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# Physiological mechanisms for underwater endurance: Canada goose (*Branta canadensis*) versus Pekin duck (*Anas platyrhynchos*)

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**Abstract** Maximum submergence time of Canada geese was 18% of that of similarly sized Pekin ducks. Due to a smaller respiratory system volume the oxygen store of Canada geese was 82% of that of Pekin ducks, accounting for approximately 33% of the difference in underwater survival times. The respiratory properties and volume of the blood were similar in both species. Both species utilised approximately 79% of the respiratory oxygen store and 90% of the blood oxygen store. Therefore, most of the species difference in survival times was due to a less effective oxygen-conserving cardiovascular response (bradycardia, peripheral vasoconstriction) in Canada geese. Duck cardiac chronotropic sensitivity to hypoxia during submergence was twice that observed in geese. Furthermore, a lower hypoxic ventilatory response was observed in geese than in ducks. Density of monoamine varicosities in hindlimb artery walls was lower in geese than ducks. However, electrical stimulation of the hindlimb muscles did not cause ascending vasodilatation during submergence in either species, perhaps due to higher levels of catecholamines in submerged geese. We conclude that the major difference between species is higher oxygen chemosensitivity in ducks which effects a much more rapid and efficacious oxygen-conserving response during forced submergence.

**Key words** Cardiovascular system · Chemosensitivity · Oxygen stores · Submergence asphyxia · Aquatic birds

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**Abbreviations** ATPS · BTPS · STPD · CNS central nervous system · *EEG* electroencephalogram · *ECG* electrocardiogram . *EDTA* ethylenediaminetetra-acetic acid · *HPLC* high performance liquid chromatography · $F_{IO_2}$ fractional oxygen concentration of inspired air · $F_{rsO_{2rest}}$ pre-immersion fractional concentration of oxygen in the respiratory system · $F_{rsO_{2sub}}$ pre-emersion fractional concentration of oxygen in the respiratory system · [Hb] haemoglobin concentration · *Hct* haematocrit · *HR* heart rate · $M_B$ body mass · $M_b$ brain mass · $M_h$ heart mass · $P_{aCO_2}$ partial pressure of carbon dioxide in arterial blood · $P_{aO_2}$ partial pressure of oxygen in arterial blood · *SPG* sucrose-potassium phosphate- glyoxylic acid · $t_d$ maximum underwater survival time · $\dot{V}_E$ respiratory minute volume · $V_{pl}$ plasma volume · $V_{rs}$ respiratory system volume · $V'_{rsO_2}$ accessible respiratory system oxygen store · $V_{taO_2}$ total non-myoglobin-bound oxygen store · $V_{tb}$ blood volume · $V_{tbO_2}$ blood oxygen store

## Introduction

In birds underwater survival time is dependent upon $O_2$ supply and demand (the magnitude of the available $O_2$ store and the rate at which it is consumed) and the hypoxic tolerance of the tissues. The Pekin duck (*Anas platyrhynchos*) is well known for its ability to survive for prolonged periods without breathing (Hudson and Jones 1986), despite the fact that it is not a habitual diver. Since the tolerance of the duck CNS to low $O_2$ levels is no greater than that of non-aquatic species (Bryan and Jones 1980) and the $O_2$ store is not particularly great (Hudson and Jones 1986), the underwater endurance of this species must be associated with an efficient $O_2$-conserving cardiovascular response (Irving 1934).

Canada geese (*Branta canadensis*) are also non-diving aquatic birds and possess the ability to invoke

an $O_2$-conserving response, as indicated by bradycardia, during involuntary head immersion (Cohn et al. 1968). As geese are often larger in $M_B$ than ducks and $O_2$ storage capacity and underwater survival time are both $M_B$ dependent (Hudson and Jones 1986; West 1981), it was predicted that Canada geese would tolerate involuntary apnoea for a longer period than Pekin ducks. In our preliminary experiments however, the underwater survival times of Canada geese were so short that it prompted us to undertake a detailed investigation of the physiological basis for the difference between species. Why are two species, sharing such apparently similar aquatic lifestyles, so different in terms of underwater endurance?

The following hypotheses were investigated: (1) that the size of the $O_2$ store is smaller in Canada geese than in Pekin ducks; (2) that Canada geese reach their maximum underwater tolerance with a higher fraction of the $O_2$ store unused; and (3) that Canada geese invoke a less effective $O_2$-conserving cardiovascular response than Pekin ducks. In addition, experiments were conducted to determine the mechanisms underlying differences in the $O_2$-conserving response in the two species.

## Materials and methods

These experiments involved a total of 21 adult white Pekin ducks of either sex and 34 adult Canada geese of undetermined sex, all housed and maintained under identical conditions. They were fed mixed corn and grower pellets and provided with water ad libitum. To avoid unnecessary use of animals and duplication of results, Pekin ducks were used only in those experiments which had not been performed previously in these laboratories.

### Maximum underwater survival time of Canada geese

This was measured in seven geese (mean ± SEM $M_B = 4.2 \pm 0.7$ kg). The method used was similar to that employed by Hudson and Jones (1986) for the Pekin duck. The geese were restrained in ventral recumbency and HR was monitored using bipolar ECG leads inserted into the skin overlying one shoulder and the contralateral leg. The ECG signal was amplified by conventional means and displayed on a chart recorder. After allowing at least 30 min of rest, the head of the bird was manually lowered into a container of cold water and submergence was maintained until a sudden sustained increase in HR (to pre-dive values or higher for several seconds) was observed, at which time the submergence was terminated. Hudson and Jones (1986) showed that this "break point" of the bradycardia coincides with the onset of an isoelectric EEG and is closely followed by death in the Pekin duck. The interval between immersion and emersion of the head was recorded as the maximum underwater survival time.

