Cardiovascular responses to progressive hypoxia in ducks native to high altitude in the Andes

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Summary Statement: An increased blood-oxygen carrying capacity provides high altitude ducks in the Andes with a greater reserve for O_2 delivery in hypoxia.

ABSTRACT

The cardiovascular system is critical for delivering O_2 to tissues. Here we examine the cardiovascular responses to progressive hypoxia in four high-altitude Andean duck species compared to four related low-altitude populations in North America, tested at their native altitude. Ducks were exposed to stepwise decreases in inspired partial pressure of O_2 while we monitored heart rate, O_2 consumption rate, blood O_2 saturation, haematocrit (Hct), and blood haemoglobin concentration [Hb]. We calculated O_2 pulse (the product of stroke volume and the arterial-venous O_2 content difference), blood O_2 concentration, and heart rate variability. Regardless of altitude, all eight populations maintained O_2 consumption rate with minimal change in heart rate or O_2 pulse, indicating that O_2 consumption was maintained by either a constant arterial-venous O_2 content difference (an increase in the relative O_2 extracted from arterial blood) or by a combination of changes in stroke volume and the arterial-venous O_2 content difference. Three high-altitude taxa (yellow-billed pintails, cinnamon teal, and speckled teal) had higher Hct and [Hb], increasing the O_2 content of arterial blood, and potentially providing a greater reserve for enhancing O_2 delivery during hypoxia. Hct and [Hb] between low- and high-altitude populations of ruddy duck were similar, representing a potential adaptation to diving life. Heart rate variability was generally lower in high-altitude ducks, concurrent with similar or lower heart rates than low-altitude ducks, suggesting a reduction in vagal and sympathetic tone. These unique features of the Andean ducks differ from previous observations in both Andean geese and bar-headed geese, neither of which exhibit significant elevations in Hct or [Hb] compared to their low-altitude relatives, revealing yet another avian strategy for coping with high altitude.

INTRODUCTION

A major role of the cardiovascular system is to maintain adequate delivery of oxygen (O_2) to the tissues to support rates of O_2 consumption ($\dot{V}o_2$) both at rest and during periods of increased activity. This becomes challenging when the environmental partial pressure of O_2 (PO_2) falls, such as at high altitude (hypobaric hypoxia). In species adapted to live under hypoxic (low O_2) conditions, cardiovascular enhancements to improve tissue O_2 transport have arisen from many sources (e.g. larger hearts and thus larger stroke volumes, expansion of vascular networks to enhance tissue diffusion capacity, increased haematocrit (Hct), changes in protein sequence and structure that increase haemoglobin (Hb)-O_2 binding affinity, and/or increases in heart rate) (Black and Tenney, 1980; Faraci, 1991; Jessen et al., 1991; Butler, 2010; Scott et al., 2015). Studies have now shown that high-altitude resident Andean geese (*Chloephaga melanoptera*) and crested ducks (*Lophonetta specularioides alticola*) have evolved fundamentally different mechanisms for maintaining O_2 supply during hypoxia than bar-headed geese (*Anser indicus*) that migrate transiently at high altitude. Bar-headed geese respond to hypoxia with robust increases in ventilation and heart rate, whereas Andean species increase lung O_2 extraction and cardiac stroke volume (Black and Tenney, 1980; Lague et al., 2016; Lague, 2017; Lague et al., 2017). Neither of these species exhibits significant elevations in Hct or blood Hb concentration compared to their low-altitude relatives (Black and Tenney, 1980; Lague et al., 2016) but both species have Hb with high O_2 affinity (Black and Tenney, 1980; Weber et al., 1993). Based on these data, it was suggested that transient high-altitude performance has favoured the evolution of robust convective O_2 transport recruitment in hypoxia (increased breathing frequency and heart rate), whereas lifelong high-altitude residency has favoured the evolution of enhancements to the lungs and heart that increase lung diffusion and stroke volume (Lague, 2017).

The extent to which these cardiovascular features of high-altitude resident Andean geese and crested ducks are common to other high-altitude resident bird species, however, is unknown. Recently populations of several duck species (family Anatidae) from multiple high-altitude sites in Perú (e.g., Lake Titicaca at ~3800 m above sea level; Chancay River at ~3000-4100 m) were compared to low-altitude populations of the same species or to closely related lowland congeners to test the effect of high-altitude colonization on the hypoxic ventilatory responses and respiratory mechanics via independent comparisons (York et al., 2017; Ivy et al., 2019). Genetically-based increases in Hb-O_2 binding affinity (McCracken et al., 2009a; Natarajan et al., 2015), reductions in work of breathing (York et al., 2017), and

increases in pulmonary ventilation (Ivy et al., 2019) were observed in some highland taxa but not in others. This suggests that the hypoxia responses of closely-related resident species that independently colonized high altitude might be divergent.

The purpose of the present study, therefore, was to compare the components of O_2 transport capacity in the same high-altitude resident species and their low-altitude resident sister populations. This approach allowed four independent comparisons of the effect of adaptation to high altitude on cardiovascular O_2 transport. All of these species are derived from low-altitude waterfowl populations (Johnson and Sorenson, 1999), but exhibit differences in genetic divergence that are suggestive of varying degrees of genetic isolation and evolutionary time at high altitude (McCracken et al., 2009b; Wilson et al., 2013; Munoz-Fuentes et al., 2013; Graham et al., 2018). We wished to determine whether the high-altitude populations would demonstrate convergent or divergent enhancements in circulatory O_2 transport during progressive hypoxia, and the extent to which their responses to hypoxia were similar or distinct from those observed in previous studies of the high-altitude resident Andean goose and the high-altitude migrant bar-headed goose (Lague et al., 2017).

MATERIALS AND METHODS

Waterfowl

Our study was conducted in conjunction with Ivy et al. (Ivy et al., 2019), and the cardiovascular measurements documented in this present manuscript were acquired simultaneously from eight of the same species. As described earlier (Ivy et al., 2019), four of the species of ducks were captured and tested at high altitude (3,812 m) at the Lake Titicaca National Reserve (near Puno, Peru) in August 2014: yellow-billed pintails (*Anas georgica*; 0.61 ± 0.02 kg, N = 12) cinnamon teals (*Spatula cyanoptera orinomus*; 0.44 ± 0.01 kg, N = 12), ruddy ducks (*Oxyura jamaicensis ferruginea*; 0.73 ± 0.07 kg, N = 6), and speckled teals (*Anas flavirostris oxyptera*; 0.37 ± 0.01, N = 10). Subsequently in July 2015, four related low-altitude taxa, were captured at low altitude in Oregon, USA (at either Summer Lake Wildlife Area at 1,260 m or Malheur National Wildlife Refuge at 1,256 m), and were tested at Summer Lake Wildlife Area: northern pintails (*Anas acuta*; 0.84 ± 0.01 kg, N = 10), cinnamon teals (*Spatula c. septentrianolium*; 0.31 ± 0.01 kg, N=11), ruddy ducks (*Oxyura j. jamicensis*; 0.42 ± 0.04 kg, N = 8), and green-winged teals (*Anas crecca carolinensis*; 0.29 ± 0.01 kg, N = 10).

Comparisons were made at either the subspecies level (high versus low-altitude populations comprising subspecies for cinnamon teal and ruddy duck) or the species level

(high-altitude yellow-billed pintail versus low-altitude northern pintail and high-altitude speckled teal versus low-altitude green-winged teal). Among these, the yellow-billed pintail and cinnamon teal are the most recent species to diverge from their low-altitude ancestor(s) and arrive at high altitude based on mitochondrial DNA divergence (McCracken et al. 2009b; Wilson et al., 2013). The speckled teal has had the longest time at altitude since diverging from its low-altitude ancestors (Graham et al., 2018). The ruddy duck likely lies more intermediate in evolutionary time since divergence from its low-altitude ancestors (Munoz-Fuentes et al., 2013).

