J. exp. Biol. **133**, 415–428 (1987)
Printed in Great Britain

RESPIRATORY CHARACTERISTICS OF MUSCLE FIBRES IN A FISH (*CHAENOCEPHALUS ACERATUS*) THAT LACKS HAEM PIGMENTS

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Accepted 15 June 1987

SUMMARY

1. Oxygen consumption, mitochondrial content and enzyme activities were determined in identified muscle fibre types of the 'haemoglobin-less' icefish *Chaenocephalus aceratus* Lönnberg.

2. Small bundles (2–12) of fast and slow fibres were isolated from the myotomal and superficial pectoral fin abductor muscles, respectively. At 0°C the time to 50% peak force and the half-relaxation time of isometric twitches were, respectively, 18 ± 1 and 38 ± 4 ms for fast and 43 ± 3 and 119 ± 21 ms for slow muscle fibres (mean ± S.E.).

3. Measurements of enzyme activities in homogenates suggest that phosphocreatine hydrolysis and oxidative phosphorylation are the main energy-supplying pathways in fast and slow muscles, respectively. Activities of glycolytic enzymes were relatively modest and showed no consistent differences between fibre types.

4. The relationship between oxygen consumption and mitochondria in slow muscle was also determined for a 'red-blooded' antarctic (*Notothenia gibberifrons*), a cold-temperate (*Myoxocephalus scorpius*) and a warm-temperate (*Oreochromis niloticus*) fish. Volume densities of mitochondria were as follows (mean ± S.D.): *C. aceratus*, 0·50 ± 0·08; *N. gibberifrons*, 0·30 ± 0·10; *M. scorpius*, 0·23 ± 0·05; and *O. niloticus*, 0·20 ± 0·05. ADP-stimulated respiration rates were measured in isolated fibre segments. In spite of their different mitochondrial contents, slow fibres from the two antarctic fish utilized pyruvate and palmitoyl-1-carnitine at similar rates (1·0–1·2 μmol O_2 g^{-1} wet mass min^{-1} at 0°C). This suggests that the high density of mitochondria in icefish muscle is related, in part, to diffusion limitations.

INTRODUCTION

The antarctic environment is characterized by extremely low temperatures and the highly seasonal nature of primary productivity. The relative importance of these factors in determining growth rates, metabolic characteristics and the activity patterns of antarctic fish is largely unknown (see Clarke, 1983). In terms of numbers of species (more than 100) and biomass, the sub-order Nototheniiformes (order Perciformes) dominates the fish fauna of Antarctica (Andriashev, 1965). They are thought to have diverged from a demersal perciform ancestor some 20 million years

Key words: antarctic fish, skeletal muscle, mitochondria, respiration, contractile properties.

ago following the establishment of the circum-antarctic current (Kennett, 1977). A long period of isolated evolutionary development and the special characteristics of the antarctic environment have given rise to a number of unique forms (Andriashev, 1965; DeVries & Eastman, 1981). For example, there is a general trend towards reduced haematocrits and haemoglobin concentrations in polar fishes (Scholander & Van Dam, 1957; Everson & Ralph, 1968). This reaches an extreme in the icefishes (family Channichthyidae) in which the number of circulating erythrocytes is reduced to very low levels (Hureau, Petit, Fine & Marneux, 1977) and the genes for haemoglobin and myoglobin are suppressed (Ruud, 1954; Walesby, Nicol & Johnston, 1982; Douglas, Peterson, Gysi & Chapman, 1985). As a result, the blood of *Chaenocephalus aceratus* (Channichthyidae) has an oxygen-carrying capacity of only 0·67 vol%, compared with 6·5 vol% for typical 'red-blooded' antarctic species such as *Notothenia gibberifrons* and 20 vol% for many temperate fish (Holeton, 1970). Factors which are thought to compensate for the absence of respiratory pigments in icefish include a high resting cardiac output and the maintenance of favourably high capillary tissue P_{O_2} gradients (Holeton, 1970; Hemmingsen & Douglas, 1977). Icefishes also have somewhat larger blood volumes (approx. 9 %) and lower systemic blood pressures than other teleosts (Twelves, 1972; Hemmingsen & Douglas, 1977).

The aim of the present study was to investigate the energy supply and contractile properties of swimming muscles in the demersal icefish *Chaenocephalus aceratus*. The respiratory characteristics of muscle fibres in channichthyids are likely to be influenced both by low temperature and by the lack of respiratory pigments. In an attempt to differentiate between these factors, the respiration rates of isolated muscle fibres were also determined in *N. gibberifrons* and two temperate species.