### Oxygen storage capacity of Canada geese

The available $V_{taO_2}$ was estimated in six Canada geese ($M_B = 4.4 \pm 0.2$ kg) by measurement of [Hb], $V_{pl}$, Hct (red cell volume/whole blood volume × 100%) and $V_{rs}$. The [Hb] of seven Pekin ducks was also measured for comparison.

The [Hb] ($g \cdot 100\ ml^{-1}$) of whole blood was measured colorimetrically using a diagnostic kit (Sigma Chemical Co., St. Louis, Mo., USA). $V_{pl}$ was measured by intravenous injection of Evans blue dye. Standard solutions and plasma samples were diluted by four volumes of 0.8% saline before spectrophotometric measurements at 620 nm. Hct was measured in eight blood samples from each animal using an International micro-capillary centrifuge. $V_{tb}$ was then derived as follows:

$$V_{tb} = \frac{100 \cdot V_{pl}}{100 - \text{Hct}}$$

$V_{tbO_2}$ at the time of immersion was estimated by assuming that one-third of the blood was arterialised, that arterial blood was 90% saturated and that venous blood was 70% saturated. It was also assumed that the $O_2$-binding capacity of Canada goose haemoglobin was similar to that of the pigeon (*Columba livia*) at 1.2 ml $O_2$ STPD $\cdot g^{-1}$ pigment (Viscor et al. 1984). Thus:

$$V_{tbO_2}\ (\text{ml STPD}) = \frac{[\text{Hb}]}{100} \cdot V_{tb} \cdot 1.2 \cdot 0.9 \cdot 0.33 + \frac{[\text{Hb}]}{100} \cdot V_{tb} \cdot 1.2 \cdot 0.7 \cdot 0.67$$

$V_{rs}$ was measured by argon gas dilution in awake and anaesthetized Canada geese using the method employed by Hudson and Jones (1986). An endotracheal tube was introduced into the glottis under local anaesthesia (lidocaine HCl endotracheal aerosol, Astra Pharmaceuticals Canada, Mississauga, Ontario). A known volume (approximately 50 ml) of a mixture of 80% argon and 20% $O_2$ was injected via a glass syringe at the end of a normal exhalation and the goose was ventilated 15 times using the syringe. Each goose was allowed to stand with minimal restraint in a cardboard box so that the respiratory system was not restricted during injection of the gas. This was repeated once more in each bird then the animals were lightly anaesthetized by intravenous injection of sodium pentobarbital (MTC Pharmaceuticals Canada, Cambridge, Ontario). The procedure was repeated twice in the anaesthetized animals except that a second respiratory gas sample was taken. As before, a gas sample was withdrawn following 15 manual ventilations of the respiratory system. Then, the anaesthetised geese were subjected to 60 manual ventilations, followed by vigorous massage of the thorax and abdomen and a further 10 ventilations before the second gas sample was taken. This was done to confirm that the injected gas had equilibrated with the air in the respiratory system within 15 ventilations to validate the measurements made in awake geese. Argon concentration was measured using a mass spectrometer (MGA 200, Twentieth Century Electronics, Croydon, UK). $V_{rs}$, calculated at ATPS (21 °C), was converted to BTPS and STPD for further analysis.

$F_{rsO_{2rest}}$ was assumed to be the same in Pekin ducks and Canada geese (Hudson and Jones 1986) and $F_{rsO_{2sub}}$ was calculated assuming that the partial pressures of $O_2$ in the air sacs and the arterial blood were equivalent at the end of the submergence. $V'_{rsO_2}$ was then calculated as follows:

$$V'_{rsO_2}\ \text{STPD} = V_{rs}\ \text{STPD} \cdot (F_{rsO_{2rest}} - F_{rsO_{2sub}})$$

The geese were killed by an overdose of anaesthetic following lung volume measurements and the heart and brain were removed. The heart chambers were emptied and any adipose or connective tissue was carefully removed. After blotting dry, the organs were weighed to 0.1 g using a Mettler model AC100 balance.

### Blood respiratory properties of ducks and geese

Blood samples (1 ml) were obtained from a cannula located in the brachiocephalic artery in two Pekin ducks and two Canada geese

and stored on ice. Two to four $O_2$ dissociation curves were obtained from each sample using an Aminco Hemoscan $O_2$ dissociation analyser at 41 °C with fractional $CO_2$ concentrations of 5% and 12% ($P_{CO_2}$ approximately 40 and 90 mmHg, respectively). The pH of all blood samples was approximately 7.42 upon withdrawal from the birds but could not be determined during scans.

### Cardiovascular response to immersion

HR and mean arterial blood pressure was recorded via a cannula located in the brachiocephalic artery during the first 2 min of head immersion in eight Pekin ducks ($M_B = 4.0 \pm 0.1$ kg) and eight Canada geese ($M_B = 4.2 \pm 0.1$ kg). The animals were size-matched as far as possible for this experiment.

Ischiadic artery blood flow was measured in four Pekin ducks ($M_B = 4.2 \pm 0.2$ kg) and four Canada geese ($M_B = 3.9 \pm 0.5$ kg). Blood flow was measured using a directional pulsed Doppler flowmeter model 545C-4 (Bioengineering, University of Iowa, Iowa City, Iowa, USA). Pulsed Doppler flow probes (1.0–3.2 mm i.d. as required; Titronics Medical Instruments, Iowa City, Iowa, USA) were placed around the ischiadic artery of the right leg under halothane anaesthesia (1–4% in 50/50 air/$O_2$ mixture). The birds were given intramuscular injections of broad spectrum antibiotic (20 mg · kg$^{-1}$ ampicillin sodium, Ayerst Laboratories, Montreal, Quebec, Canada) on five consecutive days following implantation. At least 48 h was allowed for recovery before experiments began.

The birds were restrained and the head was manually lowered into water for 2 min. Data were recorded for 1 min before, during, and 5 min following submersion. The blood flow probes were subsequently calibrated in situ under halothane anaesthesia by withdrawal of blood into a 50-ml glass syringe attached to an infusion/withdrawal pump (Harvard Apparatus, South Natick, Mass., USA).

