Control of breathing and respiratory gas exchange in ducks native to high altitude in the Andes

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Summary Statement: Distinct physiological strategies for coping with hypoxia exist across different high-altitude lineages of ducks

ABSTRACT

We examined the control of breathing and respiratory gas exchange in six species of high-altitude ducks that independently colonized the high Andes. We compared ducks from high-altitude populations in Peru (Lake Titicaca at ~3800 m above sea level; Chancay River at ~3000-4100 m) to closely related populations or species from low altitude. Hypoxic ventilatory responses were measured shortly after capture at the native altitude. In general, ducks responded to acute hypoxia with robust increases in total ventilation and pulmonary O_2 extraction. O_2 consumption rates were maintained or increased slightly in acute hypoxia, despite ~1-2°C reductions in body temperature in most species. Two high-altitude taxa – yellow-billed pintail and torrent duck – exhibited higher total ventilation than their low-altitude counterparts, and yellow-billed pintail exhibited greater increases in pulmonary O_2 extraction in severe hypoxia. In contrast, three other high-altitude taxa – ruddy duck, cinnamon teal, speckled teal – had similar or slightly reduced total ventilation and pulmonary O_2 extraction than low-altitude relatives. Arterial O_2 saturation (SaO_2) was elevated in yellow-billed pintails at moderate levels of hypoxia, but there were no differences in SaO_2 in other high-altitude taxa compared to their close relatives. This finding suggests that improvements in SaO_2 in hypoxia can require increases in both breathing and haemoglobin-O_2 affinity, because yellow-billed pintail was the only high-altitude duck with concurrent increases in both traits compared to its low-altitude relative. Overall, our results suggest that distinct physiological strategies for coping with hypoxia can exist across different high-altitude lineages, even among those inhabiting very similar high-altitude habitats.

INTRODUCTION

The air at high altitude is both cold and hypoxic. These conditions challenge the ability of birds and mammals to adequately match O_2 supply and O_2 demand, because cold increases the demand for O_2 while hypoxia restricts O_2 availability (Monge and Leon-Velarde, 1991; Storz et al., 2010). The first step in obtaining O_2 is pulmonary ventilation, and thus the hypoxic ventilatory response (HVR) is critical for O_2 uptake in the thin air at high altitude (Birchard and Tenney, 1986; Brutsaert, 2007).

Ventilation is modulated by changes in blood gas levels and metabolism. Acute exposure to hypoxia leads to a drop in arterial partial pressure of O_2 (PaO_2), which stimulates an increase in ventilation (the HVR) that helps offset the fall in PaO_2 (Powell et al., 1998). This reflex is initiated primarily by the carotid bodies, peripheral chemoreceptors that are sensitive to changes in arterial PO_2 and PCO_2/pH located in the carotid arteries supplying the brain in mammals and birds (Gonzalez et al., 1994). Prolonged exposure (days to weeks) to hypoxia leads to further increases in breathing by increasing the ventilatory sensitivity to hypoxia through the process of ventilatory acclimatization to hypoxia (VAH) (Powell et al., 1998). Ventilation is also modulated by changes in metabolism, which helps match O_2 supply to tissue O_2 demand during exercise, thermogenesis, metabolic depression, or changes in body temperature (Barros et al., 2006; Chappell, 1992; Eldridge, 1994).

Birds and mammals that live at high altitude have been shown to differ from low-altitude taxa in their ventilatory responses to hypoxia. Some species/populations native to high-altitude in the Himalayas and on the Tibetan Plateau, such as bar-headed geese (*Anser indicus*), plateau pika (*Ochotona curzoniae*), and Tibetan people, breathe more and exhibit HVRs that are equivalent or greater in magnitude than species/populations native to low altitude (Beall et al., 1997; Brutsaert, 2007; Lague et al., 2016; Moore, 2000; Pichon et al., 2009; Scott and Milsom, 2007). In contrast, in some high-altitude residents in the Andes, such as Andean goose (*Chloephaga melanoptera*), guinea pigs (*Cavia porcellus*), and Andean people, breathing and the ventilatory response to hypoxia are reduced compared to their low-altitude counterparts (Beall, 2000; Brutsaert et al., 2005; Ivy et al., 2018; Lague et al., 2017; Schwenke et al., 2007). It has been difficult to determine whether these patterns of variation result from evolved differences or from environmentally-induced plasticity (acclimatization, developmental plasticity, etc.) (Brutsaert, 2016; Laguë, 2017; Moore, 2017). Nevertheless, these intriguing results suggest that there may be convergent mechanisms for coping with hypoxia at high altitude within a given geographic region, but divergent mechanisms between species inhabiting different geographic regions. These findings, however, arise from a small number of very different species, native to different habitats, with different lifestyles, activity levels, and respiratory physiologies. It thus remains unclear whether convergent and/or divergent responses will also be observed in similar closely-related species that independently colonized high altitude in one geographic region.

The objective of this study was to investigate whether the hypoxic ventilatory response has been altered across multiple duck species from the high Andes of Peru, and if so, whether there are similar or distinct changes in each high-altitude species. Waterfowl (Order Anseriformes) native to the Andes are a powerful taxonomic group for examining the general patterns of variation across high-altitude taxa, because many species have independently colonized similar aquatic habitats at high altitude (McCracken et al., 2009a; Natarajan et al., 2015). Previous studies of haemoglobin evolution in Andean waterfowl showed that genetically-based increases in haemoglobin-O_2 binding affinity have arisen in most high-altitude waterfowl examined to date (McCracken et al., 2009a; Natarajan et al., 2015), but other aspects of respiratory physiology have not been comprehensively examined in this group. Here, we examine the HVR of six species of ducks in their native high-altitude environment in the Andes, five of which were compared to a closely related population of the same species or to a sister species in their native environment at low altitude.

MATERIALS AND METHODS

Animals

Ducks were captured and studied in July and August of 2014 and 2015. Five species were captured and tested at high altitude (3812 m above sea level) at the Lake Titicaca National Reserve (Puno, Peru) in August 2014: speckled teal (*Anas flavirostris oxyptera*; n = 12, 4 males and 8 females), Andean ruddy duck (*Oxyura jamaicensis ferruginea*; n = 12, 5 males and 7 females), yellow-billed pintail (*A. georgica*; n = 13, 10 males and 3 females), cinnamon teal (*A. cyanoptera orinomus*; n = 12, 8 males and 4 females), and puna teal (*A. puna*; n = 12, 7 males and 5 females; body mass of 404 ± 11 g). Four species, representing closely related populations of the same species or sisters species of four of these high-altitude taxa, were captured at low altitude in Oregon, USA (at either Summer Lake Wildlife Management Area at 1260 m or

