J. exp. Biol. **105**, 317–338 (1983)
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AQUATIC AND AERIAL RESPIRATION RATES, MUSCLE CAPILLARY SUPPLY AND MITOCHONDRIAL VOLUME DENSITY IN THE AIR-BREATHING CATFISH (*CLARIAS MOSSAMBICUS*) ACCLIMATED TO EITHER AERATED OR HYPOXIC WATER

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(*Received 9 November 1982—Accepted 31 January 1983*)

SUMMARY

Specimens of the African air-breathing catfish *Clarias mossambicus* were acclimated to either aerated (Pw_{O_2} 15·3 KPa) or hypoxic (Pw_{O_2} 2·4 KPa) water for up to 27 days at 20 °C. Routine respiration rate for fish acclimated to aerated water was 85·7 $ml O_2$ (kg bodyweight)$^{-1}$ h^{-1}. Gas exchange across the suprabranchial chambers accounted for 25 % of the total. In aerated water the interval between air-breaths varied from 1·4 to 30·6 min. On acute exposure to hypoxia air-breathing frequency was unaltered (6·3 h^{-1}) although aerial respiration rate increased by 70 %. This suggests that ventilation of the suprabranchial chambers is variable and that air-breathing frequency is a poor measure of air-breathing effort. Total respiration decreased by 46 % on acute exposure to hypoxia (Pw_{O_2} 2·4 KPa), reflecting a reduction in routine activity. Following acclimation to hypoxia, air-breathing frequency (8·1 h^{-1}) was higher and total routine respiration rate increased from 46·3 to 67·8 $ml O_2$ kg^{-1} h^{-1}. The increased oxygen consumption with hypoxia acclimation was largely the result of an increase in aquatic respiration from 10·4 to 27·5 $ml O_2$ kg^{-1} h^{-1}

Measurements were made of mitochondrial volume densities [Vv(mt,f)] and capillary supply to fast and slow myotomal muscles. The fraction of fibre volume occupied by mitochondria was 15 % for slow and 2·5 % for fast muscles. Values for [Vv(mt,f)] obtained for fish slow fibres are much higher than for homologous muscles in birds and mammals and show a good correlation with capillary density [NA(c,f)].

Hypoxia acclimation did not result in changes in either muscle Vv(mt,f) or NA(c,f). It is suggested that increased ventilation of the suprabranchial chambers and greater oxygen extraction across the gills obviates the need for modifications in these parameters.

Key words: Skeletal muscle, capillaries, hypoxia.

INTRODUCTION

The catfish (*Clarias mossambicus*) is common throughout the waters of East Africa. During the dry season stagnant pools evaporate and the oxygen tension is often greatly reduced as a result of rotting vegetation. *Clarias* is able to survive indefinitely under these conditions by obtaining oxygen from the atmosphere using special suprabranchial organs (Moussa, 1957).

A closely related catfish, *Clarias batrachus*, is found in similar habitats on the Asian sub-continent. Like *C. mossambicus* this species obtains oxygen from both the air and water (Singh & Hughes, 1971). These authors reported that in aerated water *C. batrachus* obtained 58 % of its oxygen from the air out of a total routine respiration rate of 94 $ml O_2 kg^{-1} h^{-1}$ at 25 °C (Singh & Hughes, 1971).

In common with many other fish, these catfish species show a reduction in metabolic rate when subject to acute hypoxia (Singh & Hughes, 1971). For example, the oxygen consumption of *Clarias batrachus* in water of P_{O_2} 5·4 KPa is only 26 % of that of catfish held in aerated water (Singh & Hughes, 1971). Under these conditions both species show a decrease in the interval between air-breaths and an increased reliance on aerial respiration (Moussa, 1957; Singh & Hughes, 1971).

In fish dependent entirely on aquatic respiration, O_2 consumption often rises following a period of acclimation to hypoxic conditions (Hughes, 1973; Van den Thillart, 1982). This is largely the result of an increased effectiveness of oxygen transfer across the gills (Wood & Johansen, 1972; Weber & Lykkeboe, 1978). The mechanisms involved are complex and include changes in ventilatory efficiency, haematocrit, erythrocyte pH, and in the concentrations of ligands affecting the haemoglobin-oxygen equilibrium (Wood & Johansen, 1972; Hughes, 1973; Greaney & Powers, 1978). For example, *Fundulus heteroclitus* acclimated to hypoxic water showed an 11 % increase in haematocrit and a 40 % decrease in ATP concentrations over a 12-day period (Greaney & Powers, 1978; Powers, 1980). Both these factors would result in a significant increase in the oxygen carrying capacity of the blood after hypoxic acclimation. The extent to which fish can compensate for a reduction in environmental oxygen availability varies between species and according to the severity of the hypoxia as well as with other environmental variables such as temperature (Hughes, 1973; Van den Thillart, 1982).

There have been few previous studies on the effects of hypoxia acclimation on the respiratory system of air-breathing fishes (Weber & Wood, 1979; Weber, Wood & Davis, 1979; Gee, 1980; Graham & Baird, 1982). In the present study *Clarias mossambicus* were acclimated to either aerated or hypoxic water for up to 27 days. Measurements were made of air-breathing frequencies, routine aerial and aquatic respiration rates, the volume densities of skeletal muscle mitochondria, and various indices of muscle capillary supply. The latter morphological parameters provide some information on oxygen demand and supply to the skeletal muscles.

MATERIALS AND METHODS

Fish

Specimens of *Clarias mossambicus* (Richter) of mean bodyweight 50·0 ± 5·2

(mean ±S.E. for 20 fish) were obtained from Lake Victoria, Kenya during June 1981. They were subsequently maintained for several weeks at 20 °C in tanks of fresh water at the University of Nairobi (altitude 1867 m). Prior to experiments, fish were transferred to small glass aquaria (60 × 25 × 25 cm) in groups of four. Except for the front panels, the sides of the tanks were covered with opaque plastic sheeting.

Fish with dissimilar characteristics e.g., spots and scars, appearance of fins etc., were chosen for each tank such that it was possible to identify and observe individual catfish. The water in two of the tanks was well aerated and had a mean Pw_{O_2} of 15·3 KPa. About one-third of the water was changed on alternate days. Two other tanks were not aerated, and the respiration of the fish was used to reduce the oxygen tension. Water was circulated between the tanks and passed through an activated charcoal filter (6·5 l capacity) to remove waste products and uneaten food. Gas-exchange at the surface of these tanks was reduced by pieces of polyethylene sheeting. The Pw_{O_2} of the water in the hypoxic tanks fell over 24 h until it reached a steady level of 2·4 KPa (range 1·5–3·2 KPa). Fish were acclimated for 27 days to either aerated or hypoxic water under natural daylight (12 h light: 12 h dark) and at room temperature (20 °C). The temperature in the laboratory was almost constant (±2 °C) over this period.

All fish were fed daily on tropical fish food flakes supplemented with chopped pig's liver.

Behavioural observations

The intervals between air-breaths were timed for each fish in the hypoxia tanks for periods during the 36 h following reduction of the oxygen tension to 2·4 KPa. Further measurements of air-breathing frequency were carried out after 10 and 13 days of hypoxia. Air-breathing frequencies of fish in the aerated tanks were measured on days 16 and 17.