### Electrical stimulation of hindlimb musculature during head immersion

In order to determine whether muscle activity reduces the efficacy of peripheral vasoconstriction, ischiadic artery blood flow was measured in the animals described above before, during and after 2 min head immersions during which the gastrocnemius muscles were electrically stimulated. Bipolar electrodes were constructed from bent 22-guage hypodermic needles and inserted under the skin on either side of the gastrocnemius muscles. The electrodes were connected to a Grass SD9 stimulator (Grass Instrument Co., Quincy, Mass., USA). The stimuli consisted of 200-ms monophasic pulses delivered at 2 Hz and 8.5 V. This was the maximum level of stimulation that could be tolerated by the awake animals. Force of contraction was not measured but the protocol provoked leg extension at a frequency similar to that observed in geese swimming at near maximum speed.

The immersion protocol was similar to that described above except that in the present experiments the hindlimb was stimulated to perform rhythmic contractions for the duration of the second half of the involuntary submergence (i.e. 60–120 s). The stimulator was switched off at the moment of emersion.

### Ventilatory response to hypoxia

Four ducks ($M_B = 2.5 \pm 0.1$ kg) and four geese ($M_B = 4.2 \pm 0.3$ kg) were each placed inside a body plethysmograph of the type described by Bouverot et al. (1979). Their heads were in a darkened chamber (volume 2.5 l) which was ventilated with $O_2/N_2$ gas mixtures at a flow rate of 7–9 l min$^{-1}$ using two gas flowmeters connected in parallel. A dental dam rubber collar formed an air-tight seal separating the body and head compartments. $F_{IO_2}$ was reduced from 21 to 9% for ducks and from 21 to 5% for geese in steps of approximately 2%. Ventilation was recorded using a pneumotachograph after 2 min at each $O_2$ level, and then $F_{IO_2}$ was lowered to the next step.

### Monoamine density in hindlimb artery walls

The density of monoamine varicosities was measured in the ischiadic and iliac arteries taken from two Canada geese and two Pekin ducks. The semi-quantitative SPG histofluorescence method was used exactly as described by De La Torre (1980). Briefly, the animals were killed by anaesthetic overdose (i.v. sodium pentobarbital) and the ischiadic and iliac arteries of both hindlimbs were carefully removed starting a few millimetres proximal to the branch-point and extending distally for 2 cm. The excised artery was cut into a total of 20 transverse segments of approximately equal length using a scalpel. Each segment was frozen to a cryostat chuck maintained at − 30°C and at least five sections (15 μm thick) were cut from each one and placed on a glass slide, allowed to melt, dipped into SPG solution and then air dried. When dry, the sections were covered with light mineral oil, incubated at 95 °C for 2.5 min, and then examined in an Orthoplan fluorescence microscope (Leitz, Wetzlar, Germany). The average number of fluorescent adrenergic profiles in the sections taken from each segment was recorded and these were then combined into a grand mean for each species.

### Canada goose plasma catecholamine analysis

Plasma norepinephrine and epinephrine concentrations were measured in eight geese ($M_B = 4.2 \pm 0.1$ kg). The geese were cannulated in both brachial arteries (PE160) under halothane anaesthesia on the day before experiments. Each goose was restrained on a padded bench with its head loose and allowed to rest quietly for at least 1 h before experiments began. Arterial blood pressure was recorded from one cannula and blood samples (5 ml) were withdrawn from the other using Vacutainer tubes containing EDTA anticoagulant. A blood sample was taken and then the head was manually submerged into water. After 2 min a second blood sample was taken and the head was released.

Blood samples were kept on ice until centrifuged (9000 rpm for 5 min) and the plasma was then stored at − 80 °C until analysis. Analysis of catecholamines was performed by HPLC as described by Tong and Baines (1993). The "2 min submerged" plasma samples were diluted 40-fold before assay. A Model Coulochem II coulometric detector was used (ESA, Bedford, Mass., USA). The guard cell was set at + 0.15 V and electrodes I, II, III and IV were set at 0, 0, + 0.2 and − 0.38 V, respectively. Data were processed using a Millenium 2010 chromatography manager (Waters Associates, Milford, Mass., USA).

### Data analysis

Results are presented as mean ± SEM. Repeated measures ANOVA, Tukey multiple comparisons test, independent samples $t$-test, paired samples $t$-test and Mann-Whitney U test were used as required and differences between groups were considered significant when $P < 0.05$.

## Results

### Maximum underwater survival time of Canada geese

The maximum underwater survival time of Canada geese was $3.0 \pm 0.1$ ($n = 7$) min. This is 18% of the

**Table 1** Non-myoglobin-bound $O_2$ store of the Canada goose (*Branta canadensis*) and the Pekin duck (*Anas platyrhynchos*). With the exception of haemoglobin content, mean values for the Pekin duck were calculated from the appropriate allometric equations reported by Hudson and Jones (1986). Body mass of the ducks was taken as 4.4 kg, equal to that of the geese. Mean values ± SEM are given for measured variables.

| Variable | Canada goose ($n = 6$) | Pekin duck |
|---|---|---|
| Plasma volume (ml) | 210.2 ± 12.3 | 256.7* |
| Haematocrit (%) | 41.5 ± 1.1 | 34.6* |
| Blood volume (ml) | 360.7 ± 25.0 | 392.7 |
| Haemoglobin content ($g \cdot 100\,ml^{-1}$ blood) | 15.9 ± 0.5 | 14.5 ± 0.7[a] |
| Respiratory system volume (ml BTPS) | 368.0 ± 35.8 | 699.2* |
| Blood $O_2$ store (ml STPD) | 52.7 ± 4.9 | 46.7 |
| Respiratory system $O_2$ store (ml STPD) | 39.1 ± 4.2 | 65.3* |
| Available non-myoglobin $O_2$ store (ml STPD) | 91.8 ± 8.1 | 112.0* |

[a] $n = 7$; * $P < 0.05$

predicted maximum dive time of similarly-sized (4.2 kg) Pekin ducks [Hudson and Jones (1986); $t_d = 6.6\ M_B^{0.64} = 16.5$ min].

