired oxygen (F\textsubscript{IO\textsubscript{2}}) for low altitude (LA) and 0.133 F\textsubscript{IO\textsubscript{2}} for high altitude (HA)) as a red bar, an orange bar for 0.06 F\textsubscript{IO\textsubscript{2}}, a yellow bar following 5-min of ambient recovery, and a green bar following 20-min of ambient recovery. All values are s.e.m. Significant differences (P<0.05) in the y-axis variable from values during exposure to ambient air within a species are indicated different letters\textsuperscript{a,b} and determined by one-way repeated measures ANOVA. Significant differences (P<0.05) in the y variable between phylogenetic pairs (A-D) are indicated by an asterisk and determined by two-way repeated measures ANOVA.
Figure 6.4: Root mean square of successive differences in RR intervals (RMSSD) in normoxia, severe hypoxia and ambient recovery.

(A-G) RMSSD is represented for all study groups as a red bar for ambient conditions (0.18 equivalent fraction of inspired oxygen (F\textsubscript{2}O\textsubscript{2}) for low altitude (LA) and 0.133 F\textsubscript{2}O\textsubscript{2} for high altitude (HA)) as a red bar, an orange bar for 0.06 F\textsubscript{2}O\textsubscript{2}, a yellow bar following 5-min of ambient recovery, and a green bar following 20-min of ambient recovery. All values are s.e.m. Significant differences (P<0.05) in the y-axis variable from values during exposure to ambient air within a species are indicated different letters\textsuperscript{a,b} and determined by one-way repeated measures ANOVA. Significant differences (P<0.05) in the y variable between phylogenetic pairs (A-D) are indicated by an asterisk and determined by two-way repeated measures ANOVA.
Figure 6.5: Mallard duck Pointcaré plots in normoxia, severe hypoxia and ambient recovery.
Representative Pointcaré plots of 500 beats for a low-altitude mallard duck in 0.13 fraction of inspired oxygen ($F_{O_2}$) (yellow), 0.06 $F_{O_2}$ (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
Figure 6.6: Gadwall Pointcaré plots in normoxia, severe hypoxia and ambient recovery.
Representative Pointcaré plots of 500 beats for a low-altitude gadwall in 0.13 fraction of inspired oxygen (F\textsubscript{I}O\textsubscript{2}) (yellow), 0.06 F\textsubscript{I}O\textsubscript{2} (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
Figure 6.7: Puna teal Pointcaré plots in normoxia, severe hypoxia and ambient recovery.
Representative Pointcaré plots of 500 beats for a high-altitude Puna teal in 0.13 fraction of inspired oxygen ($F_{O_2}$) (yellow), 0.06 $F_{O_2}$ (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
Figure 6.8: Northern pintail and yellow-billed pintail Pointcaré plots in normoxia, severe hypoxia and ambient recovery.
Representative Pointcaré plots of 500 heart beats for a low-altitude northern pintail and high-altitude yellow-billed pintail in 0.13 fraction of inspired oxygen ($F_{O_2}$) (yellow), 0.06 $F_{O_2}$ (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green). All high-altitude yellow-billed pintail data are in black.
Figure 6.9: Low- and high-altitude cinnamon teal Pointcaré plots in normoxia, severe hypoxia and ambient recovery.
Representative Pointcaré plots of 500 beats for a low-altitude cinnamon teal and high-altitude cinnamon teal in 0.13 fraction of inspired oxygen (F_{1,O_2}) (yellow), 0.06 F_{1,O_2} (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green). All high-altitude cinnamon teal data are in black.
Figure 6.10: Low- and high-altitude ruddy duck Pointcaré plots in normoxia, severe hypoxia and ambient recovery.
Representative Pointcaré plots of 500 beats for a low-altitude ruddy duck and high-altitude ruddy duck in 0.13 fraction of inspired oxygen (F$_{1}$O$_{2}$) (yellow), 0.06 F$_{1}$O$_{2}$ (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green). All high-altitude ruddy duck data are in black.
Figure 6.11: Green-winged teal and speckled teal Pointcaré plots in normoxia, severe hypoxia and ambient recovery.

Representative Pointcaré plots of 500 beats for a low-altitude green-winged teal and high-altitude speckled teal in 0.13 fraction of inspired oxygen (F\textsubscript{2}\textsubscript{O}\textsubscript{2}) (yellow), 0.06 F\textsubscript{2}\textsubscript{O}\textsubscript{2} (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green). All high-altitude speckled teal data are in black.
Figure 6.12: Mallard duck tachograms in normoxia, severe hypoxia and ambient recovery. Representative tachograms of 500 beats for a low-altitude mallard duck in 0.13 fraction of inspired oxygen (F\textsubscript{I}O\textsubscript{2}) (yellow), 0.06 F\textsubscript{I}O\textsubscript{2} (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
Figure 6.13: Gadwall tachograms in normoxia, severe hypoxia and ambient recovery.
Representative tachograms of 500 beats for a low-altitude gadwall in 0.13 fraction of inspired oxygen (FIO₂) (yellow), 0.06 FIO₂ (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
Figure 6.14: Puna teal tachograms in normoxia, severe hypoxia and ambient recovery.
Representative tachograms of 500 beats for a high-altitude Puna teal in 0.13 fraction of inspired oxygen (FIO₂) (yellow), 0.06 FIO₂ (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
Figure 6.15: Northern pintail and yellow-billed pintail tachograms in normoxia, severe hypoxia and ambient recovery.
Representative tachograms of 500 beats for a (A) low-altitude northern pintail and (B) high-altitude yellow-billed pintail in 0.13 fraction of inspired oxygen (F\textsubscript{I}O\textsubscript{2}) (yellow), 0.06 F\textsubscript{I}O\textsubscript{2} (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
Figure 6.16: Low- and high-altitude cinnamon teal tachograms in normoxia, severe hypoxia and ambient recovery.
Representative tachograms of 500 beats for a (A) low-altitude cinnamon teal and (B) high-altitude cinnamon teal in 0.13 fraction of inspired oxygen ($F_{\text{IO}_2}$) (yellow), 0.06 $F_{\text{IO}_2}$ (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
Figure 6.17: Low- and high-altitude ruddy duck tachograms in normoxia, severe hypoxia and ambient recovery.