Measurement of routine oxygen consumption

Routine oxygen consumption was measured, as described by Hughes & Singh (1971), using a closed respirometer which consisted of a water-filled chamber of 6·5 l and an air chamber of 125 ml (Fig. 1). Fish were transferred to the respirometers containing either aerated or hypoxic water at least the night before any measurements were made. During this time the fish remained undisturbed and had free access to the atmosphere. At the start of experiments the lid was placed on the respirometer such that the fish could only obtain air through a small opening (~3 cm^2) to the air-chamber (Fig. 1). Since the water and air were in direct contact the fish were able to locate the opening and breathe air normally throughout the experiment. Over the time period of the experiments the diffusion of oxygen across the opening was not significant compared with the changes caused by the respiration of the fish. Samples of air and water were collected in syringes at intervals of between 45 and 60 min. The P_{O_2} of water samples was measured using a radiometer PHM 72 Oxygen Analyser and the % O_2 content of air samples analysed with a Taylor Oxygen Meter. Measurements of respiration rates of fish from the hypoxic tanks were started after 18 days of acclimation to reduced oxygen and continued until day 27 when all the fish were killed. The initial Pw_{O_2} values in the respirometers were adjusted by bubbling either

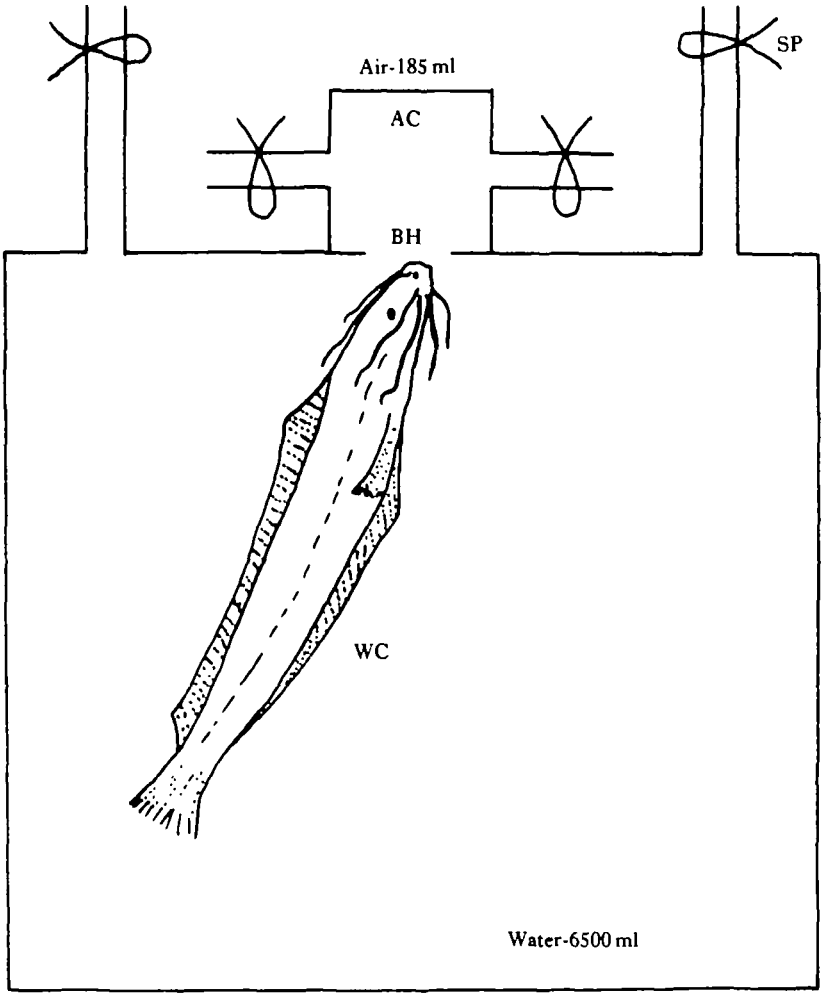

Fig. 1. Closed respirometer used for measuring routine respiration rates. AC, air-chamber 185 ml; WC, water chamber 6·5 l; SP, sampling ports for air and water; BH, 2 cm radius hole between the air and water chambers.

O_2 or N_2 to values around 1·4 KPa above that in the acclimation tanks. At least two experiments were carried out on each fish.

Opercular frequencies were counted for periods of 2–5 min at intervals throughout the experiment.

Two experiments were also carried out in a flow-through respirometer (20 cm long ×8 cm ×8 cm) in which the fish had no access to the air. Aerated water was continuously circulated (80–100 ml min^{-1}) through the box and inflow and outflow Pw_{O_2} measured at 30 min intervals for 6–8 h.

All respiration experiments were carried out at room temperature (20 °C).

Tissue glycogen concentrations

Fish were stunned and killed by pithing. Prior to the sampling of muscle fibres for electron microscopy the posterior 1/5 of the trunk muscle and the liver were exci[redacted]

[illegible]d dropped into liquid nitrogen. Subsequently, 50–100 mg samples of fast and slow muscle fibres were dissected from the partially thawed (−20 °C) tail segment. Frozen samples of liver and muscle were boiled in 30 % KOH for 15 min and glycogen precipitated with ethanol. Precipitated glycogen was collected by centrifugation and hydrolysed to glucose by boiling for 2 h in 2N-H_2SO_4. Glucose was determined spectrophotometrically in neutralized aliquots using the glucose-oxidase method (Sigma Technical Bulletin No. 510, Sigma, Poole, Dorset).

Histochemical methods

The types of muscle fibre present in the myotomal muscle of *Clarias* were determined by staining serial frozen sections for glycogen, succinic dehydrogenase activity (SDHase), and myofibrillar ATPase (m.ATPase).

Samples of muscle (~5 mm diameter) were mounted on chilled cryostat chucks in an inert embedding medium (OCT Compound, Lamb, London) and frozen in isopentane (2-methylbutane) cooled to near its freezing point (−150 °C) in liquid nitrogen. Frozen sections (8–10 μm thick) were cut from blocks equilibrated to −20 °C and mounted on dry coverslips. All incubations were performed at room temperature (20 °C). Following incubation, sections were mounted on glass slides using glycerin jelly.

Sections stained for glycogen were treated with 1 % aqueous periodic acid for 15 min and then with Schiff's reagent for 20 min as described by Pearse (1960).

Succinic dehydrogenase activity was localized by incubating sections for 30 min in 80 mM-sodium succinate, 50 mM-phosphate buffer, pH 7·4 with 1 mg ml^{-1} nitroblue tetrazolium (NBT) as the electron acceptor. Succinate was omitted from controls.

Sections were stained for myofibrillar ATPase activity following alkaline preincubation at pH 10·4 (Johnston, Patterson, Ward & Goldspink, 1974). Preincubation was for 2–20 min in a medium of 18 mM-$CaCl_2$, 50 mM-2-amino-2-methyl-1-propanol, pH 10·4 (221 buffer, Sigma, Poole, Dorset). Sections were subsequently incubated in pH 9·2 buffer, containing 20 mM-KCl, 8 mM-$CaCl_2$, 30 mM-ATP, 80 mM-2-amino-2 methyl-1-propanol for 15 min, washed in water and successively treated with 3 % cobalt chloride (3 min), washed in water again and finally stained with 1 % ammonium sulphide solution. Controls were performed in which ATP was omitted from the incubation medium.

Muscle endplates and pre-terminal axons were localized by staining fibres for acetylcholinesterase activity. Strips of muscle (2 mm^2×20 mm long) were freshly dissected and pinned onto strips of cork at their resting lengths *in situ*. Samples were fixed at 4 °C for 8 h in 10 % neutral formaline in 0·1 N-sodium acetate buffer pH 5·2. Strips were subsequently washed in water and incubated for 8 h in a freshly prepared solution containing 2·5 % $CuSO_4.5H_2O$ (0·2 ml); 3·7 % glycine (0·2 ml), 0·2 N-sodium acetate buffer, (pH 5·2) and the clear supernatant from 15 mg acetylcholine iodide, 0·3 ml of 2·5 % copper sulphate and 0·7 ml water. The strips of muscle were subsequently washed in water and cleared in glycerol. Fibre bundles were teased out from the strips under a binocular microscope and mounted on glass slides.

Histochemical studies were carried out on a single 53 g specimen of *Clarias* [illegible]*ssambicus* kindly supplied by the Institute of Aquaculture, University of Stirling.

Analyses of muscle capillary supply

Fixation of muscle samples

Small strips (3 mm^2 × 20 mm long) of superficial slow muscle were dissected from the myotomes immediately posterior to the dorsal fin in the regions of the lateral line nerve. Fast muscle strips of similar size were excised from the underlying muscle. Care was taken to exclude the fibres on the boundary between the slow and deep fast muscle zones (see below) as these represent a distinct fibre type. Strips were pinned to cork board at their resting lengths *in situ* and immersed in 3% gluteraldehyde 0·12 M-phosphate buffer, pH 7·2 at 20 °C. Initial fixation was for 3–5 h. Subsequently, small fibre bundles (100–150 slow fibres and 20–50 fast fibres) were dissected from the superficial layers of the strips and left overnight in a fresh change of the same fixative. Fibre bundles were washed in 0·12 M-phosphate buffer pH 7·2 (at 20 °C), post-fixed for 1 h in 1% osmium tetroxide, 0·12 M-phosphate buffer, dehydrated in a series of alcohols up to 100%, cleared in epoxypropane, and embedded in Araldite resin CY 212 (EM Scope, Trent, England).