## Oxygen storage capacity of Canada geese

Canada goose $O_2$ store data are summarised in Table 1 along with the corresponding values for similarly sized Pekin ducks predicted by the allometric equations of Hudson and Jones (1986). The total available $O_2$ store of the Canada goose was 82% of that of the Pekin duck. The volume of the respiratory system of the goose was 53% of that of the duck. The estimated air sac $O_2$ extraction efficacy was similar in the two species (79% in Canada geese and 76% in Pekin ducks) so the usable respiratory system $O_2$ store in the geese was estimated to be 60% of that of the ducks. Thus, the percentage of the total available $O_2$ store residing in the respiratory system was smaller in geese (43%) than in ducks (58%).

The brain of geese (10.6 ± 1.0 g; $n = 6$) was significantly larger than that of ducks [Hudson and Jones (1986); $M_b = 4.91\ M_B^{0.308} = 7.75$ g) and the goose heart (27.9 ± 4.0 g; $n = 5$) was significantly smaller than the duck heart [Hudson and Jones (1986); $M_h = 8.4\ M_B^{0.97} = 35.4$ g). Overall, therefore, the total mass of brain and heart combined (the central $O_2$-dependent tissues) was slightly (but not significantly) smaller in geese (38.5 g) than in ducks (43.1 g). This difference was balanced by the differences in $O_2$ storage capacity so that the $O_2$ store per gram of central tissue were similar at 2.4 ml STPD $\cdot g^{-1}$ in the goose and 2.6 ml STPD $\cdot g^{-1}$ in the duck.

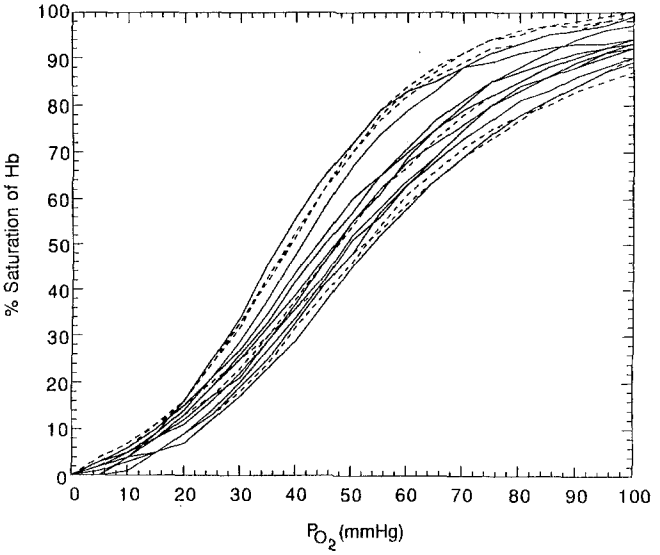


**Fig. 1** Oxygen equilibrium curves of Canada goose and Pekin duck blood. The curves were obtained using a spectrophotometric method (Hemoscan) at high (12%) and low (5%) $CO_2$. The ten *solid curves* are from Canada goose blood and the six *broken curves* are from Pekin duck blood. Blood pH was not determined during measurement of the $O_2$ equilibrium curves

## Blood respiratory properties of ducks and geese

Oxygen equilibrium curves of Canada goose and Pekin duck blood are shown in Fig. 1. There were no differences in the $O_2$ affinities of goose and duck blood. The Bohr effects also appeared to be similar but this could not be quantified because accurate pH measurements were not available. The Hill coefficient ($n$), derived from the regression of $\log(Y/(1-Y))$ on $\log P_{O_2}$ over the range of fractional saturations ($Y$) from 0.2 to 0.8, was similar in both species ($n = 2.6$ and 3.0 for goose blood, and 2.2 and 2.9 for duck blood at 5 and 12% $CO_2$, respectively).

Figure 2 illustrates blood $O_2$ content during the first 2 min of submergence in ducks and geese, showing an accelerated rate of $O_2$ depletion in the latter. Rates of depletion of blood $O_2$ content were statistically significantly different during the interval 30–60 s after onset of immersion. It should be pointed out that the $M_B$ of the Pekin ducks used in this part of the study was less than 60% of that of the Canada geese, and the difference in rate of depletion of $O_2$ content would probably have been greater if the birds had been body weight-matched.

## Cardiovascular response to immersion

There were notable species differences in HR and arterial blood pressure responses of ducks and geese to the first 2 min of head submergence (Fig. 3). Geese exhibited a delay in the onset of bradycardia compared with

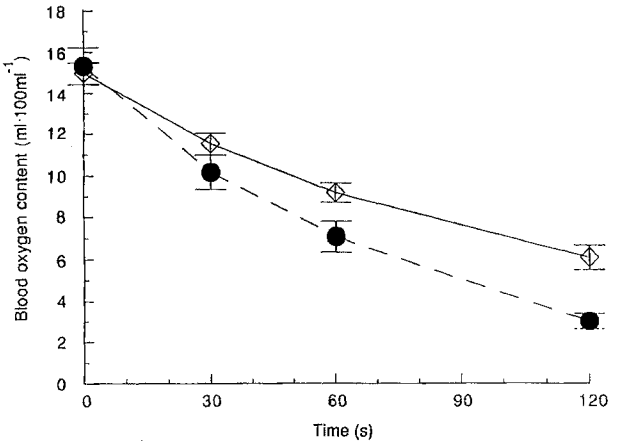


**Fig. 2** Depletion of arterial oxygen content during the first 2 min of forced submergence in Canada geese (*filled symbols*) and Pekin ducks (*open symbols*). Symbols represent the mean value ± SEM