MATERIALS AND METHODS

Fish

Icefish (*Chaenocephalus aceratus*), 21 fish, 1·4 ± 0·3 kg body mass, 52·8 ± 3·5 cm standard length and *Notothenia gibberifrons* Lönnberg, four fish, 459 ± 47 g body mass, 31·1 ± 1·2 cm standard length, were obtained from the South Orkney Islands (60°43′S, 45°36′W) during the summer, December 1985 to January 1986. Fish were caught by trammel nets at a depth of 140–200 m (water temperature −0·7°C) and maintained without food for 24–48 h in recirculated seawater aquaria at 0–2°C. Bullrouts (*Myoxocephalus scorpius* L.) were caught around the Fife coast (east Scotland) and held at 10–12°C for up to 48 h prior to experiments. The tilapia (*Oreochromis niloticus* Trewavas) used in this study were raised from brood stock by the Institute of Aquaculture, University of Stirling and kept at 25–28°C.

Determination of contractile properties

Only *C. aceratus* were used for these experiments. Slow and fast fibres were sampled from the superficial pectoral fin abductor muscle and trunk muscle. Samples of trunk muscle were taken from ventral myotomes numbers 5–10 (counting from

the head). Dissection was carried out initially in a cold-room (+4°C) and subsequently on a cooled dissection stage (0°C) illuminated with a cold-light source. Small bundles of either 2–5 fast or 5–12 slow muscle fibres were isolated in Ringer's solution containing (in mmol l^{-1}): NaCl, 142·2; KCl, 2·6; $CaCl_2$, 2·7; $MgCl_2$, 1·0; $NaHCO_3$, 18·5; Na_2PO_4, 3·2; glucose, 60·8; pH 7·5 at 0°C (after Hudson, 1968). Muscle fibre tendons were clamped with aluminium foil tags and attached between two stainless steel hooks in a Perspex chamber through which Ringer's solution was circulated at 0–2·5°C. Fibres were activated by broad-field stimulation using silver electrodes (1 ms pulses) and force was measured with a silicon beam strain gauge mounted on a micromanipulator (Akers 801, AME, Horton, Norway; sensitivity, 0·45 mN V^{-1}; compliance, <0·4 μm mN^{-1}). The initial length of fibre bundles was set to give a maximal twitch, and diameter was measured at three places with a binocular microscope. Provided 5 min was allowed between each stimulus, preparations showed little decrement in tension over several hours.

Determination of enzyme activities

Samples of fast and slow muscle (0·5 g) were isolated from *C. aceratus* as described above, minced with scissors, and homogenized in ground-glass homogenizers with ice-cold extraction buffer (1:10 w/v) containing (in mmol l^{-1}): Tris-HCl, 100; EDTA, 1; dithioerythritol (DTE), 0·5; pH 7·4. DTE was omitted for determinations of enzyme activities requiring 5′,5-dithiobis-2-nitrobenzoic acid (DTNB) in the reaction medium (citrate synthase and carnitine palmitoyl transferase). All enzymes were assayed at 0°C. Substrate concentrations and pH were set to yield optimal activities. Enzyme activities were measured by following changes in absorbance at 340 nm for adenylate kinase, creatine phosphokinase, pyruvate kinase and lactate dehydrogenase, at 412 nm for citrate synthase and at 550 nm for cytochrome oxidase. Concentrations were calculated on the basis of millimolar extinction coefficients of 6·22 (for NADH, NADPH at 340 nm) and 13·6 (for reduced DTNB at 412 nm). The extinction coefficient of reduced relative to oxidized cytochrome *c* was empirically determined on a routine basis. Assay conditions were as follows.

Creatine phosphokinase (CPK; EC 2.7.3.2) (in mmol l^{-1}): imidazole, 75; $MgCl_2$, 5; KCl, 100; glucose, 20; ADP, 2; AMP, 10; NAD^+, 0·4; phosphocreatine (PCr), 35; excess hexokinase and glucose-6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*), pH 7·5. The reaction was initiated with PCr.

Adenylate kinase (AK; EC 2.7.4.3) (in mmol l^{-1}): triethanolamine buffer, 100; phosphoenolpyruvate (PEP), 0·5; NADH, 0·2; KCl, 10; $MgCl_2$, 4; AMP, 2; ATP, 2; excess lactate dehydrogenase and pyruvate kinase, pH 7·6.