Representative tachograms of 500 beats for (A) low-altitude ruddy duck and (B) high-altitude ruddy duck in 0.13 fraction of inspired oxygen (FIO₂) (yellow), 0.06 FIO₂ (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
Figure 6.18: Green-winged teal and speckled teal tachograms in normoxia, severe hypoxia and ambient recovery.
Representative tachograms of 500 beats for a (A) low-altitude green-winged teal and (B) high-altitude speckled teal in 0.13 fraction of inspired oxygen ($F_O^2$) (yellow), 0.06 $F_O^2$ (orange), 5-min ambient recovery (yellow), and 20-min ambient recovery (green).
CHAPTER 7: DIFFERENT STRATEGIES FOR MAINTAINING OXYGEN SUPPLY DURING HYPOXIA EXPOSURE IN WATERFOWL: A SYNTHESIS

The overall objective of this thesis was to examine how waterfowl species (geese and ducks) from different altitudes (Figure 7.1) regulate their cardiovascular and respiratory systems to maintain oxygen supply during hypoxic exposures simulating altitudes equivalent to or higher than the top of Mt. Everest. I hypothesized that: (1) waterfowl groups would have more than one ventilatory and cardiovascular strategy to maintain oxygen supply during hypoxia, and (2) exposure to HA during a bird’s lifetime would alter its HVR and hypoxic cardiovascular response.

In support of my hypotheses, my research suggests that waterfowl groups exhibit different ventilatory and cardiovascular strategies to facilitate oxygen uptake and transport during hypoxic exposure, and that life at HA does modify the HVR and hypoxic cardiovascular response of waterfowl. All HA waterfowl populations studied exhibited an increased blood-oxygen carrying capacity (decreased Hb-P\textsubscript{50} and/or increased Hct and [Hb]) compared to related LA groups (Chapters 3-5). Bar-headed geese that were reared at HA exhibited a significantly reduced oxygen demand and a modest, but significant increase in oxygen uptake and delivery during progressive hypoxia compared to bar-headed geese reared at LA (Chapter 3). In addition, HA ducks exhibited lower HRV than related LA ducks (Chapter 6). I observed three ventilatory strategies for maintaining oxygen supply during hypoxia (6 study groups) mediated by predominate increases in either: breathing frequency (e.g. barnacle goose; Chapter 3), tidal
volume (e.g. HA and LA bar-headed geese; Chapters 3 and 4), or lung oxygen extraction (e.g. Andean geese and crested ducks; Chapter 4) (Figure 7.2A). Moreover, my research suggests that in waterfowl exposed to hypoxia, increases in both heart rate and stroke volume both contribute to increasing Q. In addition, it also suggests that increases in stroke volume are a critical component of the waterfowl hypoxic cardiovascular response (Chapters 3-5), contrary to previous findings that cardiac stroke volume remains largely unchanged in waterfowl exposed to hypoxia (Faraci, 1986; Fedde et al., 1989; Kiley et al., 1979; Smith et al., 2000). Furthermore, I identified three distinct cardiac strategies for maintaining oxygen supply during hypoxia (17 study groups) mediated by predominate increases in: heart rate (e.g. bar-headed geese reared at both LA and HA; Chapters 3 and 4), stroke volume (e.g. Andean geese; Chapter 4) and/or blood-oxygen carrying capacity (all HA waterfowl groups; Chapters 3-5) (Figure 7.2B). Thus, I showed that life at HA can variably impact the HVR and hypoxic cardiovascular response of waterfowl (Chapters 3 and 5), suggesting that short-term HA performance (e.g. HA migration of bar-headed geese) utilizes primarily functional enhancements (e.g. rapid heart rate and ventilation increases) to maintain oxygen supply during hypoxic exposure, whereas lifelong HA residency (e.g. Andean geese) is supported predominantly by structural enhancements (e.g. changes to lung and cardiac morphology). As a result, the 17 study groups of waterfowl (Figure 7.1) maintained oxygen supply during hypoxic exposure with different relative cardiovascular and ventilatory strategies (Figure 7.2A,B).
7.1 Ventilatory strategies for maintaining oxygen supply during hypoxia

7.1.1 Ventilatory variables contributing to oxygen uptake

The flow of oxygen through the respiratory system is both convective (ventilation) and diffusive (lung oxygen extraction). There are three ventilatory variables that can be altered to maintain or increase \( \dot{V}O_2 \) during hypoxia: breathing frequency, tidal volume, and lung oxygen extraction (Weibel, 1984b):

\[
\dot{V}O_2 = f_R \times V_T \times (F_{I}O_2 - F_EO_2)
\]

7.1.1.1 Breathing frequency and tidal volume

Breathing frequency and tidal volume can be rapidly changed, and their product is \( \dot{V}_R \). Of these three variables, there was prior evidence that greylag geese, a LA migrating species, predominantly increase breathing frequency and that LA bar-headed geese predominantly increase tidal volume to maintain oxygen supply during hypoxic exposure (Scott and Milsom, 2007).

7.1.1.2 Lung oxygen extraction

The percent of oxygen extracted from the inspired air, \((F_{I}O_2 - F_EO_2 / F_{I}O_2) \times 100\) or lung oxygen extraction, is reliant primarily upon factors that determine diffusion – namely the \( PO_2 \) gradient between the lungs and the blood, the barrier surface area, and the barrier thickness (Weibel, 1984b). As such, diffusion of oxygen across the avian lung is heavily determined by lung morphology. Morphologically, the unidirectional flow of air through the cross-current gas exchange system of the avian lungs is considered functionally more efficient than tidal ventilation in mammals (Scott, 2011). Their blood-gas exchange surface is also specialized to
enhance oxygen extraction efficiency with a larger surface area and a diffusion barrier that is exceptionally thin (2.5-fold thinner than mammals), yet still structurally robust (Powell, 2000; Scheid, 1990; West, 2009). Bar-headed geese are also known to have even larger lungs than LA geese, increasing the surface area available for oxygen diffusion (Scott, 2011). It is currently unknown to what degree breathing pattern and lung morphology are further enhanced in other HA species.