Malheur National Wildlife Refuge at 1256 m) in July 2015, and were tested at Summer Lake: green-winged teal (*A. crecca*; n = 10, 5 males and 5 females), ruddy duck (*O. j. jamaicensis*; n = 8, 4 males and 4 females), northern pintail (*A. acuta*; n = 10, 7 males and 3 females), and cinnamon teal (*A. cyanoptera septentrionalium*; n = 11, 6 males and 5 females). Torrent ducks (*Merganetta armata*) were also captured and tested in August 2015, both at high altitude (3000-4086 m above sea level; n = 8, all males) on the Chancay River Valley near Vichaycocha, Lima, Perú, and at low altitudes (1092-1665 m above sea level; n = 14, all males) on the Chillón River in Santa Rosa de Quives, Lima, Perú. Ducks were allowed to recover overnight from capture for at least 6-12 h, with unlimited access to water, before responses to acute hypoxia were measured. During this time, birds were held in large animal kennels with dry bedding. All experiments were performed within 2 days of capture, and birds were tube fed commercial duck chow if held for longer than 1 day in captivity, but food was always withheld 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-MIN AGRI-DGFFS/DGEFFS and 190-2015-SERFOR/ DGGSPFFS), U.S. Fish and Wildlife Service Region 1 Migratory Bird Permit Office MB68890B-0 (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 hypoxia responses

We measured the respiratory and metabolic responses to acute hypoxia using plethysmography and respirometry techniques similar to those used previously for Andean goose (Ivy et al., 2018; Lague et al., 2017). Ducks were held in a cradle that permitted unrestricted breathing, with their head in a 4 l opaque chamber that was sealed around the neck with a latex collar. Ducks were given 60-90 min to adjust to the apparatus (when they exhibited a noticeably relaxed and stable breathing pattern) before measurements began, with ambient air supplied to the head chamber at a flow rate of 5 l min^{-1} (volume in standard temperature and pressure of dry air, STPD). Measurements of breathing and metabolism were then recorded at several inspired O_2 tensions (PO_2), starting in ambient conditions (18 and 13 kPa at low and high altitude, respectively) and then during step-wise decreases in PO_2, for 25 min at each of the following steps: 18 (low-altitude ducks only), 13, 12, 9, 7, and 6 kPa. Dry incurrent air and nitrogen were mixed using pre-calibrated rotameters (Matheson Model 7400 Gas Mixer, E700 and E500 flowtubes, Oakville, ON, Canada) to achieve each level of hypoxia.

Metabolism, breathing, arterial O_2 saturation, and body temperature were measured continuously during the above 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, together with the flow of air through the head chamber, were used to calculate rates of O_2 consumption ($\dot{V}O_2$), as described by Lighton (2008), which we express here in volume units at STPD. Tidal volume (V_T) and breathing frequency (f_R) were determined from the flow oscillations of the biased outflow from the head chamber, measured using a pneumotachograph (8311A series, Hans Rudolph Inc, Shawnee, KS, USA) and differential pressure transducer (Validyne DP45, Cancoppas, Mississauga, ON, Canada) zeroed to baseline flow through the chamber. Body temperature (T_b) was measured continuously using a rectal probe (RET-1, Physitemp, New Jersey, USA). All of the above data were acquired using a PowerLab 16/32 and Labchart 8 Pro software (ADInstruments). Total ventilation ($\dot{V}_E$) was determined as the product of f_R and V_T. Both V_T and $\dot{V}_E$ are reported in volumes expressed at body temperature and pressure of water-saturated air (BTPS), which best reflects the air volumes moved by the animal. Air convection requirement (ACR) was calculated as the quotient of $\dot{V}_E$ and $\dot{V}O_2$. Pulmonary O_2 extraction (%) was calculated as $\dot{V}O_2$ divided by the product of $\dot{V}_E$ and the concentration of O_2 in inspired air (i.e., ml O_2 in STPD per ml air in BTPS). Arterial O_2 saturation (SaO_2) was measured using the MouseOx Plus pulse oximetry system and software (Starr Life Sciences, PA, USA) with neck collar sensors, which was enabled by plucking a small number of feathers from around the neck. We have previously demonstrated that the MouseOx Plus system is able to provide accurate measurements of SaO_2 in waterfowl (Ivy et al., 2018). Arterial O_2 saturation was measured in all low-altitude ducks and all torrent ducks, but in only a subset of high-altitude ducks (speckled teal: n = 6; Andean ruddy duck: n = 5; yellow-billed pintail: n = 6; cinnamon teal: n = 5).

Statistics

Two-factor ANOVA was generally used for most comparisons to examine the main effects and interactions of population/species altitude and acute inspired PO_2 (repeated measure) within each independent pair of closely related high-altitude and low-altitude populations/species, and we used Holm-Sidak post-tests to test for pairwise differences between populations/species within each inspired PO_2. However, for puna teal, a species for which we do not have data for a close low-altitude relative, we used one-factor ANOVA to examine the main effect of inspired PO_2. For body mass data, we used two-factor ANOVA and Holm-Sidak post-tests to test for the main effect of altitude and the pairwise differences between high- and low-altitude pairs. Values are reported as mean ± S.E.M. All statistical analysis was conducted with SigmaStat software (v. 3.5) with a significance level of $P < 0.05$.

RESULTS

Breathing and the hypoxic ventilatory response were elevated in some high-altitude ducks compared to their close relatives from low altitude (Fig. 2, Table 1). All ducks increased $\dot{V}_E$ by up to ~36-174% in response to acute hypoxia challenge (Fig. 2A), as reflected by significant main effects of inspired PO_2 for all high-low pairs in two-factor ANOVAs (Table 1). Two high-altitude taxa, torrent duck and yellow-billed pintail, exhibited higher $\dot{V}_E$ than their low-altitude counterparts across a range of inspired PO_2 (Fig. 2A), and there was a population main effect or population×PO_2 interaction for these species (Table 1). The remaining high-altitude taxa – Andean ruddy duck, cinnamon teal, and speckled teal – had similar or slightly reduced $\dot{V}_E$ when compared to closely related low-altitude taxa (Fig. 2A, Table 1). Increases in f_R (~17-170%) were the main contributor to increases in $\dot{V}_E$ in response to reductions in inspired PO_2 in nearly all species (Fig. 2B), with only modest changes in V_T (~4-50%) in some species (Fig. 2C, Table 1). The exception to this pattern was the puna teal, the only species for which we do not have data for a close lowland relative; this species increased $\dot{V}_E$ in acute hypoxia primarily by increasing V_T (Table 2). However, f_R was similar or lower in high-altitude ducks than in low-altitude ducks for all high-low comparisons (Fig. 2B, Table 1). High-altitude torrent ducks and yellow-billed pintails breathed with deeper V_T than their lowland counterparts across a range of inspired PO_2, which was the dominant contributor to the increases in $\dot{V}_E$ in these high-altitude taxa (Fig. 2C). However, this was not the case in high-altitude ruddy duck, cinnamon teal, or speckled teal, which had similar V_T to their low-altitude counterparts (Fig. 2C, Table 1).