Tissue blocks were returned to St. Andrews, Scotland, where semi-thin (0·5–1 μm) and ultrathin (0·06–0·09 μm) sections were cut on an OM U2 Reichert Ultramicrotome. Ultrathin sections were mounted on Formvar-coated 150 mesh copper grids, double stained with uranyl acetate and lead citrate, and viewed with a Phillips 301 electron microscope.

Morphometric techniques

Blocks were made from eight catfish acclimated to aerated water (Pw_{O_2} 15·3 KPa) and eight catfish acclimated to hypoxia (Pw_{O_2} 2·4 KPa). For each fibre type (i.e. slow or fast) six transversely-orientated blocks were prepared from each of the fish. Sections were cut from four blocks/fish each containing 40–90 slow fibres or 15–30 fast fibres. Low power electron micrographs (×910) were projected (×2·4) using a photographic enlarger and the outlines of fibres and capillaries traced onto white cartridge paper (Fig. 4C).

Fibre cross-sectional areas and perimeters, capillary cross-sectional areas and perimeters, and the lengths of contacts between capillaries and fibres were determined directly by digital planimetry using a Summagraphics digitizer inter-faced with an Olivetti P6060 mini-computer. In addition the numbers of capillaries/fibres were counted. From the following measured parameters:

A. Number of fibres analysed,
B. Percentage of fibres without direct capillary contact,
C. Number of capillaries in direct contact with fibres analysed,
D. Fibre cross-sectional area (μm^2),
E. Fibre circumference (μm),
F. Volume density of mitochondria (see below),
G. Average number of capillaries in contact with each fibre,
H. Length of contacts between capillaries and fibres (μm),
a(c) mean cross-sectional area of capillaries (μm^2),
b(c) mean circumference of capillaries (μm),

The following parameters were derived:

I. Percentage of fibre circumference in direct contact with capillaries (H/E × 100).

J. Capillary circumference (μm) per μm^2 of fibre cross-sectional area (H/D).

K. 'Area' of capillary wall supplying 1 μm^3 of mitochondria (J/F).

NA(c,f) Number of capillaries per unit volume of muscle fibres (mm^{-2}) [C/A × D(mm^2)].

Determination of volume density of mitochondria [Vv(mt,f)]

Negatives of micrographs (×2000–3400) of whole fibres were projected using a photographic enlarger so that the image overlaid a square test grid. In the case of fast muscle it was often necessary to photograph 2–3 overlapping fields in order to reconstruct photomicrographs of whole fibres. The grid was composed of discrete line segments (12 mm) with endpoints arranged in a regular square lattice (Weibel, 1980). Magnification (×1·6–2·6) was such that test lines corresponded to a distance of 2·3 μm on the image which is equivalent to >1 and <1·5 times the mean diameter of mitochondria (Weibel, 1980). The volume density of mitochondria [Vv(mt,f)] was determined by counting the number of intersections of test points (Pi) with mitochondria

$$Vv = Pi/Pt$$

where Pt is the total number of point falling on the fibre. The area outside a line drawn around the myofibrils was considered to represent the subsarcolemmal zone and mitochondria were scored as falling either in the subsarcolemmal or intermyofibrilar zones. Each grid consisted of 150–200 test points depending on the size of the fibre. The point-counting method used was found to give >95 % agreement with determinations of Vv using the digital planimeter described above. A total of 20 negatives were analysed for each fibre type selected at random from those prepared for the capillary counts (see above).

Statistical analyses

Measured parameters for fish acclimated to either aerated or hypoxic water were compared using a two-way analysis of variance.

RESULTS

Respiration of Clarias *in aerated and hypoxic water*

Clarias is an obligate air-breather in aerated water (Pw_{O_2} 15·3 KPa). The survival time of two fish denied access to air in flow-through respirometers was measured. The first fish of 25·6 g bodyweight survived 52 h with an inlet Pw_{O_2} of around 16 KPa. Shortly after transferring the fish to the respirometer the amplitude of ventilatory movements increased markedly and opercular frequency increased from 24 to 82 min^{-1}. The average respiration rate of this fish was 44·9 $ml O_2\, kg^{-1}\, h^{-1}$. A second fish of bodyweight 48·6 g survived 24 h. Average ventilatory frequency of this fish was also 82 min^{-1} and respiration rate 76·6 $ml O_2\, kg^{-1}\, h^{-1}$.

In the fish left undisturbed with free access to the atmosphere the interval between air-breaths was found to be quite varied even for the same individual (Fig. 2). On exposure to acute hypoxia (first 36 h) the mean air-breathing frequency of 6·1 h^{-1} was not significantly different from that of fish acclimated to aerated water (Table 1). However, following 11–13 days acclimation to hypoxia (Pw_{O_2} 2·4 KPa) both the range of intervals between breaths and air-breathing frequency (8·1 h^{-1}) decreased significantly (Table 1, Fig. 3).

Results for routine respiration rates in closed respirometers are given in Table 2.

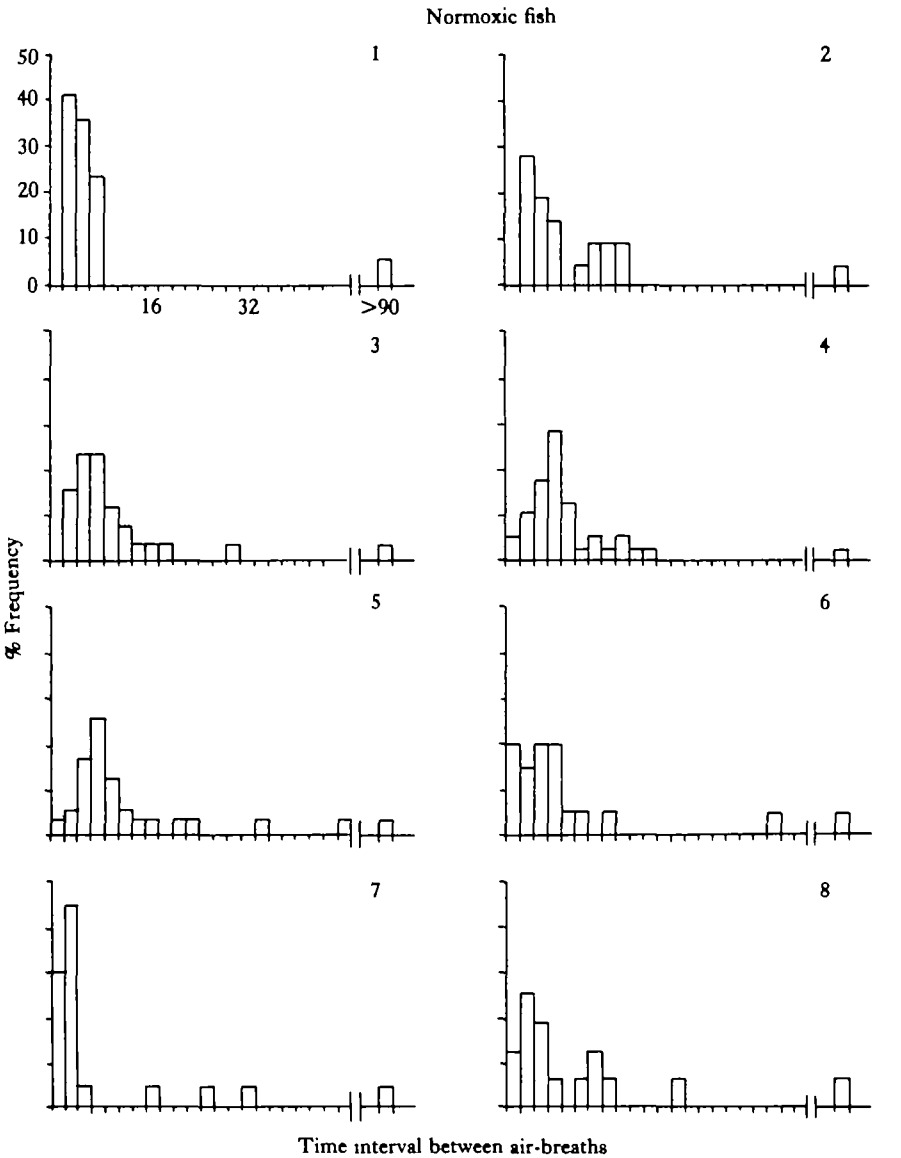

Fig. 2. Frequency histograms of the intervals between air-breaths for eight catfish acclimated to aerated water (Pw_{O_2} 15·3 KPa). Fish were observed for a total of 3·3 h. Each bar represents a 2-min time interval.