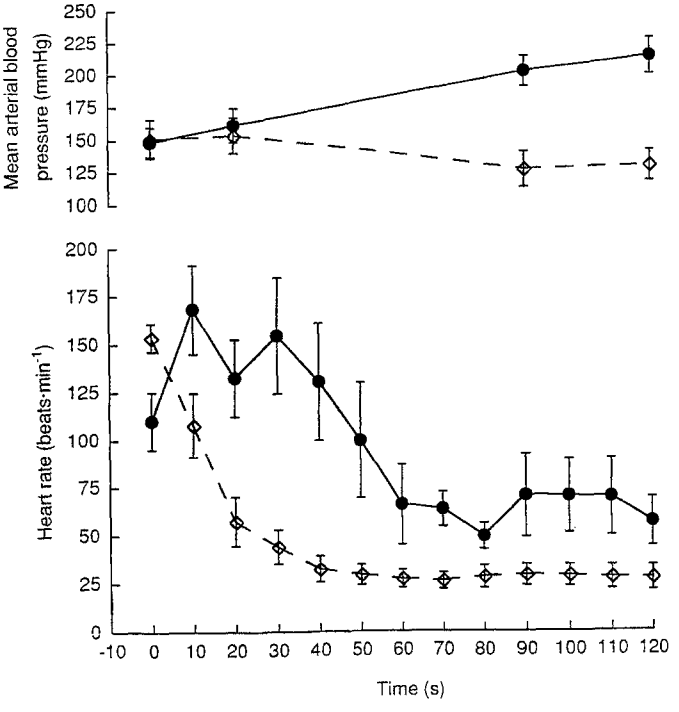


**Fig. 3** Mean ± SEM arterial blood pressure and mean ± SEM heart rate (beats · $min^{-1}$) before (time = 0 s) and during 2 min of forced submergence in Canada geese (*filled symbols, solid lines*) and Pekin ducks (*open symbols, dashed lines*)

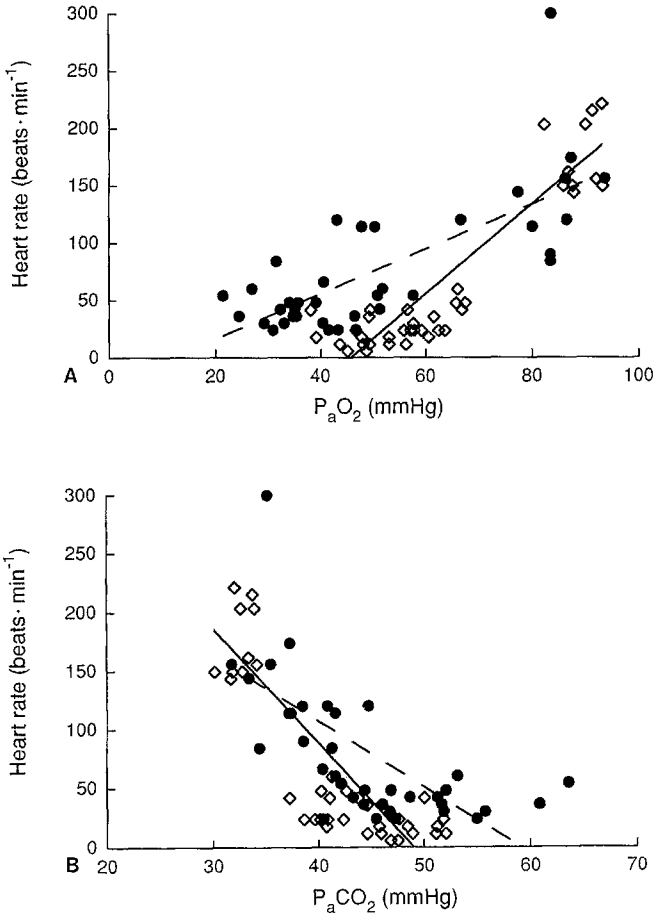


**Fig. 4A,B** Relationships between heart rate and (**A**) arterial blood $O_2$ tension ($P_{aO_2}$) and (**B**) arterial blood $CO_2$ tension ($P_{aCO_2}$), before and during head immersion in Canada geese (*filled symbols*) and Pekin ducks (open symbols). Cardiac chronotropic chemosensitivity was estimated as the change in heart rate per unit change in blood gas tension and this is illustrated as the slopes of the linear regression lines for geese (*dashed lines*) and ducks (*solid lines*)

ducks. It took 70–80 s for HR to fall to 50% of the pre-dive rate in geese but only 10–20 s for the same relative reduction in ducks. Minimum HR achieved by geese was 36 ± 6 beats · $min^{-1}$ at 101 ± 7 s of submergence, whereas that in ducks was significantly lower at 19 ± 3 beats · $min^{-1}$ at 69 ± 13 s of submergence. This represents a maximal 67% reduction in HR from pre-dive levels in geese compared with a maximal 88% reduction in ducks. Perhaps more importantly, the bradycardia was considerably more labile in geese than in ducks; very low HR tended to be sustained in ducks but was very transient in geese. The large error bars on the goose HR curve in Fig. 3 are a product of this intra-individual variability and do not signify that some geese were much better able to invoke bradycardia than others.