Mallard ducks (*Anas platyrhynchos*; 0.96 $\pm$ 0.02 kg, N = 8), and gadwalls (*Mareca strepera*; 0.75 $\pm$ 0.04 kg, N = 8) were also captured in Oregon and puna teals (*Spatula puna*; 0.40 $\pm$ 0.01 kg, N = 8) at Lake Titicaca. Since we do not have data from sister taxa for these groups the data we obtained from them is included only in the supplemental material. They do provide data for one additional high-altitude species and two other low-altitude species,

All ducks were held overnight to recover from capture (at least 6-12 h) prior to experimentation. The birds were held in large animal kennels with dry bedding and unlimited access to water. They were tube-fed a slurry of commercial duck chow if held for longer than 1 day in captivity, but deprived of food 6-12 hours before measurements took place. Ducks were collected in accordance with permits issued by the Ministerio del Ambiente del Peru (004-2014-SERNANP-DGANP-RNT/J), Ministerio de Agricultura del Peru (RD 169-2014-MINAGRI-DGFFS/DGEFFS and 190-2015-SERFOR-DGGSPFFS, U.S. Fish and Wildlife Service Migratory Bird Treaty Act I/E (MB68890B-0), and Oregon Department of Fish and Wildlife (Scientific Taking Permit 101-15). All experimental procedures followed guidelines established by the Canadian Council on Animal Care, and were approved by institutional animal care committees.

Acute hypoxic responses

We measured the cardiovascular and metabolic responses to acute hypoxia using techniques similar to those used previously for Andean geese and bar-headed geese (Lague et al., 2016; Ivy et al., 2019). Ducks were placed in a cradle that allowed unrestricted breathing, with their head in a 4 L opaque chamber that was sealed around the neck with a latex collar and provided with ambient air supplied to the head chamber at 5 l min^{-1}. Flow-through respirometry was used to measure O_2 consumption. The birds were outfitted with a three-lead ECG to measure heart rate and heart rate variability. Arterial O_2 saturation was also measured in a subset of birds, for which a 3-cm tall band was plucked free of feathers on their necks to

accommodate a MouseOx Pulse Oximeter (Starr Life Sciences, PA, USA). Ducks were given 60-90 min to adjust to the apparatus prior to beginning measurements, by which time they exhibited a stable, slow breathing pattern and routine heart rate. Measurements of heart rate and metabolism under these ambient conditions (~13 or 18 kPa O_2 at high- or low- altitude, respectively) were recorded for an additional 25 min, after which ducks were exposed to 25-min stepwise decreases in inspired O_2 tension (PO_2): 13 (for low-altitude ducks only), 12, 9, 7, and 6 kPa, followed by a 25-min recovery to ambient PO_2. Dry incurrent air and nitrogen were mixed using pre-calibrated rotameters (Matheson Model 7400 Gas Mixer, E700 and E500 flowtubes, Oakville, ON, Canada) at a flow rate of 5-10 L min^{-1} to achieve each level of hypoxia. At the end of the experiment, blood was collected from most birds (see below) by pricking a superficial leg vein with a lancet. Haematocrit (%) was determined in duplicate by a Zipocrit haematocrit centrifuge (LW Scientific, Lawrenceville, GA, USA). Haemoglobin concentration (g dl^{-1}) was acquired at the end of the experiment using a Hemocue Hb 201$^+$ System (Ängelholm, Sweden). A subset of representative individuals were euthanized with an overdose of intravenous propofol, as the number of euthanized individuals was limited due to permit regulations (3 northern pintails, 3 low-altitude cinnamon teals, 5 low-altitude ruddy ducks, 2 green-winged teals, 6 yellow-billed pintails, 10 high-altitude cinnamon teals, 8 high-altitude ruddy ducks, and 6 speckled teals), and heart mass was measured. Total heart mass is reported standardized to body mass (g kg^{-1}).

Measurements and data analysis

Heart rate, metabolism, and arterial O_2 saturation were measured continuously during the hypoxia exposures, and we report the average values across the last 10 min at each inspired PO_2. The excurrent air leaving the head chamber was subsampled at 200 ml min^{-1}, dried with silica gel (MLA6024, ADInstruments, Colorado Springs, CO, USA), and passed through CO_2 and O_2 analyzers (FOXBOX, Sable Systems, Las Vegas, NV, USA). These data were used to calculate $\dot{V}O_2$, as described by Lighton (Lighton, 2008), and expressed here in volume units at standard temperature and pressure dry (STPD). The ECG was continuously monitored 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. All of the above data were acquired using a PowerLab 16/32 and Labchart 8 Pro software (ADInstruments). A 5-min interval at the 15-minute time point of each exposure was analyzed using PowerLab heart rate variability analysis software (ADInstruments, Colorado Springs, CO, USA). RMSSD (root mean

squared of the successive differences in ms), the most common time domain measure for heart rate variability, and SDRR (standard deviation of the R-R interval in ms), a measure of total variability, were derived from this program (Khandoker et al., 2013). Cardiac O_2 pulse (ml O_2 kg^{-1}) was calculated as the quotient of $\dot{V}o_2$ and heart rate. Arterial O_2 content was calculated from measures of [Hb] and arterial O_2 saturation (Table S1). We multiplied the molar Hb concentration by 4 and by fractional O_2 saturation to get the molar O_2 content. Arterial O_2 saturation was measured in all low-altitude species. It was only measured in a subset of the high-altitude ducks (speckled teal: N = 6; Andean ruddy duck: N = 5; yellow-billed pintail: N = 6; cinnamon teal: N = 5). For the speckled teal we obtained O_2 saturation data from birds from which we did not get blood samples. As a result, for these species, we used the mean values of Hb concentration for the species and multiplied them by the individual O_2 saturation values to estimate arterial O_2 content values.

Statistical Analysis

Two-factor ANOVA was used to examine the main effects and interactions of altitude and acute inspired PO_2 (repeated measure) within each independent pair of closely related high-altitude and low-altitude taxa. We tested for pairwise differences between populations/species within each inspired PO_2 using Holm-Sidak post-tests. Comparisons between groups began at 13 kPa. Variables were transformed when they did not meet assumptions for either normality or equal variance analyzed for a two-way repeated measures ANOVA. Student t-tests were used to compare haematocrit, Hb concentration, and heart mass relative to body mass between high-low altitude pairs. Tests that failed normality were run with a Mann-Whitney Rank Sum Test.

Statistical analyses were carried out using SigmaStat (version 3.0; Systat Software) with a significance level of $P < 0.05$. Values are reported as mean ± S.E.M.

RESULTS

Progressive hypoxic exposure

Despite the progressive fall in inspired PO_2, all eight duck taxa maintained their $\dot{V}o_2$ (Fig. 1). $\dot{V}o_2$ was similar between the low- and high-altitude populations of three of the four high-low pairs. It was elevated in the high-altitude yellow-billed pintails compared to the northern pintails at the lower levels of P_IO_2 ($P = 0.007$).

All of the duck populations maintained heart rate during progressive hypoxic exposure (Fig. 2). The heart rate of low-altitude green-winged teals was significantly greater than that of high-altitude speckled teals across all PO_2 (P < 0.003). Similarly, low-altitude cinnamon teals exhibited a higher overall heart rate than their high-altitude counterparts (P = 0.019), which was particularly evident at the three most severe levels of hypoxia even though there was no significant effect of hypoxia within either population.

