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RESEARCH ARTICLE

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

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ABSTRACT

The cardiovascular system is critical for delivering O_2 to tissues. Here, we examined the cardiovascular responses to progressive hypoxia in four high-altitude Andean duck species compared with 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 (Hb) concentration. 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 concentration, increasing the O_2 content of arterial blood, and potentially providing a greater reserve for enhancing O_2 delivery during hypoxia. Hct and Hb concentration 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 concentration compared with their low-altitude relatives, revealing yet another avian strategy for coping with high altitude.

KEY WORDS: Heart rate variability, Oxygen transport, Oxygen consumption, Heart rate, Haemoglobin, Hypoxia

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INTRODUCTION

A major role of the cardiovascular system is to maintain adequate delivery of 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 (P_{O_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; Calder, 1968; 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 from those of 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; Laguë et al., 2016, 2017; Laguë et al., 2017). Neither of these species exhibits significant elevations in Hct or blood Hb concentration compared with their low-altitude relatives (Black and Tenney, 1980; Laguë et al., 2016; Laguë et al., 2017) 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 (Laguë et al., 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 Peru (e.g. Lake Titicaca at ∼3800 m above sea level; Chancay River at ∼3000–4100 m) were compared with low-altitude populations of the same species or with 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.

List of symbols and abbreviations

HA	high altitude
Hb	haemoglobin
Hct	haematocrit
HRV	heart rate variability
LA	low altitude
P_{50}	partial pressure at which Hb is 50% saturated
P_{O_2}	partial pressure of inspired oxygen
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

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; Muñoz-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 to or distinct from those observed in previous studies of the high-altitude resident Andean goose and the high-altitude migrant bar-headed goose (Laguë et al., 2017).

MATERIALS AND METHODS
Waterfowl
Our study was conducted in conjunction with Ivy et al. (2019), and the cardiovascular measurements documented in the present paper 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 (3812 m) at the Lake Titicaca National Reserve (near Puno, Peru) in August 2014: yellow-billed pintail (*Anas georgica* Gmelin 1789; 0.61±0.02 kg, *N*=12), Andean cinnamon teal (*Anas cyanoptera orinomus* Vieillot 1816; 0.44±0.01 kg, *N*=12), ruddy duck [*Oxyura jamaicensis ferruginea* (Eyton 1838); 0.73±0.07 kg, *N*=6] and speckled teal (*Anas flavirostris oxyptera* Vieillot 1816; 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 1260 m or Malheur National Wildlife Refuge at 1256 m), and were tested at Summer Lake Wildlife Area: northern pintail (*Anas acuta* Linnaeus 1758; 0.84±0.01 kg, *N*=10), northern cinnamon teal (*Anas cyanoptera septentrionalium* Vieillot 1816; 0.31±0.01 kg, *N*=11), ruddy duck [*Oxyura jamaicensis* (Gmelin 1789); 0.42± 0.04 kg, *N*=8] and green-winged teal (*Anas crecca carolinensis* Gmelin 1789; 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 (Muñoz-Fuentes et al., 2013).

Mallard ducks (*Anas platyrhynchos* Linnaeus 1758; 0.96± 0.02 kg, *N*=8) and gadwalls [*Mareca strepera* (Linnaeus 1758); 0.75±0.04 kg, *N*=8] were also captured in Oregon, and puna teals [*Spatula puna* (Tschudi 1844); 0.40±0.01 kg, *N*=8] at Lake Titicaca. As we do not have data from sister taxa for these groups, the data we obtained from them is included only in the supplementary information (Tables S1–S3). They 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 for 6–12 h 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), US 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 (Laguë et al., 2016, 2017; 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 (HRV). 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). We have previously shown that the MouseOx Pulse Oximeter system provides accurate measurements of arterial oxygen saturation in waterfowl (Ivy et al., 2018). 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 (P_{O_2}): 13 (for low-altitude ducks only), 12, 9, 7 and 6 kPa, followed by a 25 min recovery to ambient P_{O_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. Hct (%) was determined in duplicate by a Zipocrit haematocrit centrifuge (LW Scientific, Lawrenceville, GA, USA). Hb 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 (as per permit regulations) were euthanized with an overdose of intravenous propofol ($N=3$ northern pintails, $N=3$ low-altitude cinnamon teals, $N=5$ low-altitude ruddy ducks, $N=2$ green-winged teals, $N=6$ yellow-billed pintails, $N=10$ high-altitude cinnamon teals, $N=8$ high-altitude ruddy ducks and $N=6$ 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 hypoxia exposure, and we report the average values across the last 10 min at each inspired P_{O_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 analysers (FOXBOX, Sable Systems, Las Vegas, NV, USA). These data were used to calculate $\dot{V}_{O_2}$, as described by 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) and analysed using PowerLab analysis software (ADInstruments) 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 min time point of each exposure was analysed using PowerLab heart rate variability analysis software (ADInstruments). Root mean squared of the successive differences (RMSSD; in ms), the most common time domain measure for HRV, and standard deviation of the R–R interval (SDRR; 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 concentration 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 ($N=6$ speckled teal, $N=5$ Andean