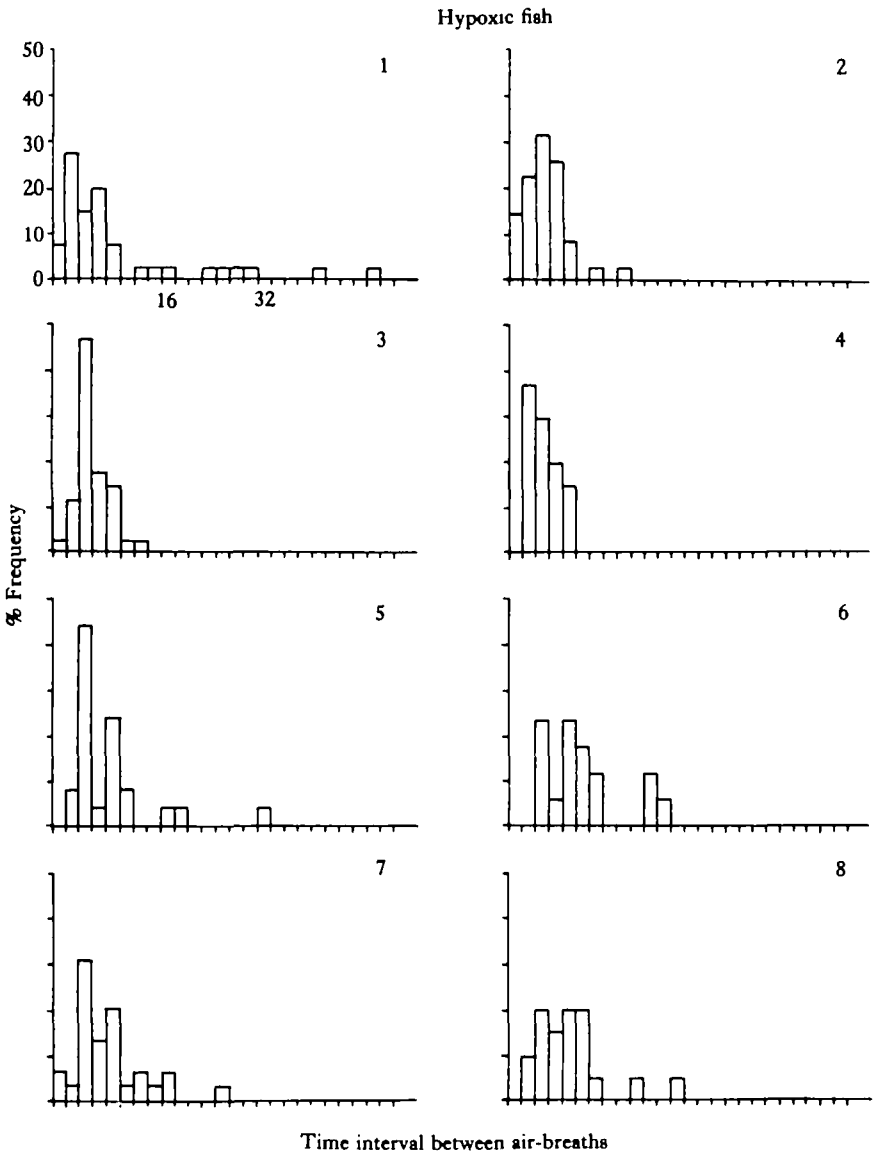

Fig. 3. Frequency histograms of the intervals between air-breaths for fish acclimated to hypoxic water (Pw_{O_2} 2·4 KPa). Fish were observed for a total of 3·7 h. Each bar represents a 2-min time interval.

Air-breathing accounted for 24·6 % of total oxygen consumption in aerated water and 77·5 % following acute hypoxia (Table 2). Total respiration in fish acclimated to 15·3 KPa fell by 54 % when exposed to 2·4 KPa largely as a result of an 84 % decrease in aquatic respiration rate ($P < 0{\cdot}001$; Table 2).

Total respiration rate was 22 % higher in fish acclimated to hypoxia than in fish subject to acute hypoxia (Table 2) ($P < 0{\cdot}05$). This was largely the result of a 164 % increase in aquatic respiration from 10·4 to 27·5 $mlO_2\,kg^{-1}\,h^{-1}$ ($P < 0{\cdot}01$).

The ventilatory movements of fish exposed to acute hypoxia were of similar

Table 1. *Air-breathing frequency of African catfish* Clarias mossambicus

Condition	No. of fish	Hours observation	Interval between air-breaths (Mean ± S.E.) (min)	Range
Fish acclimated to aerated water (P_{O_2} 15·1 KPa)	8	3·3	9·5 ± 0·8	1·4–30·6
Initial response (< 36 h) to hypoxic water (P_{O_2} 2·4 KPa)	8	5·6	9·8 ± 1·3	1·3–32·6
Fish acclimated to hypoxia (P_{O_2} 2·4 KPa) for 11–13 days	8	3·7	7·4 ± 0·8	2·3–18·4

Table 2. *Routine respiration rates of* Clarias mossambicus *acclimated for 27 days to either aerated (P_{O_2} 15·3 KPa) or hypoxic water (P_{O_2} 2·4 KPa) at 20 °C*

P_{O_2} of test water (KPa)	No. of fish	Aquatic respiration ($mlO_2\,kg^{-1}\,h^{-1}$)	Aerial respiration ($mlO_2\,kg^{-1}\,h^{-1}$)	Total respiration ($mlO_2\,kg^{-1}\,h^{-1}$)	% Air breathing
Fish acclimated to aerated water (P_{O_2} 15·3 KPa)					
15·1	7	64·6 ± 6·5	21·1 ± 1·9	85·7 ± 6·8	24·6 ± 1·9
3·5–2·0	7	10·4 ± 1·4	35·9 ± 4·0	46·3 ± 4·9	77·5 ± 2·4
Fish acclimated to hypoxic water (P_{O_2} 2·4 KPa)					
3·5–2·0	8	27·5 ± 6·3	40·3 ± 5·3	67·8 ± 7·3	59·4 ± 5·3

Values represent mean ± S.E.

frequency (25·4 ± 2·1 min^{-1}) but substantially greater amplitude than those of fish in aerated water. On acclimation to hypoxia, there were increases in both opercular frequency (to 33·3 ± 2·2 min^{-1}) and amplitude.

Muscle fibre types

Three main types of fibre can be distinguished in the myotomal muscle of *Clarias mossambicus* on the basis of staining for myofibrillar ATPase activity (Fig. 4). Following preincubation at pH 10·4, the most easily inactivated fibres (6 min) are the red fibres which form a superficial wedge of muscle adjacent to the lateral line nerve. These fibres have a complex distributed innervation with around 10–15 endplates per fibre (Fig. 4B). Histochemically they are characterized by a relatively high staining intensity for glycogen and SDHase activity. Following 12 min incubation at pH 10·4 the m.ATPase of the white fibres is inactivated. These somewhat larger diameter fibres form the bulk (>80 %) of the myotomal muscle. White fibres show a relatively weak staining reaction for glycogen and SDHase activity (Fig. 4A). They have a type of innervation which is distinct from that of red fibres. Each white fibre receives a single 'en-plaque' type endplate at one myoseptal end consisting of numerous button-like vesicles (Fig. 4B).