Cardiac O_2 pulse was calculated as the quotient of $\dot{V}O_2$ and heart rate (Fig. 3), which from the Fick equation equals the product of stroke volume and the difference between arterial and venous O_2 content. Seven of the duck populations maintained O_2 pulse during progressive hypoxic exposure, whereas O_2 pulse increased transiently during hypoxic exposure in the high-altitude yellow-billed pintails (P < 0.001). Both yellow-billed pintails and speckled teal (P = 0.028) had a higher O_2 pulse than their low-altitude counterparts at the most severe level of hypoxia.

The mean SDRR (Fig. 4) and RMSSD (Fig. 5) did not change significantly during either hypoxic exposure or ambient recovery in any group, except for a modest increase in SDRR and RMSSD in high-altitude ruddy ducks at 6 kPa O_2 and a slight drop in RMSSD in the green-winged teal at 6 kPa O_2 (P = 0.035). Nevertheless, except for the comparison between high-altitude yellow-billed pintails and low-altitude northern pintails, SDRR and RMSSD were significantly lower in the high-altitude taxa of all high-low altitude pairs. High-altitude ruddy ducks and high-altitude speckled teals exhibited a lower SDRR than their low-altitude counterparts at all exposure levels (P < 0.001), and high-altitude cinnamon teals exhibited a lower SDRR than low-altitude cinnamon teals at all exposure levels except ambient (Figs. 4, 5). Thus, the high-altitude groups generally exhibited lower SDRR and RMSSD compared to the low-altitude groups.

Blood O_2 content

Progressive hypoxia significantly decreased arterial O_2 content in all groups of ducks (Fig. 6). In three of the four high-low pairs, the high-altitude population exhibited significantly higher arterial O_2 content than its low-altitude counterpart except at the most severe levels of inspired PO_2. This was largely a result of increases in blood Hb content, as both Hb content and haematocrit were higher in the high-altitude population of each of these three high-low pairs (Table 1). In only the yellow-billed pintail did differences in arterial O_2 content also result from increases in arterial O_2 saturation (Table S1). The high-altitude ruddy

duck was the exception among the high-low pairs, with no significant differences in haematocrit, Hb concentration or O_2 content between highland versus lowland populations, and unlike the other species in our study, no significant differences in Hb-O_2 affinity between low- and high-altitude ruddy duck populations (Natarajan et al., 2015).

Heart mass relative to body mass was generally similar between high-low pairs. An exception to this was that heart mass relative to body mass was significantly greater in high-altitude speckled teals compared to low-altitude green-winged teals (P = 0.028). A similar difference in heart mass in high-altitude cinnamon teals compared to low-altitude cinnamon teals was marginally insignificant (P = 0.052) (Table 1).

Post-hypoxic recovery

In all populations arterial O_2 content returned to starting values within 5 min of return to breathing ambient air (data not shown). Heart rate, however, tended to increase during recovery in all groups (Fig. 7), reaching statistical significance in four of the eight populations (low-altitude northern pintails (P < 0.001), both high- and low-altitude cinnamon teal populations (P < 0.001), and low-altitude green winged teals (P < 0.001)). Heart rate was also significantly higher at 5- and 20-min of recovery in all low-altitude groups compared to their related high-altitude pair with the exception of high- and low-altitude ruddy ducks, whose heart rates did not differ significantly. O_2 pulse tended to decrease relative to normoxic starting values during the post-hypoxic recovery in all groups (Fig. 7). This decrease was significant in low-altitude northern pintails (P = 0.004), high-altitude yellow-billed pintails (P < 0.001), low-altitude cinnamon teals (P < 0.001), and high-altitude speckled teals (P = 0.002) (Fig. 7). Since $\dot{V}o_2$ did not change during recovery, the changes in heart rate and O_2 pulse tended to compensate each other.

The responses to stepwise hypoxia exposure in the high-altitude Puna teal, low-altitude gadwall, and low-altitude mallard, for which there were no corresponding low/high altitude pairs, are provided in the supplemental materials: arterial O_2 saturation (Table S1); $\dot{V}o_2$, heart rate, O_2 pulse, arterial O_2 content, SDRR, and RMSSD (Table S2); hematological parameters, body mass, and relative heart mass (Table S3).

DISCUSSION

The present study compared the cardiovascular responses to progressive hypoxia exposure in four species of high-altitude duck to those of four closely related low-altitude ducks that represent sister populations/subspecies or congeneric species. In general, all eight taxa maintained O_2 consumption rate with little, if any, change in heart rate or O_2 pulse during hypoxia. Heart rates were significantly lower in the high-altitude taxon in half of the species pairs. The most overarching difference between high- and low-altitude populations was in blood O_2 content and hence in blood O_2 carrying capacity, which was higher in all high-altitude species except for the ruddy duck. Finally, there were intriguing differences between species pairs that are discussed in terms of convergence/divergence and time at altitude in the sections that follow.

Progressive hypoxic exposure

During progressive hypoxic exposure all birds maintained $\dot{V}o_2$ (Fig. 1) indicating that O_2 delivery was likely not limiting $\dot{V}o_2$ even at 6 kPa PO_2. Heart rate also generally remained constant during progressive hypoxia in all taxa (Fig. 2). This is similar to what has been reported in previous studies on other Andean waterfowl, such as Andean geese and crested ducks (Lague et al., 2017). By contrast, several studies on bar-headed geese identified a strong tachycardia in response to hypoxia (Faraci, 1991; Black and Tenney, 1980; Lague et al., 2016). Heart rate variability was unaffected by hypoxia in any species.

The O_2 pulse (the product of stroke volume and arterial-venous O_2 content difference) also did not change with progressive hypoxia (except in the yellow-billed pintail at 9 and 7 kPa O_2, but not 6 kPa O_2), despite declines in arterial O_2 content of more than 50%. If stroke volume and the arterial-venous O_2 content difference remained constant, venous PO_2 must have been very low. It is also possible that the arterial-venous difference in O_2 content was reduced, ameliorating the reduction in venous PO_2 and helping retain the diffusion gradient, while the stroke volume increased to offset this decline and maintain a constant O_2 pulse. This is highly speculative but would be consistent with recent studies on Andean geese, crested ducks, bar-headed geese, and barnacle geese (*Branta leucopsis*) (Lague et al., 2016; Lague et al., 2017).

Comparisons between low- and high-altitude species

For the ruddy ducks, there were no differences between high and low-altitude populations for any variable measured or calculated other than heart rate variability. Ruddy ducks were the only diving species in this study, however, and many diving mammals and birds exhibit enhanced Hb concentration and Hb-O_2 affinity commensurate with the use of blood as an O_2 store for diving (Butler, 2001; Kooyman and Ponganis,1998; Meir and Ponganis, 2009). In ruddy ducks for example, the low- and high-altitude populations exhibit a relatively high Hb-O_2 affinity that does not differ significantly between populations, nor does it differ from the other high-altitude waterfowl populations (P_{50} = 28.5-30.0 at standard concentrations of KCl and IHP; Table S2 in Natarajan et al. 2015). The diving lifestyle thus may account for the elevated Hb concentration and Hb-O_2 affinity and arterial O_2 content seen in even low-altitude ruddy ducks. Note that the responses of the high-altitude ruddy ducks were similar to those of all the other high-altitude species; it is the low-altitude population that differs.

For two of the other three matched pairs, $\dot{V}o_2$ was similar between high- and low-altitude counterparts. $\dot{V}o_2$ was higher in the yellow-billed pintail than in the northern pintail, this was because the northern pintail had a relatively low $\dot{V}o_2$ compared to all other species. The $\dot{V}o_2$ of the yellow-billed pintails was similar to that of all the other species. Interestingly, the heart rate was lower in the high-altitude cinnamon teals and speckled teals compared to their low-altitude counterparts but this was not the case for the yellow-billed pintails compared to the northern pintails. This, however, again reflects the low heart rates in the northern pintails. The heart rates of the yellow-billed pintails were similar to those of the other high-altitude species.

