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# THE AEROBIC CAPACITY OF LOCOMOTORY MUSCLES IN THE TUFTED DUCK, *AYTHYA FULIGULA*

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## SUMMARY

The locomotory muscles of the tufted duck, *Aythya fuligula* (L.), were analysed for mass, aerobic and anaerobic enzyme activities, fibre-type proportions, capillarity, mitochondrial and myoglobin content. The estimated aerobic capacity of the muscles correlated well with the muscles' maximal oxygen uptake both when measured during swimming and when predicted for steady-state flight. The results suggest that exercise performance in birds cannot be predicted purely on the basis of muscle mass (see Butler & Woakes, 1985); the specific enzyme complement of each muscle must also be taken into account.

The delivery of oxygen to mitochondria is facilitated by the dense capillarity and high myoglobin content of the muscles.

## INTRODUCTION

Because flight is the major form of locomotion in most birds, anatomical studies on avian muscle have mainly been concentrated on the pectoral muscles. The leg muscles have received much less attention, even though they may be important in locomotion in aquatic species such as the tufted duck, *Aythya fuligula*, which is capable of a variety of types of locomotion (flying, walking, swimming and diving). Underwater activity is particularly strenuous in this species; oxygen consumption during diving is similar to that during surface swimming at maximal sustainable speed (Woakes & Butler, 1983).

Although oxygen consumption during flight is some 2·2 times higher than maximal oxygen consumption during swimming in most birds, running cursorial birds may have maximal oxygen consumptions close to those of flying birds (Butler, 1982). As the mass ratio of flight and leg musculature is similar to the ratio of maximum oxygen consumption during flight and running (Prange & Schmidt-Nielsen, 1970), it has been suggested that the limitation to exercise performance in birds may be the mass of muscle involved and, in particular, the volume of the

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oxidative machinery (Butler & Woakes, 1985). Cursorial birds have a relatively large leg muscle mass and this would explain their ability to attain high levels of oxygen consumption whilst running.

The pectoral muscle of many flying birds has been shown to be capable of continuous activity for long periods of flight without fatiguing (Goldspink, Mills & Schmidt-Nielsen, 1978; Rothe, Biesel & Nachtigall, 1987). It has a very high oxidative capacity (Marsh, 1981; Suarez, Brown & Hochachka, 1986) and substantial substrate stores of glycogen and lipid (Ashhurst, 1969) in order to maintain such prolonged exercise. The high mechanical power requirement necessary for flight in many birds is provided by an exclusive complement of fast twitch fibres (Talesara & Goldspink, 1978; Swatland, 1984), most of which are highly oxidative. However, there are exceptions (e.g. herring gull) where there may be a small proportion of slow twitch oxidative (SO) fibres present (Talesara & Goldspink, 1978).

Leg muscles in birds have a similar distribution of fibre types (Suzuki, Tsuchiya, Ohwada & Tamate, 1985; Swatland, 1985; Talesara & Goldspink, 1978) to that found in mammals (Ariano, Armstrong & Edgerton, 1973; Armstrong & Phelps, 1984). In addition to slow twitch fibres, which rely predominantly on oxidative metabolism for ATP production, there are fast twitch fibres, some of which rely predominantly on glycolytic metabolism (FG fibres), whereas others rely equally on oxidative and glycolytic metabolism (FOG fibres).

The oxidative capacity of the heart in birds should be higher than that in non-flying mammals as it must be able to increase cardiac output to meet, at least partly, the extremely high levels of oxidative metabolism during flight (Butler, West & Jones, 1977). Indeed, allometric studies indicate that the mass of heart muscle in birds (and bats) is greater than that in non-flying mammals (Jurgens, Bartels & Bartels, 1981; Grubb, 1983).

A detailed analysis of mitochondrial composition, aerobic and anaerobic enzyme complements, and substrate content of the flight, leg and heart muscles of one species of bird has not been performed, so it is not possible to compare the oxidative capacities of different muscle masses involved in different activities.

The primary aim of this study was to assess the oxidative capacities of the pectoral, leg and cardiac muscles of the tufted duck. The secondary aim was to use the results to test whether the oxygen consumption, measured or calculated, during swimming (Woakes & Butler, 1983) and flying (from Butler, 1982) could be predicted from an estimate of the oxidative capacity of the muscles involved.

## MATERIALS AND METHODS

### *Animals*

Six tufted ducks, *Aythya fuligula*, of either sex were raised from eggs and housed in an indoor aviary, 3·3 m × 1·2 m, with a pool area of 3·3 m × 1·0 m × 0·4 m deep. The shallow depth plus overhead netting prevented prolonged diving or flying. Mixed corn and growers' pellets (Heygate & Sons, Ltd), supplemented by Vionate (E. R. Squibb & Sons, Ltd), were available on a dry area; mixed corn was

additionally thrown onto the pool. This diet was varied by adding cornshoots, pondweed and fresh grass when available. No attempt was made to control photoperiod and the ducks were exposed to the normal annual cycle of daylight. The temperature of the air in the aviary was 10–25 °C and that of the water 8–20 °C.