The remaining fibres situated between the red and white muscle layers lose their

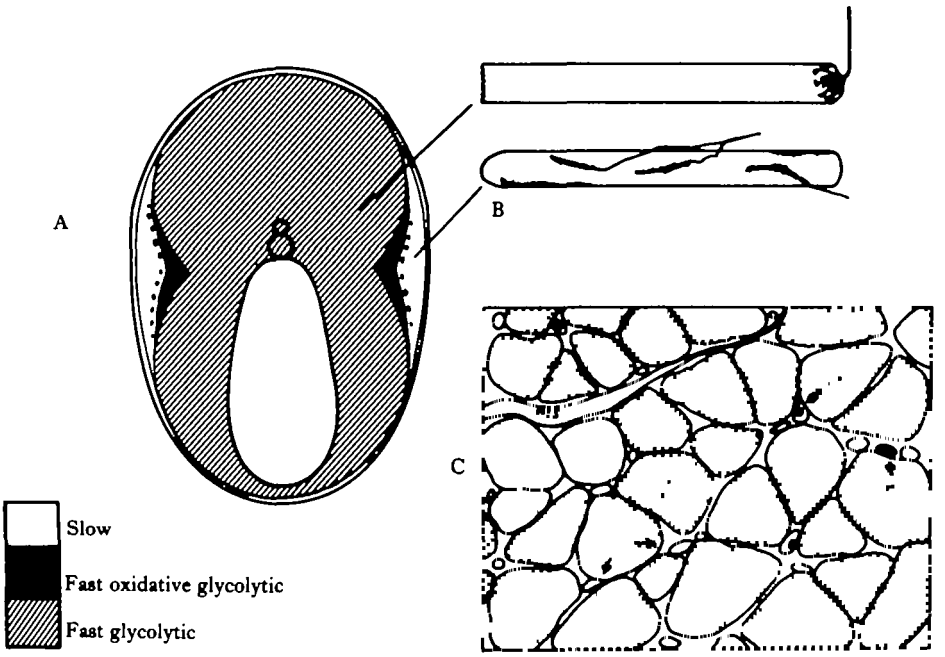

Fig. 4. Diagram illustrating (A) the distribution of different fibre types and (B) the type of innervation to the myotomal muscles of *Clarias mossambicus* L. (C) Shows part of a projected tracing of fibres and capillaries used for the capillary analyses. *ms*, myoseptum; *c*, capillary; *f*, fibre; *cr*, capillary containing red cell; *n*, nerve ending.

m.ATPase activity after 15 min preincubation at pH 10·4. A few fibres with an alkali-stable m.ATPase are also found scattered amongst the adjacent red fibres (Fig. 4A). This intermediate zone of muscle consists of a wide range of fibre sizes with a staining for glycogen and SDHase activity which is slightly less than that of red fibres. These three classes of fibre correspond closely to those observed in some other fish e.g., carp and cod (see Bone, 1978; Johnston, 1981). The maximum speed contraction of white myotomal muscle fibres is around 2–3 times that of red fibres (Altringham & Johnston, 1981*a*; Johnston, 1982*a*). In carp, white and intermediate fibres have a similar myosin light chain sub-unit composition (Johnston, Davison & Goldspink, 1977). It seems likely that red, intermediate and white fibres in *Clarias* correspond to the slow, fast oxidative glycolytic and fast glycolytic fibre types described for other species (see Bone, 1978; Johnston, 1981, 1982*a*; van Raamsdonk, *et al.* 1982 for further discussion of muscle fibre types in fish).

Glycogen concentrations

Tissue glycogen concentrations [μmol glucose equivalents (g wet wt tissue)$^{-1}$] for *Clarias* acclimated to aerated and hypoxic water are shown in Table 3. Hepatic glycogen concentrations in normoxic fish were around three and five times higher than those in slow and fast myotomal muscles respectively. On acclimation of the fish to hypoxia, glycogen concentrations increased 78% in slow muscle and 76% in fast muscle fibres ($P < 0{\cdot}05$) (Table 3).

Table 3. *Glycogen concentrations [mg · (100 g wt muscle)$^{-1}$] in tissues of* Clarias mossambicus *acclimated to either aerated (P_{O_2} 15·3 KPa) (7 fish) or hypoxic (P_{O_2} 2·4 KPa) water (8 fish)*

Tissue	Acclimation condition: Aerated water	Acclimation condition: Hypoxic water
Slow muscle	*65·2 ± 7·6*	*116·0 ± 12·9*
Fast muscle	*35·0 ± 3·2*	*61·7 ± 8·8*
Liver	*179·2 ± 63·6*	*154·6 ± 15·5*

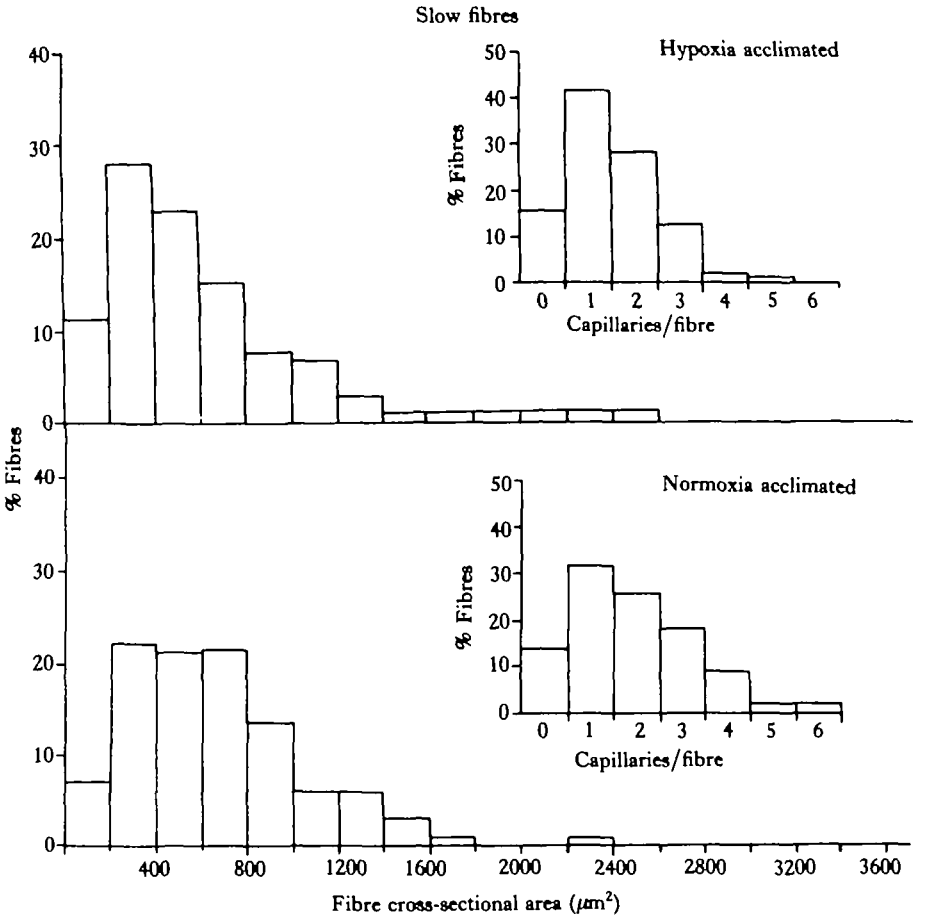

Fig. 5. Frequency histograms of the distribution of fibre size in slow muscle of *Clarias* acclimated to either aerated or hypoxic water for 27 days. 162 fibres were analysed at random from eight fish acclimated to aerated water and 158 fibres from eight fish acclimated to hypoxic water. The insets show frequency distribution of the number of capillaries in direct contact with muscle fibres.

Muscle mitochondrial volume density and capillary supply

The distribution of fibre size in fast and slow muscle fibre populations is shown in Figs 5 and 6. Acclimation to hypoxia resulted in a 36% decrease in the mean cross-sectional area of fast muscle fibres ($P < 0·05$) (Table 5).