7.1.2 Different ventilatory strategies observed for maintaining oxygen supply in waterfowl

I applied the Fick equation to investigate the relative contributions of ventilatory variables to the maintenance of oxygen supply during progressive hypoxic exposure in six groups of waterfowl (LA barnacle geese, LA bar-headed geese, two populations of HA bar-headed geese, HA Andean geese, and HA crested ducks) (Figure 7.1). Prior to this study, the only group investigated here whose HVR had been previously studied was the LA bar-headed goose. While all three ventilatory variables contributed to the overall maintenance of oxygen supply during hypoxia, I found evidence of three distinct strategies that served to maintain oxygen supply during hypoxic exposure in these groups. These include greater relative contributions of either breathing frequency, tidal volume, or lung oxygen extraction to \( \dot{V}_{O_2} \) maintenance (Figure 7.2A).

7.1.2.1 Breathing frequency

Barnacle geese, a LA migrating species, increased \( \dot{V}_R \) by a combination of tidal volume and breathing frequency increases (Chapter 3). This was distinct from the response of another
LA migrating species, the greylag goose, that was previously shown to rely almost exclusively on increases in breathing frequency to increase $V_R$ (Scott and Milsom, 2007). Thus, even within LA-migrating geese there are different HVR patterns.

### 7.1.2.2 Tidal volume

Bar-headed geese, a species that migrates transiently at HA, also increased $V_R$ by a combination of increases in tidal volume and breathing frequency but, as previously shown by Scott and Milsom (Scott and Milsom, 2007), supported initial $V_R$ increases with large tidal volume increases (Chapters 3 and 4). HA rearing in bar-headed geese did impact the HVR. Most strikingly, HAR bar-headed geese exhibited lower $\dot{V}O_2$ than LAR bar-headed geese (Chapter 3), as well as greater increases in tidal volume and $V_R$ than LAR bar-headed geese when measured at BTPS (Chapter 3).

### 7.1.2.3 Lung oxygen extraction

Lung oxygen extraction remained constant in barnacle geese and all populations of bar-headed geese around 30-50% (Chapters 3 and 4). I identified a third distinct ventilatory strategy in Andean geese and crested ducks, both HA resident species, however. These species did not increase $V_R$, but rather increased lung oxygen extraction. Indeed, I measured lung oxygen extraction increases of ~90% during progressive hypoxia with no significant $V_R$ increases (Chapter 4).
Thus the waterfowl employed different relative contributions of increases in ventilatory variables (frequency, volume, and extraction) to maintain oxygen supply during hypoxia (Figure 7.2A).

### 7.1.3 Implications associated with different ventilatory strategies

There are potential tradeoffs that could be associated with the predominant use of increases in frequency, tidal volume, or lung oxygen extraction to maintain oxygen supply during hypoxia.

#### 7.1.3.1 Breathing frequency and tidal volume

A benefit of increasing breathing frequency or tidal volume is that these variables can be rapidly recruited and altered to support $\dot{V}O_2$. These changes are not as constrained by morphology as lung oxygen extraction. Of the two, increasing tidal volume is hypothesized to be more efficient than increasing breathing frequency because it decreases ventilation of respiratory dead space (Scott and Milsom, 2007). This would be especially beneficial in a hypoxic environment because it would ensure maximal ventilation of oxygen exchange surfaces and increase the proportion of each breath available for gas exchange, thereby enhancing the amount of oxygen that can potentially be exchanged per breath during conditions of decreased oxygen. Depending on pulmonary mechanics, however, slow deep breathing could be mechanically expensive. It remains to be seen whether the HA geese have more compliant respiratory systems reducing the elastic work associated with large tidal volumes. Regardless, the act of breathing incurs mechanical costs (Otis, 1954), and regardless of which variable contributes most to
increases in $\dot{V}_R$, the act of increasing $\dot{V}_R$ represents an acute energetic cost. Increased lung oxygen extraction, however, does not.

### 7.1.3.2 Lung oxygen extraction

The basis of increased lung oxygen extraction in Andean geese and crested ducks is unknown, but may be attributable to any combination of the following functional (decreased Hb-$P_{50}$, increased perfusion of the blood-gas surface, and increased capillary recruitment) and morphological (increased lung capillarity, increased lung surface area, and decreased barrier thickness) enhancements. Morphological changes, however, incur energetic costs during development and require lifelong maintenance. Such morphological and functional changes would also impact all other exchange processes at the lungs, and could lead to increased evaporative water loss. Ultimately, these enhancements would facilitate the maintenance of oxygen supply under chronic hypoxic conditions through increased lung oxygen extraction, allowing recruitment of short-term increases in convective ventilatory variables to serve as a reserve for more metabolically demanding activities, such as flight.

While the ultimate factors determining which strategy a species exhibits remain unknown, they may include life history (migrator versus resident) and duration of hypoxia exposure (transient versus lifelong). I propose that demanding performance (e.g. HA migration of bar-headed geese and LA long-distance migration of barnacle geese) predominantly necessitates rapid recruitment of convective flow (rapid physiological increases in tidal volume and breathing frequency), while permanent HA residency (e.g. Andean geese and crested ducks) allows for an arguably more sustainable strategy long-term that is primarily supported by alterations in diffusive capacity (e.g. changes to lung morphology) (Chapters 3 and 4).
7.2 Cardiovascular strategies for transporting oxygen during hypoxia

7.2.1 Cardiovascular variables contributing to oxygen delivery

Circulatory convection describes the mass transport of oxygen as determined by \( \dot{Q} \) and the quantity of oxygen carried per unit volume of blood (Weibel, 1984b):

\[
\dot{V}_O_2 = f_H \times V_S \times \beta_{O_2} \times (P_{aO_2}-P_{VO_2}) \tag{7.2}
\]

The product of the heart rate and stroke volume is \( \dot{Q} \). Blood-oxygen carrying capacity can be modulated by a variety of factors, including Hb-P_{50}, as well as changes in Hct, [Hb], or allosteric modulators of Hb-oxygen binding. The convective cardiovascular Fick equation can also be expressed using terms that already encompass \( \beta_{O_2} \), such as oxygen content (the product of PO_{2} and \( \beta_{O_2} \)):

\[
\dot{V}_O_2 = f_H \times V_S \times (C_{aO_2}-C_{VO_2}) \tag{7.3}
\]

Tissue oxygen extraction describes the percent change in oxygen content between the arterial and venous systems, and is primarily reliant upon factors that determine diffusion (Weibel, 1984b). Finally, this equation can be further simplified by using cardiac oxygen pulse, the product of stroke volume and the arterial-venous oxygen content difference, when variables such as stroke volume, \( C_{aO_2} \), and \( C_{VO_2} \) are unknown:

\[
\dot{V}_O_2 = f_H \times O_2 \text{ pulse} \tag{7.4}
\]