### *Histochemistry*

All the ducks were killed either by an overdose of an inhalation mixture of halothane (25 % oxygen/air) or by an intravenous injection of sodium pentobarbitone (May & Baker). Two leg muscles, one from the thigh (semitendinosus) and one from the calf (lateral gastrocnemius), which undergo representative increases in blood flow during swimming (Butler, Turner, Al-Wassia & Bevan, 1988), and the pectoralis major muscle were isolated, cleaned of blood and connective tissue, and weighed before samples were quenched in liquid nitrogen. Storage was at −80 °C in an ultra-low-temperature freezer (Sanyo, MDF190). Serial cross-sections (10–20 $\mu$m) were cut on a freezing microtome at −20 °C and stained for myofibrillar ATPase (mATPase) after acid and alkaline preincubation using the method of Guth & Samaha (1970) and for succinate dehydrogenase (SDH) using the method of Nachlas *et al.* (1957). Fibre diameters were measured using a calibrated eyepiece on a light microscope and fibre cross-sectional areas calculated from the measurements. Fibre-type proportions were calculated from at least 150 fibres using alkaline stability and SDH staining intensity as descriptive criteria. SO fibres had low alkaline mATPase stability/high SDH staining; FOG fibres had high alkaline mATPase stability/high SDH staining and FG fibres had high alkaline mATPase stability/low SDH staining.

The estimated proportional area (%) of a particular fibre type in a muscle was calculated, and for calculation of the absolute mass (g) of a particular fibre type in a muscle it was assumed that fibres make up 85 % of muscle mass (Gollnick, Timson, Moore & Riedy, 1981; Armstrong & Phelps, 1984). Capillary density, the number of capillaries around a fibre, and the capillary/fibre ratio were calculated from counts made on at least 10 fields (>200 fibres) of slides stained for mATPase after an acid preincubation (Sillau & Banchero, 1977). On sections stained for mATPase after acid preincubation, capillaries appear as small black deposits between fibres. The number of capillaries in a field of view can be converted to capillary density ($mm^{-2}$), knowing the area of the field. The number of capillaries around each fibre of a specific type can be counted, and if the number of capillaries in view is divided by the number of fibres in view, this gives the capillary/fibre ratio.

### *Morphometry*

Small (2 mm × 2 mm × 15 mm) superficial tissue samples were collected from all the chosen muscles of three ducks and pinned at approximately resting length on cork and immediately placed in ice-cold fixative (2·5 % paraformaldehyde; 5 % glutaraldehyde in 0·1 mol $l^{-1}$ sodium cacodylate buffer at pH 7·4) for at least 8 h. The samples were then cut into 1 $mm^3$ blocks and postfixed with osmium tetroxide for

1·5 h. The fixed tissue was then dehydrated in graded alcohols, finishing with absolute alcohol dried over anhydrous copper sulphate. Once infiltrated with propylene oxide, blocks were embedded in resin and left to polymerize in an oven overnight at 60°C. Ultra-thin sections (60–90 nm) of both longitudinally and transversely aligned blocks were cut on an ultramicrotome (LKB), and mounted on carbon-coated 200-mesh copper grids (Gilder grids). The sections were then stained with uranyl acetate (1 h) and lead citrate (5 min).

Six micrographs of transverse or longitudinal sections from each of five blocks (a total of 30 micrographs) of a muscle from each of three ducks were taken at a final magnification of 25 000×. A points-test grid with 168 points was placed over contact prints of the micrographs and the number of points falling on myofibrils, mitochondria and other structures was counted. Assuming that volume estimates can be made from estimated areas on planar sections and that the number of points used exceeded that needed for statistical validity at the 95 % confidence limit (Weibel, 1979), the volume density of a component may be estimated with respect to a reference volume, in this case the fibre. Results are given as the mean ± S.E. for each of the muscles in three ducks, with the sample unit being the single muscle. The volume densities of mitochondria and myofibrillar structures were determined by this method. By multiplying volume densities by muscle mass and dividing by the density of muscle tissue (1·06 g $ml^{-1}$; Mendez & Keys, 1960), the absolute volumes (ml) were calculated. The maximal oxygen consumption of mitochondria has been estimated to be 4·9 ml $O_2$ $min^{-1}$ $ml^{-1}$ (Hoppeler *et al.* 1984*b*), and by multiplying this by the volume of mitochondria in a muscle, an estimate of maximal aerobic capacity of the muscle can be made.

### *Enzyme analysis*

Cross-sectional samples of all the chosen muscles from between four and six ducks were taken and stored for no more than 1 week at −80°C. Muscle samples were thawed to 0°C and homogenized in 20 volumes of 100 mmol $l^{-1}$ phosphate buffer, 5 mmol $l^{-1}$ EDTA and 1 % Triton-X 100 at pH 7·3. The homogenate was centrifuged at 18 000 rev. $min^{-1}$ for 70 min, and the clear supernatant diluted with 1 % bovine serum albumin (Sigma). The activities of citrate synthase (CS; EC 4.1.3.7), 3-hydroxyacyl-CoA-dehydrogenase (HAD; EC 1.1.1.35) and lactate dehydrogenase (LDH; EC 1.1.1.27) were determined by standard techniques (Srere, 1969; Marsh, 1981). Muscle homogenates were placed in a dual-beam spectrophotometer (Cecil Instruments), with a 1 cm light path, at a temperature of 25°C. The changes in light absorbance with the production of mercaptide ions (CS) or the utilization of NADH (HAD, LDH) were followed on a pen recorder (Cecil Instruments) for at least 3 min, using appropriate controls with substrate depletion. Activities are expressed as μmol $min^{-1}$ $g^{-1}$ fresh mass of muscle (U $g^{-1}$).