Cardiac chemosensitivity was estimated as the change in HR (pre-immersion–immersion) per unit change in blood gas tension (Fig. 4). Cardiac oxygen chemosensitivity ($\Delta HR/\Delta P_{aO_2}$) in submerged ducks (4.64 ± 0.11 beats · $mmHg^{-1}$) was twice that in submerged geese (2.18 ± 0.12 beats · $mmHg^{-1}$). This difference was statistically significant. However, $CO_2$ sensitivity ($\Delta HR/\Delta P_{aCO_2}$) was statistically similar in ducks (13.7 ± 0.4 beats · $mmHg^{-1}$) and geese (10.9 ± 0.81 beats · $mmHg^{-1}$). The correlations between HR and $P_{aO_2}$ as well as $P_{aCO_2}$ were significant for ducks. However, in geese only the relation between HR and $P_{aCO_2}$ was significant. As expected, $P_{aO_2}$ and $P_{aCO_2}$

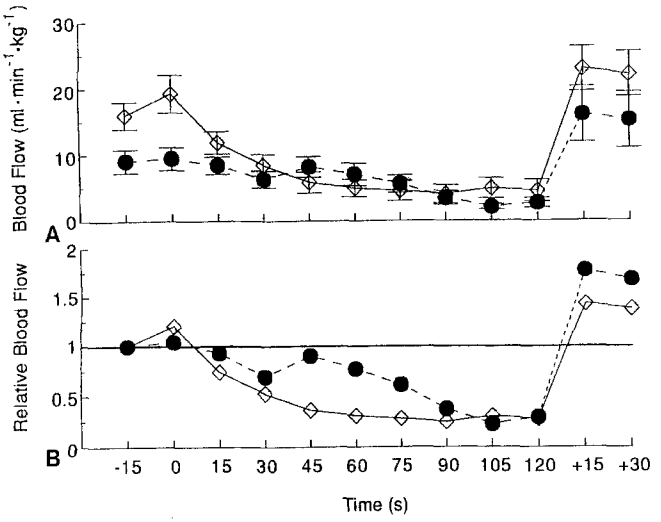


**Fig. 5** A Mean ± SEM ischiadic artery blood flow before and during head immersion in Canada geese (*filled symbols*) and Pekin ducks (*open symbols*). **B** Changes in blood flow expressed as a fraction of the pre-submerged value ( − 15 s)

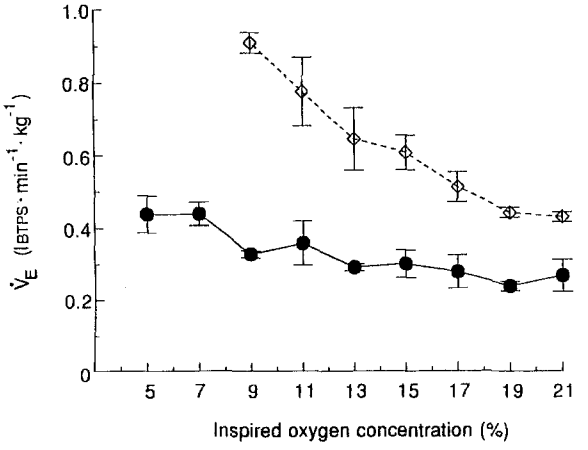


**Fig. 6** The hypoxic ventilatory responses of Canada geese (*filled symbols*) and Pekin ducks (*open symbols*). Mean ( ± SEM) respiratory minute ventilation ($\dot{V}_E$; lBTPS·min$^{-1}$·kg$^{-1}$) is plotted as a function of inspired oxygen concentration (%)

during submergence were significantly negatively correlated in both ducks ($r = 0.79$) and geese ($r = 0.66$) which precluded a multiple linear regression analysis of HR, $P_{aO_2}$ and $P_{aCO_2}$ in either species.

Mean arterial blood pressures of ducks and geese were not significantly different before immersion but differed significantly during submergence. In geese, mean arterial blood pressure was significantly higher than the pre-submerged value after 20 s of immersion, increasing by 44% after 2 min. In ducks, mean arterial blood pressure decreased gradually during submergence and was significantly lower than pre-submerged levels after 90 s of submersion. Duck mean arterial blood pressure had decreased by 14% after 2 min (Fig. 3).

Electrical stimulation of the gastrocnemius muscle group had no statistically significant effects on ischiadic artery blood flow. Mean blood flow during the second minute of submergence was 3.7 ± 0.9 and 5.2 ± 1.1 ml·min$^{-1}$·kg$^{-1}$ in non-stimulated and stimulated ducks, respectively. The corresponding values for geese were 3.2 ± 0.5 and 3.6 ± 0.9 ml·min$^{-1}$·kg$^{-1}$. All data from stimulated and unstimulated trials were therefore pooled for further analysis of species differences (Fig. 5).

Pre-submergence blood flow was a significant 76% higher in Pekin ducks (16.0 ± 2.1 ml·min$^{-1}$·kg$^{-1}$) than in Canada geese (9.1 ± 1.8 ml·min$^{-1}$·kg$^{-1}$). Post-submergence blood flow was 43% higher in ducks than geese but this difference was not statistically significant. Blood flow during the second minute of submergence was similar in both species (4.4 ± 0.7 ml·min$^{-1}$·kg$^{-1}$ in ducks and 3.4 ± 0.5 ml·min$^{-1}$·kg$^{-1}$ in geese; Fig. 5A). The time-course of changes in blood flow are obscured by the differences in pre-submergence values so the relative changes are plotted in Fig. 5B. The onset of vasoconstriction was delayed in Canada geese compared with Pekin ducks; time to decrease to 50% of pre-submerged value was 75–90 s in geese and 30–45 s in ducks.

## Ventilatory response to hypoxia

$\dot{V}_E$ is plotted as a function of $F_{IO_2}$ in Fig. 6, and it appears that Canada geese had a lower hypoxic ventilatory threshold than Pekin ducks. In ducks $\dot{V}_E$ increased significantly ($P < 0.05$) at a $F_{IO_2}$of 15%, whereas in geese $\dot{V}_E$ remained statistically unchanged until $F_{IO_2}$ had decreased to 5%. Furthermore, the respiratory hypoxic sensitivity (estimated as the slopes of the curves which were virtually linear in this study; Fig. 5) of the ducks (0.039 l·min$^{-1}$·kg$^{-1}$·%$F_{IO_2}{}^{-1}$) was nearly four times that of the geese (0.01 l·min$^{-1}$·kg$^{-1}$·%$F_{IO_2}{}^{-1}$).