In the discussion to follow, heart rate, stroke volume, tissue oxygen extraction, and oxygen pulse will be used to compare how waterfowl species maintain oxygen supply during hypoxic exposure (Figure 7.2B), and differences in \( \beta_{O_2} \) will be discussed where appropriate.
7.2.1.1 Heart rate and stroke volume

When considering which changes in the above variables might contribute most to $\dot{V}O_2$ maintenance during hypoxic exposure, existing enhancements to the avian cardiovascular system need to be acknowledged. Birds have larger hearts than mammals, and thus compared to a mammal of similar body size they can exhibit a larger $Q$ and larger stroke volume (Calder, 1968; Grubb, 1983; Smith et al., 2000). It is generally accepted that waterfowl increase $Q$ during hypoxic exposure (Faraci, 1986; Shams and Scheid, 1989; Smith et al., 2000) (Chapters 3 and 4). Bar-headed geese have been reported to increase $Q$ from 2.5-fold (Chapters 3 and 4) to 7-fold (Black and Tenney, 1980a) when exposed to severe hypoxia ($<0.07 F_O_2$) at rest. Previous studies on geese and ducks reported increases in heart rate as the primary contributor to increases in $Q$ with stroke volume remaining largely unchanged (Faraci, 1986; Fedde et al., 1989; Kiley et al., 1979; Smith et al., 2000).

7.2.1.2 Tissue oxygen extraction

While the flight muscle, heart, and brain of birds have an enhanced capacity for tissue oxygen diffusion compared to mammals due to their higher capillary to muscle fibre ratio (Faraci, 1991; Mathieu-Costello et al., 1998; Scott, 2011), changes in tissue oxygen diffusion are constrained by the same basic morphological parameters as lung oxygen diffusion. Examples of enhancements for tissue oxygen diffusion in HA birds are the increased capillarity in the left cardiac ventricle of bar-headed geese (Scott et al., 2010), and the positioning of mitochondria closer to the capillaries in the flight muscle of bar-headed geese (Scott et al., 2009). Tissue thickness and surface area cannot be rapidly changed, however, and it is unknown to what degree
they can be further enhanced in this species or to what degree they are already enhanced in other HA species.

7.2.1.3 **Blood-oxygen carrying capacity**

The components of $\beta_o$ most likely to be altered in HA populations include Hb-$P_{50}$, Hct, and [Hb]. Many HA populations have been shown to have intrinsic (unmodified by allosteric modulators) Hb-$P_{50}$ values that are lower than those found in LA populations (Storz et al., 2010), thereby increasing blood-oxygen affinity. While increased Hct and [Hb] will increase blood-oxygen carrying capacity, increases beyond a certain threshold will increase blood viscosity, increasing resistance to blood flow and placing greater demands on the heart (Smith et al., 2000). Thus, based on the dynamic components of the Fick equation and described in the literature, the variables that could be changed during a hypoxic stress are heart rate and stroke volume, tissue oxygen extraction, oxygen pulse, and $\beta_o$.

7.2.2 **Different cardiovascular strategies observed for maintaining oxygen supply in waterfowl**

I applied the Fick equation to investigate the relative contributions of heart rate, stroke volume, tissue oxygen extraction, and oxygen pulse in maintaining oxygen supply during progressive hypoxic exposure in all 17 groups of waterfowl (Figure 7.1). Prior to this study, the only groups investigated here for which there was already some cardiovascular data available during hypoxia were LA bar-headed geese (Black and Tenney, 1980a; Fedde et al., 1989; Hawkes et al., 2014) and LA barnacle geese (Hawkes et al., 2014). Other than information on Hb-$P_{50}$ available for select groups, these are the first cardiovascular measurements reported for
the other 15 waterfowl groups. While each cardiovascular variable contributed to the overall maintenance of oxygen supply during hypoxia in all groups, I found evidence of three main strategies that served to maintain oxygen supply during progressive hypoxic exposure. These include greater relative contributions of predominant increases in: (1) heart rate, (2) stroke volume, and/or (3) blood-oxygen carrying capacity achieved by either decreased Hb-P50, increased Hct, and/or increased [Hb] (Figure 7.2B).

7.2.2.1 Heart rate and stroke volume

For those birds in which stroke volume could be directly calculated (Figure 7.1: all bar-headed geese, barnacle geese, Andean geese, and crested ducks), all birds used a combination of increases in heart rate and stroke volume to increase \( \dot{Q} \) (Chapters 3 and 4). Some species, however, relied more on (1) increases in heart rate, as seen in bar-headed geese regardless of rearing altitude (Chapters 3 and 4), or (2) increases in stroke volume, as seen in Andean geese (Chapter 4) (Figure 7.2B). For the 11 groups of ducks in which heart rate was measured and oxygen pulse calculated (Figure 7.1: yellow-billed pintail, HA and LA cinnamon teal, HA and LA ruddy duck, speckled teal, Puna teal, northern pintail, green-winged teal, gadwall, and mallard duck), there were only modest increases in heart rate during progressive hypoxic exposure in some groups and oxygen pulse generally remained unchanged (Chapter 5). Both contributed almost equally to the maintenance of \( \dot{V}O_2 \) in severe hypoxia (Figure 7.2B). Because all groups maintained oxygen pulse during hypoxia exposure while arterial oxygen saturation (and hence \( \dot{C}_2O_2 \)) decreased by ~50%, either stroke volume must have increased or \( \dot{C}_2O_2 \) must have decreased appreciably (see Equation 7.3). Given that there is a finite capacity for \( \dot{C}_2O_2 \) to decrease and that stroke volume has been known to increase in all other waterfowl in this present
study (Figure 7.2B), increases in stroke volume to maintain oxygen pulse and thus $\dot{V}o_2$ are likely. In further support of this hypothesis, tissue oxygen extraction, measured as the percent of oxygen extracted from the blood, did not change significantly during hypoxia exposure in all other groups measured. Thus, I hypothesize that any $\dot{Q}$ increases in these 11 groups (Chapter 5) would have arisen primarily by the second strategy - increases in stroke volume.