Hematocrit and Hb concentration were significantly higher in all high-altitude groups except the ruddy ducks. Also, the Hb-O_2 affinity values from the literature are higher (i.e. P_{50} values are lower) in the two high-altitude groups studied here compared to their close low-altitude relatives for which there are data (Natarajan et al., 2015) (Table 1). The increased Hb-O_2 binding affinity in the high-altitude birds is consistent with data for many animals endemic to high altitude (Natarajan et al., 2015; Storz, 2010; Storz, 2016). The net result was that the arterial O_2 content was elevated in all of the high-altitude birds with the exception of ruddy duck giving the high-altitude populations a larger reserve for increasing circulatory O_2 supply during hypoxia.

Despite the benefit to O_2 carrying capacity, increases in haematocrit and Hb concentration increase blood viscosity, and when large enough (polycythemia) are thought to be maladaptive because the increased blood viscosity increases resistance to blood flow and places greater demands on the heart (Smith et al., 2000; Dempsey and Morgan, 2015). The relative contributions of evolutionary adaptation and environmentally-induced phenotypic plasticity to the variation in haematology are unclear. Increases in haematocrit and Hb concentration are a common occurrence in sojourners that acclimatize to altitude, as well as for many native highlanders with widely different durations of hypoxic exposure in their ancestry (e.g. from 3 to 4 generations in the natives of Leadville, Colorado to 40 to 50 generations in Andean natives) (Dempsey and Morgan, 2015). They are not present, however, in Tibetan natives with 30,000 years of exposure to altitudes above 3,000 m (Dempsey and Morgan, 2015), potentially due to allelic differences at the *EPAS1* locus, which encodes hypoxia inducible factor 2α (HIF2α) (Simonson et al., 2010). High-altitude acclimation increased haematocrit and Hb concentration in populations of both high- and low-altitude deer mice raised at low altitude, but less so in the high-altitude populations (Lui et al., 2015). Low-altitude Pekin ducks (domestic mallard) acclimated to simulated high altitude (5,640 m) increased haematocrit from 45.4% to 55.9% (Black and Tenney, 1980); however, in bar-headed geese, haematocrit and Hb concentration were unchanged when acclimated to the same simulated high-altitude conditions (5,640 m) (Black and Tenney, 1980) or when reared at high altitude (3,200 m) (Lague et al., 2016; Lague et al., 2017).

Heart Rate Variability

High-altitude ducks generally exhibited less heart rate variability (HRV, as quantified by SDRR and RMSSD) than closely-related low-altitude waterfowl species or populations. The only exception was the lack of significant difference between the HRV of low-altitude northern pintails and high-altitude yellow-billed pintails. This finding was contrary to our hypothesis, as well as to findings in the literature for rats and humans acclimated to high altitude (Cornolo et al., 2004; Melin et al., 2003; Sharshenova et al., 2006). The relationship between heart rate and HRV is complex and generally understudied even in mammals (Coumel et al., 1994k). 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). Indeed, studies on mammals suggest that the increases in heart rate during acute hypoxia result from 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 ventricle of rats (Kacimi et al., 1992), as well as to a decrease in adrenoreceptor density in the right ventricle (Morel et al., 1999). The net result is to reduce the impact of hypoxia on HRV and restore chronotropic sensitivity. The lower HRV with normal or lower heart rates in the high-altitude 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 that high-altitude pikas (*Ochotona* spp.) exhibit blunted parasympathetic and sympathetic modulation of heart rate, in association with reduced expression of muscarinic m2 receptors and β_1-adrenoreceptors in the cardiac ventricles (Pichon et al., 2013). A reduction in the importance of the sympathetic nervous system may also have evolved in high-altitude deer mice, in which catecholamine release from the adrenal medulla is blunted in association with reductions in the expression of enzymes involved in catecholamine biosynthesis (Scott et al. 2019; Schweizer et al. 2019). Although the mechanisms underlying the observed changes in HRV in high-altitude ducks remain to be discovered, our results here are consistent with these previous studies suggesting that changes in autonomic tone may be involved in coping with the challenges at high altitude.

Post-hypoxic recovery

Although heart rate and O_2 pulse did not change significantly during progressive hypoxia, recovery from severe hypoxia was associated with a general tachycardia and reduced O_2 pulse. Since $\dot{V}o_2$ did not change, the two compensated for each other. These changes were present up to 20 min after the animals had returned to breathing ambient levels of O_2. This suggests that the changes were not in response to the progressive hypoxia, raising the possibility that they were a response to a metabolic acidosis that may have developed during exposure to the most severe levels of hypoxia, a suggestion that remains to be explored.

Time at altitude

The high-altitude speckled teal diverged from their low-altitude ancestors approximately one million years ago or more, as probably did the Andean goose (Graham et al., 2018; McCracken unpubl. data). In contrast, the populations of yellow-billed pintails, cinnamon teal, and ruddy ducks have likely only been established at altitude for multiples of tens of thousands of years, as estimated by population divergence times calculated from genetic data (McCracken et al., 2009b; Wilson et al., 2013; Munoz-Fuentes et al., 2013). Ignoring the ruddy ducks again, the data suggest that the primary adaptations seen here (increased [Hb] and Hb-O_2 affinity) evolved quickly. While the yellow-billed pintail/northern pintail pair exhibit differences not seen in the cinnamon teals and the speckled teal/green winged teal pair, this was due to differences in the responses of the low-altitude populations.

CONCLUSIONS

The purpose of the present study was to compare the effects of hypoxia on the circulatory O_2 transport in the high-altitude populations of four different duck species with low-altitude populations of the same species or closely related low-altitude congeners. Specifically, we asked whether the high-altitude species/populations would demonstrate convergent patterns of enhanced circulatory O_2 transport during progressive hypoxia similar to that of the resident Andean goose and distinct from that of the migratory bar-headed goose, or whether these species would exhibit divergent responses to high altitude.

Surprisingly, all species, low and high-altitude residents, were able to maintain relatively constant levels of O_2 consumption down to 6 kPa inspired O_2 and did so with no change in heart rate. The O_2 pulse also did not change indicating that O_2 consumption was maintained by either maintaining a constant a-vO_2 difference (which would represent an increase in the relative % O_2 extracted from arterial blood) or by some combination of changes in stroke volume and the arterial-venous O_2 content difference.

What differentiated high- vs low-altitude ducks for three of the four high-altitude duck taxa was their higher arterial O_2 content in association with higher blood Hb concentrations and Hb-O_2 affinities. A different three of four highland duck taxa exhibited lower heart rate variability, which when combined with heart rates that were similar or lower than those in lowland taxa, suggest potential reductions in both vagal tone and sympathetic tone. The exception was the ruddy ducks for which no differences were found in any variable measured between the low and high-altitude populations, which is hypothesized to reflect

adaptation of the low-altitude population for diving (or exaptation in the case of the high-altitude population). Therefore, several high-altitude resident populations exhibited similar responses to high altitude reflecting a high degree of convergence. However, they differed in some respects from Andean geese, crested ducks, and bar-headed geese (Lague et al., 2017), none of which exhibit increased haematocrit or total Hb concentration at high altitude (Black and Tenney, 1980; Lague et al., 2016; Lague et al., 2017), reflecting some divergence across waterfowl in the strategies used to cope with chronic hypoxia. In sum, across all these species, we have identified a surprisingly diverse set of physiological trait combinations, further supporting the hypothesis that evolution is both predictable and idiosyncratic. It can converge on the same basic phenotype via a multitude of different pathways. The unique responses of these Andean ducks reveal yet another avian strategy for coping with high altitude.