Pyruvate kinase (PK; EC 2.7.1.40) (in mmol l^{-1}): imidazole, 50; KCN, 1; KCl, 150; $MgSO_4$, 10; NADH, 0·15; ADP, 5; phosphoenolpyruvate, 2·5; excess lactate dehydrogenase, pH 7·2. The reaction was initiated with PEP.

Lactate dehydrogenase (LDH; EC 1.1.1.27) (in mmol l^{-1}): imidazole, 50; KCN, 2·5; NADH, 0·15; pyruvate, 10; pH 7·5.

Citrate synthase (CS; EC 4.1.3.7) (in mmol l^{-1}): Tris, 75; oxaloacetate, 0·5; DTNB, 0·25; acetyl CoA, 0·4; pH 8·0.

Cytochrome oxidase (CO; EC 1.9.3.1) (in mmol l^{-1}): K_2HPO_4/KH_2PO_4, 10; 0·065 % reduced cytochrome *c*, pH 7·3 (see Hansen & Sidell, 1983 for recording conditions and substrate preparation).

Electron microscopy

Small bundles of the slow fibres isolated for oxygen consumption measurements (see below) were pinned *via* their tendonous insertions to cork strips and immersion-fixed for 24 h in 3 % glutaraldehyde, 0·15 mol l^{-1} phosphate buffer, pH 7·4 (at 4 °C). Samples were subsequently post-fixed in phosphate-buffered 1 % osmium tetroxide, washed in distilled water, dehydrated in acetone and embedded in Araldite CY212 resin (EMscope, Ashford, England). For each muscle type, 20 blocks were prepared per fish and five selected at random for sectioning. Ultrathin sections (60 nm) were cut on a Reichert OM U2 ultramicrotome and mounted on 150-mesh pyroxyline-coated copper grids. All sections were cut transverse to the longitudinal axis of the fibres. Sections were stained with uranyl acetate and Reynolds lead citrate and viewed with a Phillips 301 transmission electron microscope at 60 kV. Volume densities (Vv) of mitochondria (mt), myofibrils (my) and lipid droplets (l) were measured using a point-counting method (Weibel, 1980). Quarter-plate negatives (magnification 1500–4500) of four whole fibres selected at random from each block sectioned were projected onto a 1 cm square-lattice counting grid at various magnifications (2·5–8·5 times), such that the grid spacing was >1 and <1·5 times the average dimensions of the organelle being measured. The volume density of muscle fibre organelles was determined by point counting the intercepts on a 200-point test grid (Weibel, 1980).

Oxygen uptake of isolated muscle fibres

Slow fibres were isolated from the superficial pectoral fin abductor muscle of *C. aceratus* and *N. gibberifrons* and the myotomal muscle of *M. scorpius* and *O. niloticus*. Bundles of fibres were transferred to a cooled dissection dish containing silicon oil (MS 550, BDH, Poole, England). Single fibres or small bundles (2–3 fibres) were isolated, cut into approximately 0·5 mm lengths and transferred to a solution containing (in mmol l^{-1}): sucrose, 25; mannitol, 75; KCl, 100; piperazine-*N*,*N*′-bis(2-ethanesulphonic acid), 20; K_2HPO_4, 10; ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*′,*N*′-tetra acetic acid, 1; 0·1 % (w/v) bovine serum albumin, pH 7·5 at 0 °C. Oxygen uptake was measured in a stirred, water-jacketed glass chamber with a Rank Bros oxygen electrode (Bottisham, Cambridge, England). The following substrates were investigated; 10 mmol l^{-1} pyruvate + 10 mmol l^{-1} malate, 10 mmol l^{-1} glutamate + 10 mmol l^{-1} malate, 40 μmol l^{-1} palmitoyl-1-carnitine + 1 mmol l^{-1} malate, 10 mmol l^{-1} α-ketoglutarate, 15 mmol l^{-1} α-glycerophosphate. Steady-state respiration rates were measured in a final volume of 1 ml following sequential additions of substrate and ADP (0·8 mmol l^{-1}) (dissolved in incubation medium) using a 20 μl Hamilton syringe. The oxygen content of air-saturated

incubation medium was determined at each temperature studied using the Winkler method (Fox & Wingfield, 1938). At the end of each experiment the fibre segments were transferred to a clean vial and dried to constant weight at 60°C. Water content was determined in a similar manner using larger pieces of tissue.