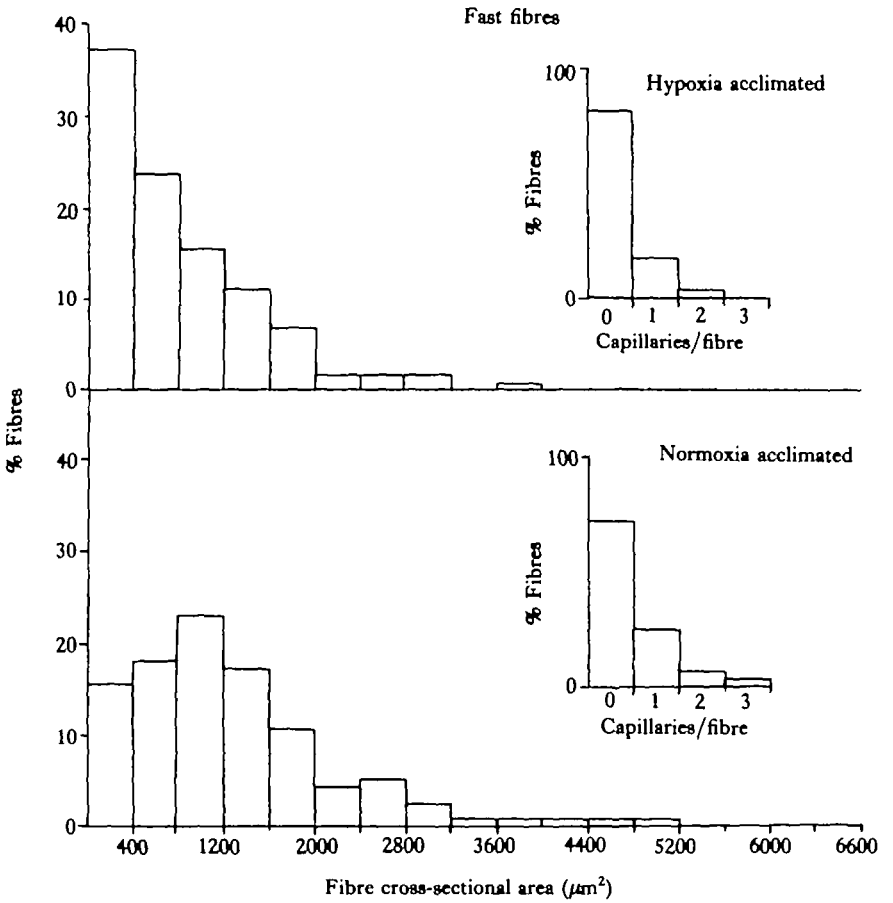

Fig. 6. Frequency histograms of the distribution of fibre size in fast glycolytic muscle of *Clarias* acclimated to either aerated or hypoxic water for 27 days. 122 fibres were analysed at random from eight fish acclimated to aerated water and 118 fibres from eight fish acclimated to hypoxic water. The insets show frequency distributions of the number of capillaries in direct contact with muscle fibres.

In fish acclimated to aerated water, the number of capillaries per mm^2 of fibre cross-sectional area [NA(c,f)] was 10 times greater for slow than fast muscle (Tables 4, 5). Hypoxia acclimation did not result in significant changes in the various indices of capillary supply investigated (Tables 4, 5). Frequency histograms of the number of capillaries per muscle fibre for fish acclimated to aerated and hypoxic water are shown as insets to Figs 5 and 6.

Total mitochondrial volume densities were 0·16 for slow and 0·025 for fast muscle fibres. These values were similar for fish acclimated to either aerated or hypoxic water (Table 6). Almost all the mitochondria were found to be situated within the sub-sarcolemmal zone (Table 6).

DISCUSSION

Capillary supply to the swimming muscles

Many factors other than capillary density affect tissue oxygenation: these include

Table 4. *Capillary supply to slow myotomal muscle fibres of African catfish* (Clarias mossambicus) *acclimated to either aerated (P_{O_2} 15·1 KPa) or hypoxic water (P_{O_2} 2·4 KPa)*

	Symbol	Acclimation condition: Aerated water	Acclimation condition: Hypoxic water
No. of fibres analysed	A	162	158
Percentage of fibres without direct capillary contact	B	13·6	8·9
No. of capillaries associated with fibres analysed	C	174	151
Fibre cross-sectional area (μm^2)	D	660 ± 30	581 ± 33
Fibre circumference (μm)	E	106 ± 3	96 ± 3
Volume density of mitochondria	F	0·16 ± 0·01	0·19 ± 0·01
Average number of capillaries touching each fibre	G	1·9 ± 0·1	1·4 ± 0·1
Fibre circumference in direct contact with capillaries (μm)	H_1*	14·3 ± 0·8	11·6 ± 0·7
	H_2†	12·4 ± 0·8	9·6 ± 0·7
Percentage of fibre circumference in direct contact with capillaries	I_1	13·3 ± 0·7	12·2 ± 0·7
	I_2	11·5 ± 0·7	10·2 ± 0·7
Capillary circumference (μm) per μm^2 of fibre cross-sectional area (H/D)	J_1	0·021	0·019
	J_2	0·019	0·018
Area of capillary wall supplying 1 μm^3 of mitochondria (J/F)	K_1	0·13	0·10
	K_2	0·12	0·09
Cross-sectional area of capillaries (μm^2)	a(c)	20·8 ± 1·5	18·3 ± 1·1
Circumference of capillaries (μm)	b(c)	18·7 ± 0·7	17·7 ± 0·8
No. of capillaries per cross sectional area of muscle fibres (mm^{-2})	NA(c,f)	1633	1657

* 1. Mean values calculated for vascularized fibres only.
† 2. Mean values for all fibres.

mitochondrial respiration rates, myoglobin concentrations, blood flow, perfusion distribution and various factors influencing the haemoglobin-oxygen equilibrium such as blood P_{CO_2} and ATP concentrations (see Prosser, 1973; Weber, 1983). There is, however, a good correlation between capillary density [NA(c,f)] and the volume density of mitochondria [Vv(mt,f)] for the slow muscle fibres of various shallow water fish (Fig. 7). This would suggest that the surface area of the capillary network available for gas-exchange is a limiting factor in determining the size of the mitochondrial compartment and hence aerobic capacity. A similar, though more scattered, relationship between NA(c,f) and Vv(mt,f) has been demonstrated for the muscles of African mammals, which contain a mixture of different fibre types (Hoppeler *et al.* 1981*a*,*b*).

Values of Vv(mt,f) for fish slow fibres are generally 3–10 times higher than for mammalian muscles containing primarily slow or fast oxidative glycolytic motor units (Eisenberg, Kuda & Peter, 1974; Hoppeler *et al.* 1981*a*,*b*). This may reflect the relatively high proportion of cardiac output that fish slow muscle fibres receive during sustained swimming. Daxboeck, Randall & Jones (1983) have studied the redistribution of blood flow to tissues of the rainbow trout using ^{141}Cerium ar

Table 5. *Capillary supply to fast myotomal muscle fibres of African catfish* (Clarias mossambicus) *acclimated to either aerated (P_o, 15·1 KPa) or hypoxic water (P_o, 2·4 KPa)*

	Symbol	Acclimation condition Aerated water	Hypoxic water
No. of fibres analysed	A	122	118
Percentage of fibres without direct capillary contact	B	72·2	80·5
No. of capillaries associated with fibres analysed	C	29	15
Fibre cross-sectional area (μm^2)	D	1265 ± 83	809 ± 63
Fibre circumference (μm)	E	149 ± 6	114 ± 4
Volume density of mitochondria	F	0·025 ± 0·002	0·024 ± 0·002
Average number of capillaries touching each fibre	G	0·3 ± 0·05	0·2 ± 0·04
Fibre circumference in direct contact with capillaries (μm)	H_1*	6·99 ± 0·31	5·89 ± 0·22
	H_2†	1·94 ± 0·33	1·14 ± 0·23
Percentage of fibre perimeter in direct contact with capillaries (H/E × 100)	I_1	5·0 ± 0·3	5·7 ± 0·2
	I_2	1·39 ± 0·25	1·11 ± 0·25
Capillary circumference per μm^2 of fibre cross-sectional area (H/D)	J_1	0·0055	0·0073
	J_2	0·0015	0·0014
Area of capillary wall supplying 1 μm^3 of mitochondria (J/F)	K_1	0·22	0·30
	K_2	0·061	0·059
Cross-sectional area of capillaries (μm^2)	a(c)	20·3 ± 2·5	19·7 ± 2·1
Circumference of capillaries (μm)	b(c)	18·7 ± 1·0	18·5 ± 0·9
No. of capillaries per unit cross-sectional area of muscle fibres (mm^{-2})	NA(c,f)	189	136

* 1. Mean values calculated for vascularized fibres only.
† 2. Mean values for all fibres.

