

Body temperature depression and peripheral heat loss accompany the metabolic and ventilatory responses to hypoxia in low and high altitude birds

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SUMMARY

The objectives of this study were to compare the thermoregulatory, metabolic and ventilatory responses to hypoxia of the high altitude bar-headed goose with low altitude waterfowl. All birds were found to reduce body temperature (T_b) during hypoxia, by up to 1–1.5°C in severe hypoxia. During prolonged hypoxia, T_b stabilized at a new lower temperature. A regulated increase in heat loss contributed to T_b depression as reflected by increases in bill surface temperatures (up to 5°C) during hypoxia. Bill warming required peripheral chemoreceptor inputs, since vagotomy abolished this response to hypoxia. T_b depression could still occur without bill warming, however, because vagotomized birds reduced T_b as much as intact birds. Compared to both greylag geese and pekin ducks, bar-headed geese required more severe hypoxia to initiate T_b depression and heat loss from the bill. However, when T_b depression or bill warming were expressed relative to arterial O_2 concentration (rather than inspired O_2) all species were similar; this suggests that enhanced O_2 loading, rather than differences in thermoregulatory control centres, reduces T_b depression during hypoxia in bar-headed geese. Correspondingly, bar-headed geese maintained higher rates of metabolism during severe hypoxia (7% inspired O_2), but this was only partly due to differences in T_b . Time domains of the hypoxic ventilatory response also appeared to differ between bar-headed geese and low altitude species. Overall, our results suggest that birds can adjust peripheral heat dissipation to facilitate T_b depression during hypoxia, and that bar-headed geese minimize T_b and metabolic depression as a result of evolutionary adaptations that enhance O_2 transport.

Key words: thermoregulation, thermal windows, respiration, breathing pattern.

INTRODUCTION

In order to survive in hypoxic environments, animals must continue to balance cellular oxygen supply and demand. In many hypoxia-adapted animals the capacity to maintain this balance is enhanced by a combination of physiological and biochemical responses that increase O_2 supply and/or reduce O_2 demand (Hochachka, 1985). Coordinated metabolic depression appears to be the most pervasive strategy for reducing O_2 demand and surviving in severe hypoxia, and occurs through concerted responses by individual cells and whole physiological control systems (Hochachka et al., 1996; Guppy and Withers, 1999; Boutilier, 2001).

Reductions in body temperature (T_b) should facilitate metabolic depression during hypoxia by reducing temperature-dependent O_2 demands. T_b depression is believed to result from a decrease in T_b setpoint that is regulated by thermoregulatory control regions in the hypothalamus and/or spinal cord (Crawshaw et al., 1985; Simon et al., 1986; Wood and Gonzales, 1996; Bicego et al., 2007), presumably by altering the balance between metabolic heat generation and heat loss. Peripheral heat loss is regulated by controlling blood flow to specific regions of the body surface, which alters surface temperature and thus the temperature differential driving heat dissipation (Klir and Heath, 1994; Mauck et al., 2003). These ‘thermal windows’ are typically poorly insulated, and include the ears, feet and nose of mammals (Klir and Heath, 1992), or the bill and feet of birds (Kilgore and Schmidt-Nielsen, 1975; Baudinette et al., 1976; Hagan and Heath, 1980). Despite its known importance for thermoregulation in general, the role and control of peripheral

heat loss from thermal windows during hypoxic T_b depression has received very little attention (cf. Tattersall and Milsom, 2003).

In addition to reductions in O_2 demand *via* metabolic depression, O_2 supply during hypoxia can be improved. The O_2 transport pathway from environment to mitochondria has several components, including ventilation, pulmonary diffusion, circulation and tissue diffusion (Weibel, 1984). Control of this O_2 supply pathway is well understood in vertebrates (Bouverot, 1978; Taylor et al., 1999), but the relative importance of alterations in O_2 supply *versus* O_2 demand during hypoxia is unclear. Hypoxia adaptation could enhance the capacity for either O_2 transport or metabolic depression, depending on the selective pressure driving hypoxia tolerance. For example, the bar-headed goose (*Anser indicus*) flies over the Himalayas on its migratory route between South and Central Asia, at altitudes of up to 9000 m, where O_2 pressures are five times lower than at sea level (Swan, 1970; Javed et al., 2000). O_2 consumption must concurrently increase 10- to 15-fold above resting levels in this species to sustain flight (Ward et al., 2002). Metabolic depression is clearly not feasible in bar-headed geese while flying in hypoxia, and it is conceivable that this species should minimize heat loss and T_b depression during hypoxia.

Little comparative data exist concerning the use of T_b depression as a strategy for matching O_2 supply and demand. In the present study we investigated heat loss and T_b depression during hypoxia in birds, and examined the relationship between these thermoregulatory variables and the metabolic and ventilatory responses to hypoxia. Bar-headed geese were compared with two low altitude waterfowl

species, the closely related greylag goose (*Anser anser*) and the more distantly related pekin duck (*Anas platyrhynchos*). We hypothesized that thermal windows would be used to help depress T_b in birds during hypoxia, and that the degree of T_b depression would be inversely related to the capacity for maintaining O_2 supply. We also hypothesized that bar-headed geese would minimize metabolic depression in hypoxia, and would therefore reduce heat loss and T_b depression compared with low altitude birds.

MATERIALS AND METHODS

Experimental animals

Experiments were performed on seven bar-headed geese *Anser indicus* Bechstein (2.1–3.1 kg), four greylag geese *Anser anser* L. (3.8–4.8 kg), and 13 pekin ducks *Anas platyrhynchos* L. (2.7–3.9 kg). All animals were bred and raised at sea level, either at the Animal Care Facility of the University of British Columbia (UBC) or by local suppliers. Animals were housed outdoors at UBC, and were fasted for 1 day before each experiment but continued to receive free access to water. All animal care and experimentation was conducted according to UBC animal care protocol #A04-1013.

Surgical procedures

Six of the ducks were bilaterally vagotomized to determine the responses of waterfowl to hypoxia in the absence of stimulation by peripheral chemoreceptor afferents. A surgical plane of anaesthesia was maintained with isoflurane, and local analgesia (Lidocaine) was applied to the site of incision. Both vagi were isolated in the upper region of the neck and then cut, after which the skin was sutured.

Measurements

Body plethysmography was used to measure breathing, as described previously (Dodd and Milsom, 1987; Dodd et al., 2007; Scott and Milsom, 2007). The plethysmograph consisted of two parts, a body compartment and a head compartment, separated from each other by a flexible latex collar. The head compartment was used to administer specific gas mixtures, using calibrated N_2 and O_2 flowmeters, which were monitored with an oxygen analyzer (Raytech, Vancouver, BC, Canada). Changes in body volume (due to ventilatory movements) were detected with a pneumotachograph (Fleisch, Richmond, VA, USA) connected to a differential pressure transducer (Validyne, Northridge, CA, USA) to yield a measurement of ventilatory flow. Ventilatory flow, T_b (measured with a flexible rectal thermometer), fractional O_2 composition of gas entering and leaving the head compartment, and airflow through the head compartment were recorded using Windaq® data acquisition software (Dataq Instruments, Akron, OH, USA).

Bill surface temperatures (T_{bill}) were measured using a portable infrared thermal imaging camera (Model 7515; Mikron Instruments, Oakland, NJ, USA). The camera was mounted directly above the head compartment of the plethysmograph, which was sealed with transparent polyvinylidene chloride (PVDC) film (Saran Wrap®, S. C. Johnson and Son, Brantford, ON, Canada) to provide a window with minimal absorption for infrared radiation to pass. Commercial software (MikroSpec RT®, Mikron Instruments) was used to determine average T_{bill} from thermal images. Data were corrected for the slight decrease in detected temperature ($\sim 0.2^\circ\text{C}$) that was caused by heat absorption by the PVDC film.