Metabolic rates (as reflected by $\dot{V}O_2$) were stable or increased under hypoxic conditions across species (Fig. 3A, Table 1). $\dot{V}O_2$ was largely similar between high and low altitude, with the exception that high-altitude yellow-billed pintail had higher $\dot{V}O_2$ than closely related low-altitude northern pintail. This was reflected by a main effect of population in two-factor ANOVA (Table 1) and the differences were particularly evident as hypoxia became more severe (Fig. 3A). Increases in both $\dot{V}_E$ and pulmonary O_2 extraction with deepening hypoxia likely helped avoid any falls in $\dot{V}O_2$ across species. This was reflected by main effects of inspired PO_2 on ACR (Fig. 3B) and pulmonary O_2 extraction (Fig. 3C) for nearly all high-low pairs (Table 1). The increases in ACR during hypoxia were similar in high-altitude ruddy duck, cinnamon teal, and speckled teal when compared to their close low-altitude relatives, and there were no main effects of population on this variable. However, ACR was lower in the deepest levels of hypoxia (but not at the intermediate levels) in the other two high-altitude taxa, torrent duck and yellow-billed pintail, when compared to their close lowland relatives, and there were significant population×PO_2 interactions for these high-low comparisons (Table 1) High-altitude yellow-billed pintail appeared to counterbalance this decline in ACR in the most severe levels of hypoxia with an increase in pulmonary O_2 extraction compared to low-altitude northern pintails (Fig. 3C, Table 1).

T_b declined by up to ~1-2°C in response to acute hypoxia in most duck species (Fig. 4), as previously observed in many other birds (Kilgore et al., 2008; Novoa et al., 1991; Scott et al., 2008). The exception to this pattern were the two diving ducks from low altitude – torrent duck and ruddy duck – but these low-altitude populations differed from their conspecific high-altitude populations, which did exhibit a reduction in T_b in hypoxia (Fig. 4, Table 1). The variation in T_b did not appear to be clearly associated with any comparable variation in $\dot{V}O_2$ (Fig. 3A).

There were surprisingly few differences in SaO_2 in hypoxia between high-altitude and low-altitude ducks. As expected, SaO_2 decreased progressively with increasing severity of acute hypoxia, and the main effect of inspired PO_2 was seen across species (Fig. 5, Table 1). However, yellow-billed pintail was the only high-altitude duck that had a higher SaO_2 than their low-altitude counterpart, which was reflected by higher saturations than low-altitude northern pintails at levels of moderate hypoxia that are environmentally realistic in the high Andes (Fig. 5, Table

1). SaO_2 was similar between high- and low-altitude taxa for all other high-low comparisons. These data suggest that the differences in SaO_2 between the high- and low-altitude yellow-billed pintails may be a product of differences in both breathing and haemoglobin-O_2 affinity, as will be examined in more detail in the Discussion.

Body mass often differed between high- and low-altitude taxa (Fig. 6). There was variation between species pairs, with teals tending to be smaller than ruddy ducks and pintails. Body mass was greater in high-altitude speckled teal (30%), cinnamon teal (43%), and Andean ruddy duck (62%) compared to their close relatives from low altitude (Fig. 6A). In contrast, yellow-billed pintail was 27% smaller than northern pintail, and torrent duck was of similar body mass between high and low altitudes (Fig. 6A). The variation in body mass across pairs accounted for some of the variation in metabolic rate, particularly when excluding torrent ducks, in which case mass-specific $\dot{V}O_2$ (measured at a common PO_2 of 13 kPa) was related to body mass (M_b) with a scaling exponent of -0.33 (i.e., $\dot{V}O_2 \propto M_b^{-0.33}$) (Fig. 6B). However, there was no significant relationships between body mass and air convection requirement (data not shown), suggesting that body mass had no effect on breathing that was independent of its effect on metabolic rate.

DISCUSSION

Previous work has shown that breathing and the hypoxic ventilatory response of high-altitude natives differ from that of their low-altitude counterparts in distinct ways (see Introduction). Here, we show that breathing and pulmonary gas exchange in different lineages of high-altitude ducks can differ in distinct ways from their close relatives from low altitude, even among similar species inhabiting similar high-altitude habitats. Based on these and previous findings, high-altitude ducks differ from those from low altitude in at least two possible ways – (i) increases in $\dot{V}_E$ and the hypoxic ventilatory response and/or (ii) an increased haemoglobin-O_2 binding affinity (Natarajan et al., 2015). However, only when both occurred together, as in the yellow-billed pintail, did these differences lead to any net benefit in increasing SaO_2.

Increases in the hypoxic ventilatory response exist in some, but not all, high-altitude ducks

All ducks, from both low and high altitude, responded to acute hypoxia with robust increases in $\dot{V}_E$ and pulmonary O_2 extraction. The magnitude of these responses appear to be similar to previous studies of other waterfowl species, being somewhat intermediate between bar-headed geese (which exhibit pronounced increases in $\dot{V}_E$ but only modest increases in extraction in hypoxia) and Andean geese (which exhibit very modest increases in $\dot{V}_E$ but large increases in pulmonary O_2 extraction in hypoxia) (Scott et al., 2007; Lague et al., 2017). Increases in breathing frequency contributed the majority to increases in $\dot{V}_E$ in hypoxia, as is typical of many species of bird and mammal, with smaller to negligible increases in tidal volume (Fig. 2) (Scott et al., 2007; Lague et al., 2017). In contrast, puna teal increased $\dot{V}_E$ primarily by increasing tidal volume in hypoxia (Table 2). This hypoxia response of puna teal may be particularly beneficial for gas exchange, because increases in tidal volume are more effective at increasing parabronchial ventilation than are increases in breathing frequency (the former reduces the ratio of dead space gas in the air ventilating parabronchioles). Puna teal is endemic to high altitude and has likely been established in the high Andes for over one million years (McCracken et al., 2009b), and further examination of high-altitude adaptations in this species may be a fruitful direction for future research.

In comparison to close relatives from low altitude, the increase in $\dot{V}_E$ was elevated in only two duck taxa native to high altitude – torrent ducks and yellow-billed pintails (Fig. 2). One possible explanation for the differences in these two high-altitude groups is that chronic exposure to hypobaric hypoxia led to plasticity in the underlying neural networks controlling breathing, increasing $\dot{V}_E$ and enhancing the hypoxic ventilatory response, as has been observed in some (Black and Tenney, 1980; Lague et al., 2016) but not all (Powell et al., 2004) previous studies. A second possible explanation is that increases in $\dot{V}_E$ and the hypoxic ventilatory response arose from evolutionary adaptation to high altitude, as appears to have occurred in the bar-headed goose (Scott and Milsom, 2007). For this latter possibility to occur, the effects of selection would have to be very strong, because gene flow still occurs between high- and low-altitude populations of most of the species we examined (McCracken et al., 2009a; McCracken et al., 2009c; Natarajan et al., 2015).