Table 6. *Distribution of mitochondria in the slow and fast muscle fibres of African catfish* (Clarias mossambicus) *acclimated to either aerated or hypoxic water*

	Slow muscle		Fast muscle	
	Acclimation condition			
	Aerated water	Hypoxic water	Aerated water	Hypoxic water
Sub-sarcolemmal zone	0·12 ± 0·01	0·14 ± 0·02	0·023 ± 0·002	0·023 ± 0·001
Intermyofibrillar	0·03 ± 0·003	0·05 ± 0·005	0·002 ± 0·0007	0·0016 ± 0·0011

Mean values of mitochondrial volume density, Vv(mt,f) ± S.E. See text for a description of the sampling protocol and numbers of fish used.

^{95}Niobium-labelled microspheres. They found that at 80% of maximum oxygen uptake slow muscle receives around half of the cardiac output, although it only constitutes 5–7% of the body weight (Daxboeck *et al.* 1983).

Capillary supply to fast muscle shows considerable variation between species and

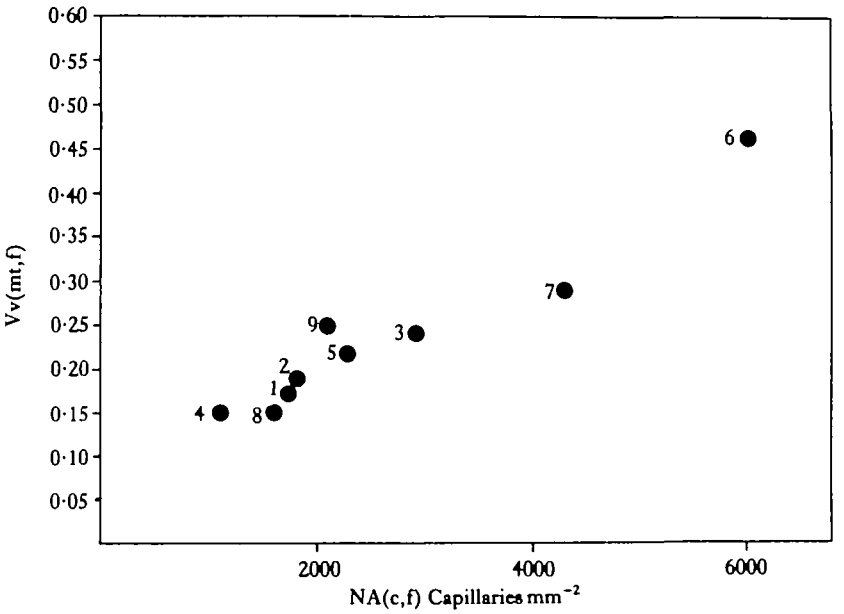

Fig. 7. Relationship between mitochondrial volume density [Vv(mt,f)] and the number of capillaries per mm^2 of muscle fibre cross-sectional area [NA(c,f)] for fish slow fibres. (1) Catfish (*Clarias mossambicus*) acclimated to aerated water (this study); (2) Catfish (*Clarias mossambicus*) acclimated to hypoxic water (this study); (3) Tench (*Tinca tinca*) acclimated to aerated water Pw_{O_2} 17·6 KPa at 15 °C for 6 weeks (Johnston & Bernard, 1982*a*,*b*); (4) Tench (*Tinca tinca*) acclimated to hypoxic water (Pw_{O_2} 1·5 KPa at 15 °C for 6 weeks (Johnston & Bernard, 1982*a*,*b*); (5) Elver (*Anguilla anguilla*) (Egginton & Johnston, 1982*a*,*b*); (6) European anchovy (*Engraulis encrasicolus*), (Johnston, 1982*c*); (7) Silver Dollar (*Myleus rosevetti*) acclimated to 24 °C in aerated water (J. Salamonski & I. A. Johnston, unpublished results); (8) Crucian carp (*Carassius carassius*) acclimated to aerated water Pw_{O_2} 17·6 KPa at 15 °C (Johnston & Bernard, 1983); (9) Crucian carp acclimated to hypoxic water at 15 °C Pw_{O_2} 1.5 KPa for 6 weeks (Johnston & Bernard, 1983*b*).

appears to be correlated with the type of motor innervation and pattern of fibre recruitment (Table 7). In general, mitochondrial volume densities and capillary densities are 2–10 times higher in multiply than focally innervated fast fibres (Table 7). Electromyographical studies of dogfish (*Scyliorhinus canicula* L.) and Pacific herring (*Clupea harengus pallesi*) have shown that focally innervated fast fibres are reserved for high intensity effort of short duration (Bone, 1966; Bone, Kiceniuk & Jones, 1978). The energy for contraction during burst swimming comes from the degradation of phosphorylcreatine and from anaerobic glycogenolysis (see Johnston, 1981). In the short term, provision of ATP is not dependent on the capillary circulation. It might be predicted that, following activity, muscles with very low capillary densities would require an extended recovery period in order to remove lactate and replenish muscle glycogen stores.

In contrast, electromyograms can be recorded from the muscles of fish with multiply innervated fast muscles at sustainable as well as burst swimming speeds (Hudson, 1973; Johnston *et al.* 1977; Bone, 1978; Johnston & Moon, 1980*a*,*b*). The higher capillary densities found for multiply innervated fast muscles may reflect both a diversification of the fuels used to support contraction (Johnston & Moon, 1981) and a capacity for a more rapid rate of recovery following activity.

Table 7. *Relationship between the pattern of fast muscle innervation and capillary density [NA(c,f)] and mitochondrial volume density [Vv(mt,f)] for different species*

Species (bodyweight or length if given)	Mean fibre cross-sectional area (μm2)	NA(c,f)	Vv(mt,f)	Type of innervation	Reference
ELASMOBRANCHS					
Chimaera montrosa	11 150	8	0·003	Focal	Totland, Kryvi, Bone & Flood (1981)
Etmopterus spinax	7000	3	0·005	Focal	Totland, Kryvi, Bone & Flood (1981)
Galeus melastomus (Rafinesque)	12 200	11	0·01	Focal	Totland, Kryvi, Bone & Flood (1981)
Scyliorhinus canicula	15 100	111	0·01	Focal	Totland, Kryvi, Bone & Flood (1981)
CHONDROSTEANS					
Acipenser stellatus (Pallas) (25–40 cm)	340	91	0·007	Focal	Kryvi, Flood & Guljaev (1980)
TELEOSTEANS					
Clarias mossambicus (Richter) (50 g)	1265	188	0·025	Focal	this study
Tinca tinca (36 g)	838	893	0·045	Multiple	Johnston & Bernard (1982*a*)
Carassius carassius					
acclimated to 28 °C	1258	1113	0·016	Multiple	Johnston (1982*b*)
acclimated to 2 °C	1528	1898	0·061	Multiple	Johnston (1982*b*)

Effects of hypoxia acclimation

Our results on the effects of acute exposure to hypoxia on survival time, air-breathing frequency and respiration rate largely confirm those of Moussa (1957) and Singh & Hughes, (1971) on the African and Asian species of *Clarias* respectively.

Clarias mossambicus is entirely dependent for survival on its capacity to breath atmospheric oxygen. In his more extensive study of survival times, Moussa (1957) observed that the importance of aquatic respiration declined with increasing body size. For example, mean survival time in anoxic water was 45 h for 32·5 cm fish compared with only 15 h for 10·5 cm fish. The greater dependence of immature fish on aquatic respiration was found to be correlated with the development and maturation of the suprabranchial respiratory organs (Moussa, 1957). The individual variability in the intervals between air-breaths (Table 2, Figs 2, 3) suggests that the ventilation of the suprabranchial chambers is also variable. This is supported by the observation that air-breathing frequency was unchanged on acute exposure to hypoxia whereas aerial respiration rate was significantly increased (Tables 1, 2). The intervals between air-breaths therefore provides a relatively poor measure of air-breathing effort.

Species which are relatively tolerant of anoxia and which have well-developed anaerobic capacities usually have liver glycogen concentrations in excess of 500 μmol (g wet wt tissue)$^{-1}$ (Van den Thillart, Kesbeke & Waard, 1976; Smith & Heath, 1980). For example, Van den Thillart *et al.* (1976) found that goldfish were able to survive 12 h anoxia at 20 °C during which time liver glycogen concentrations decreased from 746 to 357 μmol (g wet wt)$^{-1}$. The relatively low concentrations of hepatic and slow muscle glycogen stores in *Clarias* are consistent with only a limited capacity for sustained anaerobiosis. The evolution of accessory air-breathing organs in this species may, therefore, be viewed as an alternative rather than a complimentary strategy to anaerobic metabolism as a means of surviving in oxygen deficient water.