Experimental protocols

For all experiments on intact birds, the animals were placed in the water-jacketed plethysmograph (held between $11\text{--}13^\circ\text{C}$) and allowed 60–90 min to adjust to their surroundings. This temperature

is well within the thermoneutral zone of all the species (V.C., G.R.S., W.K.M. and G.J.T., unpublished), and birds can be held at this temperature for several hours in normoxia and exhibit no significant changes in T_b , metabolism or breathing. In the first experiment (stepwise hypoxia), seven bar-headed geese, seven pekin ducks, and four greylag geese were used. Birds were exposed to progressive step reductions in the fractional O_2 composition of inspired gas ($F_{I_{O_2}}$: 21%, 12%, 9%, 7%, and in bar-headed geese only, 5%) with each step lasting 15 min. The most severe level of hypoxia was followed

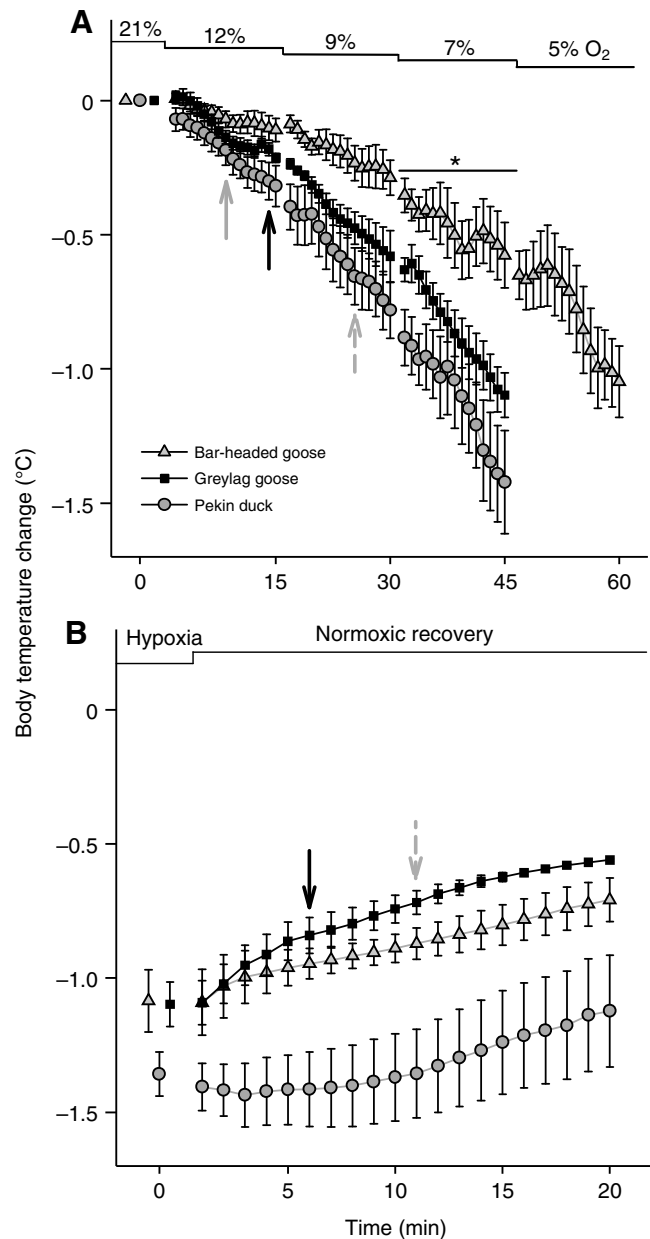


Fig. 1. (A) Body temperature (T_b) depression during stepwise hypoxia was less severe in bar-headed geese (light grey triangles) than in greylag geese (black squares) or pekin ducks (dark grey circles). Significant T_b depression began earlier in greylag geese (black arrow) and pekin ducks (dark grey arrow) than in bar-headed geese (light grey dashed arrow), and bar-headed geese reduced T_b less during 7% inspired O_2 (asterisk). (B) T_b increased significantly during 20 min of recovery in normoxia in bar-headed geese (light grey dashed arrow) and greylag geese (black arrow) but not in pekin ducks. The body temperature change in each individual was determined by subtracting its pre-hypoxia T_b value (see Results).

by a 20 min normoxic recovery period. In the second experiment (prolonged hypoxia), birds (five bar-headed geese and four greylag geese) were exposed to 9% O₂ for 60 min, followed by a 30 min recovery in normoxia. At the end of each experiment the bird was returned to the Animal Care Facility.

The first experiment was also performed on vagotomized ducks. However, many bird species do not tolerate chronic bilateral vagotomy (Fedde and Burger, 1963), so these ducks were placed directly into the experimental apparatus after surgery to minimize the chronic effects of vagotomy. Birds were kept in normoxia for 60–90 min to adjust to their surroundings and allow time for the residual effects of isoflurane anaesthesia to diminish. Three vagotomized ducks were then exposed to the stepwise hypoxia protocol described above. Three other vagotomized ducks were exposed to normoxia for the same duration to control for the effects of vagotomy and anaesthesia alone (in absence of hypoxia). Birds were sacrificed with an intramuscular overdose of sodium pentobarbital after completing the protocol.

Data and statistical analyses

All data acquired in Windaq were analyzed using a specially written Matlab[®] (version 7, Mathworks) program. Inspiratory tidal volume (V_T), breathing frequency (f_L), total ventilation (product of V_T and f_L), oxygen consumption rate (\dot{V}_{O_2}), and air convection requirement (quotient of total ventilation and \dot{V}_{O_2}) were determined as previously described (Scott and Milsom, 2007). Due to the effect of temperature on metabolism, we also calculated what mean \dot{V}_{O_2} would have been in absence of hypoxic T_b depression (\dot{V}_{O_2} , corrected):

$$\dot{V}_{O_2, \text{ corrected}} = \dot{V}_{O_2} \times Q_{10}^{\Delta T_b/10}. \quad (1)$$

In Eqn 1, ΔT_b is the magnitude of body temperature depression (using values measured after 5, 10 or 15 min at each $F_{I_{O_2}}$, as appropriate), and we made these calculations assuming Q_{10} values of both 2 and 3.

Data are reported as means \pm s.e.m. Two factor (species and time) repeated-measures analysis of variance (ANOVA) and Holm–Sidak *post-hoc* tests were used to determine statistical significance within and between species (using a significance level of $P < 0.05$). Least-

squares linear regression was used to assess the relationships between arterial O₂ concentration, which was measured in our previous study (Scott and Milsom, 2007) and T_b , T_{bill} or \dot{V}_{O_2} . Statistical tests were performed using Sigmaplot software (version 4, Systat Software Inc., San Jose, CA, USA).