ruddy duck, $N=6$ yellow-billed pintail, $N=5$ cinnamon teal). 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.

Statistical analysis

Two-factor ANOVA was used to examine the main effects and interactions of altitude and acute inspired P_{O_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 P_{O_2} using Holm–Šidák post hoc tests. Comparisons between groups began at 13 kPa. Variables were transformed when they did not meet assumptions for either normality or equal variance analysed for a two-way repeated measures ANOVA. Student's t-tests were used to compare Hct, 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 means±s.e.m.

RESULTS

Progressive hypoxic exposure

Despite the progressive fall in inspired P_{O_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-altitude pairs. It was elevated in the high-altitude yellow-billed pintails compared with the northern pintails at the lower levels of inspired P_{O_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 P_{O_2} ($P<0.003$). Similarly,
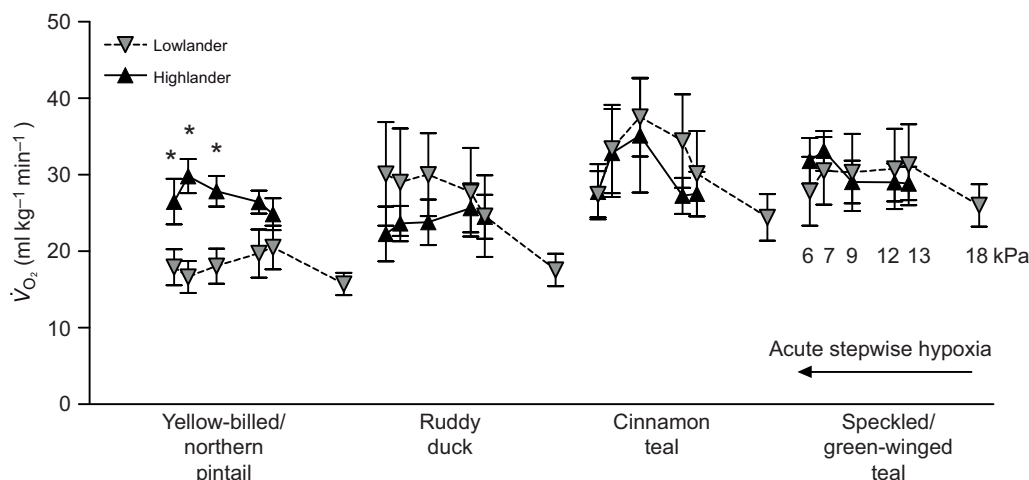