The incubation solution for the determination of CS activity contained 0·1 mmol $l^{-1}$ 5,5′-dithiobis-(2-nitrobenzoic acid), 0·3 mmol $l^{-1}$ acetyl-CoA and

0·5 mmol $l^{-1}$ oxaloacetate (the last of these was omitted from the control solution). The incubation solution for the determination of HAD activity contained 0·1 mmol $l^{-1}$ triethanolamine, 5 mmol $l^{-1}$ EDTA, 0·225 mmol $l^{-1}$ NADH and 0·1 mmol $l^{-1}$ acetoacetyl-CoA (the last of these was omitted from the control solution). The incubation solution for the determination of LDH activity contained 483·3 $\mu$l of prepared substrate + NADH (Boehringer Mannheim GmbH) and 16·6 $\mu$l of muscle supernatant. Chemicals were obtained from Sigma Chemicals (Poole, Dorset).

### *Myoglobin determination*

Cross-sectional samples of all the chosen muscles from five ducks were thawed and homogenized in 19·25 volumes of 0·04 mol $l^{-1}$ phosphate buffer, pH 6·6. The homogenate was centrifuged at 16 000 rev. $min^{-1}$ for 70 min and the supernatant kept on ice. Each sample was placed into a tonometer and carbon monoxide was bubbled through the supernatant for 12–15 min. Sodium dithionate was not added as detailed by Reynafarje (1963) as it caused a precipitate to cloud the solution. The supernatant was placed in a cuvette and the absorbance of the reduced myoglobin and haemoglobin read at 538 nm in a spectrophotometer with a 1 cm lightpath (Beckman). The absorbance of haemoglobin was read at 568 nm, and the absorbance of myoglobin obtained by subtraction. Myoglobin concentration was calculated as described by Reynafarje (1963) and expressed in mg $g^{-1}$.

### *Statistics*

Mean values are given ±S.E. of the mean. The number of observations is represented by $N$. Statistical analyses were performed using a microcomputer (BBC Model B) and a statistical package (Unistat, Unisoft Ltd). Significant differences between mean values were determined using a one-way analysis of variance and Student's $t$-tests for samples of unknown variances. Differences between means are considered significant at the 95 % ($P < 0·05$) confidence level.

## RESULTS

The lateral gastrocnemius muscle had two distinct areas differentiated by colour. The part of the muscle nearer the tibia was much redder than the larger part further from the bone. Thus in histochemical studies, the lateral gastrocnemius was split into two portions: red and white. The semitendinosus muscle did not have such a significant distinction between deeper and more superficial parts. The heart (both ventricles and both atria) represented 0·92 ± 0·07 % of total body mass (Table 1). The lateral gastrocnemius and semitendinosus muscles represented much smaller proportions of body mass; in fact the sum of all leg muscles (left and right) only represented 6·88 ± 0·11 % of body mass and only approximately 26·4 % of all locomotory limb muscle masses assessed. The flight muscle mass (left and right

Table 1. *Absolute and relative masses of various skeletal muscles and the heart in the tufted duck*

| | Absolute (g) | Relative (% body mass) |
|---|---|---|
| Body mass | 638 ± 26 | |
| Heart mass | 5·84 ± 0·50 | 0·9 ± 0·07 |
| Left gastrocnemius mass | 1·76 ± 0·10 | 0·3 ± 0·01 |
| Left semitendinosus mass | 2·50 ± 0·14 | 0·4 ± 0·01 |
| Left pectoralis mass | 47·40 ± 2·00 | 7·4 ± 0·31 |
| Total hindlimb muscle mass | 43·84 ± 1·24 | 6·9 ± 0·11 |
| Total flight muscle mass (pectoral + supracoracoideus) | 121·80 ± 6·00 | 18·5 ± 1·00 |
| Total flight muscle mass/total hindlimb muscle mass | 2·63 ± 0·16 | |

Values are means ± S.E. ($N = 6$ animals).

pectoral and supracoracoideus muscles) was 2·63 ± 0·16 times that of the legs and represented the bulk of the measured locomotory muscle mass.

*Fibre-type distribution*

The anatomically defined red portion of the lateral gastrocnemius was the only muscle part to contain SO fibres (Table 2; Fig. 1A,B). FOG fibres made up the remainder of this portion of the muscle. At the border of the red and white portions, there was a gradual reduction in the proportion of SO fibres and a progressive increase in FG fibres (Fig. 1C), giving rise to an uneven distribution of fibre types throughout the cross-section of the lateral gastrocnemius. FOG and FG fibres made up the vast proportion of muscle mass of the whole lateral gastrocnemius, with SO fibres weighing 0·03 g and representing only 2·3 % of the total muscle fibre bulk. FOG and FG muscle fibres represented approximately equal proportions of the remaining muscle bulk (45·1 % FOG, 52·6 % FG). The relative proportions of oxidative fibres (FOG+SO) and glycolytic fibres (FG only) were similar in the lateral gastrocnemius (Table 2).

The semitendinosus contained no SO fibres throughout its cross-section and there was no significant uneven distribution of FOG and FG fibres (Fig. 2A,B). Each fibre type made up approximately equal proportions (by number) of the semitendinosus. However, because FG fibres were approximately double the cross-sectional area of FOG fibres, they made up approximately 64·5 % of the muscle fibre mass within the whole muscle. Both FOG and FG fibres in the lateral gastrocnemius and semitendinosus were significantly larger in cross-sectional area than similar fibre types in the pectoral muscle (Table 2).