### 7.2.2.2 Blood-oxygen carrying capacity

With regards to the third strategy, increasing blood-oxygen carrying capacity, all HA groups had an enhanced $\beta_o$ compared to closely related LA groups, and thus also an enhanced capacity of the blood to carry oxygen. Bar-headed geese and Andean geese had lower Hb-P$_{50}$ values, and thus higher blood-oxygen affinity (and $\beta_o$) than barnacle geese (Black and Tenney, 1980a; McCracken et al., 2010; Weber et al., 1993). HA Andean ducks (speckled teal, cinnamon teal, and yellow-billed pintail) had higher Hct and [Hb] compared to closely related LA taxa, but whether this enhanced blood-oxygen carrying capacity was accompanied by pathological increases in blood viscosity is unknown. Ruddy ducks, the only diving bird in this comparison, exhibited high Hct and [Hb] (Chapter 5) and low Hb-P$_{50}$ regardless of altitude (Natarajan et al., 2015). This may be because many diving mammals and birds regularly rely on their blood as an oxygen store during dives, and natural selection has enhanced [Hb] and blood volume, and decreased P$_{50}$ values (Butler, 2001; Kooymans and Ponganis, 1998; Meir and Ponganis, 2009).

### 7.2.3 Implications associated with different cardiovascular strategies

Of the three hypoxic cardiovascular response strategies observed in this thesis, almost all species predominantly increased stroke volume (Chapters 3-5) and, in the case of the HA taxa,
blood-oxygen carrying capacity (Chapters 3-5). Only the bar-headed geese showed large
increases in heart rate during progressive hypoxic exposure (Chapters 3 and 4), and no species
significantly increased tissue oxygen extraction (Chapters 3-5).

7.2.3.1 Heart rate

There are potential tradeoffs with the use of predominant increases in either heart rate or
stroke volume in the maintenance of oxygen delivery during hypoxia. One benefit of relying on
heart rate as a primary modulator of cardiovascular oxygen supply is its capacity to be rapidly
changed by altering sinoatrial node depolarization (chronotropy) (Klabunde, 2012). However,
increases in heart rate also result in proportional increases in myocardial oxygen demand because
the myocytes must generate proportional increases in the number of contraction cycles per
minute (e.g. 3-fold increases in heart rate result in ~3-fold increases in myocardial oxygen
demand because the myocytes must generate triple the amount of contraction cycles per minute)
(Klabunde, 2012). Conversely, increases in ventricular volume (e.g. increases in end diastolic
volume and thus increases in stroke volume) have a relatively small affect on myocardial oxygen
consumption due to the small role that volume plays in determining wall stress, which is the
primary determiner of myocardial oxygen consumption (Klabunde, 2012). In addition, increasing
heart rate beyond certain frequencies will decrease cardiac filling time (Farrell, 1991b;
Klabunde, 2012; Olson and Farrell, 2006), potentially at the expense of producing sufficient
stroke volume. Furthermore, in mammals coronary blood flow primarily occurs during diastole
(Klabunde, 2012). Assuming that this is true in part for birds (Smith et al., 2000), large increases
in heart rate could compromise coronary flow and thus myocardial oxygenation.
7.2.3.2 Stroke volume

Alternatively, modulating cardiovascular oxygen supply primarily by increasing stroke volume would allow for delivery of more blood (and thus more oxygen) per heartbeat. Increasing \( Q \) by predominant increases in stroke volume would be more energetically efficient and require less myocardial oxygen demand primarily because of the decreased number of contractions required to produce the same \( Q \), as discussed above (Klabunde, 2012). In addition, having greater reliance on stroke volume allows the heart to capitalize on the Frank-Starling effect, which describes the intrinsic ability of the heart to increase its force of ventricular contraction whenever preload or end diastolic volume increase (Farrell, 1991b; Klabunde, 2012). Moreover, assuming that most coronary blood flow in birds occurs during diastole, as it does in mammals (Klabunde, 2012), relying on increases in stroke volume rather than heart rate to achieve a given \( Q \) would theoretically allow greater time for coronary blood flow and myocardial oxygenation to occur as high heart rates can reduce coronary flow while also increasing myocardial oxygen demand (Klabunde, 2012). This may present a critical advantage when \( P_{aO_2} \) is decreased, such as at HA. One major detriment of stroke volume-modulated increases in oxygen supply is that, while stroke volume can be altered in some systems by venous return, the ability to increase stroke volume (and the magnitude to which a heart can actually achieve this) is ultimately determined and constrained by ventricular morphology - factors such as ventricular lumen volume, level of trabecularity, and cardiac muscle fibre contractility and compliance.

Ultimately, however, achieving levels of \( Q \) sufficient to sustain high oxygen-demanding activities in hypoxia such as flight would be most efficiently achieved by a combination of an increase in heart rate and stroke volume, thus providing blood circulation that is both high in
frequency and volume. Indeed, use of both heart rate and stroke volume (as likely implied by the constant oxygen pulse) to increase $\dot{Q}$ is seen in all of the 17 groups I studied (Figure 7.2B). Of these groups, bar-headed geese (which need to rapidly mount a cardiovascular response to support short-term HA performance) rely more heavily on increases in heart rate, whereas Andean geese (which are lifelong HA residents) rely more heavily on increases in stroke volume. I propose that short-term demanding HA performance (e.g. HA migration of bar-headed geese) necessitates rapid recruitment of convective flow (rapid physiological increases in heart rate) (Chapters 3-4), while permanent HA residency (e.g. Andean geese and potentially the 11 groups of waterfowl studied in Chapter 11) allows an arguably more efficient strategy potentially supported by changes in cardiac morphology (greater reliance on and capacity to increase stroke volume) (Chapters 4-5).