RESULTS

Thermoregulatory responses to stepwise hypoxia

All three species depressed body temperature in response to step reductions in inspired O₂, and the extent of T_b depression increased with the severity of hypoxia (Fig. 1A). Initial body temperatures were not significantly different between species (bar-headed geese, $41.3 \pm 0.2^\circ\text{C}$; greylag geese, $41.6 \pm 0.2^\circ\text{C}$; and pekin ducks, $41.9 \pm 0.3^\circ\text{C}$; $P > 0.05$). Unlike the low altitude species, however, whose initial reductions in T_b occurred after 9 min (pekin ducks) and 14 min (greylag geese) of 12% inspired O₂ ($F_{I_{O_2}}$), bar-headed geese did not significantly reduce T_b until 10 min into 9% $F_{I_{O_2}}$. Furthermore, bar-headed geese experienced less body temperature depression than both low altitude species during 7% $F_{I_{O_2}}$ (Fig. 1A). Both low altitude species had reduced T_b by more than 1°C during 7% $F_{I_{O_2}}$, but the same degree of T_b depression did not occur in bar-headed geese until the later stages of 5% $F_{I_{O_2}}$. We did not expose greylag geese or pekin ducks to this lowest level of hypoxia since it is not well tolerated by these species, unlike bar-headed geese which can survive much deeper levels of hypoxia (Black and Tenney, 1980; Scott and Milsom, 2007).

All species increased bill surface temperature (T_{bill}) in response to stepwise hypoxia (Fig. 2 and Fig. 3A). Bill warming tended to begin at the end or along the midline of the bill, then spread over the rest of the bill surface. The statistically significant onset of bill warming occurred after 4 min and 3 min of 9% $F_{I_{O_2}}$ in greylag geese and pekin ducks, respectively, but not until 4 min of 7% $F_{I_{O_2}}$ in bar-headed geese. As a result, bar-headed geese demonstrated significantly less bill warming during the majority of exposure to 9% $F_{I_{O_2}}$ (Fig. 3A). There was also small but statistically insignificant bill warming in greylag geese and pekin ducks at 12% $F_{I_{O_2}}$, and after 6–7 min of 12% $F_{I_{O_2}}$, T_{bill} was higher in greylag geese than in bar-headed geese. Initial T_{bill} values were similar between species

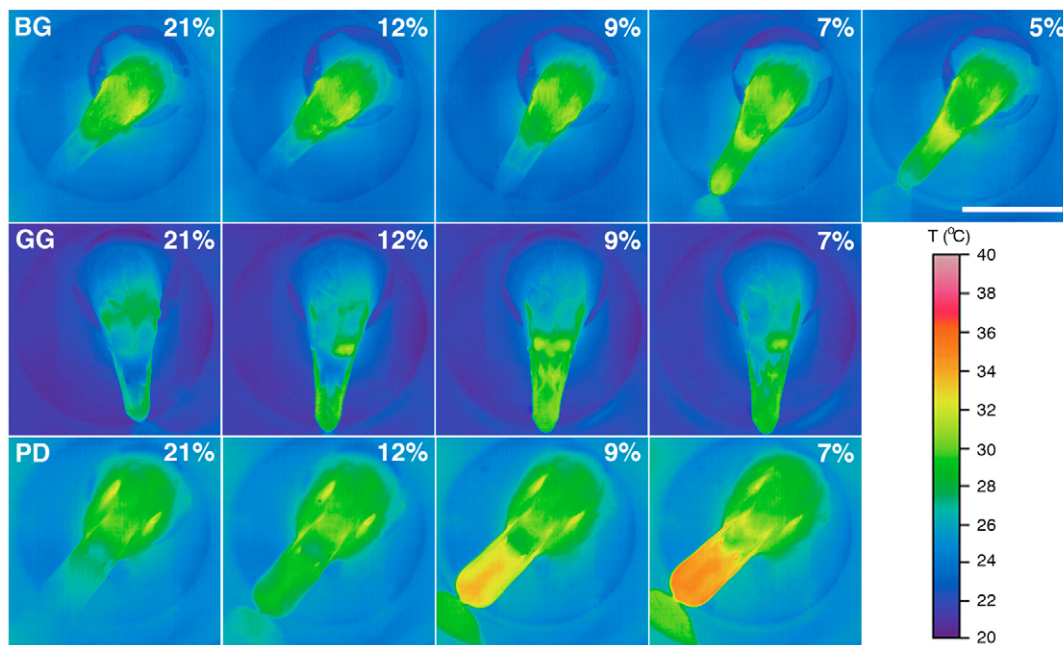


Fig. 2. Surface temperatures (T) of the bill increased in bar-headed geese (BG, top row), greylag geese (GG, middle row) and pekin ducks (PD, bottom row) during stepwise hypoxia. Inspired O₂ was reduced from 21% to 12%, 9%, 7%, and then in bar-headed geese only to 5%. Each level of hypoxia was sustained for 15 min. Scale bar, 10 cm.

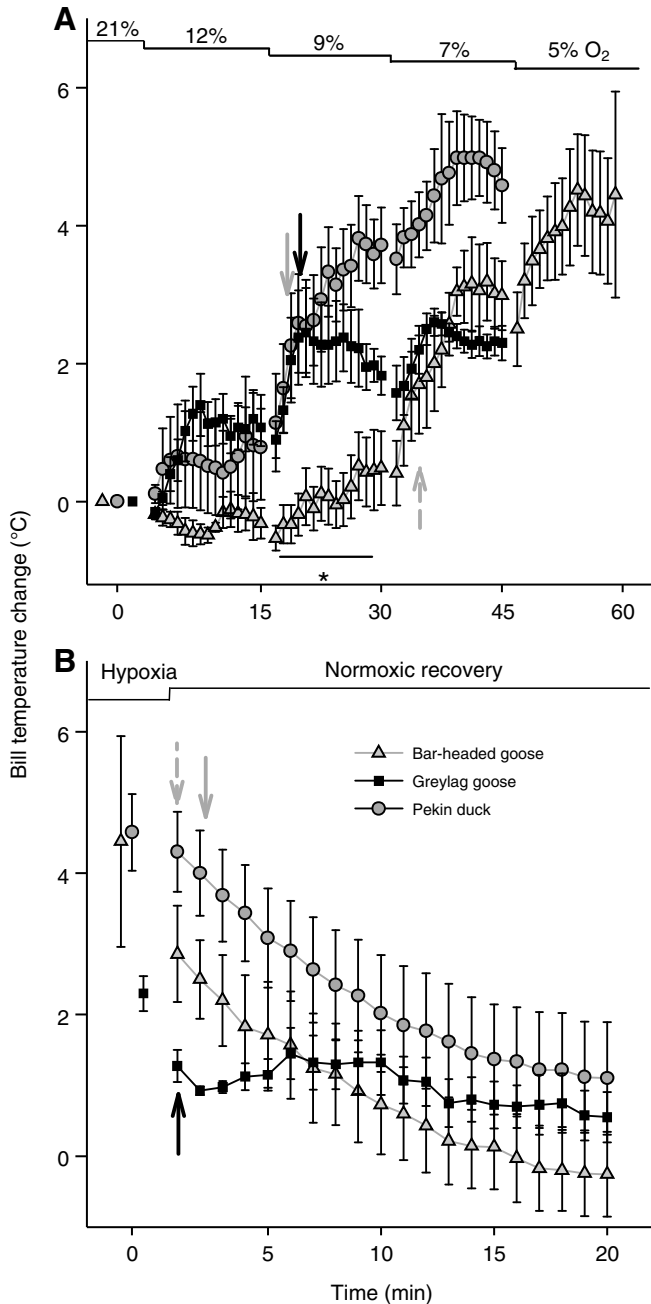


Fig. 3. (A) Bill surface temperatures increased during stepwise hypoxia in bar-headed geese (light grey triangles), greylag geese (black squares) and pekin ducks (dark grey circles). This increase became significant much earlier in greylag geese (black arrow) and pekin ducks (dark grey arrow) than in bar-headed geese (light grey broken arrow), and was lower in bar-headed geese during 9% inspired O₂ (asterisk). (B) Bill temperature decreased after the first minute of normoxic recovery in bar-headed geese (light grey broken arrow) and greylag geese (black arrow) and after the second minute in pekin ducks (dark grey arrow). The bill temperature change in each individual was determined by subtracting its pre-hypoxia value (see Results). Representative thermal images are shown in Fig. 2.