There were no SO fibres in the pectoral muscle of the tufted duck. There was an uneven distribution of fast twitch fibres. FG fibres were more frequent in the more

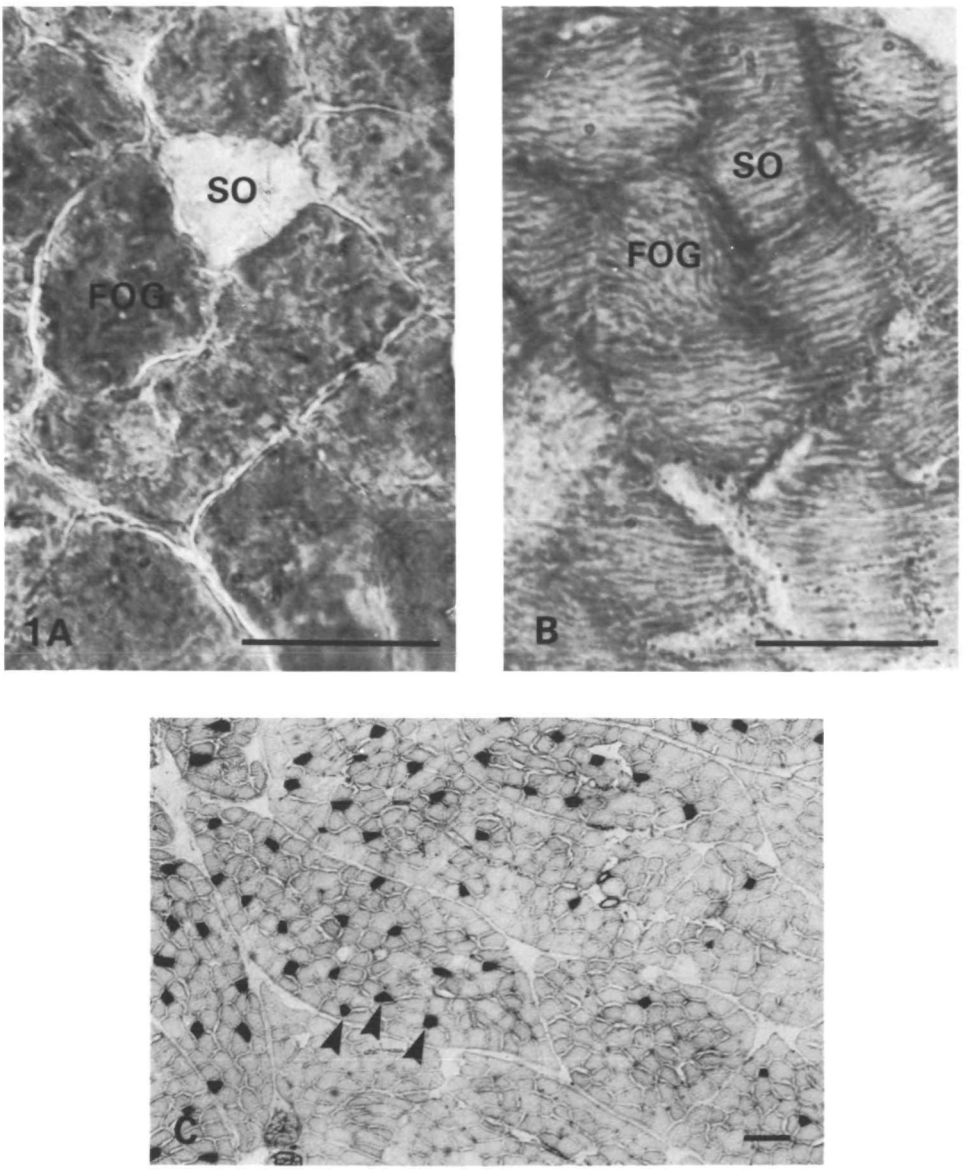


Fig. 1. Serial cross-sections of muscle fibres in the red (A,B) and transitional area (C) of the lateral gastrocnemius stained for mATPase after a preincubation at pH 10·4 (A) or pH 3·9 (C) and stained for SDH activity (B). In A and B, SO represents a slow oxidative fibre and FOG represents a fast oxidative glycolytic fibre. The arrowheads in C indicate SO fibres. Scale bars, 50 $\mu$m A,B; 100 $\mu$m C.

superficial regions of the muscle (Fig. 3C) and, furthermore, FG fibres appeared to be more common at the periphery of a muscle fascicle. On either a numerical or mass basis, the proportion of FG fibres was smaller than that of FOG fibres (Table 2). The FOG fibres in the pectoral muscle stained particularly intensely for the aerobic

Table 2. *Mean values of fibre-type proportions and their relative (%) and absolute (g) contributions to the overall mass of a number of locomotor muscles in the tufted duck*