7.2.3.3 Blood-oxygen carrying capacity

Just as the convective components of the Fick equation have trade-offs, the same is true for the factors determining blood-oxygen carrying capacity. Factors altering blood-oxygen carrying capacity include the intrinsic affinity of Hb for oxygen (represented by $P_{50}$), Hct, [Hb], and various allosteric modulators. A benefit of having a low Hb-$P_{50}$ (or increased intrinsic blood-oxygen affinity) includes the capacity of blood to remain saturated at lower levels of $P_{\text{a}O_2}$, and thus also for blood to acquire oxygen in hypoxic environments. A natural disadvantage of having a low Hb-$P_{50}$ is that it would be more difficult to off-load oxygen. Physiological conditions in working endothermic muscles help mediate this apparent drawback by factors such as increased temperature and increased $[H^+]$ (decreased pH), among others – all of which serve to decrease Hb-oxygen affinity. The reverse of this effect was evident in hyperventilating HA bar-headed
geese in 0.05 F\(_2\)O\(_2\), where their respiratory alkalosis enhanced Hb-oxygen loading via a Bohr shift such that, despite having a lower intrinsic Hb-oxygen affinity than Andean geese, \textit{in vivo} bar-headed geese achieved a comparable higher blood-oxygen affinity (Chapter 4). Increased Hct and [Hb] will certainly serve to increase blood-oxygen carrying capacity, allowing each ml of blood to carry more oxygen; however, within every animal there is a level beyond which increasing these factors would increase blood viscosity and resistance to blood flow, thereby also increasing cardiac oxygen demand (Smith et al., 2000). Thus, the cardiovascular components of the Fick equation are all intrinsically related, with \(\beta\)\(_{O_2}\) determining the amount of oxygen in a ml of blood and ultimately the level at which the convective components (heart rate and stroke volume) will need to be elevated in order to maintain oxygen supply during hypoxia.

7.3 Research implications

From the data in this thesis I obtain a comprehensive understanding of the cardiovascular and ventilatory strategies used by waterfowl to maintain oxygen supply during hypoxic exposure at rest. Two unexpected findings from this study were the prominent role of increases in stroke volume to increases in \(\dot{Q}\) and overall oxygen supply (Chapters 3-5), as well as the impressive capacity of Andean geese and crested ducks to increase lung oxygen extraction during hypoxic exposure (Chapter 4).

Studies of how \(\dot{Q}\) changes occur in birds in response to hypoxia are relatively few in number, but have generally found that increases in heart rate were the primary contributor to increases in \(\dot{Q}\) with stroke volume remaining largely unchanged (Fedde et al., 1989; Kiley et al., 1979) (for reviews see: Dzal et al., 2015; Faraci, 1986; Smith et al., 2000). In contrast, I found that stroke volume increased in all studies in which it could be directly calculated (N=6 groups)
(Chapters 3 and 4), reaching very appreciable volumes in Andean geese (Chapter 4). While stroke volume was not calculated in the 11 HA and LA ducks studied in Chapters 5 and 6, I suspect it increased based on the reasoning discussed above in Section 7.2.2.1. Indeed, while oxygen pulse does not change appreciably during hypoxia in most of these 11 groups, it does contribute ~30-60% to the maintenance of $\dot{V}O_2$ upon exposure to severe hypoxia (Figure 7.2B). These increases in stroke volume could be mediated either by extrinsic factors (circulating hormones, neurotransmitters, or sympathetic innervation) or intrinsic factors (ventricular lumen volume, level of ventricular trabecularity, pericardium compliance, cardiac muscle fibre contractility and compliance, and cardiac muscle fiber contractile properties associated with the Frank-Starling response) (for reviews see: Dzal et al., 2015; Smith et al., 2000). These findings highlight the need to measure changes in stroke volume rather than just heart rate when investigating the contribution of changes in $\dot{Q}$ in waterfowl exposed to hypoxia. Without considering this information, using heart rate as a proxy for calculating metabolic rate, as is the practice in many avian telemetry studies, may fail to capture an integral component of the waterfowl response to hypoxia.

In addition, I observed increases in lung oxygen extraction up to ~90% in Andean geese and up to ~80% in crested ducks with no significant $\dot{V}R$ increases when exposed to progressive hypoxia (Chapter 4). This was unexpected because, despite the already substantially increased surface area and thinner diffusion barrier present in avian lungs (Powell, 2000; Scheid, 1990; West, 2009), bar-headed geese maintain lung oxygen extraction <40% during hypoxic exposure (Chapters 3 and 4). While the mechanisms underlying this extraordinary increase in lung oxygen extraction are unknown, possible explanations are a combination of any of the following functional (decreased Hb-P50, increased perfusion of the blood-gas surface area and increased
capillary recruitment) and morphological (increased lung capillarity, increased lung surface area, and decreased barrier thickness) enhancements. Because the increases in $\dot{Q}$ are not accompanied by similar increases in $\dot{V}_R$, the lungs of the Andean goose experience a decreased $\dot{V}_R/\dot{Q}$ in hypoxia (Chapter 4). If also associated with increased lung capillary recruitment, this would result in a functional increase in the surface area available for gas exchange. Increased diffusion capacity could also be achieved through increasing total lung capillarity. Of the two possible morphological changes – increased surface area and decreased barrier thickness, conceptually the most likely is increased surface area. While the already thin gas-perfusion barrier in birds is mechanically robust, further decreasing this thickness might compromise its structural integrity. Increased lung surface area has been documented in bar-headed geese, which have larger lungs and thus a larger lung surface area than LA birds (Scott, 2011). Thus, morphological increases in surface area could be achieved by increases in overall lung size as well in lung capillarity. As such, I hypothesize that the exceptional increases in lung oxygen extraction observed in Andean geese will be explained by a combination of functional factors (low Hb-P$_{50}$ of Andean geese, increased capillary recruitment with the decreased $\dot{V}_R/\dot{Q}$ present during hypoxia) and morphological factors (increased lung surface area, increased capillarity, and/or decreased barrier distance) acting in tandem. Mechanisms aside, such an enhanced ability to extract oxygen during hypoxic exposure at rest is an incredible physiological phenomenon that has not yet been described in any bird.