(bar-headed geese, $25.3 \pm 0.5^\circ\text{C}$; greylag geese, $27.0 \pm 1.5^\circ\text{C}$; and pekin ducks, $26.8 \pm 0.4^\circ\text{C}$; $P > 0.05$).

Normoxic recovery of T_b after severe hypoxia was slightly different in pekin ducks than in either goose species (Fig. 1B). Both geese started recovering T_b within 20 min, but no significant

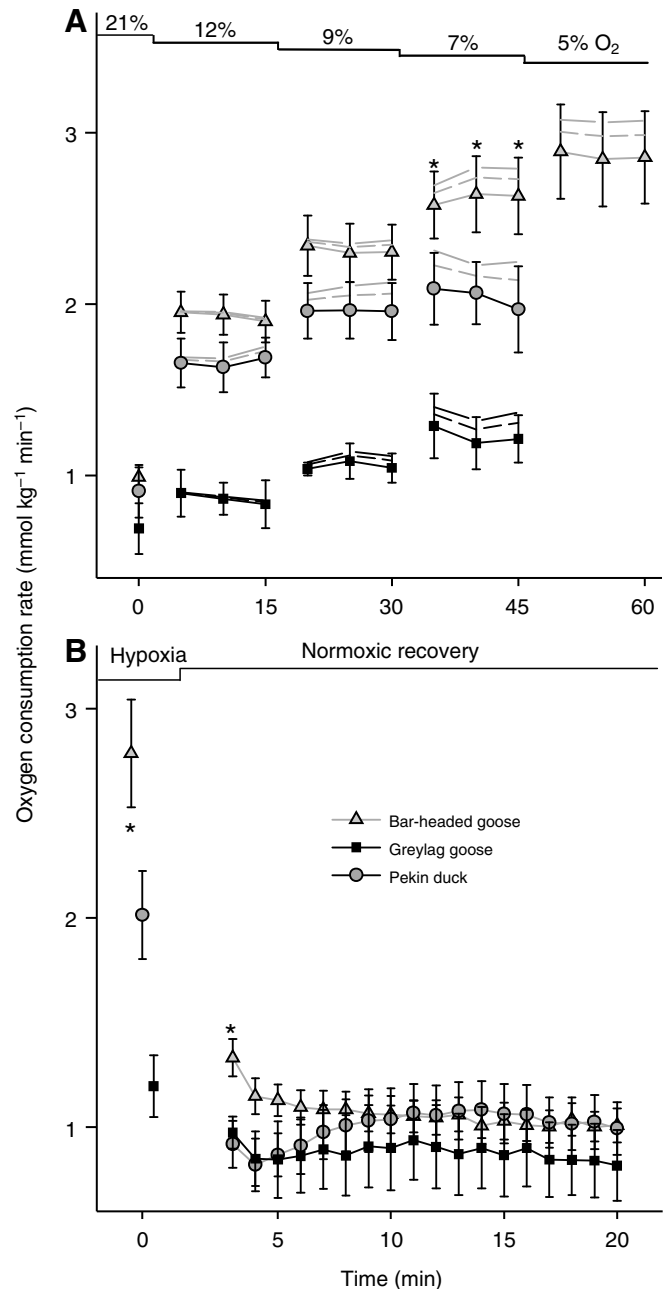


Fig. 4. (A) Oxygen consumption rate increased during stepwise hypoxia in bar-headed geese (light grey triangles), greylag geese (black squares) and pekin ducks (dark grey circles). Bar-headed geese had higher oxygen consumption rates than both other species during 7% inspired O₂ (asterisks). Differences between species persisted after mean oxygen consumption rates were corrected for differences in body temperature depression (see Materials and methods), using Q_{10} values of either 2 (short broken lines) or 3 (long broken lines). (B) Oxygen consumption rates decreased rapidly during normoxic recovery in all species.

recovery occurred in ducks. Consistent with this difference, bar-headed geese and greylag geese immediately reduced T_{bill} with the onset of normoxia (thus favouring heat retention), but in pekin ducks there was not an immediate reduction (Fig. 3B). After this initial reduction, T_{bill} generally continued to decline throughout recovery.

Greylag geese exhibited the greatest T_b recovery during the 20 min recovery, possibly because of their slightly larger size (which should favour heat retention).

Metabolic responses to stepwise hypoxia

Oxygen consumption rates were similar between species in normoxia, and generally increased with each successive decrease in $F_{I_{O_2}}$ (Fig. 4A). The duration of exposure to each level of hypoxia (assessed at 5, 10 and 15 min) did not alter the hypoxic metabolic responses. Bar-headed geese generally maintained higher metabolic rates during hypoxia: metabolism in this species was higher than in greylag geese for all levels of hypoxia, and higher than both low altitude species during 7% $F_{I_{O_2}}$. However, the differences in

metabolism could not be entirely explained by differences in T_b . After correcting oxygen consumption rates for the differences in T_b depression between species, using Q_{10} values of either 2 or 3 (short or long dashed lines, respectively, in Fig. 4A), the mean rates were still elevated in bar-headed geese compared with the low altitude species.

Oxygen consumption rates recovered rapidly to pre-hypoxia levels in all species after they were returned from hypoxia to normoxia (Fig. 4B; the first 2 min of recovery could not be measured because of methodological issues). Even though metabolism during hypoxia was different between species, these differences were abolished after 4 min of recovery. Small changes in metabolism also appeared to occur in all species throughout recovery.