Fig. 1. O$_2$ consumption during progressive hypoxic exposure. O$_2$ consumption rate ($\dot{V}_{O_2}$) was maintained in all groups throughout progressive decreases in partial pressure of inspired O$_2$ (P_{O_2}). Values represent means±s.e.m. High-altitude populations of several duck species were compared with low-altitude populations of the same species or with closely related low-altitude congeners. Responses to acute hypoxia are shown from right to left for stepwise reductions in inspired P_{O_2}: 18 (lowlanders only), 13, 12, 9, 7 and 6 kPa O$_2$. There were no significant differences ($P<0.05$) in the y-axis variable within a species, as determined by one-way repeated measures ANOVA. Significant differences ($P<0.05$) in the y-axis 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.
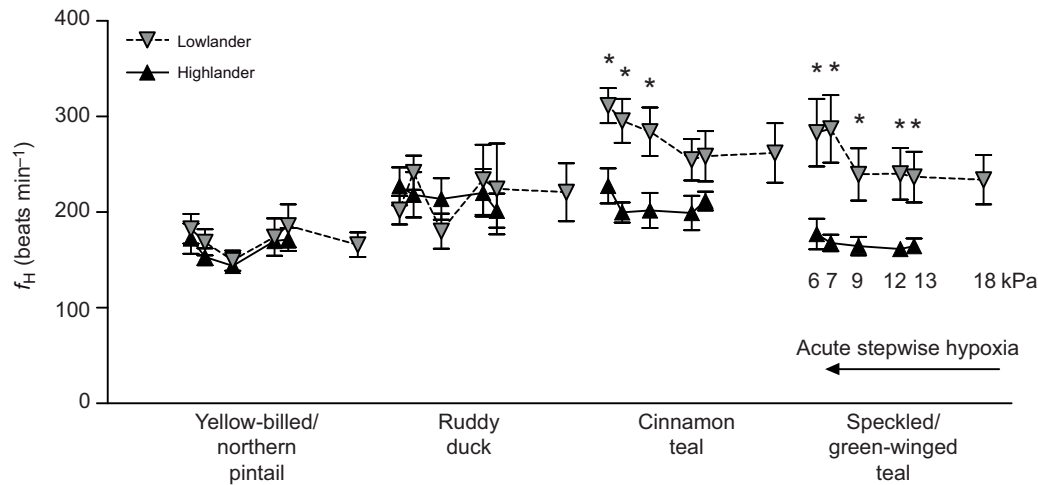
Fig. 2. Heart rate during progressive hypoxic exposure. Heart rate (f_H) was maintained in all groups throughout progressive decreases in inspired partial pressure of O_2 (P_{O_2}). Values represent means±s.e.m. High-altitude populations of several duck species were compared with low-altitude populations of the same species or with closely related low-altitude congeners. Responses to acute hypoxia are shown from right to left for stepwise reductions in inspired P_{O_2}: 18 (lowlanders only), 13, 12, 9, 7 and 6 kPa O_2. There were no significant differences ($P<0.05$) in the y-axis variable within a species, as determined by one-way repeated measures ANOVA. Significant differences ($P<0.05$) in the y-axis 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.

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 low-altitude green-winged teal at 6 kPa O_2 ($P=0.046$). 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
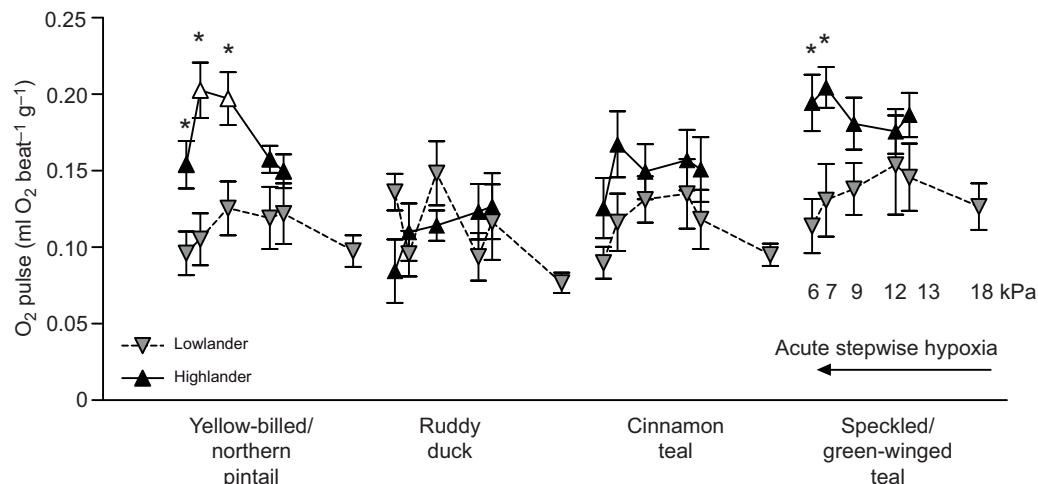