| | | Red gastrocnemius | White gastrocnemius | Whole gastrocnemius | Semitendinosus | Pectoralis |
|---|---|---|---|---|---|---|
| Muscle mass (g) | | 0·40 ± 0·04 | 1·35 ± 0·09 | 1·76 ± 0·10 | 2·50 ± 0·14 | 47·40 ± 2·00 |
| Estimated fibre mass of muscle (g) | | 0·34 | 1·15 | 1·50 | 2·13 | 40·29 |
| Fibre type (%) | SO | 15 ± 0·6 | | | | |
| | FOG | 85 ± 0·6 | 46 ± 4·5 | | 51 ± 3·3 | 88 ± 2·3 |
| | FG | | 54 ± 4·5 | | 49 ± 3·3 | 12 ± 2·3 |
| Fibre diameter (μm) | SO | 34·2 ± 1·8 | | | | |
| | FOG | 44·9 ± 2·3 | 38·9 ± 2·8 | | 38·2 ± 2·0 | 20·1 ± 1·5 |
| | FG | | 55·9 ± 4·3 | | 55·9 ± 2·1 | 37·6 ± 2·4 |
| Fibre cross-sectional area ($\mu m^2$) | SO | 962 ± 86 | | | | |
| | FOG | 1691 ± 151 | 1320 ± 228 | | 1253 ± 189 | 389 ± 48 |
| | FG | | 2382 ± 359 | | 2405 ± 221 | 1141 ± 123 |
| Estimated mass of fibre type (g) | SO | 0·034 | | 0·034 | | |
| | FOG | 0·306 | 0·368 | 0·674 | 0·756 | 28·687 |
| | FG | | 0·782 | 0·782 | 1·374 | 11·603 |
| Estimated mass of fibre type as a percentage of total mass of muscle (%) | SO | 10·0 | | 2·3 | | |
| | FOG | 90·0 | 32·0 | 45·1 | 35·5 | 71·2 |
| | FG | | 68·0 | 52·6 | 64·5 | 28·8 |

SO, slow oxidative fibre; FOG, fast oxidative glycolytic fibre; FG, fast glycolytic fibre.
Body mass = 638 ± 26 g ($N = 6$).
Values are means ± S.E.

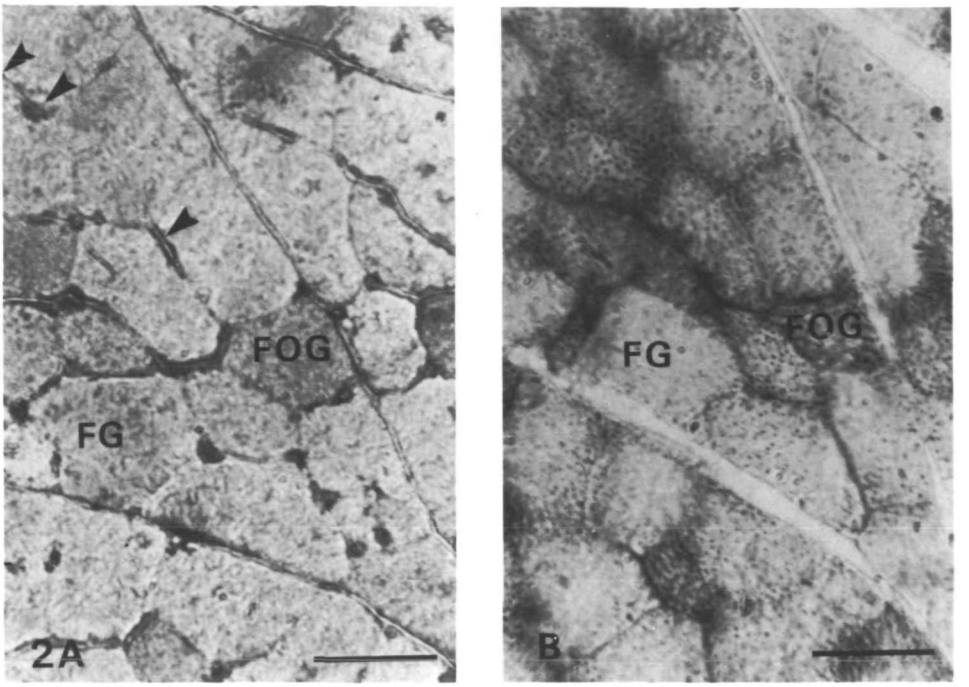


Fig. 2. Serial cross-section of muscle fibres in the semitendinosus stained (A) for mATPase after a preincubation at pH 4·1 and (B) for SDH activity. FOG represents a fast oxidative glycolytic fibre: FG represents a fast glycolytic fibre. The arrowheads in A indicate capillaries. Scale bars, 50 $\mu$m.

enzyme SDH, in comparison with FOG fibres in the red portion of the lateral gastrocnemius and semitendinosus (Figs 1B, 2B, 3B).

### *Capillarity*

The number of capillaries around a fibre in muscles varied from $4{\cdot}5 \pm 0{\cdot}1$ (semitendinosus; FOG fibre) to $5{\cdot}5 \pm 0{\cdot}4$ (pectoralis; FG fibre). The pectoralis had a higher capillary density than either of the leg muscles that were studied. Within the lateral gastrocnemius, both the red and white portions had higher capillary/fibre ratios than that in the semitendinosus, but all were significantly lower than the capillary/fibre ratio measured in the pectoralis (Table 3).

### *Ultrastructure*

The volume density of mitochondria, absolute volume of mitochondria, and the ratio of mitochondrial and myofibrillar volumes were not significantly different in the lateral gastrocnemius and semitendinosus (Table 4). The heart had the highest mitochondrial volume density, but the pectoralis had by far the largest absolute volume of mitochondria because of its larger mass. Consequently the pectoralis had the highest estimated maximal capacity for oxygen consumption (Table 4).