LIST OF SYMBOLS AND ABBREVIATIONS

HA	high altitude
Hb	haemoglobin
HIF2A	hypoxia inducible factor 2 alpha
HRV	heart rate variability
kPa	kilo pascals
LA	low altitude
P_{50}	partial pressure at which Hb is half saturated
PO_2	partial pressure of inspired oxygen
STPD	standard temperature and pressure dry
RMSSD	root mean square of the standard deviation
SDRR	standard deviation of the R-R interval
$\dot{V}o_2$	whole animal oxygen consumption rate

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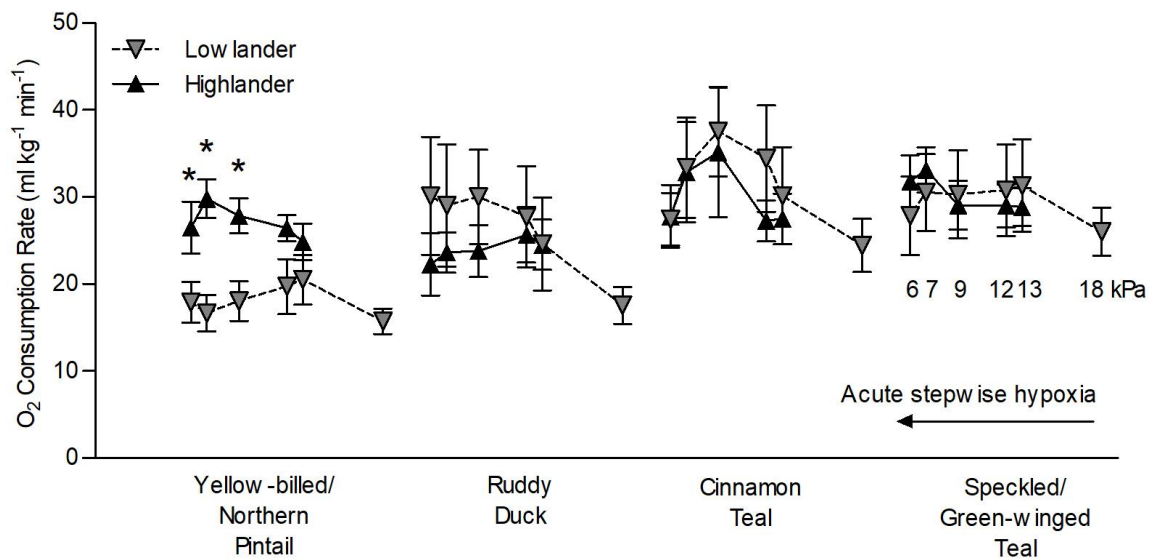
Figures

Figure 1: O_2 consumption during progressive hypoxic exposure.

O_2 consumption was maintained in all groups throughout progressive decreases in partial pressure of inspired O_2 (PO_2). Values represent means $\pm$ s.e.m. High-altitude populations of several duck species are compared to low-altitude populations of the same species or to closely-related low-altitude congeners. Responses to acute hypoxia are shown from right to left for stepwise reductions in inspired O_2 tension (PO_2): 18 (lowlanders only), 13 12, 9, 7, and 6 kPa O_2. 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 are indicated by an asterisk and determined by two-way repeated measures ANOVA. N=12 high-altitude yellow-billed pintails, n=10 low-altitude northern pintails, n=6 high-altitude ruddy ducks, n=8 low-altitude ruddy ducks, n=12 high-altitude cinnamon teals, n=11 low-altitude cinnamon teals, n=10 high-altitude speckled teals, and n=10 low-altitude green-winged teals.

Figure 2: Heart rate during progressive hypoxic exposure. Heart rate was maintained in all groups throughout progressive decreases in partial pressure of inspired O_2 (PO_2). Values represent means $\pm$ s.e.m. High-altitude populations of several duck species are compared to low-altitude populations of the same species or to closely-related low-altitude congeners. Responses to acute hypoxia are shown from right to left for stepwise reductions in inspired O_2 tension (PO_2): 18 (lowlanders only), 13 12, 9, 7, and 6 kPa O_2. 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 are indicated by an asterisk and determined by two-way repeated measures ANOVA. N=12 high-altitude yellow-billed pintails, n=10 low-altitude northern pintails, n=6 high-altitude ruddy ducks, n=8 low-altitude ruddy ducks, n=12 high-altitude cinnamon teals, n=11 low-altitude cinnamon teals, n=10 high-altitude speckled teals, and n=10 low-altitude green-winged teals.
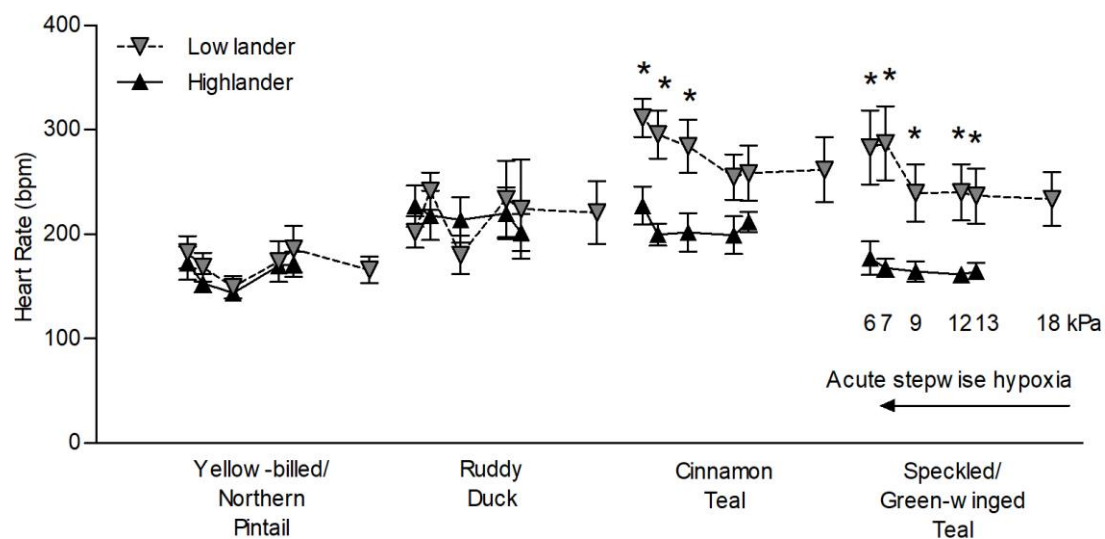
O$_2$ Pulse (ml O$_2$ beat^{-1} g^{-1})

0.25
0.20
0.15
0.10
0.05
0.00

Low lander
Highlander

6 7 9 12 13 18 kPa

Acute stepwise hypoxia

Yellow-billed/ Northern Pintail

Ruddy Duck

Cinnamon Teal

Speckled/ Green-winged Teal

Figure 3: O$_2$ pulse during progressive hypoxic exposure.