Statistical analyses

Results from different muscle types and species were compared using a one-way analysis of variance.

RESULTS

Contractile properties

Red and white muscle regions are composed of slow and fast twitch fibres, respectively (Fig. 1; Table 1). The isometric contractile properties of both muscle fibre types are summarized in Table 1. Maximum tetanic tension and the rates of force development and relaxation were significantly higher in fast than in slow muscle fibres ($P < 0{\cdot}01$; Fig. 1; Table 1). The rate of force development during isometric contractions was found to be independent of stimulation frequency for both fibre types (Fig. 1). Values for maximum (tetanic) tension were similar to those

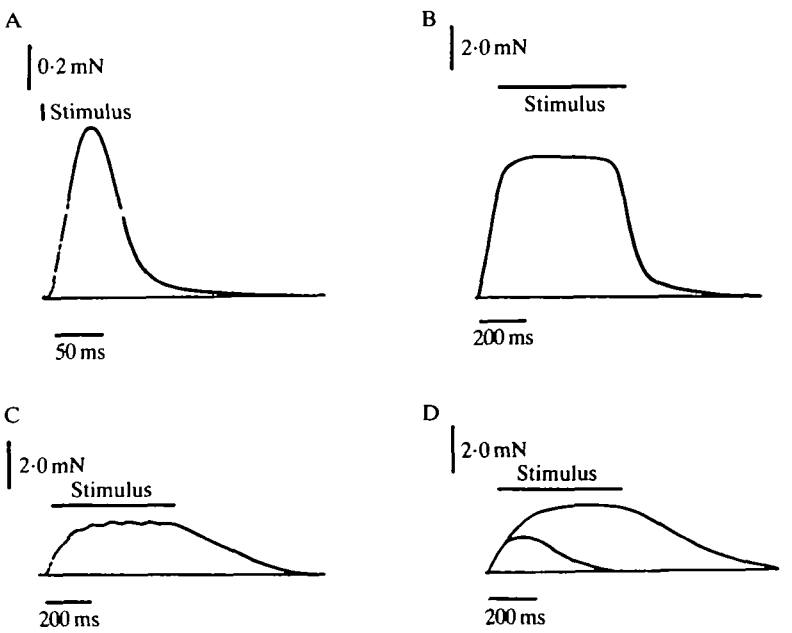

Fig. 1. Records illustrating the contractile properties of fast and slow muscle fibre types in *Chaenocephalus aceratus*. (A) Isometric twitch of a bundle of three fast fibres isolated from anterior myotomes. (B) Short tetanus of fibres shown in A stimulated at 80 Hz. (C) Isometric tetanus of a small bundle of slow fibres isolated from the superficial pectoral fin abductor muscle. The stimulation frequency was 8 Hz which is close to that required to give a fused tetanus. (D) 20 Hz tetanus and superimposed twitch for the same fibres shown in C. All experiments were carried out at 0–2·5°C.

Table 1. *Contractile properties of live fibre bundles isolated from the swimming muscles of the demersal icefish,* Chaenocephalus aceratus

Parameter (mean ± S.E.)	Units	Muscle type Slow	Fast
Twitch		$N = 7$	$N = 9$
$t_{1/2}$ tension development	ms	45·8 ± 2·7	18·1 ± 1·3**
$t_{1/2}$ relaxation	ms	119·2 ± 21·1	37·7 ± 4·4**
Contraction time	ms	354 ± 39	123 ± 13**
Tetanus		$N = 5$	$N = 7$
Fusion frequency	Hz	8–10	25
Stimulation frequency for maximum tension (P_0)	Hz	20	80
$t_{1/2}$ tension development	ms	119 ± 19	54 ± 6**
$t_{1/2}$ relaxation	ms	248 ± 43	101 ± 13**
P_0	$kN\,m^{-2}$	60·4 ± 6·9	189·0 ± 21·4**
dP/dt	$kN\,m^{-2}s^{-1}$	272 ± 47	1804 ± 260**

** Significantly different at the $P < 0{\cdot}01$ level.
All experiments were carried out at 0–2·5 °C.

reported for fully activated demembranated muscle fibres in this species (Johnston & Harrison, 1985). Fused tetani were produced at stimulation frequencies of 8–10 Hz for slow fibres and 25 Hz for fast fibres (Fig. 1; Table 1). Fast muscle fibres gave their maximum tension response at 80 Hz and were observed to fatigue rapidly at higher stimulation frequencies.