Fig. 3. O_2 pulse during progressive hypoxic exposure. O_2 pulse was maintained or increased throughout progressive decreases in inspired partial pressure of O_2 (P_{O_2}). Values represent means±s.e.m. High-altitude populations of several duck species were compared with low-altitude populations of the same species or with closely related low-altitude congeners. Responses to acute hypoxia are shown from right to left for stepwise reductions in inspired P_{O_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-axis 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.
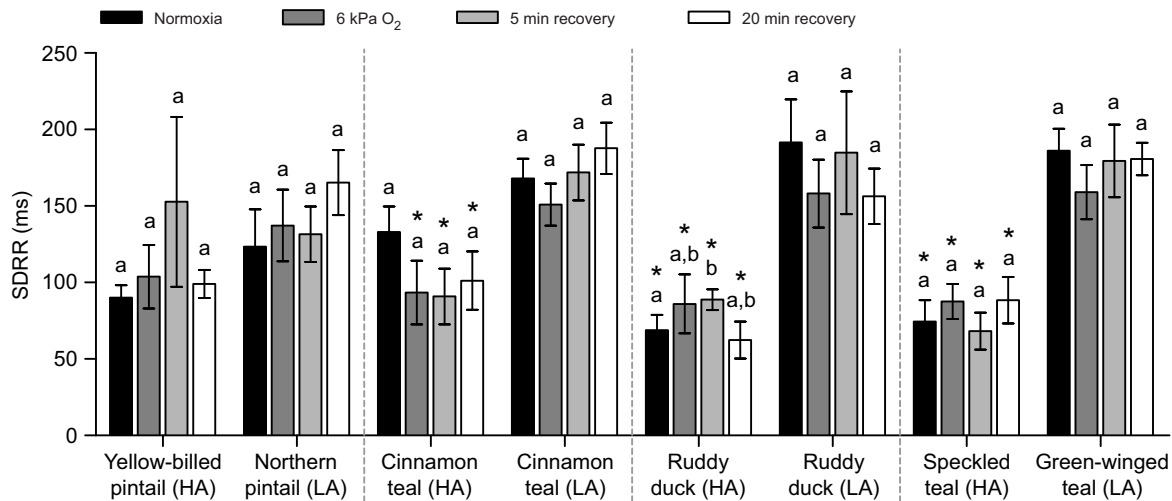
Fig. 4. Standard deviation of R–R interval (SDRR) in normoxia, severe hypoxia and ambient recovery. SDRR is represented for all study groups under ambient conditions [18 kPa for low altitude (LA) and 13 kPa for high altitude (HA)], at 6 kPa inspired partial pressure of O_2 (P_{O_2}), following 5 min 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 by different letters and determined by one-way repeated measures ANOVA. Significant differences ($P<0.05$) in the y-axis 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, $n=12$ high-altitude cinnamon teals, $n=11$ low-altitude cinnamon teals, $n=6$ high-altitude ruddy ducks, $n=8$ low-altitude ruddy ducks, $n=10$ high-altitude speckled teals and $n=10$ low-altitude green-winged teals.

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 and 5). Thus, the high-altitude groups generally exhibited lower SDRR and RMSSD compared with 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-altitude 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 P_{O_2}. This was largely a result of increases in blood Hb content, as both Hb content and Hct were higher in the high-altitude population of each of these three high-/low-altitude 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-altitude pairs, with no significant differences in Hct, 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).
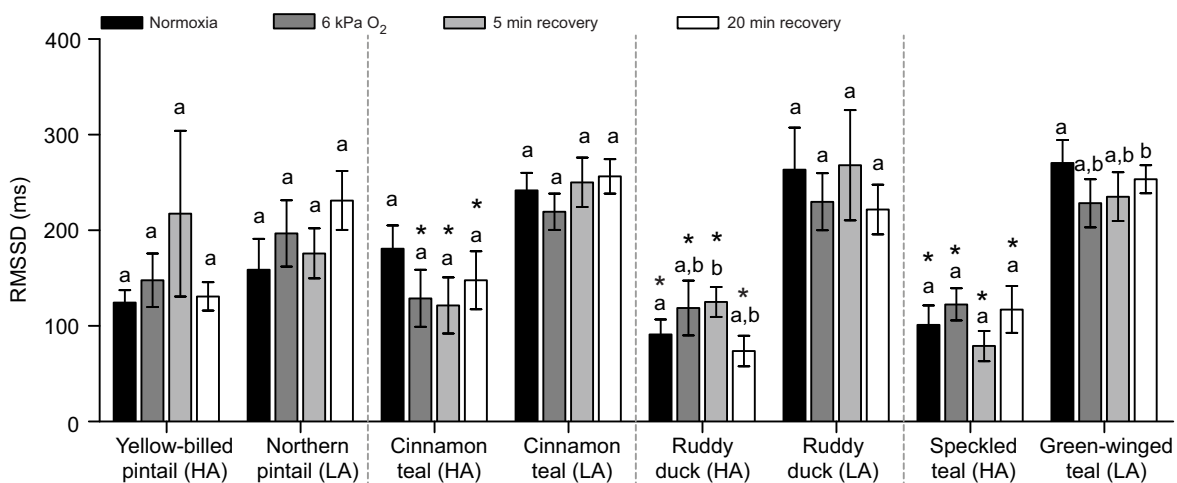