### 7.3.1 Summary

Altogether, this comprehensive study investigated how 17 species and subspecies of waterfowl (N=162), 15 groups (N=147) of which were studied in the wild at a range of altitudes
on three different continents (Chapters 3-6), regulate their cardiovascular and respiratory systems to maintain oxygen supply during hypoxic exposure. My research supports the hypothesis that multiple ventilatory and cardiovascular strategies are used to maintain oxygen supply during hypoxic exposure in waterfowl. While all ventilatory variables (breathing frequency, tidal volume, or lung oxygen extraction) contributed to the maintenance of oxygen supply (Figure 7.2A), I observed three distinct strategies, each primarily modulated by a different variable (Chapters 3 and 4). Indeed, while heart rate, stroke volume (or oxygen pulse, in the case of Chapter 5), and blood-oxygen carrying capacity all contributed to the waterfowl hypoxic cardiovascular response (Figure 7.2B), almost all species predominantly increased stroke volume (Chapters 3-5) and, in the case of the HA taxa, blood-oxygen carrying capacity (decreased $P_{50}$, increased Hct, and/or increased [Hb]; Chapters 3-5). Only the bar-headed geese increased heart rate substantially during progressive hypoxic exposure (Chapters 3 and 4). Moreover, my research also supports the hypothesis that lifelong exposure to HA can impact the cardiovascular and ventilatory responses to hypoxia, as seen with the lower $\dot{V}O_2$ and higher $\dot{V}_R$ of bar-headed geese reared at HA compared with those reared at LA (Chapter 3). Here I also provide the first HRV data for waterfowl, demonstrating that HA duck species generally have lower HRV than closely related LA duck species (Chapter 6). Finally, my research furthers the growing literature on the physiology of bar-headed geese (Chapters 3 and 4; Table 7.1). I propose that short-term demanding HA performance (e.g. HA migration of bar-headed geese) necessitates primarily functional enhancements (e.g. rapid recruitment of increases in heart rate and ventilation) (Chapters 3-4), while permanent HA residency (e.g. Andean geese and potentially the 11 groups of waterfowl studied in Chapter 11) is supported predominantly by structural enhancements (e.g. changes to lung and cardiac morphology permitting large increases in lung oxygen diffusion and
stroke volume) (Chapters 3-5). In summary, this thesis constitutes a large, comprehensive and comparative study indicating that there are multiple strategies by which waterfowl maintain oxygen supply by their ventilatory and cardiovascular systems during hypoxia, and that HA rearing and lifetime performance demands can impact these responses.

7.4 Future directions

This thesis has identified numerous directions for further research surrounding HA waterfowl physiology and the cardiovascular and ventilatory responses to hypoxia in waterfowl.

First, further studies are required to determine the underlying mechanisms of the metabolic and ventilatory differences reported in Chapter 3 between bar-headed geese reared at HA and LA, and the extent to which these differences may also facilitate HA flight. Common garden experiments should be conducted that incubate goose eggs and rear geese derived from the same population under controlled conditions at different altitudes and investigate their hypoxic responses. These experiments should also vary the severity of hypoxia exposure (i.e. level of altitude) and examine how hypoxic responses change throughout development. Because all baseline data in this thesis were collected at rest, it would be logical to conduct experiments that investigate similar cardiovascular and ventilatory responses during flight to see whether the different HVR and hypoxic cardiovascular response strategies are maintained during and serve to enhance HA performance.

Second, a number of studies remain to be conducted on the Andean waterfowl discussed in Chapters 5 and 6. In particular, after observing that the hypoxic cardiovascular response is mediated only in part by heart rate, it would be informative to conduct experiments measuring \( \text{C}_{\text{a}}\text{O}_2 \) and \( \text{C}_{\text{v}}\text{O}_2 \) to allow quantification of \( \dot{Q} \), stroke volume, and tissue oxygen extraction. In
addition to expanding these existing data sets, these would also serve to support or reject the hypothesis that $\text{Vo}_2$ is being maintained in part by increases in stroke volume rather than tissue oxygen extraction. It would also be informative to conduct these experiments on a greater number of Himalayan and Andean waterfowl species with carefully selected phylogenetic pairings to try to elucidate the physiological differences between HA and LA waterfowl and whether factors such as time at altitude or activity level impact the HVR and hypoxic cardiovascular response.

Third, the identification of increases in stroke volume as a major component of the cardiovascular response of waterfowl to hypoxia lends itself to a myriad of questions. The most obvious of these questions is: what are the mechanisms underlying such large stroke volume increases? Experiments could be conducted to target potential contributing external factors such as circulating hormones or neurotransmitters, as well as intrinsic cardiac factors, such as ventricular lumen volume, level of ventricular trabecularity, pericardium compliance, cardiac muscle fibre contractility and compliance, and cardiac muscle fiber contractile properties associated with the Frank-Starling response. Limited information is available on the avian cardiomyocyte Frank-Starling response (Shiels and White, 2008), a phenomenon known to facilitate substantial stroke volume increases in fish exposed to hypoxia (Farrell, 1991a; Shiels and White, 2008). Future studies of the underlying mechanisms that allow force development over such large ventricular volume changes under conditions of oxygen limitation would also be of value. In addition, it would be useful to compare the values of stroke volume obtained in these studies to those obtained through direct stroke volume measurement using a flow probe on the pulmonary artery or aorta or to those obtained through noninvasive indirect measurement using ultrasound. Validation of a portable, noninvasive technique such as ultrasound would greatly
reduce many logistical elements associated with measuring avian stroke volume in the field. As seen in Chapters 5 and 6, ECG measurements capture only a snapshot into the avian cardiovascular response to hypoxia. Future in vivo cardiovascular waterfowl studies should have a capacity to either directly measure or calculate stroke volume, and any studies intending to rely solely upon heart rate for calculating parameters such as metabolic rate should address the potentially substantial role of stroke volume.

Fourth, the data on HRV for waterfowl collected in this study would be much more informative in the context of studies examining the roles of vagal versus sympathetic inputs conducted using sympathetic and parasympathetic agonists and antagonists. This would inform what levels of sympathovagal balance exist during normoxia and hypoxia, in addition to providing information about whether overall generalities described in human Pointcaré plots (e.g. decreased HRV or less scatter in RR interval length is attributable to greater sympathetic tone) (Task Force of the European Society of Cardiology, 1996) apply to waterfowl. In addition, these data would also provide threshold values for very low frequency, low frequency, and high frequency that could be used to generate meaningful power spectra. Finally, reporting a greater variety of HRV parameters, or at least some of the most commonly used parameters, would also aid greatly in intra- and interspecies comparisons, as well as comparison with the literature.