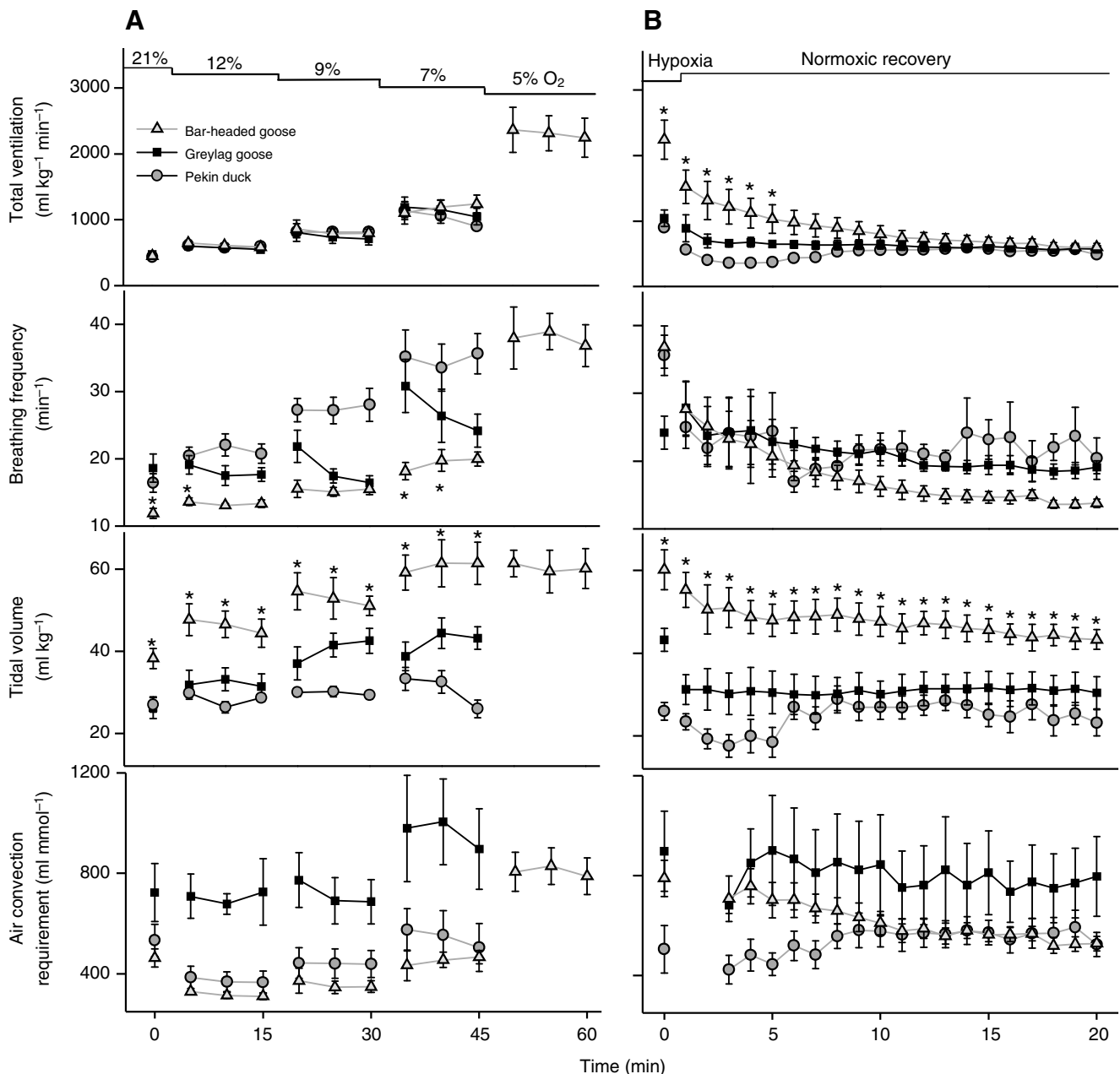


Fig. 5. Total ventilation, breathing frequency, tidal volume and air convection requirements (total ventilation divided by oxygen consumption rate) during stepwise hypoxia (A) and subsequent normoxic recovery (B) in bar-headed geese (light grey triangles), greylag geese (black squares) and pekkin ducks (dark grey circles). *Significant difference between bar-headed geese and both greylag geese and pekkin ducks.

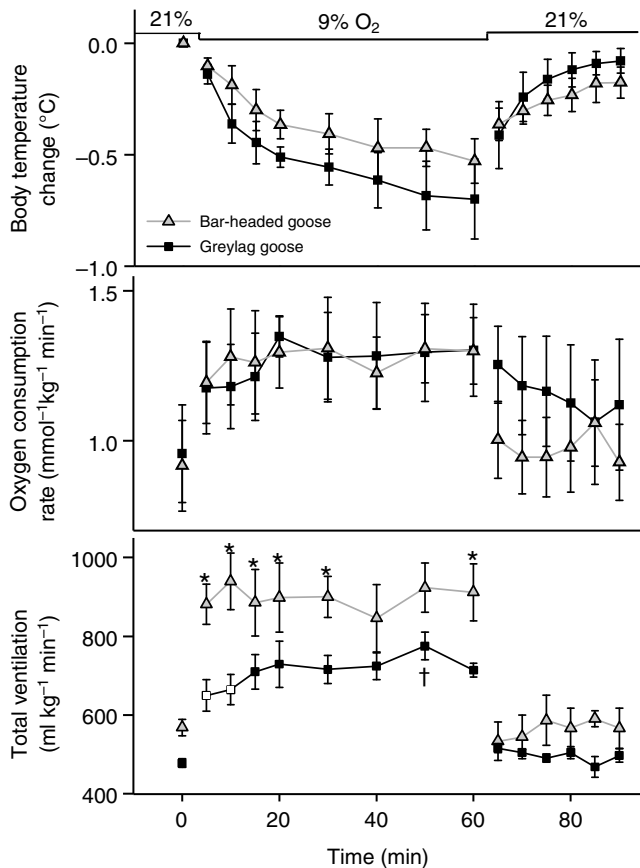


Fig. 6. Body temperature change, oxygen consumption rate and total ventilation during 9% inspired O_2 that was sustained for 60 min, followed by 30 min normoxic recovery, in bar-headed geese (light grey triangles) and greylag geese (squares). *Significant difference between bar-headed geese and greylag geese. †Significant difference from 5 min and 10 min time points (white squares) in greylag geese.

Ventilatory responses to stepwise hypoxia

Breathing increased in all species in response to stepwise poikilocapnic (uncontrolled CO_2) hypoxia, due to increases in both tidal volume and breathing frequency (Fig. 5A). As the severity of hypoxia increased, time-dependent changes in the ventilatory responses became apparent in the low altitude species. In greylag geese breathing frequency declined over time at each FI_{O_2} , whereas in pekin ducks tidal volume decreased over time at 7% FI_{O_2} . These changes were partially offset by changes in tidal volume or breathing frequency, respectively, so total ventilation changed only slightly with duration. Unlike the low altitude species however, the ventilatory response of bar-headed geese to poikilocapnic hypoxia was extremely stable. Bar-headed geese also exhibited different breathing patterns than the low altitude species, generally having higher tidal volumes and lower breathing frequencies.

Total ventilation was generally matched to metabolism in all species, because air convection requirements (quotient of total ventilation and oxygen consumption rate) changed very little during exposure to moderate levels of hypoxia (Fig. 5A). At more severe levels of hypoxia air convection requirements increased in bar-headed geese and greylag geese (5% and 7% FI_{O_2} , respectively). All breathing variables generally returned to pre-hypoxic levels within 20 min of normoxic recovery (Fig. 5B).

Responses to prolonged hypoxia

During prolonged hypoxia (9% FI_{O_2}), the greatest changes in body temperature, metabolism and breathing occurred during the first 15–20 min of exposure (Fig. 6). Slight reductions in T_b continued throughout 60 min of hypoxia in both bar-headed geese and greylag geese, but appeared to approach a stable value (at least in bar-headed geese). Species differed in the overall T_b response to hypoxia ($P < 0.05$ for species \times time interaction), and greylag geese tended to exhibit more profound reductions in T_b ; however, both species recovered T_b at similar rates after being returned to normoxia. Oxygen consumption increased immediately in bar-headed geese, was sustained for the duration of hypoxia, and then returned rapidly to control levels during recovery; curiously, this increase in metabolism was less than the increase during stepwise hypoxia (compare Fig. 4 with Fig. 6). O_2 consumption appeared to increase immediately in greylag geese as well (although this was not significant until 20 min), but the recovery occurred more slowly. Breathing increased immediately in response to 9% O_2 in bar-headed geese, and was very stable throughout prolonged hypoxia. Bar-headed geese breathed substantially more than greylag geese, particularly early in hypoxia exposure, as the initial (measured after 5 min) increase in breathing was larger in bar-headed geese (1.6-fold) than in greylag geese (1.3-fold; Fig. 6). However, breathing increased progressively over time in greylag geese as a result of increases in tidal volume (Table 1). The higher total ventilation in bar-headed geese was due to both an overall higher tidal volume and a more pronounced hypoxic breathing frequency response (Table 1). Ventilation was well matched to metabolism: there were only small insignificant changes in air convection requirements (Table 1).