O$_2$ pulse was maintained or increased throughout progressive decreases in partial pressure of inspired O$_2$ (PO$_2$). Values represent means $\pm$ s.e.m. High-altitude populations of several duck species are compared to low-altitude populations of the same species or to closely-related low-altitude congeners. Responses to acute hypoxia are shown from right to left for stepwise reductions in inspired O$_2$ tension (PO$_2$): 18 (lowlanders only), 13 12, 9, 7, and 6 kPa O$_2$. 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 are indicated by an asterisk and determined by two-way repeated measures ANOVA. N=12 high-altitude yellow-billed pintails, n=10 low-altitude northern pintails, n=6 high-altitude ruddy ducks, n=8 low-altitude ruddy ducks, n=12 high-altitude cinnamon teals, n=11 low-altitude cinnamon teals, n=10 high-altitude speckled teals, and n=10 low-altitude green-winged teals.
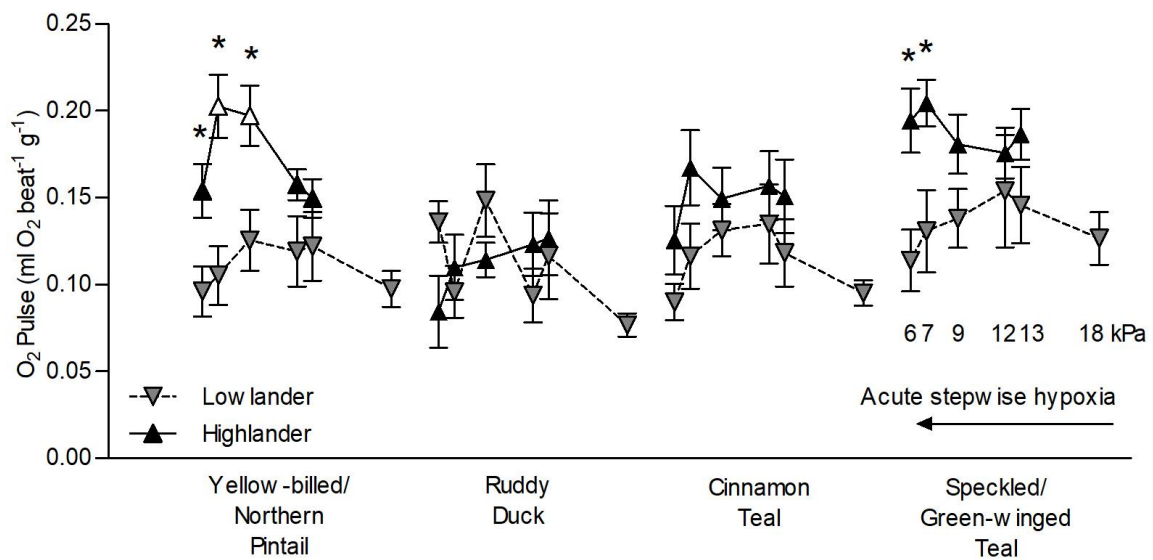
Figure 4: Standard deviation of RR intervals (SDRR) in normoxia, severe hypoxia and ambient recovery.

SDRR is represented for all study groups under ambient conditions (18 kPa for low altitude (LA) and 13 kPa for high altitude (HA)), at 6 kPa inspired O_2, following 5-min of ambient recovery, and following 20-min of ambient recovery. All values are means ± 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 and determined by one-way repeated measures ANOVA. Significant differences ($P<0.05$) in the y variable between phylogenetic pairs are indicated by an asterisk and determined by two-way repeated measures ANOVA. N=12 high-altitude yellow-billed pintails, n=10 low-altitude northern pintails, n=6 high-altitude ruddy ducks, n=8 low-altitude ruddy ducks, n=12 high-altitude cinnamon teals, n=11 low-altitude cinnamon teals, n=10 high-altitude speckled teals, and n=10 low-altitude green-winged teals.
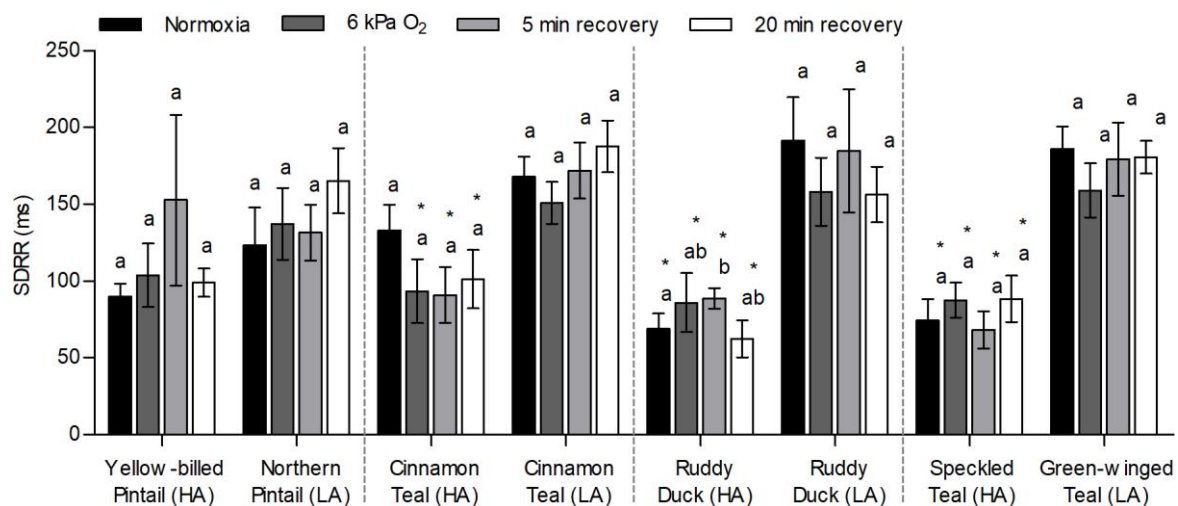
Figure 5: Root mean square of successive differences in RR intervals (RMSSD) in normoxia, severe hypoxia and ambient recovery. RMSSD is represented for all study groups for ambient conditions (18 kPa for low altitude (LA) and 13 kPa for high altitude (HA)), at 6 kPa inspired O_2, following 5-min of ambient recovery, and following 20-min of ambient recovery. All values are means ± 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 and determined by one-way repeated measures ANOVA. Significant differences ($P<0.05$) in the y variable between phylogenetic pairs are indicated by an asterisk and determined by two-way repeated measures ANOVA. N=12 high-altitude yellow-billed pintails, n=10 low-altitude northern pintails, n=6 high-altitude ruddy ducks, n=8 low-altitude ruddy ducks, n=12 high-altitude cinnamon teals, n=11 low-altitude cinnamon teals, n=10 high-altitude speckled teals, and n=10 low-altitude green-winged teals.
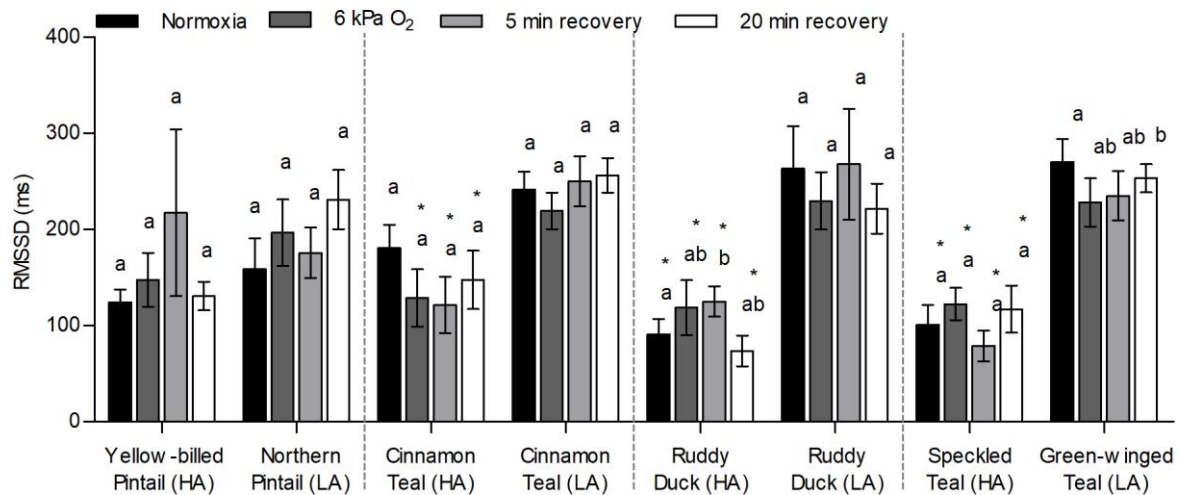
Figure 6: Arterial O₂ content during progressive hypoxic exposure.