Fig. 5. Root mean square of successive differences in R–R interval (RMSSD) in normoxia, severe hypoxia and ambient recovery. RMSSD is represented for all study groups for ambient conditions [18 kPa for low altitude (LA) and 13 kPa for high altitude (HA)], at 6 kPa inspired partial pressure of O_2 (P_{O_2}), 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 by different letters and determined by one-way repeated measures ANOVA. Significant differences ($P<0.05$) in the y-axis 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, $n=12$ high-altitude cinnamon teals, $n=11$ low-altitude cinnamon teals, $n=6$ high-altitude ruddy ducks, $n=8$ low-altitude ruddy ducks, $n=10$ high-altitude speckled teals and $n=10$ low-altitude green-winged teals.
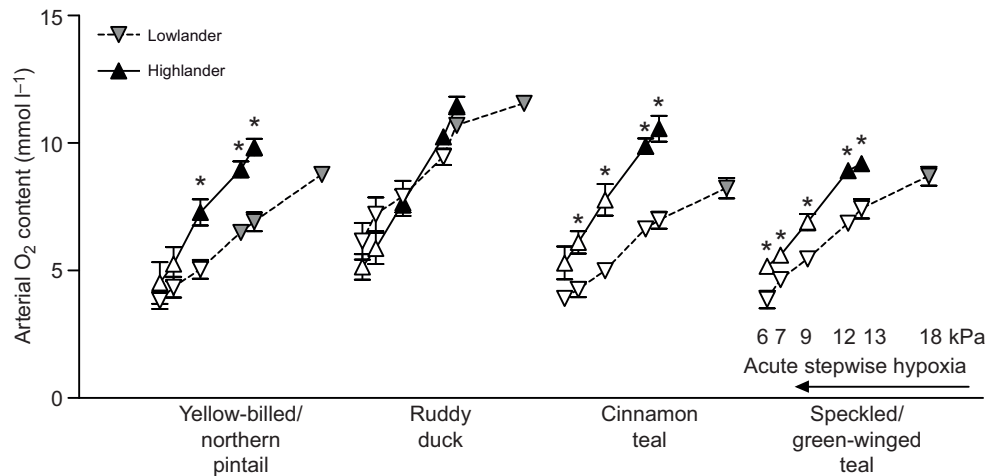
Fig. 6. Arterial O₂ content during progressive hypoxic exposure. Arterial O_2 content (mmol l^{-1}) decreased with progressive decreases in inspired partial pressure of O_2 (P_{O_2}). Values represent means±s.e.m. High-altitude populations of several duck species were compared with low-altitude populations of the same species or with closely related low-altitude congeners. Responses to acute hypoxia are shown from right to left for stepwise reductions in inspired P_{O_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-axis 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.