The increases in $\dot{V}_E$ and the hypoxic ventilatory response in the yellow-billed pintail (but not torrent duck) from high altitude could also be a consequence of elevated metabolic rate (Fig. 3). Yellow-billed pintail are ~25% smaller than northern pintail (Fig. 6A), so the allometric effects of body mass on resting metabolic rate could explain some of this difference between species (Fig. 6B) (White et al., 2006), but the more substantial ~2-fold differences in metabolic rate at lower PO_2 cannot be explained by these more subtle effects of allometry. Nevertheless, the observation that yellow-billed pintail from high altitude had higher $\dot{V}_E$ (Fig. 2) but not higher ACR (Fig. 3) than their relatives from low altitude supports the suggestion that differences in metabolic rate could drive the differences in total ventilation. In fact, ACR was slightly less in this high-altitude taxon during severe hypoxia. Under these conditions, the higher metabolic rates in high-altitude pintails are matched by increases in pulmonary O_2 extraction in severe hypoxia as compared to low-altitude pintails (Fig. 3). This could reflect a high O_2 diffusing capacity in the lungs of high-altitude pintails, as previously observed for Andean geese (Maina et al., 2017).

However, it is also possible that the increases in metabolic rate in yellow-billed pintail from high altitude arise as a consequence of the increases in $\dot{V}_E$. Metabolic savings have been suggested as one possible advantage of blunting $\dot{V}_E$ and the HVR in some other high-altitude taxa, so long as O_2 demands can still be met (Powell, 2007). By a similar rationale, it is possible that increases in $\dot{V}O_2$ due to respiratory muscle activity could consume any added O_2 taken up into the blood as a result of breathing more in hypoxia. However, we have previously shown that the metabolic cost of breathing is quite low (~1-4% of resting metabolic rate) in all of the species studied here (York et al., 2017). We also found that the dynamic compliance of the respiratory system is greater in high-altitude yellow-billed pintail and in some other high-altitude ducks than in their low-altitude counterparts, such that many high-altitude ducks have higher breathing efficacy and can move a greater volume of air for a given power output of breathing (York et al., 2017).

Three high-altitude taxa – ruddy duck, cinnamon teal, and speckled teal – on the other hand, had similar levels of $\dot{V}_E$ as their low-altitude relatives (Fig. 2). In all three comparisons between high and low altitude pairs, increases in ACR were similar during progressive hypoxia. Although this lack of variation could reflect a lack of any plasticity or evolved changes in these high-altitude taxa, it could also reflect concurrent but opposing effects of plasticity and evolution on the control of breathing. The latter scenario could reflect counter-gradient variation, a term that describes situations in which the effects of phenotypic plasticity on a trait are opposed by local adaptation, thus minimizing phenotypic change along an environmental gradient (Conover and Schultz, 1995). Although increases in breathing improve O_2 uptake, they also result in excessive rates of CO_2 excretion and/or respiratory water loss (Powell, 2007). In this case, there could be opposing selective forces at high altitude for enhanced O_2 delivery versus preservation of acid-base homeostasis and/or water balance. This may also explain why some other species of high-altitude waterfowl, such as the Andean goose and crested duck, have evolved a blunted hypoxic ventilatory response (Ivy et al., 2018; Lague et al., 2017).

Increases in haemoglobin-O_2 affinity have evolved in some, but not all, high-altitude ducks

Some of the high-altitude duck species studied here have evolved an increased haemoglobin-O_2 binding affinity compared to their close relatives from low altitude (McCracken et al., 2009a; Natarajan et al., 2015). Birds co-express two major haemoglobin isoforms – HbA and HbD – that express different α-chain subunits, encoded by two distinct α-globin genes (Storz, 2016a). HbA is the major isoform, comprising ~70-80% of all blood Hb, and this isoform has a higher affinity (lower P_{50}, the PO_2 at 50% saturation) in high-altitude yellow-billed pintail, cinnamon teal, speckled teal, and puna teal when measured in the presence of physiologically relevant concentrations of allosteric modifiers (Natarajan et al., 2015). In contrast, Hb-O_2 affinity is very similar in high-altitude torrent duck and ruddy duck as compared to their low-altitude relatives. However, the Hb-O_2 affinity of the low-altitude populations of each of these two diving species is already characteristic of the high-altitude populations, not low-altitude populations, of non-diving species (Natarajan et al., 2015). It is possible that selection for traits that improve breath holding underwater increased Hb-O_2 affinity in these diving duck species, as observed in some other diving birds (Meir and Ponganis, 2009), such that they may have been “preadapted” to life at high-altitude. Then upon colonizing high altitude, these diving species may have since been constrained in increasing Hb-O_2 affinity any further, or there may have been weaker selective pressure for doing so. Nevertheless, in general, there is very strong evidence for convergent evolution of increased Hb-O_2 affinity in high-altitude taxa, making a strong case for the adaptive value of this trait for life in hypoxic environments (Storz, 2016b). However, the physiological implications of this evolved trait for SaO_2 and respiratory gas exchange have seldom been examined in high-altitude natives.

Improvements in arterial O_2 saturation in hypoxia are only present in high-altitude taxa with concurrent increases in both ventilation and haemoglobin-O_2 affinity

Our current findings suggest that the relative advantage of increasing Hb-O_2 affinity for improving arterial O_2 saturation is contingent upon the relative levels of ventilation. Of the three species of high-altitude ducks that have evolved an increased Hb-O_2 affinity that we examined (yellow-billed pintail, cinnamon teal, speckled teal), only one – the yellow-billed pintail – maintained higher SaO_2 in hypoxia than their close relative from low altitude, and only in moderate hypoxia (Fig. 5). The yellow-billed pintail was also the only one of this group of high-altitude ducks in which $\dot{V}_E$ was elevated compared to its low-altitude counterpart during hypoxia. The other two high-altitude ducks that have evolved higher Hb-O_2 affinities – cinnamon teal and speckled teal – had similar or lower $\dot{V}_E$ as compared to their close low-altitude relatives and SaO_2 in hypoxia was not enhanced. In high-altitude torrent duck, where $\dot{V}_E$ was higher but Hb-O_2 affinity was similar to its low-altitude relative, SaO_2 in hypoxia was also not enhanced. These findings suggest that evolved increases in Hb-O_2 affinity may only be expected to improve SaO_2 in high-altitude taxa that also exhibit higher $\dot{V}_E$ than their low-altitude relatives. Therefore, interactions between multiple respiratory traits in the O_2 transport cascade affect the integrated systems-level function of animals native to high altitude.

COMPETING INTERESTS

The authors declare no competing or financial interests.