Arterial O_2 content (mmol l^{-1}) to acute hypoxia are shown from right to left for stepwise reductions in inspired partial pressure of O_2 (PO$_2$): Values represent means $\pm$ s.e.m. High-altitude populations of several duck species are compared to low-altitude populations of the same species or to closely-related low-altitude congeners. Responses to acute hypoxia are shown from right to left for stepwise reductions in inspired O_2 tension (PO$_2$): 18 (lowlanders only), 13 12, 9, 7, and 6 kPa O_2. 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 are indicated by an asterisk and determined by two-way repeated measures ANOVA. Only the ruddy duck shows no difference in arterial O_2 content at low to moderate inspired partial pressure. N=12 high-altitude yellow-billed pintails, n=10 low-altitude northern pintails, n=6 high-altitude ruddy ducks, n=8 low-altitude ruddy ducks, n=12 high-altitude cinnamon teals, n=11 low-altitude cinnamon teals, n=10 high-altitude speckled teals, and n=10 low-altitude green-winged teals.
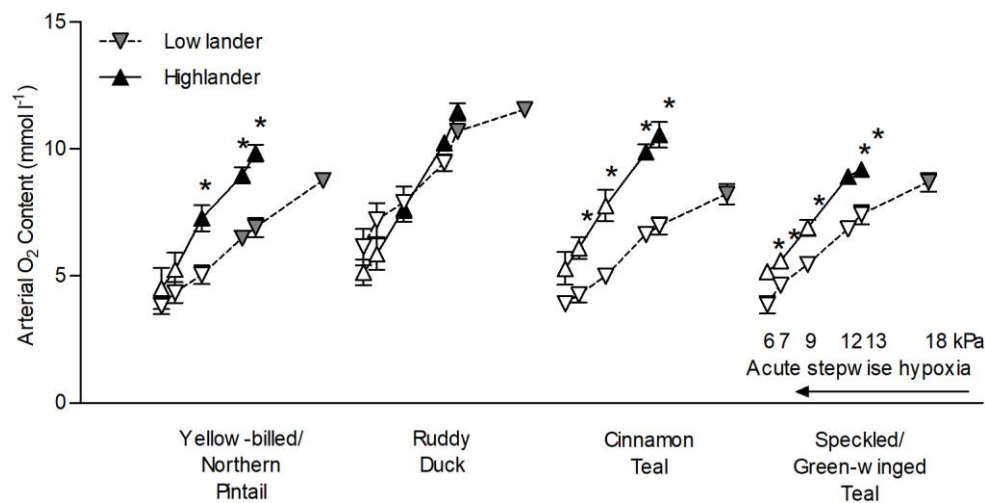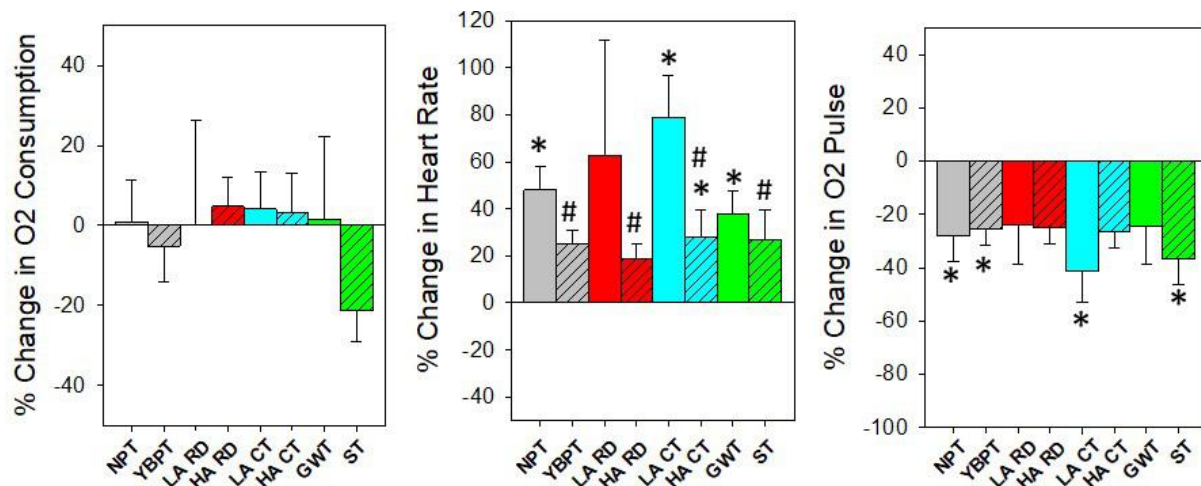
Figure 7: Percent change in heart rate and O$_2$ pulse during post-hypoxic recovery.
Percent change from starting levels of O$_2$ consumption, heart rate and the O$_2$ pulse 20-min post-hypoxic recovery. Values represent means $\pm$ s.e.m with low-altitude (LA) groups indicated by solid bars: gray (northern pintail; NPT; n=10), red (ruddy duck; LA RD; n=8), light blue (cinnamon teal; LA CT; n=12), and green (green-winged teal; GWT; n=10). High-altitude (HA) groups are indicated by dashed bars: gray (yellow-billed pintail; YBPT; n=12), red (ruddy duck; HA RD; n=6), light blue (cinnamon teal; HA CT; n=12), and green (speckled teal; ST; n=10). Significant differences ($P < 0.05$) in the y-axis variable from values during exposure to ambient air within a species are indicated by an asterix and determined by one-way ANOVA. Significant differences between low- and high-altitude pairs are indicated by # and determined by two-way ANOVA.

Tables

Table 1: Differences in haematological parameters, body mass, and relative heart mass in high- and low-altitude ducks. Hb, haemoglobin; P_{50}, the PO_2 at which Hb is half-saturated with O_2 (values obtained from Natarajan et al. (Natarajan et al., 2015). Significant differences ($P<0.05$) in the variable between phylogenetic pairs are indicated by an asterisk and determined by two-way repeated measures ANOVA. N=10 low-altitude northern pintails, n=11 low-altitude cinnamon teals, n=8 low-altitude ruddy ducks, n=10 green-winged teals, n=12 yellow-billed pintails, n=12 high-altitude cinnamon teals, n=6 high-altitude ruddy ducks, and n=10 speckled teals.