## Hindlimb artery monoamine profiles

The SPG method for histofluorescent analysis of tissue monoamines did not demonstrate any consistent trends in number of fluorescent adrenergic profiles as a function of sample site for ducks or geese. Therefore, all samples were pooled and the overall profile count was 118.8 ± 6.5 per section in duck arteries and 53.6 ± 3.7 per section in goose arteries, a significant difference between species.

## Canada goose plasma catecholamines

Resting plasma norepinephrine concentration was 8.9 ± 4.1 nmol·l$^{-1}$ ($n = 8$) and this increased by 87-fold to 774 ± 185 nmol·l$^{-1}$ ($n = 8$) after 2 min of

submergence. Resting plasma epinephrine concentration was $3.7 \pm 1.6$ nmol·l$^{-1}$ ($n = 7$) and increased by 168-fold to $621 \pm 50$ nmol·l$^{-1}$ ($n = 8$) after 2 min of submergence. For comparison, Hudson and Jones (1982) found that Pekin duck plasma norepinephrine concentration increased from a resting value of $7.1 \pm 3.8$ nmol·l$^{-1}$ ($n = 4$) to $63 \pm 25$ nmol·l$^{-1}$ ($n = 5$) after 2 min and $1017 \pm 95$ nmol·l$^{-1}$ ($n = 3$) after 10 min of head immersion. Duck plasma epinephrine concentration increased from a resting value of $3.6 \pm 1.1$ nmol·l$^{-1}$ ($n = 4$) to $39 \pm 24$ nmol·l$^{-1}$ ($n = 4$) after 2 min and $884 \pm 128$ nmol·l$^{-1}$ ($n = 3$) after 10 min of immersion (Hudson and Jones 1982).

## Discussion

Pekin ducks can withstand involuntary submergence for 5.5 times longer than Canada geese of equal $M_B$. We have shown that this species difference in maximum underwater survival time is caused by both a smaller available $O_2$ store and a less effective $O_2$-conserving cardiovascular response in Canada geese. Both species can utilise their $O_2$ stores to approximately the same extent.

The usable $O_2$ store of the Canada goose was estimated to be about 82% of that of the Pekin duck (Table 1). However, this 18% deficit in stored $O_2$ accounts for more than 18% of the difference in underwater survival times because $O_2$ consumption decreases progressively and non-linearly during submergence. In order to estimate the contribution of reduced $O_2$ storage capacity to reduced underwater endurance time, it was assumed that the minimum rate of $O_2$ consumption corresponds to the maximum intensity of the $O_2$-conserving cardiovascular response (i.e. bradycardia and hindlimb blood flow), which occurs within about 1 min in the birds used in this study [Figs. 3, 5; cf. Hudson and Jones (1986)]. Approximately 40% of the total $O_2$ store of the Pekin duck (i.e. 45 ml $O_2$) was used within the first minute of submergence (Fig. 2) before the cardiovascular response was fully developed. Assuming that the initial rate of $O_2$ consumption would be little affected by limited changes in the absolute size of the $O_2$ store, an 18% reduction in the total store would leave only 70% of the store that is usually present after 1 min of submergence. Thus, a Pekin duck which can normally survive for 16.5 min would survive for only $1 + (15.5 \cdot 0.7) = 12$ min if the usable $O_2$ store were reduced to 82% of its usual level. Since Canada geese were capable of enduring only 3 min of submergence, the smaller $O_2$ storage capacity of this species accounts for approximately 33% of the difference between species.

Canada geese were capable of extracting 79% of the respiratory system $O_2$ store and approximately 90% of the blood $O_2$ store during prolonged submersions. This was similar to Pekin ducks, which extracted 76% of the $O_2$ stored in the respiratory system and 96% of the $O_2$ bound to haemoglobin in the blood (Hudson and Jones 1986). The similar efficacy of $O_2$ store utilization is explained by the similar blood respiratory properties of the two species (Fig. 1). The $O_2$ affinity and Hill coefficients were similar to those measured in other studies of these species (Christensen and Dill 1935; Scheipers et al. 1975; Petschow et al. 1977; Black and Tenney 1980).

Our estimate of Canada goose blood volume (i.e. blood constitutes about 8.2% of body weight) is in the upper range of published estimates for the domestic goose, *Anser* spp. [6.4–8.4%; Hanwell et al. (1971); Hunsaker (1968)]. Haematocrit and haemoglobin content of the Canada geese in this study confirm other observations in this species (Williams and Trainer 1971; Petschow et al. 1977; Ronald and George 1988) and are also similar to those measured in the bar-headed goose, *Anser indicus* (Black et al. 1978; Black and Tenney 1980; Faraci et al. 1984) and the snow goose, *Chen caerulescens* (Williams and Trainer 1971).

We are unaware of any previous measurements of respiratory system volume in geese with which we could compare our results. However, we could confirm the values for Pekin duck respiratory system volume reported by Hudson and Jones (1986) in two preliminary tests using the same technique. The fact that the same volumes were obtained in lightly anaesthetized as in awake geese, despite a far more thorough mixing of the gases in the respiratory system in the former, also supports the accuracy of our results. These checks were important because the entire deficiency in the total $O_2$ storage capacity of the Canada goose, compared with that of the Pekin duck, was a result of the much smaller respiratory system volume in the former.

Since approximately 67% of the deficiency in underwater survival time cannot be explained by a smaller $O_2$ store, nor by an impaired ability to utilise the store, the capacity of Canada geese to redistribute the blood and thereby selectively to consume the $O_2$ must have been inferior to that of Pekin ducks. This suggestion was supported by measurements of HR. The delay in onset, reduced final intensity of the bradycardia and greater variability of HR in submerged geese all indicate that the $O_2$-conserving cardiovascular response was less effective in geese than in ducks. The onset of hindlimb vasoconstriction was also delayed in geese compared with ducks, although the final absolute blood flow rate through the ischiadic artery was the same in both species. The resting ischiadic artery flow was significantly higher in ducks than in geese. Whether this indicates a lower total rate of hindlimb blood flow in geese or simply a difference in the proportion of the hindlimb that is supplied by the ischiadic artery cannot be concluded from this study.