Interactions of O_2 loading with thermoregulation and metabolism

During stepwise hypoxia when body temperature and bill temperature were expressed as a function of the O_2 concentration in arterial blood (see Scott and Milsom, 2007) instead of time (which allows comparison of different species at each inspired O_2), differences between bar-headed geese and the low altitude species were much less prominent (Fig. 7). Body temperature fell by approximately 0.5°C for every 1 mmol l^{-1} fall in arterial O_2 content in all three species (see legend of Fig. 7A). Bill temperature increased by 2–2.5°C for every 1 mmol l^{-1} fall in O_2 content in bar-headed geese and pekin ducks (Fig. 7B). Although T_{bill} in greylag geese increased less as a function of O_2 content overall ($\sim 1^\circ C$ mmol l^{-1}), this species was similar to the other two if the deepest level of hypoxia is excluded ($\sim 2^\circ C$ mmol l^{-1}). In contrast to T_b and T_{bill} , the relationships between O_2 consumption rates and arterial O_2 content were different between species (Fig. 7C). Metabolism increased the most in bar-headed geese as O_2 content fell, followed by pekin ducks, then greylag geese.

Effects of vagotomy on the responses to stepwise hypoxia

Removing vagal chemoafferent input to the central nervous system did not abolish body temperature depression during stepwise hypoxia in pekin ducks. Ducks that were bilaterally vagotomized reduced T_b more than intact ducks during hypoxia, such that by the end of hypoxia exposure T_b fell by greater than 2°C in these birds (Fig. 8A). This contrasted with the effect of vagotomy on bill surface temperatures during hypoxia. Vagotomized ducks did not change T_{bill} during hypoxia, unlike the increase in intact ducks, though there appeared to be a slight decrease in T_{bill} during the most severe hypoxia (Fig. 8B). These results were not non-specific effects of vagotomy, because T_b and T_{bill} were constant in time-matched vagotomized normoxic controls (Fig. 8).

Table 1. Respiratory variables during prolonged hypoxia (9% inspired O₂)

Oxygen content	Time (min)	Breathing frequency (min ⁻¹)	Tidal volume (ml kg ⁻¹)	Air convection requirement (ml mmol ⁻¹)
Bar-headed geese				
21% O ₂	0	11.9±0.6	47.8±1.9*	690±110
9% O ₂	5	16.7±1.7	54.1±4.0*	789±116
	10	16.4±1.5	58.5±4.7*†	777±96
	15	16.5±1.9	54.5±3.6*	744±104
	20	17.2±2.6†	54.1±4.8*	715±92
	30	16.2±1.4	56.8±4.1*	740±107
	40	16.5±2.0	52.6±5.2*	737±132
	50	17.4±1.4†	53.9±4.4*	735±95
	60	16.8±1.7†	54.9±3.3*	731±99
21% O ₂	65	12.1±0.9*	44.2±3.8*	566±89
	70	12.1±0.6*	44.7±3.5*	617±105
	75	12.9±0.9*	45.5±4.0*	680±131
	80	12.7±0.8	44.5±3.6*	620±91
	85	13.0±0.6	45.9±2.3*	606±91
	90	12.8±0.5	44.3±3.6*	655±104
Greylag geese				
21% O ₂	0	16.2±2.4	29.5±4.7	560±126
9% O ₂	5	16.4±0.8	36.8±1.2	579±84
	10	15.3±1.1	39.7±0.8	595±102
	15	16.0±0.9	40.4±0.5	624±121
	20	16.1±0.9	40.7±0.5†	541±36
	30	16.4±1.1	42.0±1.1†	582±76
	40	17.8±2.0	39.0±1.0	592±68
	50	16.1±1.1	42.9±1.2†	643±124
60	15.9±1.2	42.8±1.2†	577±85	
21% O ₂	65	17.8±1.1	30.8±2.7	425±45
	70	17.3±1.7	29.5±2.6	458±79
	75	17.5±2.4	29.0±3.0	466±98
	80	17.1±2.0	29.3±2.6	524±149
	85	17.0±2.1	28.1±2.4	551±196
90	16.3±1.2	30.2±1.6	533±159	

Values are means ± s.e.m. ($N \geq 4$).

*Significant difference from greylag geese ($P < 0.05$); †significant difference from pre-hypoxic control (within species).

Vagotomy attenuated the ventilatory responses to hypoxia (Table 2). Although ventilatory responses appeared to be greater in hypoxic vagotomized ducks, total ventilation tended to increase in both hypoxic ducks and normoxic control ducks over time. Hypoxic and normoxic groups were statistically indistinguishable, but this may have been due to high inter-individual variation or the low sample size ($N=3$). Interestingly, vagotomized ducks exposed to the deepest level of hypoxia reduced oxygen consumption rates slightly compared to pre-hypoxia controls (Table 2).

DISCUSSION

Body temperature depression occurs across all vertebrate classes during hypoxia, and is believed to reduce O₂ demands and thus help balance O₂ supply and demand (Wood and Gonzales, 1996; Bicego et al., 2007). We show here that T_b depression (Figs 1, 6) during hypoxia occurs in concert with increases in the surface temperature of thermal windows in the bill (Figs 2, 3), supporting the idea that hypoxia leads to specific physiological adjustments that reduce the T_b setpoint. Hypoxic T_b depression also appeared to relate inversely to O₂ supply: T_b depression and bill warming occurred at lower inspired O₂ in bar-headed geese than in low altitude waterfowl, but this could be explained by higher blood O₂ loading in this species (Fig. 7). This ability of bar-headed geese to minimize the depressive effects of hypoxia on T_b and metabolism is undoubtedly essential

for maintaining the high metabolic rates necessary for flight at high altitude, and suggests that enhancing O₂ supply is adaptive for some species in hypoxia.

Hypoxic responses of waterfowl

The generality of hypoxic T_b depression across bird species (e.g. Novoa et al., 1991; Kilgore et al., 2007) and other vertebrate classes suggests that the mechanisms responsible for T_b depression are widespread; however, responses of the thermoregulatory control system to hypoxia are still poorly understood (Bicego et al., 2007). Previous studies in mammals indicate that hypothalamic O₂ sensors may initiate T_b depression during hypoxia, and can operate in absence of peripheral chemoreceptor inputs (Iriki and Kozawa, 1976; Fewell et al., 1997; Barros et al., 2006; Gargaglioni et al., 2006). Our findings suggest that this could also be the case in birds: vagotomy, which in birds eliminates afferent input from all arterial and pulmonary chemoreceptors, did not eliminate T_b depression during hypoxia in ducks (Fig. 8A). Consistent with this hypothesis, metabolic O₂ limitation in the brain of ducks (Bryan and Jones, 1980) occurs at similar levels of hypoxia to that initiating T_b depression in the present study. However, vagotomy did eliminate bill warming during hypoxia (Fig. 8B), so at least part of the hypoxic thermoregulatory response relies on information from peripheral chemoreceptors or thermoafferents carried by the vagus. Regardless of the sensors involved, our conclusions agree with work in pigeons showing that the magnitude of T_b depression

is strongly influenced by changes in O₂ loading (Barnas and Rautenberg, 1990). Enhancement of O₂ supply to neural sensors can therefore alleviate the occurrence of T_b depression during hypoxia.

The increases in bill surface temperature observed during hypoxia (Figs 2, 3) strongly suggest that perfusion of thermal windows is specifically controlled to facilitate heat loss during hypoxia, similar to what occurs during heat stress (Bech et al., 1982). Local regulation of blood flow to the bill in response to cellular O₂ limitation could explain our results. Preferential perfusion of this tissue is unlikely, however, because blood flow is redistributed to the heart and brain during hypoxia, and away from less hypoxia-sensitive tissues (Faraci et al., 1984b; Faraci et al., 1985).