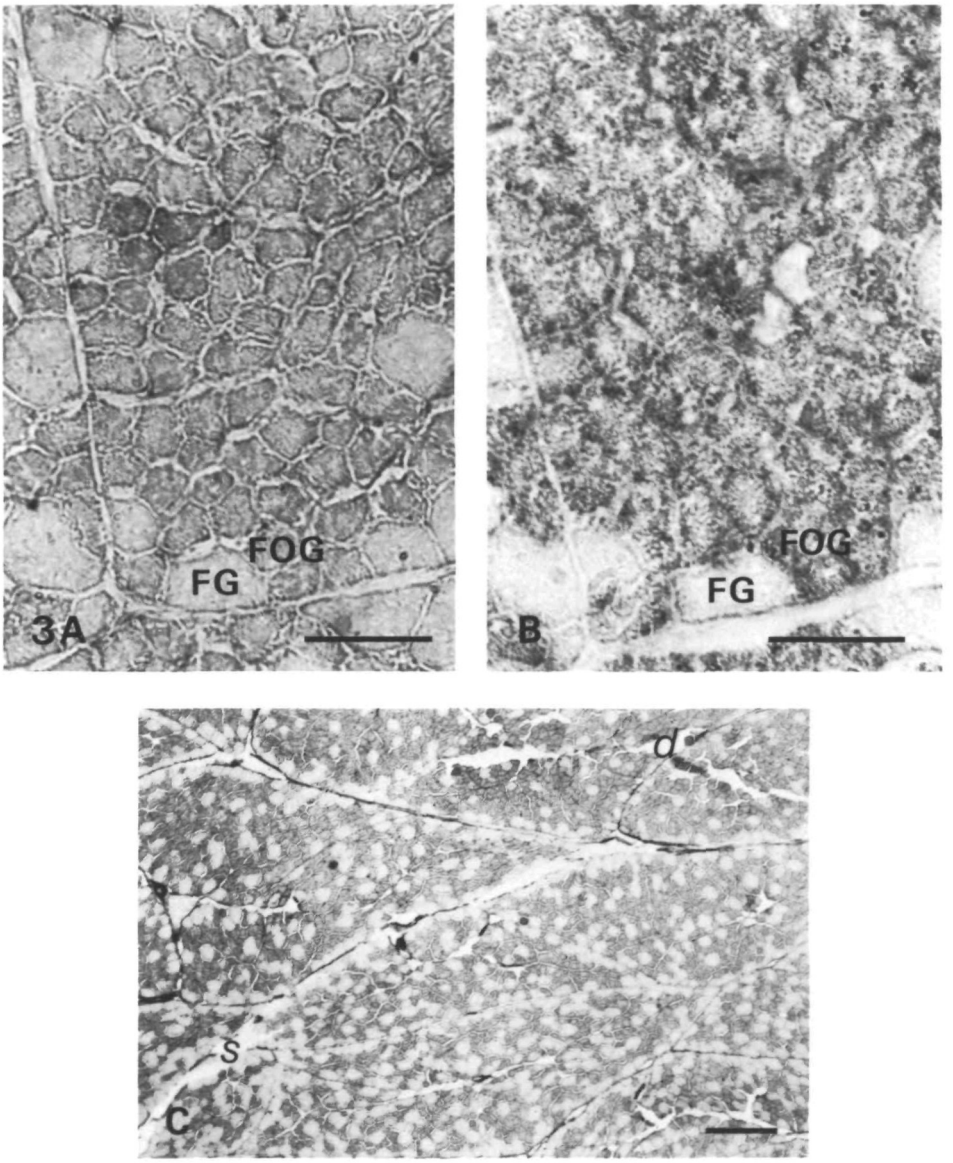


Fig. 3. Serial cross-sections of muscle fibres in the pectoral muscle, stained for mATPase after a preincubation at pH 3·9 (A,C) and for SDH activity (B). FG and FOG as in Fig. 2. *s* and *d* represent superficial and deep portions of the pectoral muscle. Scale bars 50 $\mu$m A,B; 100 $\mu$m C.

### *Enzyme activities*

There were no significant differences in any measured enzyme activities between the two leg muscles. In contrast, the pectoralis had significantly higher CS and HAD activities than both the lateral gastrocnemius and semitendinosus. The heart had similar CS and HAD activities to the pectoralis, was significantly less reliant on

Table 3. *Mean values of capillary density, the number of capillaries around a fibre and capillary/fibre ratio in various locomotor muscles from tufted ducks*

| | | Red gastrocnemius | White gastrocnemius | Semi-tendinosus | Pectoralis |
|---|---|---|---|---|---|
| Number of capillaries around a fibre | SO | 4·53 ± 0·13 | | | |
| | FOG | 4·83 ± 0·14 | 5·03 ± 0·15 | 5·50 ± 0·10 | 4·80 ± 0·20 |
| | FG | | 5·05 ± 0·19 | 5·20 ± 0·21 | 5·50 ± 0·40 |
| Capillary density ($mm^{-2}$) | | 1313 ± 211 | 895 ± 111 | 1106 ± 84 | 3361 ± 399 |
| Capillary/fibre ratio | | 1·83 ± 0·08 | 1·66 ± 0·03 | 1·46 ± 0·09 | 2·20 ± 0·11 |

Fibre types as described in Table 2.
Body mass = 638 ± 26 g ($N = 6$).
Values are means ± S.E.