The final major area of further research this thesis highlights is that of investigating how Andean geese and crested ducks are able to achieve such large increases in lung oxygen extraction. The preliminary studies currently being conducted provide intriguing insights and it would be prudent to compare the results from Andean geese to bar-headed geese (two species that use entirely different HVR strategies).
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Species</th>
<th>Scientific Name</th>
<th>Altitude</th>
<th>Study Type</th>
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<td>Barnacle goose</td>
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<td>HVR and HCVR</td>
</tr>
<tr>
<td>3</td>
<td>Bar-headed goose</td>
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<td>HVR and HCVR</td>
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<tr>
<td>4</td>
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<td>HCVR</td>
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<tr>
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<td>5 and 6</td>
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<td><em>Anas platyrhynchos</em></td>
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**Figure 7.1: A compilation of waterfowl studied in this thesis.**

For each waterfowl group this compilation summarizes the chapter containing the data of the group, the originating altitude of the group, as well as which response was studied: the hypoxic ventilatory response (HVR) and/or the hypoxic cardiovascular response (HCVR).
**Ventilatory Response**

\[ \dot{V}_{O_2} = f_R \times V_T \times E_{LO_2} \]

- Barnacle goose (LA)
- Bar-headed goose (LA)
- Bar-headed goose (HA) (Ch.3)
- Bar-headed goose (HA) (Ch.4)
- Andean goose (HA)
- Crested duck (HA)

**Cardiovascular Response**

\[ \dot{V}_{O_2} = f_H \times V_S \times E_{TO_2} \]

\[ \dot{V}_{O_2} = f_H \times O_2 \text{ pulse} \]

- Northern Pintail (LA)
- Cinnamon Teal (LA)
- Green-Winged Teal (LA)
- Gadwall (LA)
- Mallard (LA)
- Ruddy Duck (LA)
- Puna Teal (HA)
- Yellow-billed Pintail (HA)
- Cinnamon Teal (HA)
- Speckled Teal (HA)
- Ruddy Duck (HA)
Figure 7.2: A synthesis comparing the relative contributions of ventilatory and cardiovascular variables to oxygen consumption.

Data from Chapters 3-5 is compiled to compare the relative percent contribution of fold changes in Fick equation variables to the maintenance of oxygen consumption (VO₂) from ambient fractional inspired levels of oxygen (FIO₂) to the most severe level of hypoxia (0.07 FIO₂ for crested ducks and the cardiovascular parameters of low-altitude (LA) bar-headed geese; 0.05 FIO₂ for both populations of high-altitude (HA) bar-headed geese, barnacle goose, Andean goose, and the ventilatory variables of LA bar-headed geese; 0.06 FIO₂ for all remaining birds). Fick equation variables include: f as breathing frequency, VT as tidal volume, EO₂ as lung oxygen extraction or FIO₂-FEO₂, fH as heart rate, Vₜ as stroke volume, and ETₐO₂ as tissue oxygen extraction or CaO₂-CvO₂. The coloured bars represent the relative fold contribution of the variable of like-colour to the maintenance of VO₂ during severe hypoxia in terms of percent contribution. (A) Percent contributions of ventilatory variables to VO₂ maintenance during hypoxia, including breathing frequency (fₐ), tidal volume (VT), and lung oxygen extraction (EO₂). (B) Percent contributions of cardiovascular variables to VO₂ maintenance during hypoxia, including heart rate (fH), cardiac stroke volume (Vₜ), and tissue oxygen extraction (ETₐO₂). The Fick equation is also expressed in terms of percent contributions of heart rate and cardiac oxygen pulse (the product of Vₜ and the difference in arterial and venous oxygen content) for the birds in which cardiac output was not measured. The relative percent contribution of changes in Fick equation variables to VO₂ was calculated as follows: (1) the fold change in a variable from 0.12 FIO₂ to the most severe hypoxic exposure was calculated as X_H/X_0.12, where X_H is the value of the variable in hypoxia and X_0.12 is the value at 0.12 FIO₂; (2) the “hypoxic ratio” was calculated as being equal to the following: (0.12 x VO₂_0.12)/(FIO₂_H x VO₂_H) where VO₂_0.12 is the VO₂ under 0.12 FIO₂, (FIO₂_H) is the FIO₂ of the most severe hypoxic exposure (H), and VO₂_H is the VO₂ during the most severe hypoxic exposure (H); (3) the ratio of the fold change (1) and hypoxic ratio (2) were calculated to equal Y for each contributing variable; (4) the sum of Y for all contributing variables to VO₂ was calculated and represented by Z (e.g. Yfₐ+YVT+YET₀₂=Z); and (4) the relative percent contribution of fold changes in a given Fick equation component to VO₂ (the value plotted in this figure) was calculated for each component as Y/Z x 100.
Table 7.1: Ventilatory, metabolic, and cardiovascular variables during progressive hypoxia exposure in bar-headed geese.
Mean data from the literature (1: Black and Tenney, 1980a; 2: Fedde et al., 1989; 3: Scott and Milsom, 2007; 4: Hawkes et al., 2014) for a suite of variables in low-altitude bar-headed geese are compared when provided with data presented in (5) Chapter 3 on low-altitude-reared (LAR) bar-headed geese and (6) high-altitude-reared (HAR) bar-headed geese and the data for (7) high-altitude (HA) bar-headed geese presented in Chapter 4. Note that the ventilatory data and cardiovascular data for LAR bar-headed geese are taken from two different study groups, as presented in Chapter 3. Ventilatory data are expressed in either standard temperature and pressure, dry (STPD) or body temperature and pressure, saturated (BTPS) and are labeled accordingly in the table when this information was disclosed in the paper. Variables expressed in the table are as follows: arterial partial pressure of oxygen (P$_{O_2}$), arterial partial pressure of carbon dioxide (P$_{CO_2}$), arterial pH (pH$_a$), arterial oxygen content (C$_{O_2}$a), venous partial pressure of oxygen (P$_{O_2}$v), venous partial pressure of carbon dioxide (P$_{CO_2}$v), venous pH (pH$_v$), venous oxygen content (C$_{O_2}$v), hematocrit (Hct), total blood hemoglobin concentration ([Hb]), oxygen consumption (V$_{O_2}$), carbon dioxide production (V$_{CO_2}$), respiratory quotient (RQ; V$_{CO_2}$/V$_{O_2}$), total ventilation (V$_R$), tidal volume (V$_T$), breathing frequency (f$_R$), air convection requirement (ACR), lung oxygen extraction (E$_l$O$_2$) cardiac output (Q), stroke volume (V$_S$), heart rate (f$_H$), blood convection requirement (BCR), tissue oxygen extraction (E$_t$O$_2$), difference in arterial and venous oxygen content (C$_{O_2}$a-C$_{O_2}$v), total peripheral resistance (TPR), and the ventilation-perfusion ratio (V/Q).

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164
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<td>184 323 -- 261 515</td>
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<td>\textit{f}\textsubscript{hu}</td>
<td>min\textsuperscript{-1}</td>
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