Increasing heat loss from the bill was probably not the only means of T_b depression during hypoxia in our study. T_b depression occurred without any change in bill temperature in vagotomized ducks (Fig. 8); hypoxic T_b depression in these animals may have therefore occurred by reducing heat generation or by increasing heat loss from routes not dependent on vagal feedback. Biochemical adjustments that decrease proton leak across the mitochondrial inner membrane could reduce thermogenesis in hypoxia, which would have the added effect of reducing temperature-independent rates of metabolism and O₂ demand (Gnaiger et al., 2000; St-Pierre et al., 2000). Evaporative heat loss from respiratory surfaces could increase during hypoxia

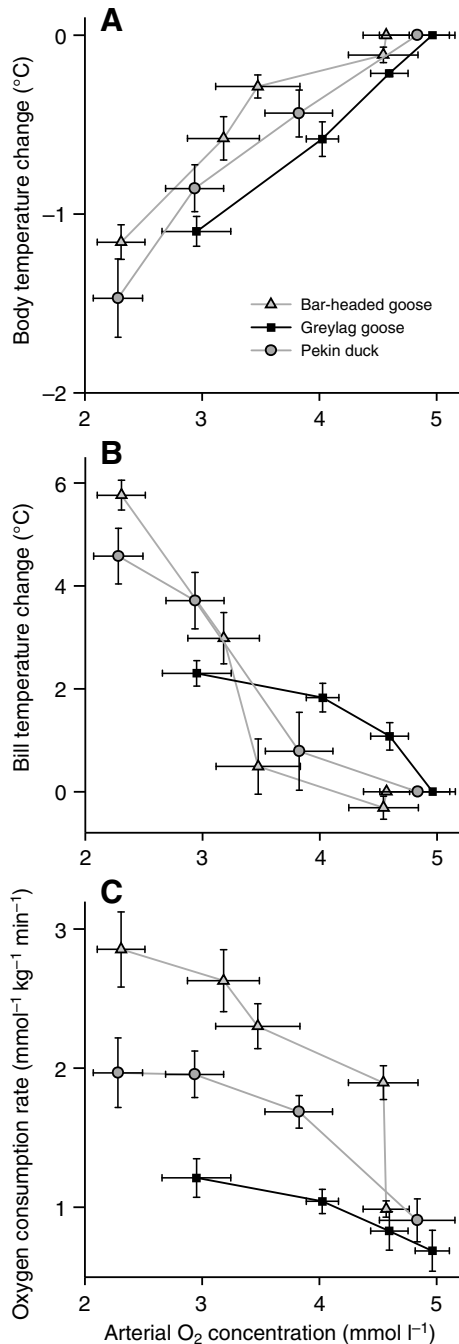


Fig. 7. Relationships between arterial oxygen loading and (A) body temperature change, (B) bill temperature change, or (C) oxygen consumption rate during stepwise hypoxia in bar-headed geese (light grey triangles), greylag geese (black squares) and pekin ducks (dark grey circles). Arterial O₂ concentration data were measured in our previous study (Scott and Milsom, 2007), which used a very similar stepwise hypoxia protocol to the present study. Temperature and O₂ consumption data are the values after 15 min at each level of hypoxia. Slopes of linear regressions of body temperature against content were similar between species (bar-headed geese, 0.46 ± 0.08 ; greylag geese, 0.55 ± 0.03 ; pekin ducks, 0.56 ± 0.07). Slopes of bill temperature against content were similar between bar-headed geese and pekin ducks (bar-headed geese, -2.5 ± 0.6 ; greylag geese, -0.80 ± 0.25 ; pekin ducks, -2.0 ± 0.4). The slope of O₂ consumption rate against content was highest in bar-headed geese (-0.68 ± 0.21 ; significantly higher than greylag geese), followed by pekin ducks (-0.42 ± 0.12), and greylag geese (-0.26 ± 0.04). All regressions were statistically significant.

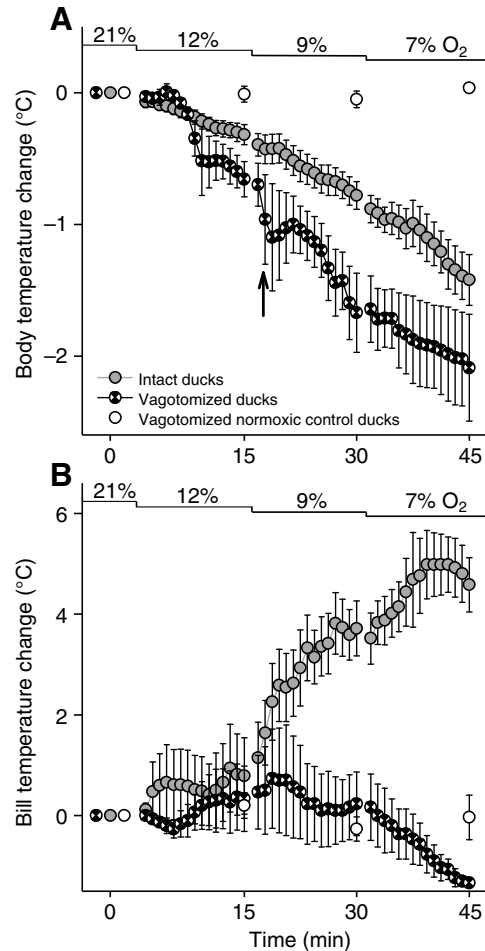


Fig. 8. (A) Bilateral vagotomy did not eliminate body temperature (T_b) depression in response to stepwise hypoxia. Vagotomized ducks exposed to hypoxia (black and white circles) began reducing T_b during 9% inspired O₂ (black arrow), and reached lower mean T_b than intact ducks (dark grey circles). (B) Bilateral vagotomy abolished the increase in bill surface temperature that occurs in intact ducks during hypoxia. Vagotomized ducks kept in normoxia for equivalent durations (white circles) did not alter T_b or bill temperature; data for these controls are only shown at the end of each 15 min period for clarity.

(Tattersall and Gerlach, 2005; Hoffman et al., 2007), particularly when total ventilation increases (although ventilatory responses are attenuated by vagotomy). Heat loss from the feet probably occurs in hypoxia as well, and it is unclear whether this route of heat loss requires intact vagi. The relative contributions of the various mechanisms for reducing heat generation and increasing heat loss remain unclear.