Heart mass relative to body mass was generally similar between high-/low-altitude pairs. An exception to this was that heart mass relative to body mass was significantly greater in high-altitude speckled teals compared with low-altitude green-winged teals ($P=0.028$). A similar difference in heart mass in high-altitude cinnamon teals compared with 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 with 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). As $\dot{V}_{O_2}$ 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 supplementary information: arterial O_2 saturation (Table S1); O_2, heart rate, O_2 pulse, arterial O_2 content, SDRR and RMSSD (Table S2); haematological 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 ducks with those of four closely related low-altitude ducks that represent sister populations/subspecies or congeneric species. In

Table 1. Differences in haematological parameters, body mass and relative heart mass in high- and low-altitude ducks

Group	Hct (%)	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±1.3*	15.1±0.5*	–	0.842±0.01	10.7±0.1
Cinnamon teal	43.1±1.5*	14.0±0.5*	4.95	0.305±0.01	9.2±0.4
Ruddy duck	55.6±4.0	19.6±0.3	3.81	0.450±0.03	11.4±0.4
Green-winged teal	45.9±1.7*	15.2±0.5*	–	0.343±0.05	10.3±0.5*
High altitude					
Yellow-billed pintail	56.3±1.7*	18.4±0.3*	4.68	0.605±0.02	10.4±0.6
Cinnamon teal	56.1±1.2*	19.4±0.4*	3.88	0.435±0.01	11.1±0.7
Ruddy duck	55.1±1.8	20.4±0.7	4.01	0.728±0.06	9.6±0.4
Speckled teal	53.7±2.3*	17.8±0.8*	4.12	0.372±0.01	12.8±0.5*

Hct, haematocrit; Hb, haemoglobin; P_{50}, the P_{O_2} at which Hb is 50% saturated with O_2 (values obtained from 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.
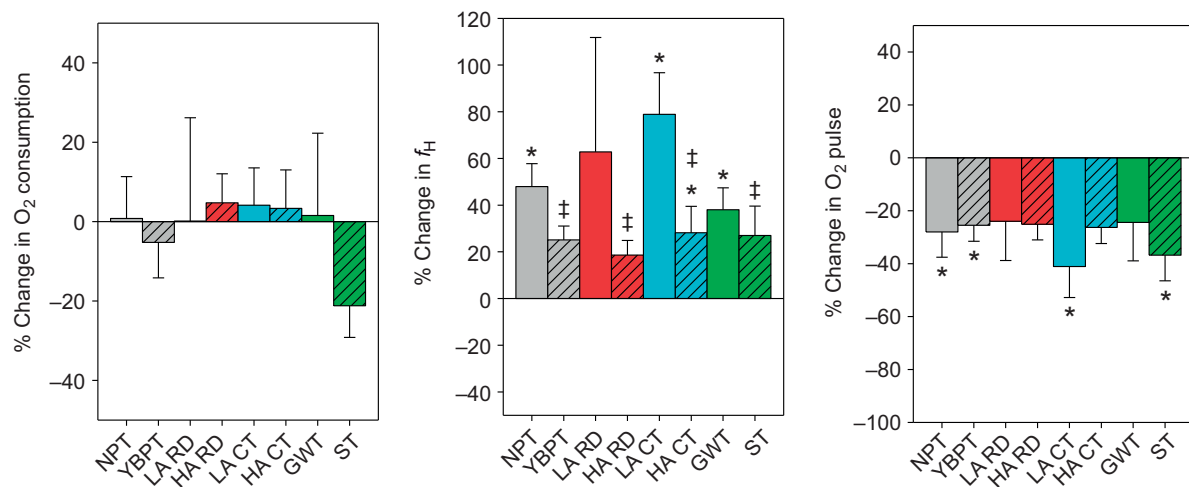
Fig. 7. Percentage change in O$_2$ consumption, heart rate (f_H) and O$_2$ pulse during post-hypoxic recovery. Percentage change from starting levels of inspired partial pressure of O$_2$ (P_{O_2}), 20 min post-hypoxic recovery is shown. Values represent means±s.e.m. with low-altitude (LA) groups indicated by non-hatched bars: NPT, northern pintail (n=10); LA RD, low-altitude ruddy duck (n=8); LA CT, low-altitude cinnamon teal (n=12); and GWT, green-winged teal (n=10). High-altitude (HA) groups are indicated by hatched bars: YBPT, yellow-billed pintail (n=12); HA RD, high-altitude ruddy duck (n=6); HA CT, high-altitude cinnamon teal (n=12); and ST, speckled teal (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 asterisk and determined by one-way ANOVA. Significant differences between low- and high-altitude pairs are indicated by a double-dagger and determined by two-way ANOVA.