The level of acute hypoxia which results in a shortfall in oxygen delivery to the tissues varies widely between species and with temperature (Hughes, 1973). In some species acclimation to hypoxia results in a compensatory increase in oxygen consumption (Wood & Johansen, 1972; Hughes, 1973; Van den Thillart, 1982). The mechanisms involved are complex and include adjustments in ventilatory efficiency, haematocrit, erythrocyte pH, and in the concentrations of ligands such as ATP and GTP which affect the oxygen-affinity of haemoglobin (Hughes, 1973; Weber & Lykkeboe, 1978; Weber, 1983).

There have been few previous studies of hypoxia acclimation in air-breathing fishes (Gee, 1980; Weber *et al.* 1979; Graham & Baird, 1982). Graham & Baird (1982) measured air-breathing frequencies in two species of armoured loricariid catfishes (*Ancistrus chagresi* and *Hypostomus plecostomus*) both of which utilize their stomachs as accessory air-breathing organs. They found that acclimation to water P_{O_2} of 2·0–5·4 KPa for 14–21 days reduced air-breathing frequency but did not alter the threshold P_{WO_2} for air-breathing (Graham & Baird, 1982). Weber *et al.* (1979) have provided evidence that hypoxia acclimation increases O_2 affinity and reduces the magnitude of the Bohr effect in *Hypostomus* blood. The increase in O_2 affinity of blood from fish acclimated to hypoxic water was correlated with a decrease in bo

GTP (48 to 1·5 mmol l^{-1} cells) and ATP concentrations (3·1 to 2·4 mmol l^{-1} cells) (Weber *et al.* 1979). Lowered nucleoside triphosphate concentrations are thought to reduce blood O_2 affinity directly through reduced allosteric interaction and indirectly through a decreased erythrocyte pH resulting from an altered Donnan distribution of protons across the red cell membrane (Wood & Johansen, 1973; Weber & Wood, 1979). These results suggest that, even though *Hypostomus* can breathe air, the oxygen delivery to its tissues during hypoxia was sufficiently reduced so as to promote some adjustment in erythyrocyte NTP concentrations (Weber *et al.* 1979; Weber & Wood, 1979).

Aquatic respiration rate of *Clarias mossambicus* was reduced markedly on acute exposure to P_{O_2} of 2·4 KPa but increased significantly following a period of acclimation to around 43 % of the value for fish kept in aerated water (Table 2). Enhanced oxygen extraction during hypoxia acclimation could result from an increased blood O_2 carrying capacity, and/or circulatory and ventilatory responses that favour O_2 loading at the gills and/or O_2 unloading in the tissues. Observations on the amplitude and frequency of ventilatory movements suggest that the increased oxygen extraction from the water at P_{O_2} 2·4 KPa may be due in part to adjustments in gill perfusion and irrigation. Lomholt & Johansen (1979) obtained a somewhat different result for carp acclimated to hypoxia. In this species, increased oxygen extraction rates occurred despite lower gill ventilation rates and were correlated with a reduction in red cell NTP concentrations. It is not known whether adjustments in cofactor concentrations are important in increasing oxygen extraction in hypoxia-acclimated *Clarias*. However, it is of interest that Fish (1956) reported that the oxygen-dissociation curve of *Clarias* haemoglobin was much steeper and relatively insensitive to carbon dioxide compared to that of other East African fish, such as *Lates* and *Tilapia*, which are limited to open water. This latter property of *Clarias* haemoglobin is likely to be important in view of the accumulation of CO_2 in the air surrounding the suprabranchial organ during intervals between air-breaths.

Maximal rates of oxygen consumption during activity exceed those at rest by around 10 times (Bennett, 1978). Under these conditions, oxygen uptake largely reflects the oxygen demand of mitochondria in the exercising muscles. Factors such as temperature and/or environmental P_{O_2} that affect maximal rates of oxygen consumption have been shown to produce changes in both mitochondrial volume density and capillary supply to the swimming muscles (Johnston & Maitland, 1980; Johnston, 1982*b*; Johnston & Bernard, 1982*a*,*b*, 1983).

The way in which muscle capillary supply is affected by chronic hypoxia varies between species according to the behavioural, respiratory and cardiovascular adjustments made to lowered $P_{W_{O_2}}$ (Fig. 8). For example, slow muscle mitochondrial volume densities increase in the crucian carp, and decrease in tench following acclimation to hypoxia (Fig. 8). The lack of significant change in these parameters in hypoxia-acclimated *Clarias* suggests that maximal oxygen demands can be met by a combination of increased ventilation of the suprabranchial chamber and adjustments to the efficiency of oxygen extraction at the gills.

Fibre cross-sectional area was found to decrease in fast muscle following hypoxia acclimation (Table 5, Fig. 6). Fibre size is thought to be controlled in part by muscle [illegible]sion (Lomo, 1976). Training regimes which involve isometric exercises, e.g.

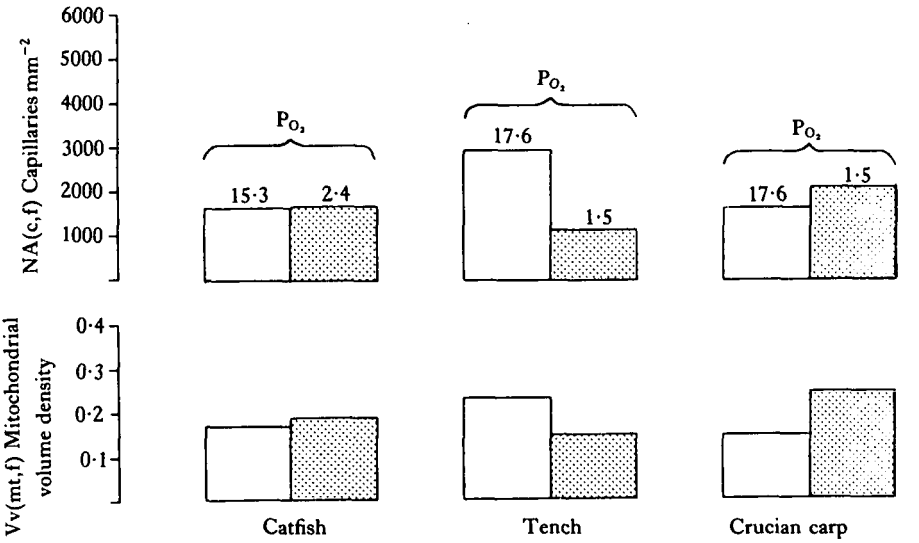

Fig. 8. Histograms showing the effects of acclimation to chronic hypoxia on slow muscle mitochondrial volume density [Vv(mt,f)] and capillary density [NA(c,f)] for three species of freshwater fish. The figures above each bar represent the Pw_{O_2} at which the fish were acclimated. The data on carp and tench are taken from Johnston & Bernard (1982*a*,*b*) and Johnston & Bernard (1983).

weight-lifting in man, tend to result in muscle fibre hypertrophy. Alexander (1969) has presented evidence that fast fibres in teleost myomeres need only contract by 2–3 % of their resting lengths to produce large bending movements of the trunk. This may explain why enforced swimming is such a powerful stimulus for muscle fibre hypertrophy in fish (Greer-Walker, 1971; Johnston & Moon, 1980*a*,*b*). Although hypoxia-acclimated *Clarias* show an increase in air-breathing frequency relative to fish from oxygenated water, their general spontaneous locomotory activity was somewhat reduced. A reduction in swimming activity would not only reduce routine oxygen requirements but might also provide a mechanism for decreasing fibre cross-sectional area.

I.A.J. and L.M.B. are grateful to the authorities of the University of Nairobi for the facilities to carry out the experimental part of this work in Kenya. The support of travel grants from the Browne Fund of the Royal Society (IAJ) and Carnegie Trust (LMB) and project grant (GR3/4084) from the Natural Environment Research Council (IAJ) is gratefully acknowledged.

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