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LIST OF ABBREVIATIONS

ACR – air convection requirement

BTPS – body temperature pressure, saturated

f_R – breathing frequency

Hb-O_2 – haemoglobin-oxygen

HVR – hypoxic ventilatory response

M_b – body mass

PaO_2 – arterial partial pressure of oxygen

PCO_2 – partial pressure of carbon dioxide

PO_2 – partial pressure of oxygen

SaO_2 – arterial oxygen saturation

STPD – standard temperature and pressure, dried

T_b – body temperature

VAH – ventilatory acclimatization to hypoxia

$\dot{V}_E$ – total ventilation

$\dot{V}O_2$ – oxygen consumption rate

V_T – tidal volume

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Figures

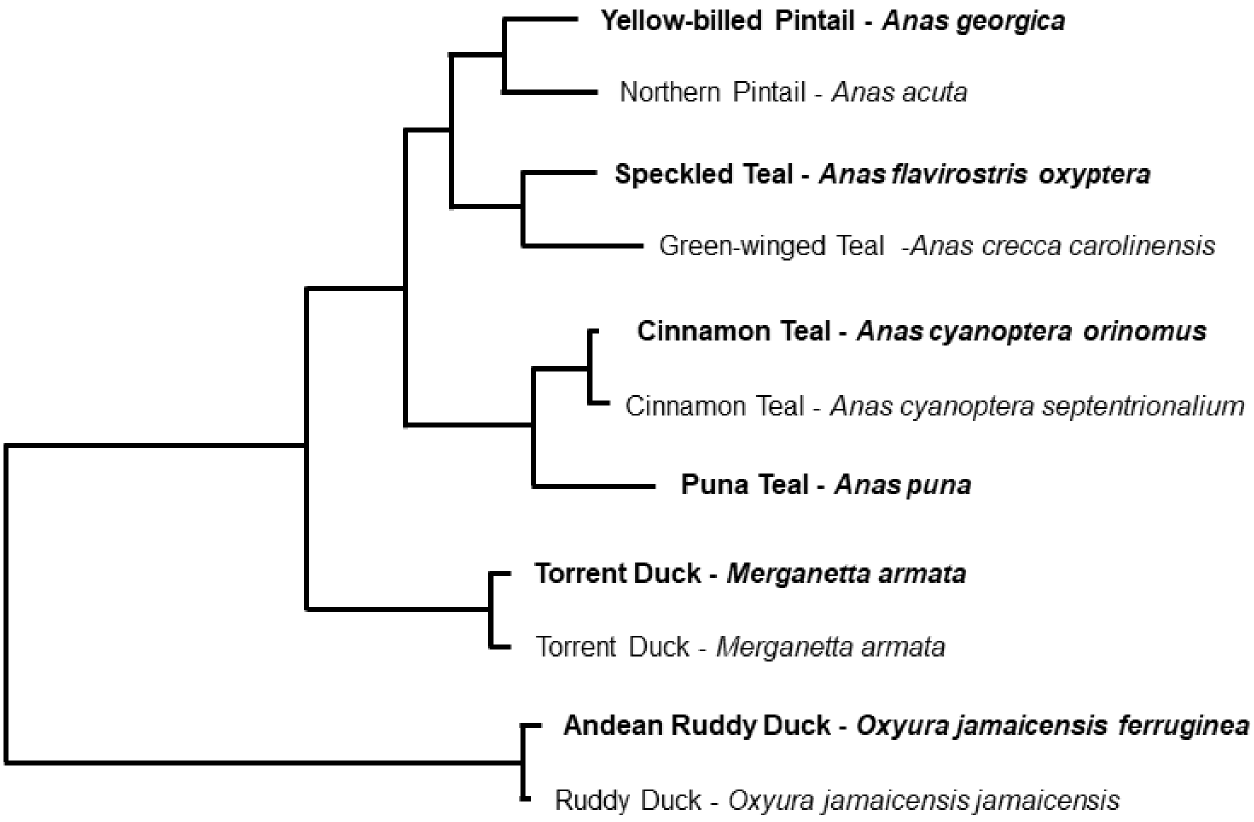

Figure 1. Phylogeny of duck species compared in this study. Bolded names denote high-altitude species. Adapted from (Bulgarella et al., 2010; Gonzalez et al., 2009; Johnson et al., 1999).

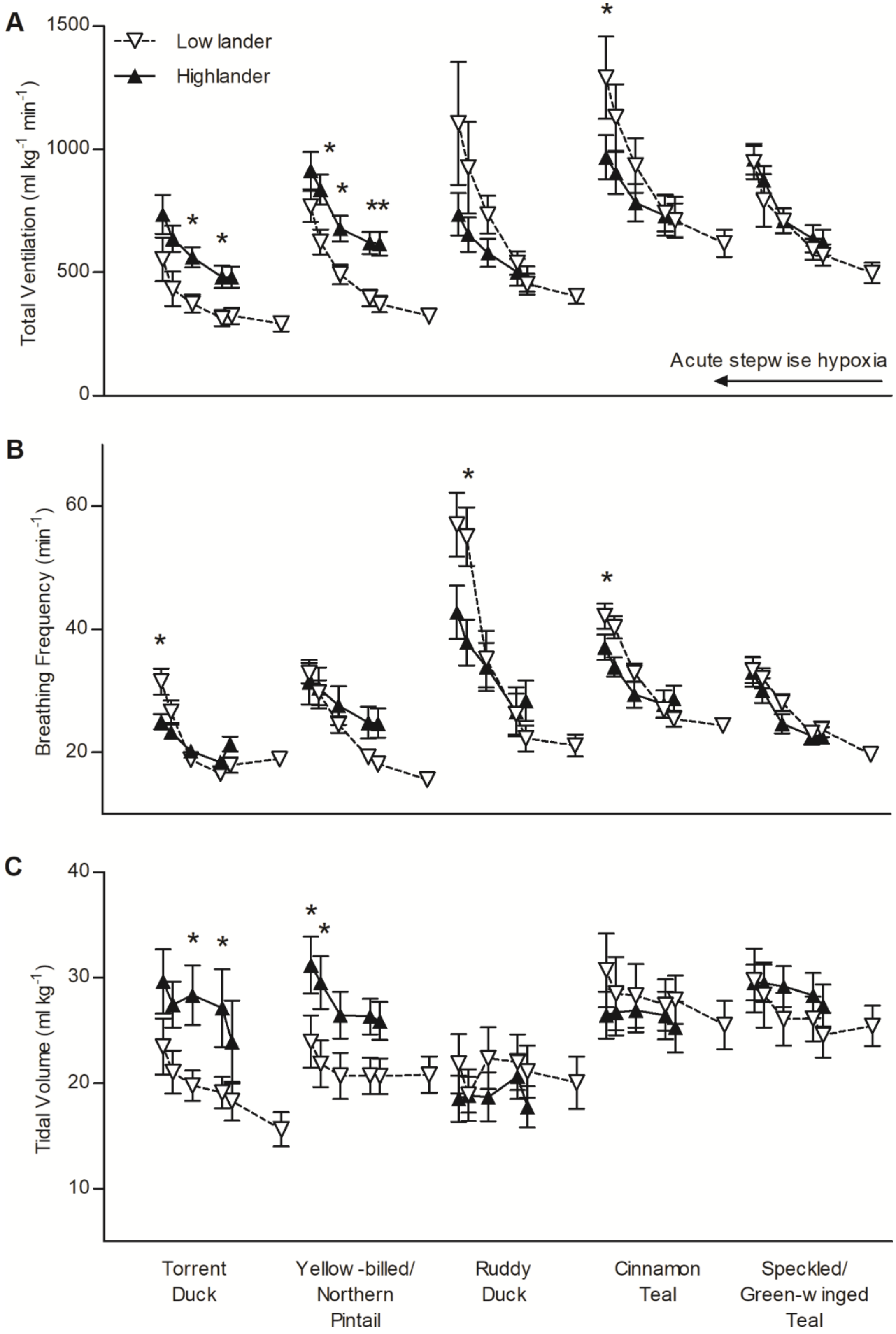
A
1500
1000
500
0
Total Ventilation (ml kg^{-1} min^{-1})
Low lander
Highlander
Acute stepw ise hypoxia
*
B
60
40
20
Breathing Frequency (min^{-1})
C
40
30
20
10
Tidal Volume (ml kg^{-1})
Torrent Duck
Yellow -billed/ Northern Pintail
Ruddy Duck
Cinnamon Teal
Speckled/ Green-w inged Teal