general, all eight taxa maintained O$_2$ consumption rate with little, if any, change in heart rate or O$_2$ pulse during hypoxia. Heart rate was 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 probably not limiting $\dot{V}_{O_2}$ even at 6 kPa P_{O_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 (Laguë 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; Laguë et al., 2016). HRV 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 P_{O_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 P_{O_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*) (Laguë et al., 2016, 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 HRV. 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_{O_2} at which Hb is 50% saturated, P_{50}=28.5–30.0 at standard concentrations of KCl and inositol hexaphosphate (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 with that of all other species. The $\dot{V}_{O_2}$ of the yellow-billed pintails was similar to that of all the other species. Interestingly, heart rate was lower in the high-altitude cinnamon teals and speckled teals compared with that of their low-altitude counterparts but this was not the case for the yellow-billed pintails compared with the northern pintails. This, however, again reflects the low heart rate in the northern pintails. The heart rate of the yellow-billed pintails was similar to that of the other high-altitude species.

Hct 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 were higher (i.e. P_{50} values were lower) in the two high-altitude groups studied here compared with their low-altitude close 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, 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 Hct 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 (Schuler et al., 2010; Simonson et al., 2015; 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 Hct 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, CO, USA, 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 3000 m (Dempsey and Morgan, 2015), potentially as a result of allelic differences at the *EPAS1* locus, which encodes hypoxia inducible factor 2α (HIF2α) (Simonson et al., 2010). High-altitude acclimation increased Hct 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 (5640 m) increased Hct from 45.4% to 55.9% (Black and Tenney, 1980); however, in bar-headed geese, Hct and Hb concentration were unchanged when acclimated to the same simulated high-altitude conditions (5640 m) (Black and Tenney, 1980) or when reared at high altitude (3200 m) (Laguë et al., 2016, 2017).

Heart rate variability

High-altitude ducks generally exhibited less 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., 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 (Axelsson, 2005; Bernardi et al., 2001; Brennan et al., 2002; Hughson et al., 1994; Perini et al., 1996; Valance et al., 2008; Wolfel and Levine, 2001). 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 a reduction in the impact of hypoxia on HRV and restoration of chronotropic sensitivity. The lower HRV with normal or lower heart rate 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. As $\dot{V}_{O_2}$ 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; K.G.M., unpublished data). In contrast, the populations of yellow-billed pintails, cinnamon teal and ruddy ducks have probably only been established at altitude for tens of thousands of years, as estimated by population divergence times calculated from genetic data (McCracken et al., 2009b; Wilson et al., 2013; Muñoz-Fuentes et al., 2013). Ignoring the ruddy ducks again, the data suggest that the primary adaptations seen here (increased Hb concentration 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 either by maintaining a constant arterial–venous O_2 difference (which would represent an increase in the relative percentage 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- versus 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 concentration and Hb–O_2 affinity. A different three of the four highland duck taxa exhibited lower HRV, which when combined with heart rate that was similar or lower than that in lowland taxa, suggesting 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 (Laguë et al., 2017), none of which exhibit increased Hct or total Hb concentration at high altitude (Black and Tenney, 1980; Laguë et al., 2016, 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.

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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$_I$O$_2$) 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	$261.17 \pm 16.55*$	186.50 ± 23.44	228.51 ± 24.76
6	$278.84 \pm 15.48*$	239.65 ± 24.55	$305.73 \pm 33.30*$
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	$0.09 \pm 0.01*$	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	$7.20 \pm 0.37*$	6.86 ± 0.41
12	8.20 ± 0.06	$6.88 \pm 0.26*$	6.43 ± 0.40
9	6.56 ± 0.35	$5.34 \pm 0.45*$	$5.40 \pm 0.36*$
7	$4.65 \pm 0.55*$	$4.38 \pm 0.33*$	$4.69 \pm 0.44*$
6	$4.23 \pm 0.55*$	$4.45 \pm 0.35*$	$4.16 \pm 0.51*$
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 $\pm$ 1.9	15.3 $\pm$ 0.4	----	0.754 $\pm$ 0.04	----
Mallard	46.5 $\pm$ 2.4	15.1 $\pm$ 0.6	----	0.947 $\pm$ 0.02	7.4 $\pm$ 0.4
High altitude					
Puna teal	49.8 $\pm$ 1.3	17.0 $\pm$ 0.3	3.61	0.404 $\pm$ 0.01	9.8 $\pm$ 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.