Table 4. *Mean values of the ultrastructural composition of various locomotor muscles and heart of tufted ducks*

| | Whole left gastrocnemius | Left semi-tendinosus | Left pectoralis | Heart |
|---|---|---|---|---|
| Muscle volume ($cm^3$) | 1·59 ± 0·08 | 1·96 ± 0·02 | 40·89 ± 0·48 | 4·80 ± 0·36 |
| Volume density of mitochondria ($cm^3\,cm^{-3}$) | 0·085 ± 0·001 | 0·079 ± 0·012 | 0·188 ± 0·017 | 0·341 ± 0·040 |
| Volume density of myofibrils ($cm^3\,cm^{-3}$) | 0·764 ± 0·027 | 0·800 ± 0·008 | 0·690 ± 0·008 | 0·546 ± 0·036 |
| Volume of mitochondria (ml) | 0·135 ± 0·007 | 0·155 ± 0·024 | 7·696 ± 0·776 | 1·670 ± 0·308 |
| Volume of mitochondria/volume of myofibrils | 0·112 ± 0·001 | 0·100 ± 0·016 | 0·273 ± 0·025 | 0·647 ± 0·116 |
| Estimated maximal oxygen consumption of mitochondria in whole muscle ($ml\,O_2\,min^{-1}$) | 0·662 | 0·760 | 37·710 | 8·183 |

Estimated maximal oxygen consumption of a muscle was calculated assuming that maximal mitochondrial oxygen consumption = 4·9 $ml\,O_2\,min^{-1}\,ml\,mitochondria^{-1}$ (Hoppeler *et al.* 1984*b*).
Body mass = 583 ± 7 g ($N = 3$).
Values are means ± S.E.

glycolytic ATP production and had a significantly lower LDH activity and a higher CS/LDH activity ratio than the pectoralis muscle. Although there were differences in absolute activities of CS and HAD in the muscles studied, there appeared to be a relatively constant degree of reliance on fatty acid oxidation, as indicated by a relatively constant ratio of HAD/CS activities in the different muscles (Table 5).

Table 5. *Mean ± S.E. from (N) animals of the enzyme activities and paired enzyme activity ratios in various locomotor muscles and heart of tufted ducks*

| | Whole gastrocnemius | Semi-tendinosus | Pectoralis | Heart |
|---|---|---|---|---|
| Citrate synthase (CS) | 30·8 ± 6·3 | 23·8 ± 4·6 | 86·5 ± 17·0 | 108·0 ± 6·8 |
| 3 hydroxyacyl-CoA-dehydrogenase (HAD) | 9·0 ± 1·0 | 8·6 ± 1·7 | 19·6 ± 3·7 | 23·1 ± 4·2 |
| Lactate dehydrogenase (LDH) | 488 ± 68 | 505 ± 54 | 627 ± 50 | 334 ± 31 |
| HAD/CS | 0·388 ± 0·103 | 0·330 ± 0·050 | 0·323 ± 0·089 | 0·226 ± 0·055 |
| CS/LDH | 0·090 ± 0·016 | 0·062 ± 0·012 | 0·157 ± 0·042 | 0·304 ± 0·008 |
| *N* | 5 | 5 | 6 | 4 |

Activities are presented as $\mu$mol min$^{-1}$ g$^{-1}$ fresh wet mass of muscle (U g$^{-1}$).
Body mass = 638 ± 26 g (6).

The glycolytic nature of the different muscles appeared to be variable, as indicated by a variable CS/LDH activity ratio (Table 5).

### *Myoglobin content*

The highest myoglobin contents (mg g$^{-1}$; $N = 5$) were measured in the lateral gastrocnemius (7·13 ± 0·35) and heart (7·01 ± 0·69) and these values were not significantly different from each other. Significantly lower myoglobin contents were measured in the semitendinosus (4·83 ± 0·51) and the pectoralis (4·74 ± 0·59).

## DISCUSSION

The staining patterns for mATPase and SDH activities in leg muscles of the tufted duck resemble those found in other avian species (Talesara & Goldspink, 1978; Suzuki *et al.* 1985; Swatland, 1985) and many mammals (Ariano *et al.* 1973; Armstrong & Phelps, 1984). In many hindlimb muscles of birds and mammals, there is an uneven distribution of fibre types. Resting blood flow is predominantly directed to areas of muscles containing SO fibres (Laughlin & Armstrong, 1982) and the functional significance of this is thought to be the maintenance of posture (Armstrong & Laughlin, 1985). In the hindlimb of the tufted duck, however, SO fibres are scarce, which is consistent with the fact that this species spends a large proportion of its time floating on water, thus obviating the need for long-term support by 'postural' muscles.

A large proportion of the pectoral muscle mass in the tufted duck is, as in other birds (Rosser & George, 1986), made up of very small diameter FOG fibres which are highly oxidative in nature. Indeed, the pectoral muscle has activities of aerobic enzymes that match or surpass those found in the heart, which is itself regarded as an extremely oxidative muscle (Suarez *et al.* 1986). The aerobic enzymes of the pectoral

muscle in birds have activities that are amongst the highest measured in vertebrates (Marsh, 1981), and therefore, to describe the majority of fibres as simply FOG fibres understates this extremely high oxidative capacity. However, to describe the pectoral muscle fibres as simply fast oxidative fibres (see Armstrong, Ianuzzo & Kunz, 1977) would be equally misleading, because the pectoral muscle also has an LDH activity that is just as high as that in the leg muscles studied, indicating that they also have a high glycolytic capacity. So, although it is justifiable to describe the vast majority of fibres in the pectoral muscle as FOG fibres it is, nonetheless, important to emphasize their extraordinarily high oxidative capacity. This specific adaptation, which is found only in birds and bats, is clearly related to the particularly high levels of aerobic metabolism during flight (Butler, 1982).

The larger, less oxidative fibres (FG) are more numerous in the superficial area of the pectoral muscle and on the periphery of muscle fascicles. This pattern of muscle fibres, which is found in the pectoral muscle of many birds (Rosser & George, 1986) and in the muscle fascicles of humans (Sjostrom, Downham & Lexell, 1986), has an unknown functional significance. There are no SO fibres in the pectoral muscle of the tufted duck, as is the case in most flying birds (Kiessling, 1977; Talesara & Goldspink, 1978; Swatland, 1984).