Figure 2. Total ventilation (A), breathing frequency (B), and tidal volume (C) during hypoxia for high-altitude populations of several duck species, compared to low-altitude populations of the same species or to low-altitude congeners. Responses to acute hypoxia for each high-low pair 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. * represents a significant pairwise difference between highlanders and lowlanders within an inspired PO_2 using Holm-Sidak post-tests. n = 14 low-altitude torrent ducks, n = 8 high-altitude torrent ducks, n = 10 low-altitude northern pintails, n = 13 high-altitude yellow-billed pintails, n = 8 low-altitude ruddy ducks, n = 12 high-altitude ruddy ducks, n = 11 low-altitude cinnamon teal, n = 12 high-altitude cinnamon teal, n = 10 low-altitude green-winged teal, n = 12 high-altitude speckled teal.

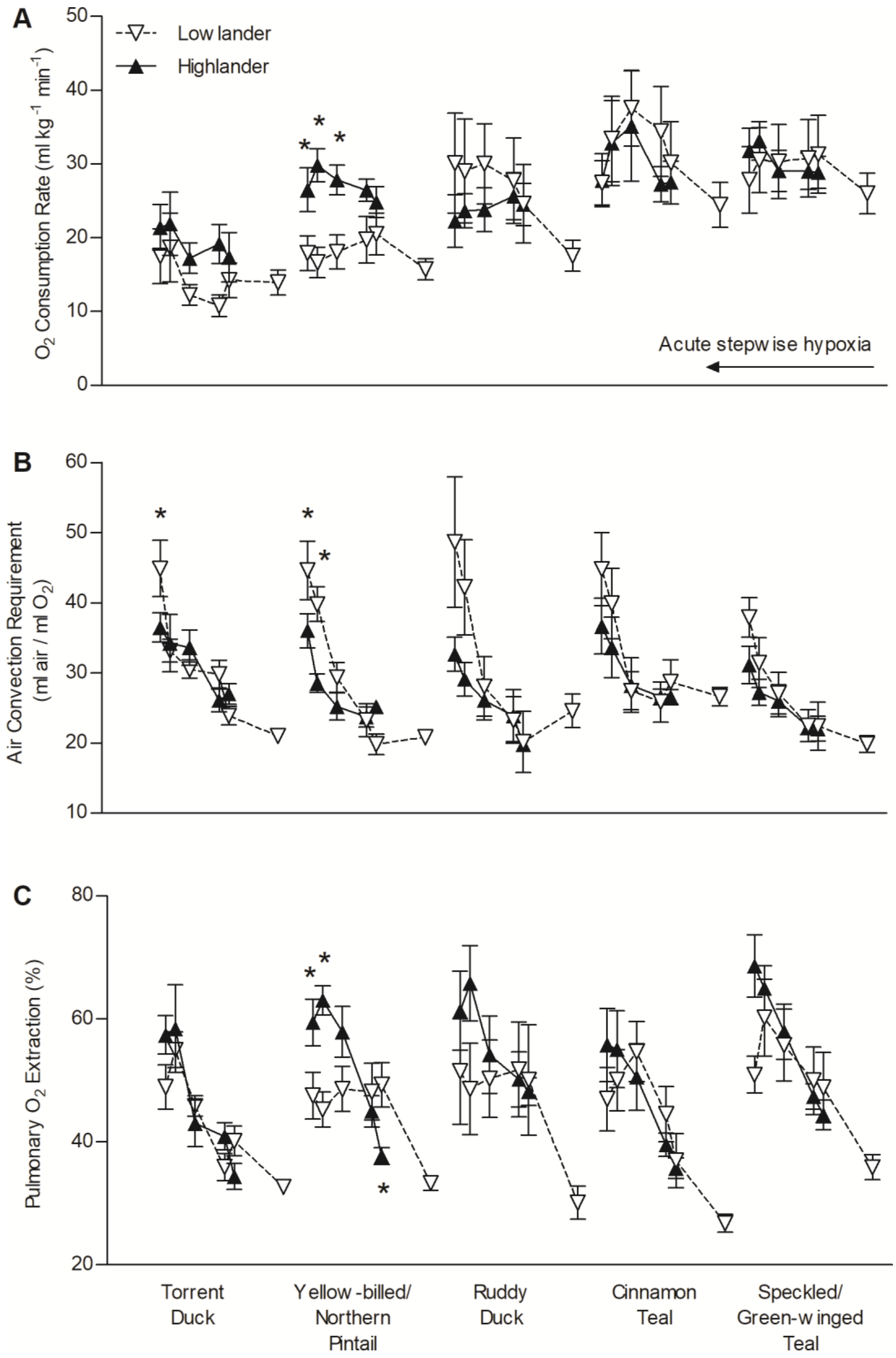
A
B
C
Lowlander
Highlander
O2 Consumption Rate (ml kg-1 min-1)
50
40
30
20
10
0
Acute stepwise hypoxia
Air Convection Requirement (ml air / ml O2)
60
50
40
30
20
10
Pulmonary O2 Extraction (%)
80
60
40
20
Torrent Duck
Yellow-billed/ Northern Pintail
Ruddy Duck
Cinnamon Teal
Speckled/ Green-winged Teal
*

Figure 3. (A) Rate of O_2 consumption, (B) air convection requirement, and (C) pulmonary O_2 extraction during hypoxia for high-altitude populations of several duck species, compared to low-altitude populations of the same species or to lowland congeners. Responses to acute hypoxia for each high-low pair 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. * represents a significant pairwise difference between high and low altitude within an inspired PO_2 using Holm-Sidak post-tests. N as for Figure 2.