Group	Haematocrit (%)	Blood Hb content (g dl^{-1})	Hb P_{50} (kPa)	Body mass (kg)	Relative heart mass (g kg^{-1})
Low altitude					
Northern pintail	43.7 $\pm$ 1.3*	15.1 $\pm$ 0.5*	----	0.842 $\pm$ 0.01	10.7 $\pm$ 0.1
Cinnamon teal	43.1 $\pm$ 1.5*	14.0 $\pm$ 0.5*	4.95	0.305 $\pm$ 0.01	9.2 $\pm$ 0.4
Ruddy duck	55.6 $\pm$ 4.0	19.6 $\pm$ 0.3	3.81	0.450 $\pm$ 0.03	11.4 $\pm$ 0.4
Green-winged teal	45.9 $\pm$ 1.7*	15.2 $\pm$ 0.5*	----	0.343 $\pm$ 0.05	10.3 $\pm$ 0.5*
High altitude					
Yellow-billed pintail	56.3 $\pm$ 1.7*	18.4 $\pm$ 0.3*	4.68	0.605 $\pm$ 0.02	10.4 $\pm$ 0.6
Cinnamon teal	56.1 $\pm$ 1.2*	19.4 $\pm$ 0.4*	3.88	0.435 $\pm$ 0.01	11.1 $\pm$ 0.7
Ruddy duck	55.1 $\pm$ 1.8	20.4 $\pm$ 0.7	4.01	0.728 $\pm$ 0.06	9.6 $\pm$ 0.4
Speckled teal	53.7 $\pm$ 2.3*	17.8 $\pm$ 0.8*	4.12	0.372 $\pm$ 0.01	12.8 $\pm$ 0.5*

Group	Partial Pressure of Inspired O_2 (kPa)					
	21	13	13	9	7	6
Arterial O_2 Saturation – Low-Altitude Populations (%)						
Northern pintail	94 ± 1	74 ± 3	70 ± 1	54 ± 4	47 ± 4	42 ± 4
Cinnamon teal	95 ± 1	80 ± 2	76 ± 2	57 ± 1	49 ± 3	45 ± 2
Ruddy duck	95 ± 1	87 ± 1	78 ± 1	65 ± 4	57 ± 5	51 ± 5
Green-winged teal	92 ± 1	80 ± 3	73 ± 1	58 ± 2	49 ± 1	41 ± 3
Gadwall	94 ± 2	75 ± 2	72 ± 2	56 ± 3	46 ± 3	47 ± 3
Mallard	94 ± 1	73 ± 3	69 ± 3	58 ± 3	50 ± 4	44 ± 4
Arterial O_2 Saturation – High-Altitude Populations (%)						
Yellow-billed pintail	---	85 ± 2*	77 ± 2	63 ± 4	45 ± 5	38 ± 4
Cinnamon teal	---	86 ± 3	80 ± 1	63 ± 4	50 ± 3	43 ± 5
Ruddy duck	---	90 ± 2	80 ± 3	60 ± 4	46 ± 5	41 ± 5
Speckled teal	---	83 ± 2	81 ± 2	62 ± 3	51 ± 2	47 ± 2
Puna teal	---	83 ± 1	77 ± 1	64 ± 3	46 ± 6	42 ± 6

Table S1: Arterial oxygen saturation (%) during stepwise hypoxia exposure for the different species of high- and low-altitude ducks. Significant differences ($P<0.05$) in arterial O_2 saturation between high- and low-altitude pairs at a given partial pressure of inspired oxygen (P_IO_2) are indicated by an asterisk as determined by Holm-Sidak post hoc tests. N=10 northern pintails, n=8 low-altitude ruddy ducks, n=11 low-altitude cinnamon teals, n=10 green-winged teals, n=8 gadwalls, n=8 mallard ducks, n=12 yellow-billed pintails, n=6 high-altitude ruddy ducks, n=12 high-altitude cinnamon teals, n=10 speckled teals, and n=8 Puna teals.

PO$_2$ (kPa)	Puna teal	Gadwall	Mallard
O$_2$ Consumption (ml kg^{-1} min^{-1})			
18	---	17.56 ± 1.89	15.17 ± 3.00
13	26.95 ± 1.79	20.07 ± 2.38	18.58 ± 3.27
12	25.08 ± 2.75	17.70 ± 3.23	19.34 ± 2.28
9	28.77 ± 4.00	19.21 ± 3.44	21.41 ± 3.40
7	24.43 ± 3.12	19.37 ± 2.51	21.87 ± 3.08
6	22.78 ± 2.71	16.84 ± 2.71	20.67 ± 4.65
Heart Rate (min^{-1})			
18	---	206.67 ± 25.53	191.65 ± 22.33
13	225.16 ± 10.37	181.95 ± 24.79	184.28 ± 22.74
12	214.85 ± 10.27	158.44 ± 15.38	187.47 ± 22.30
9	238.38 ± 10.58	173.93 ± 16.84	215.40 ± 31.66
7	261.17 ± 16.55*	186.50 ± 23.44	228.51 ± 24.76
6	278.84 ± 15.48*	239.65 ± 24.55	305.73 ± 33.30*
O$_2$ Pulse (ml O$_2$ beat^{-1} g^{-1})			
18	---	0.09 ± 0.01	0.08 ± 0.02
13	0.13 ± 0.01	0.12 ± 0.02	0.10 ± 0.01
12	0.12 ± 0.01	0.12 ± 0.02	0.11 ± 0.02
9	0.13 ± 0.02	0.12 ± 0.02	0.10 ± 0.02
7	0.10 ± 0.02	0.11 ± 0.02	0.11 ± 0.020
6	0.09 ± 0.01*	0.07 ± 0.01	0.07 ± 0.02
Arterial O$_2$ Content (mmol l^{-1})			
18	---	8.89 ± 0.26	8.83 ± 0.33
13	8.81 ± 0.09	7.20 ± 0.37*	6.86 ± 0.41
12	8.20 ± 0.06	6.88 ± 0.26*	6.43 ± 0.40
9	6.56 ± 0.35	5.34 ± 0.45*	5.40 ± 0.36*
7	4.65 ± 0.55*	4.38 ± 0.33*	4.69 ± 0.44*
6	4.23 ± 0.55*	4.45 ± 0.35*	4.16 ± 0.51*
SDRR (ms)			
Normoxia	107.60 ± 15.85	126.60 ± 22.17	150.46 ± 30.88
6 kPa O$_2$	79.62 ± 24.18	101.72 ± 24.28	145.24 ± 43.43
5-min recovery	96.37 ± 19.06	137.68 ± 19.53	139.35 ± 29.81
20-min recovery	83.97 ± 16.52	149.95 ± 23.06	123.34 ± 24.10
RMSSD (ms)			
Normoxia	136.13 ± 23.10	175.63 ± 33.94	198.76 ± 43.76
6 kPa O$_2$	103.13 ± 34.38	143.89 ± 34.53	211.53 ± 63.85
5-min recovery	126.45 ± 31.48	185.43 ± 29.12	203.36 ± 44.52
20-min recovery	99.44 ± 24.78	195.35 ± 30.02	174.92 ± 32.91

Table S2: Measurements of oxygen consumption, heart rate, oxygen pulse, arterial oxygen content, SDRR (standard deviation of the R-R interval), and RMSSD (root mean square of the standard deviation) during stepwise hypoxia exposure in the Puna teal, gadwall and mallard. These represent the species recorded in our study for which there were no low/high-altitude pair. Significant differences ($P<0.05$) in the y variable between phylogenetic pairs are indicated by an asterisk and determined by two-way repeated measures ANOVA. N=8 high-altitude Puna teal, n=8 low-altitude gadwall, and n=8 low-altitude mallard duck.

Species	Haematocrit	Blood Hb content	Hb P_{50}	Body mass	Relative heart mass
	(%)	(g dl⁻¹)	(kPa)	(kg)	(g kg⁻¹)
Low altitude					
Gadwall	44.6 ± 1.9	15.3 ± 0.4	----	0.754 ± 0.04	----
Mallard	46.5 ± 2.4	15.1 ± 0.6	----	0.947 ± 0.02	7.4 ± 0.4
High altitude					
Puna teal	49.8 ± 1.3	17.0 ± 0.3	3.61	0.404 ± 0.01	9.8 ± 0.6

Table S3: Differences in haematological parameters, body mass, and relative heart mass in Puna teal, gadwall, and mallard ducks. Hb, haemoglobin; P_{50}, the PO_2 at which Hb is half-saturated with O_2 (values obtained from Natarajan et al. (Natarajan et al., 2015). N=8 high-altitude Puna teal, n=8 low-altitude gadwall, and n=8 low-altitude mallard duck.