Since most of the cardiovascular response to forced submergence occurs as a result of peripheral chemoreceptor stimulation in the Pekin duck (Jones and Purves 1970a) we attempted to compare the

"sensitivities" of these chemoreflexes in the two species. The ventilatory responses of the Pekin duck to hypoxia were similar to those published previously for this species (Black et al. 1978; Butler 1970; Jones and Purves 1970b; Jones and Holeton 1972; Colacino et al. 1977; Bouverot et al. 1979; Van Nice et al. 1980). The hypoxic ventilatory response of the Canada goose, however, was notably different from that of the Pekin duck, with much lower levels of hypoxia being tolerated by the geese before significant increases in minute ventilation occurred (Fig. 6). Indeed, the ventilatory response of the Canada goose, a species that lives at low altitudes, was similar to that reported for the bar-headed goose, a species that is often considered to be adapted for high altitude environments (Black et al. 1978; Black and Tenney 1980; Van Nice et al. 1980). This is surprising in view of the fact that the $O_2$ affinity of Canada goose blood was similar to that of the Pekin duck and significantly lower than that of the bar-headed goose (Petschow et al. 1977; Black and Tenney 1980).

The lower sensitivity of the respiratory system of the Canada goose to inhalation of hypoxic gases suggests that the chemoreceptors, particularly the carotid body chemoreceptors (Jones and Purves 1970b; Bouverot et al. 1979), may be less responsive in this species which may in turn explain the delayed and less intense cardiovascular response to forced submergence. However, the hypoxic ventilatory response may not be an adequate test of the effects of chemoreceptor input to the cardiovascular system during apnoea. To investigate this further we related change in HR to change in blood gas tensions in ducks and geese during head immersion. The sensitivity of HR to hypoxia was twice as great in ducks as in geese. On the other hand, the HR sensitivities of both species to hypercapnia were similar. Since the cardiovascular adjustments in ducks, and probably also in geese (Cohn et al. 1968), are primarily set in train by hypoxic stimulation of peripheral chemoreceptors (Jones and Purves 1970a), the enhanced $O_2$ chemosensitivity of ducks is likely to be a major contributor to the greater efficacy of $O_2$ conservation during submergence compared with geese.

Folkow et al. (1966) studied factors affecting hindlimb vasculature in ducks, turkeys and cats and concluded that ducks are capable of a more pronounced vasoconstriction both in the presence and absence of muscle activity. They reported a higher number of fluorescent adrenergic profiles in the femoral artery of the duck and, assuming that this was indicative of the density of adrenergic innervation, suggested that this may be at least partly responsible for the more pronounced response to involuntary submergence in that species. Folkow et al. (1966) were unfortunately constrained by available techniques to use a protocol in which the "diving" vasoconstriction was simulated in anaesthetised animals by administration of 20% $CO_2$ combined with vagotomy and controlled bleeding. In an attempt to verify their results we repeated aspects of their experiment using awake animals fitted with chronically implanted pulsed Doppler flow probes, and an improved method for histofluorescent assay of tissue monoamines (De La Torre 1980). Our results essentially confirmed those of Folkow et al. (1966) with respect to the duck. Monoamine profiles in the duck hindlimb artery walls were approximately twice as numerous as those in the geese. However, electrically-stimulated muscle activity did not cause ascending vasodilatation in either species, as predicted for the duck but contrary to expectations for the Canada goose.

It may be that the extremely high levels of circulating catecholamines in geese are sufficient to maintain peripheral vasoconstriction in spite of diminished numbers of monoamine varicosities (Lacombe and Jones 1991a,b). Resting values for epinephrine and norepinephrine were statistically similar in geese and ducks (Hudson and Jones 1982; Lacombe and Jones 1991a), while after 2 min submergence catecholamine concentrations were a statistically significant 12–16 times greater in geese than in ducks. In fact, the plasma catecholamine concentrations measured after 2 min submergence in geese were statistically similar to those measured after 10 min submergence in Pekin ducks (Hudson and Jones 1982). Interestingly, 2 and 10 min are about 66% of maximum underwater endurance in geese and ducks, respectively. The high levels of circulating catecholamines may have been responsible for the mild hypertension observed during immersion in geese, and for the lack of ascending vasodilatation during muscle stimulation.

In contrast to the situation during electrical stimulation, voluntary muscle contractions initiated during struggles were associated with transient increases in ischiadic blood flow. These increases were difficult to quantify due to non-steady flow before and following each struggle, but they did not appear to be augmented by concurrent electrical stimulation of the gastrocnemius muscles. Furthermore, it is unknown how much of the increased blood flow reached the capillary exchange surfaces, and whether there were any differences between species in this regard. The frequency of struggling was slightly higher in geese than in ducks but this difference was not statistically significant.

Although the two species are closely related both in terms of phylogeny and ecology, there is one aspect of their behaviour that might have a bearing on the evolution of the physiological differences described in this paper. During the reproductive season, mallards (of which the Pekin is a domesticated variety) frequently engage in forced copulations on water, often with several males mating a single female in succession (McKinney et al. 1983). The females are often fully submerged during these episodes and Huxley (1912) estimated that as many as 10% of the females may drown as a result. Thus, this behaviour probably represents a significant natural cause of adult mortality in mallards. Even a much lower average mortality rate would constitute

a substantial selective pressure for the evolution of extended breath-hold capacities in this species (Florin-Christensen et al. 1986). Canada geese do not engage in this behaviour and this may explain their relatively poor underwater endurance capacity. A broader comparative study is needed to test this hypothesis conclusively (Garland and Adolph 1994).

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