A regulated decline in T_b during hypoxia undoubtedly reduces O₂ demands, and probably facilitates metabolic depression in tissues that need not remain active. However, higher workloads of respiratory and cardiac muscles (and possibly tissues involved in acid-base regulation) should increase their metabolic requirements during hypoxia. The response of a whole animal is therefore the sum of factors that either increase or decrease global metabolism. In the current study and previous studies of birds during hypoxia (Tucker, 1968; Bouverot and Hildwein, 1978; Black and Tenney, 1980; Novoa et al., 1991; Scott and Milsom, 2007), this sum caused a net increase in whole animal metabolism. This is unlike the situation in mammals (e.g. Barros et al., 2001;

Table 2. Respiratory variables during stepwise hypoxia in vagotomized ducks

	Time (min)	$F_{I_{O_2}}$	\dot{V}_{Tot} (ml kg ⁻¹ min ⁻¹)	f_L (min ⁻¹)	V_T (ml kg ⁻¹)	\dot{V}_{O_2} (mmol kg ⁻¹ min ⁻¹)	$\dot{V}_{Tot}/\dot{V}_{O_2}$ (ml mmol ⁻¹)
Hypoxia	0	21%	591±105	7.6±1.0	78.1±11.1	1.64±0.07	363±72
	15	12%	700±148	8.6±1.9	81.9±3.0	1.70±0.09	409±82
	30	9%	914±179	12.2±4.2	86.3±16.1	1.65±0.10	564±119
	45	7%	1504±411†	16.5±6.0	104.2±17.9	1.46±0.08†	1080±333*†
Control	0	21%	473±137	5.5±1.8	89.5±8.6	1.50±0.30	301±44
	15	21%	637±193	6.9±3.1	103.8±14.9	1.62±0.33	388±73
	30	21%	743±240	8.1±3.5	100.8±12.9	1.52±0.34	468±72
	45	21%	857±312	10.9±4.9	84.8±8.7	1.54±0.32	524±102

$F_{I_{O_2}}$, inspired O₂ fraction; \dot{V}_{Tot} , total ventilation; f_L , breathing frequency; V_T , tidal volume; \dot{V}_{O_2} , oxygen consumption rate; $\dot{V}_{Tot}/\dot{V}_{O_2}$, air convection requirement.

Values are means (± s.e.m.) after 10–15 minutes at each $F_{I_{O_2}}$ ($N=3$).

*Significant difference between hypoxia and normoxia-control treatments ($P<0.05$); †significant difference from time 0 (within species).

Tattersall et al., 2002), which may relate to the exceptional ability of birds to increase ventilation (with its associated metabolic costs) (Scheid, 1990). Birds primarily experience hypoxia when flying, so it is conceivable that metabolic depression was selected against during the origins of flight (but see Bucher and Chappell, 1997). Nevertheless, depression of T_b during hypoxia will lead to reductions in metabolism that may not decrease whole animal O₂ consumption, but certainly diminish the global metabolic demands that would exist without this response.

Interspecific differences in hypoxia responses

Many previous studies of bar-headed geese suggest that this species is exceptional at maintaining O₂ supply to mitochondria during hypoxia. Bar-headed geese have an enhanced poikilocapnic hypoxic ventilatory response (HVR), particularly during severe hypoxia (Scott and Milsom, 2007). Poikilocapnic hypoxia is environmentally realistic, but the decrease in blood CO₂ that occurs because of the initial ventilatory response reflexly inhibits breathing. Because the isocapnic HVR (when CO₂ is experimentally maintained) of bar-headed geese is the same as that of other species, the enhanced poikilocapnic HVR appears to be partly caused by a ventilatory insensitivity to hypocapnia (Scott and Milsom, 2007). This, along with potential differences in how the pulmonary circulation is controlled during hypoxia (Faraci et al., 1984a), may substantially increase pulmonary O₂ uptake in this species (Scott and Milsom, 2007). Bar-headed geese also have an increased haemoglobin–O₂ affinity (Petschow et al., 1977; Weber et al., 1993), which should further enhance O₂ loading during hypoxia. Based on our previous theoretical calculations, these adaptations in the O₂ transport pathway of bar-headed geese should impart considerable benefit for maintaining high metabolic rates during flight at high altitude (Scott and Milsom, 2006).

In the current study we confirm our previous findings on the hypoxic ventilatory response of bar-headed geese (Scott and Milsom, 2007). This species breathes with much larger tidal volumes than low altitude species (Fig. 5; Table 1), which should reduce dead space ventilation, improve effective ventilation of the gas exchange surface, and enhance O₂ loading (Scott and Milsom, 2007). In addition, total ventilation in bar-headed geese was higher than in greylag geese during prolonged hypoxia at 9% inspired O₂ (Fig. 6). This is inconsistent with the results at 9% O₂ of acute stepwise hypoxia experiments (Fig. 5) (Scott and Milsom, 2007), but consistent with previous observations that bar-headed geese breathe substantially more than low altitude species at 5% inspired O₂ (Scott and Milsom, 2007).

Time domains of the ventilatory response to poikilocapnic hypoxia appear to differ between bar-headed geese and low altitude waterfowl. Total ventilation, as well as its components, breathing frequency and tidal volume, increased rapidly and then changed very little throughout the duration of hypoxia in bar-headed geese, in both the stepwise and prolonged protocols (Figs 5, 6; Table 1). By contrast, greylag geese and pekin ducks exhibited time-dependent changes in breathing pattern. During the stepwise protocol, the acute response to hypoxia was followed by either decreases in breathing frequency (greylag geese) or tidal volume (pekin ducks at 7% O₂), and in greylag geese this appeared to be offset by increases in tidal

volume (Fig. 5). Tidal volume also increased progressively during the prolonged hypoxia protocol in greylag geese (Table 1), but was not offset by declines in breathing frequency in this experiment, such that a gradual increase in total ventilation occurred (Fig. 6). Unfortunately, we do not at present have sufficient information to define these changes in terms of established time domains of the hypoxic ventilatory response (Powell et al., 1998; Mitchell et al., 2001).

The onset of T_b depression and bill warming did not occur until more severe levels of hypoxia in bar-headed geese, but the relationships between body or bill temperature change and arterial O₂ content were similar between species. Previous research supports this finding, having shown that bar-headed geese have higher arterial O₂ content and reduce T_b less than pekin ducks during hypoxia (Black and Tenney, 1980; Faraci et al., 1984b; Scott and Milsom, 2007). Rather than being because of differences in how thermoregulatory control centres respond to changes in arterial O₂, the reduced T_b depression of bar-headed geese may result from a lower magnitude of hypoxaemia at any given inspired O₂. However, the extent of T_b depression may decrease at higher ambient temperatures (Faraci et al., 1984b), suggesting that the relationship between O₂ loading and T_b depression depends on the thermal environment.

The higher rates of metabolism in bar-headed geese during hypoxia could not be explained by differences in O₂ loading or the Q₁₀ effects of changes in T_b (Figs 4 and 7), but could reflect a higher metabolic cost of O₂ transport or less metabolic depression in this species. Assuming the latter, adaptations at multiple steps in the pathway of O₂ transport and utilization could help sustain higher metabolic rates in bar-headed geese. For example, some preliminary evidence suggests that this species may have a higher capacity for O₂ diffusion from the blood to mitochondria (Snyder et al., 1984; Fedde et al., 1985). Mitochondria of bar-headed geese could also be better at maintaining rates of ATP supply when intracellular O₂ is low. These possibilities could be especially important for maintaining high rates of metabolism for flight during hypoxia, and are currently being investigated.

High altitude adaptations in bar-headed geese

Bar-headed geese and greylag geese have a close phylogenetic relationship within the genus *Anser*, whereas ducks are more distantly related (Donne-Goussé et al., 2002). Therefore, if differences between species were caused by neutral evolutionary processes, greylag geese and bar-headed geese should have been more alike one another than to pekin ducks. This was generally not

the case: bar-headed geese often responded differently to hypoxia than both low altitude species, suggesting that these unique phenotypes are related to hypoxia adaptation.

Because bar-headed geese must increase metabolic rates substantially during flight at high altitude, any suppressive effect of hypoxia on their metabolism will be detrimental to performance. The ability of bar-headed geese to minimize body temperature and metabolic depression during hypoxia could therefore be essential to this species' extraordinary migration. We believe this results from the enhanced capacity of bar-headed geese to load O₂ into the blood, which probably has immense adaptive value for high altitude flight.

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