The pectoral muscle of the tufted duck clearly represents a locomotory muscle with a much higher oxidative capacity than either the lateral gastrocnemius or the semitendinosus muscles. It weighs more, contains a higher proportion of oxidative fibres, has a higher mitochondrial content and has aerobic enzymes with higher activities than either of the two leg muscles studied. As a result of the greater potential oxygen demand in the pectoral muscle, the capillarity of this flight muscle is far greater than that of the leg muscles studied.

Having measured the oxidative capacity of a flight muscle and two leg muscles, it is possible to estimate the maximal oxygen consumption by muscles involved in either flying or swimming. Assuming that the pectoral muscle is representative of all the flight muscles and the chosen leg muscles are representative of all hindlimb muscles, the maximal oxygen consumption by either flight or hindlimb muscle masses can be estimated from anatomical data (see Materials and Methods section for details) (Table 6).

The measured resting and maximal oxygen consumptions during swimming in the same three ducks that were used for morphometry, were $10{\cdot}4 \pm 1{\cdot}6$ and $31{\cdot}0 \pm 0{\cdot}6\,\mathrm{ml\,O_2\,min^{-1}}$, respectively (Turner, 1986). The difference between these two values ($20{\cdot}4 \pm 1{\cdot}0\,\mathrm{ml\,O_2\,min^{-1}}$) is mainly the result of the increased aerobic metabolism of the hindlimb muscles during swimming. The discrepancy between this value and that derived from the anatomical analysis (Table 6) can be accounted for by a contribution from increased oxygen consumption of cardiac muscle when the birds were swimming at their maximum sustainable speed.

The oxygen consumption during steady-state flight in the same three ducks can be estimated from the allometric equation of Butler (1982). The difference between this and the resting value is $90{\cdot}5 \pm 2{\cdot}2\,\mathrm{ml\,O_2\,min^{-1}}$ and is mainly the result of the increased aerobic metabolism of the pectoral muscles during flight. This value is

Table 6. *The aerobic capacity of leg, flight and cardiac muscle masses of tufted ducks estimated from anatomical data (see Table 4; Hoppeler* et al. *1984*b*)*

| | Total hindlimb muscle | Total flight muscle | Total heart muscle |
|---|---|---|---|
| Muscle mass (g) | 40·1 ± 0·5 | 107·9 ± 1·3 | 5·10 ± 0·38 |
| Muscle volume ($cm^3$) | 37·8 ± 0·5 | 101·8 ± 1·4 | 4·80 ± 0·36 |
| Volume of mitochondria ($cm^3$) | 3·26 ± 0·08 | 19·33 ± 1·90 | 1·67 ± 0·31 |
| Estimated maximal oxygen consumption of muscle ($ml\,O_2\,min^{-1}$) | 16·0 ± 0·4 | 94·0 ± 9·5 | 8·2 ± 1·5 |
| Measured or calculated maximal oxygen consumption while swimming or during flight ($ml\,O_2\,min^{-1}$) | 31·0 ± 0·6 (swimming) | 101·1 ± 0·85 (flight) | |
| Maximal oxygen consumption during flight or swimming–resting oxygen consumption ($ml\,O_2\,min^{-1}$) | 20·4 ± 1·0 | 90·5 ± 2·2 | |

Also presented are estimates of oxygen consumption during steady state flight (Butler, 1982) and maximal swimming (Turner, 1986).
Body mass = 583 ± 7 g ($N = 3$).

similar to the maximum oxygen consumption of the pectoral musculature as predicted from anatomical data (Table 6).

The closeness of anatomically and physiologically determined oxygen consumptions for flying and swimming (at maximum sustainable velocity), which involve both different muscle masses and specific muscle oxidative capacities, substantiates the accuracy of the calculation for maximum mitochondrial oxygen consumption ($4{\cdot}9\,ml\,O_2\,min^{-1}\,ml^{-1}$ mitochondria) made by Hoppeler *et al.* (1984*b*). Pennycuick & Rezende (1984) derived a similar estimate for maximal oxygen consumption of mitochondria based on more mechanical considerations.

Of the mammals, only the bats can match the exercise performance of most birds (Butler, 1981) and may therefore be expected to have flight muscles similar to those of birds. The fibres in the pectoral muscle of an active bat species, like the fibres in the pectoral muscle in the tufted duck, stain intensely for aerobic enzymes and are much smaller in diameter than those of typical mammalian locomotory muscles (Armstrong *et al.* 1977). The capillary density and C/F ratio measured in pectoral muscles from a number of active bat species are similar to the very high values measured in the tufted duck and in pigeons (Rakusan, Ostadal & Wachtlova, 1971), and all are greater than in hindlimb muscles of the tufted duck, bats and other non-flying mammals (Pietschmann, Bartels & Fons, 1982). The activities of aerobic enzymes measured in the pectoral muscle of bats are also very high (Yacoe, Cummings, Myers & Creighton, 1982).

The extremely high oxygen demand of flying birds and bats must be matched by an adequate oxygen supply. The volume density of cardiac muscle mitochondria, cardiac muscle mass and, therefore, total mitochondrial volume are all higher in the tufted duck than the values predicted for a non-flying mammal of a similar mass (Hoppeler *et al.* 1984*a*). The total volume of cardiac mitochondria has not been determined for bats, but is probably similar to that in birds.

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