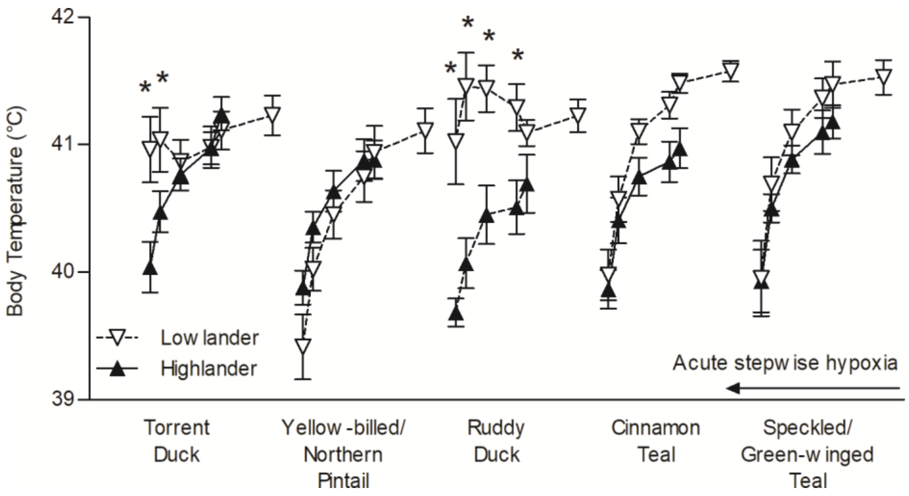

Figure 4. Body temperature during hypoxia for high-altitude populations of several duck species, compared to low-altitude populations of the same species or to lowland congeners. Responses to acute hypoxia for each high-low pair are shown from right to left for stepwise reductions in inspired O_2 tension (PO_2): 18 (lowlanders only), 13, 12, 9, 7, and 6 kPa O_2. * represents a significant pairwise difference between high and low altitude within an inspired PO_2 using Holm-Sidak post-tests. N as for Figure 2.

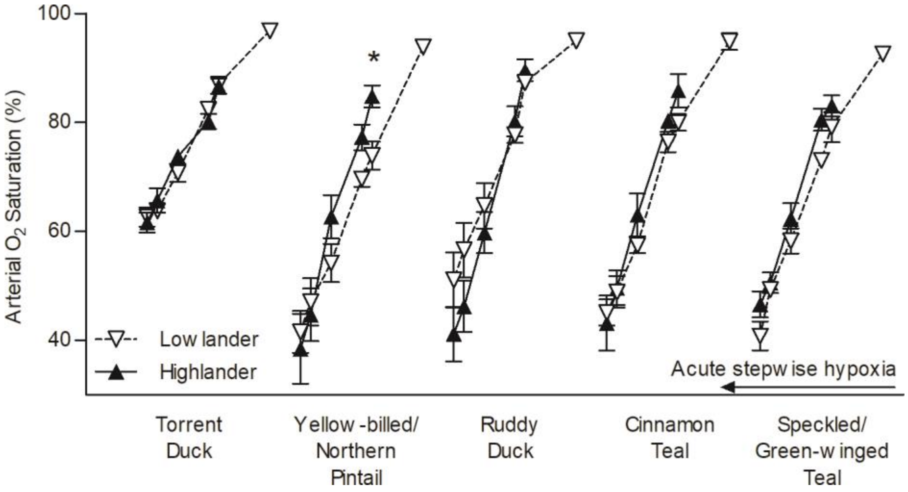

Figure 5. Arterial O_2 saturation during hypoxia for high-altitude populations of several duck species, compared to low-altitude populations of the same species or to lowland congeners. Responses to acute hypoxia for each high-low pair are shown from right to left for stepwise reductions in inspired O_2 tension (PO_2): 18 (lowlanders only), 13 12, 9, 7, and 6 kPa O_2. * represents a significant pairwise difference between highlanders and lowlanders within an inspired PO_2 using Holm-Sidak post-tests. N = 10 low-altitude torrent ducks, n = 9 high-altitude torrent ducks, n = 10 low-altitude northern pintails, n = 6 high-altitude yellow-billed pintails, n = 8 low-altitude ruddy ducks, n = 7 high-altitude ruddy ducks, n = 10 low-altitude cinnamon teal, n = 5 high-altitude cinnamon teal, n = 9 low-altitude green-winged teal, n = 6 high-altitude speckled teal.

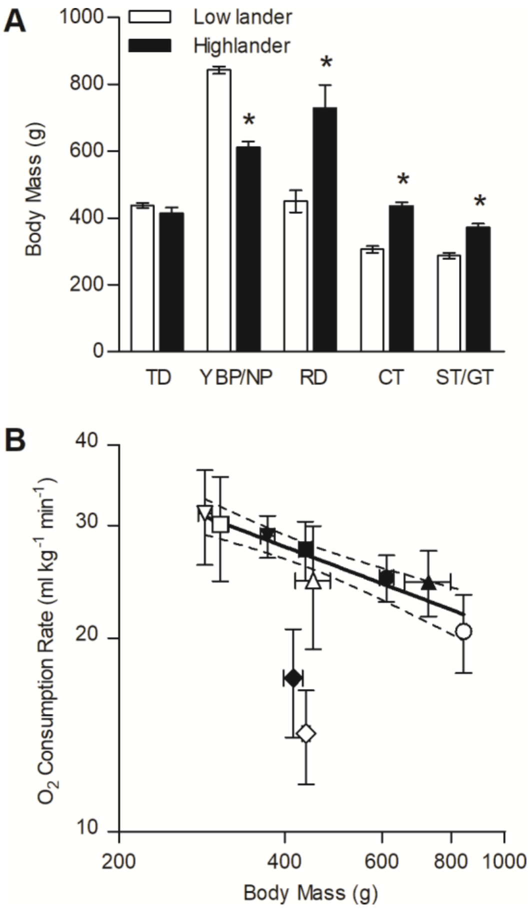

Figure 6. Body mass (M_b) and its relationship to O_2 consumption rate ($\dot{V}O_2$) across high-altitude and low-altitude ducks. (A) There were significant main effects of species pair ($F_{4,100}$=71.857, P<0.001) and altitude ($F_{1,100}$=7.171, P=0.009) on M_b in two-factor ANOVA. * represents a significant pairwise difference between highlanders and lowlanders within a high-low pair. N as in Figure 2. (B) There was a strong allometric relationship between $\dot{V}O_2$ (measured at 13 kPa for all taxa) and M_b (both shown on a log scale) across taxa when torrent ducks were excluded ($\dot{V}O_2 = 195\ M_b^{-0.33}$; black line, with dotted lines representing 95% confidence intervals of the regression). Data points are mean ± S.E.M. and symbols are as follows: black, highland taxon; white, lowland taxon; diamonds, torrent ducks (TD); circles, yellow-billed pintail (YBP) or northern pintail (NP); upwards triangles, ruddy ducks (RD); squares, cinnamon teal (CT); downwards triangles, speckled teal (ST) or green-winged teal (GT).

Table 1. Statistical results of two-way ANOVA of acute hypoxia responses

	Highland species	Population effect		Inspired PO_2 effect		Interaction	
		F-value	P-value	F-value	P-value	F-value	P-value
Total ventilation	Torrent duck	2.725	0.115	44.79	**<0.001**	3.213	**0.017**
	Yellow-billed pintail	8.237	**0.009**	77.76	**<0.001**	1.268	0.289
	Ruddy duck	4.004	0.060	40.96	**<0.001**	11.38	**<0.001**
	Cinnamon teal	1.116	0.303	38.58	**<0.001**	6.130	**<0.001**
	Speckled teal	0.026	0.874	60.75	**<0.001**	0.369	0.830
Breathing frequency	Torrent duck	0.208	0.653	35.35	**<0.001**	7.431	**<0.001**
	Yellow-billed pintail	0.687	0.417	58.50	**<0.001**	7.400	**<0.001**
	Ruddy duck	0.792	0.385	55.51	**<0.001**	10.33	**<0.001**
	Cinnamon teal	0.975	0.335	49.85	**<0.001**	6.626	**<0.001**
	Speckled teal	0.577	0.457	25.96	**<0.001**	0.456	0.767
Tidal volume	Torrent duck	5.236	**0.034**	9.891	**<0.001**	1.489	0.214
	Yellow-billed pintail	4.495	**0.046**	12.55	**<0.001**	1.077	0.373
	Ruddy duck	0.982	0.335	1.466	0.222	1.099	0.364
	Cinnamon teal	0.388	0.540	1.724	0.152	1.218	0.309
	Speckled teal	0.316	0.581	7.597	**<0.001**	1.766	0.145
O_2 consumption rate	Torrent duck	1.745	0.206	4.876	**0.002**	0.754	0.559
	Yellow-billed pintail	8.993	**0.007**	0.363	0.834	3.605	**0.009**
	Ruddy duck	2.144	0.160	0.656	0.625	1.571	0.192
	Cinnamon teal	0.224	0.641	1.864	0.124	0.347	0.846
	Speckled teal	0.017	0.897	0.246	0.911	0.723	0.579
Air convection requirement	Torrent duck	0.216	0.649	14.58	**<0.001**	3.508	**0.012**
	Yellow-billed pintail	2.776	0.110	41.55	**<0.001**	8.564	**<0.001**
	Ruddy duck	0.061	0.807	2.494	0.051	0.410	0.801
	Cinnamon teal	1.052	0.317	10.317	**<0.001**	1.158	0.335
	Speckled teal	1.184	0.291	9.400	**<0.001**	0.776	0.544
Pulmonary O_2 extraction	Torrent duck	0.309	0.586	17.23	**<0.001**	2.295	0.070
	Yellow-billed pintail	1.807	0.193	7.701	**<0.001**	11.48	**<0.001**
	Ruddy duck	0.052	0.822	1.438	0.231	0.792	0.534
	Cinnamon teal	0.025	0.877	8.224	**<0.001**	1.395	0.243
	Speckled teal	0.515	0.482	4.873	**0.002**	2.058	0.096
Body temperature	Torrent duck	1.312	0.266	10.77	**<0.001**	11.17	**<0.001**
	Yellow-billed pintail	0.882	0.358	81.51	**<0.001**	3.070	**0.021**
	Ruddy duck	10.99	**0.003**	3.274	**0.016**	3.070	**0.022**
	Cinnamon teal	3.240	0.086	60.99	**<0.001**	1.624	0.176
	Speckled teal	0.605	0.447	37.18	**<0.001**	3.16	0.867
Arterial O_2 saturation	Torrent duck	0.008	0.932	107.8	**<0.001**	1.361	0.256
	Yellow-billed pintail	1.237	0.285	66.89	**<0.001**	2.485	0.054
	Ruddy duck	0.399	0.547	45.33	**<0.001**	1.133	0.361
	Cinnamon teal	2.071	0.174	85.79	**<0.001**	0.812	0.523
	Speckled teal	3.795	0.073	102.2	**<0.001**	1.032	0.402

See text for the low-altitude population/species that were compared to each high-altitude species.

Significant values are bolded.

Table 2. Hypoxia responses of the high-altitude puna teal

	Inspired PO_2 (kPa)					Main effect of inspired PO_2	
	13	12	9	7	6	F-value	P-value
Total ventilation (ml kg^{-1} min^{-1})	651.5 ± 54.8	655.6 ± 54.4	743.7 ± 64.4*	830.7 ± 60.7*	903.6 ± 62.6*	59.83	**<0.001**
Breathing frequency (min^{-1})	22.96 ± 1.54	21.65 ± 1.37	22.10 ± 1.50	23.19 ± 1.55	24.48 ± 1.59	4.296	**0.005**
Tidal volume (ml kg^{-1})	28.88 ± 2.29	30.87 ± 2.47	34.16 ± 2.55*	36.80 ± 2.75*	38.03 ± 2.88*	41.93	**<0.001**
O_2 consumption rate (ml kg^{-1} min^{-1})	26.95 ± 1.79	25.08 ± 2.75	28.77 ± 4.00	24.43 ± 3.12	22.78 ± 2.71	1.695	0.168
Air convection requirement (ml air ml^{-1} O_2)	12.34 ± 0.37	14.61 ± 1.49	15.01 ± 1.44	19.55 ± 1.67*	22.89 ± 2.35*	8.894	**<0.001**
Pulmonary O_2 extraction (%)	39.23 ± 1.25	40.09 ± 3.56	51.55 ± 4.23	50.92 ± 4.93	51.28 ± 5.06	3.356	**0.018**
Body temperature (°C)	40.59 ± 0.14	40.45 ± 0.13	40.24 ± 0.09*	39.92 ± 0.09*	39.50 ± 0.10*	72.07	**<0.001**
Arterial O_2 saturation (%)	83.23 ± 0.86	77.44 ± 0.53	61.97 ± 3.28*	43.93 ± 5.17*	40.01 ± 5.21*	57.26	**<0.001**

PO_2, partial pressure of O_2; values are mean ± SEM, * represents a significant difference compared to 13 kPa O_2