Enzyme activities

The fast muscle of icefish is characterized by high activities of immediate energy supply enzymes (CPK, AK) and low activities of aerobic enzymes (CS, CO) relative to those in slow muscle (Table 2). The activities of glycolytic enzymes are relatively

Table 2. *Activities (μmol substrate utilized g^{-1} wet mass min^{-1}) of enzymes of energy metabolism in fast and slow muscles from the demersal icefish,* Chaenocephalus aceratus

Enzyme	Slow muscle fibres	Fast muscle fibres
Immediate energy supply		
Creatine phosphokinase	112 ± 24	825 ± 56**
Adenylate kinase	28·0 ± 6·7	71·6 ± 10·7**
Glycolysis		
Pyruvate kinase	29·2 ± 4·8	10·2 ± 1·8**
Lactate dehydrogenase	69·5 ± 7·5	115·0 ± 18·3
Aerobic metabolism		
Citrate synthase	16·8 ± 1·0	0·39 ± 0·11**
Cytochrome oxidase	24·6 ± 4·2	0·53 ± 0·16**

** Significantly different at the $P < 0{\cdot}01$ level.
Values represent mean ± S.E. of determinations from six fish.
All assays were performed at 0 °C.

modest and show no consistent differences between fibre types (Table 2). On the basis of these results it would appear that phosphocreatine hydrolysis and aerobic metabolism are the main energy-supplying pathways in fast and slow muscles, respectively.

Ultrastructural characteristics

Table 3 shows comparative data on the volume densities (Vv) of organelles in slow fibres from two antarctic, a temperate and a tropical fish species (see Materials and Methods for a description of muscles sampled). The highest density of mitochondria (mt) (50%) was found in fibres from the icefish (Fig. 2; Table 3). This value was 67% higher than for homologous fibres in *Notothenia gibberifrons* ($P < 0{\cdot}01$) (Fig. 2; Table 3). The myofibrils in *C. aceratus* were highly irregular in cross-section and occurred in isolated clumps surrounded by mitochondria (Fig. 2). The subsarcolemmal mitochondrial compartment was particularly well-developed in icefish fibres (Fig. 2). Icefish fibres also contained the lowest volume density of myofibrils (my) such that the ratio $Vv_{(mt,f)}/Vv_{(my,f)}$ increased in the ratio 1·4: 0·6: 0·4: 0·3 for *C. aceratus*, *N. gibberifrons*, *M. scorpius* and *O. niloticus*, respectively (Table 3).

Oxygen consumption of isolated muscle fibres

The rate of oxygen utilization by isolated fibre segments increased on addition of substrates and ADP but was largely inhibited by oligomycin (5 $\mu g\,ml^{-1}$) (an inhibitor of the mitochondrial F1 ATPase) (Fig. 3). Respiration rate was increased by the addition of uncoupling reagents e.g. 0·4 $\mu mol\,l^{-1}$ carbonyl cyanide *m*-chlorophenyl hydrozone (FCCP) (Fig. 3). Oxygen consumption rates were found to be unaffected by the order of addition of substrate or ADP (Fig. 3). It was not possible to determine respiratory control ratios since once ADP had been added it was regenerated within the preparation. This suggests that endogenous ADP and substrates diffuse out of the segments during the preparative procedure (see Materials and Methods). The rate of oxygen utilization varied with the substrate used and decreased in the order pyruvate + malate = palmitoyl-1-carnitine > glutamate = α-ketoglutarate > α-glycerophosphate (Table 4). Similar respiration rates per gram wet mass of muscle were found for slow fibres from the red-blooded antarctic species *N. gibberifrons* (Table 4). Since slow fibres from this species

Table 3. *Volume densities (Vv) of mitochondria (mt), myofibrils (my) and lipid droplets (l) in slow muscle fibres*

Species	$Vv_{mt,f}$	$Vv_{my,f}$	$Vv_{l,f}$
Chaenocephalus aceratus	0·50 ± 0·082	0·35 ± 0·085	0·020 ± 0·012
Notothenia gibberifrons	0·30 ± 0·097	0·51 ± 0·11	0·13 ± 0·022
Myoxocephalus scorpius	0·23 ± 0·054	0·65 ± 0·086	—
Oreochromis niloticus	0·20 ± 0·052	0·68 ± 0·068	—

Values represent mean ± S.D. of 20 fibres.

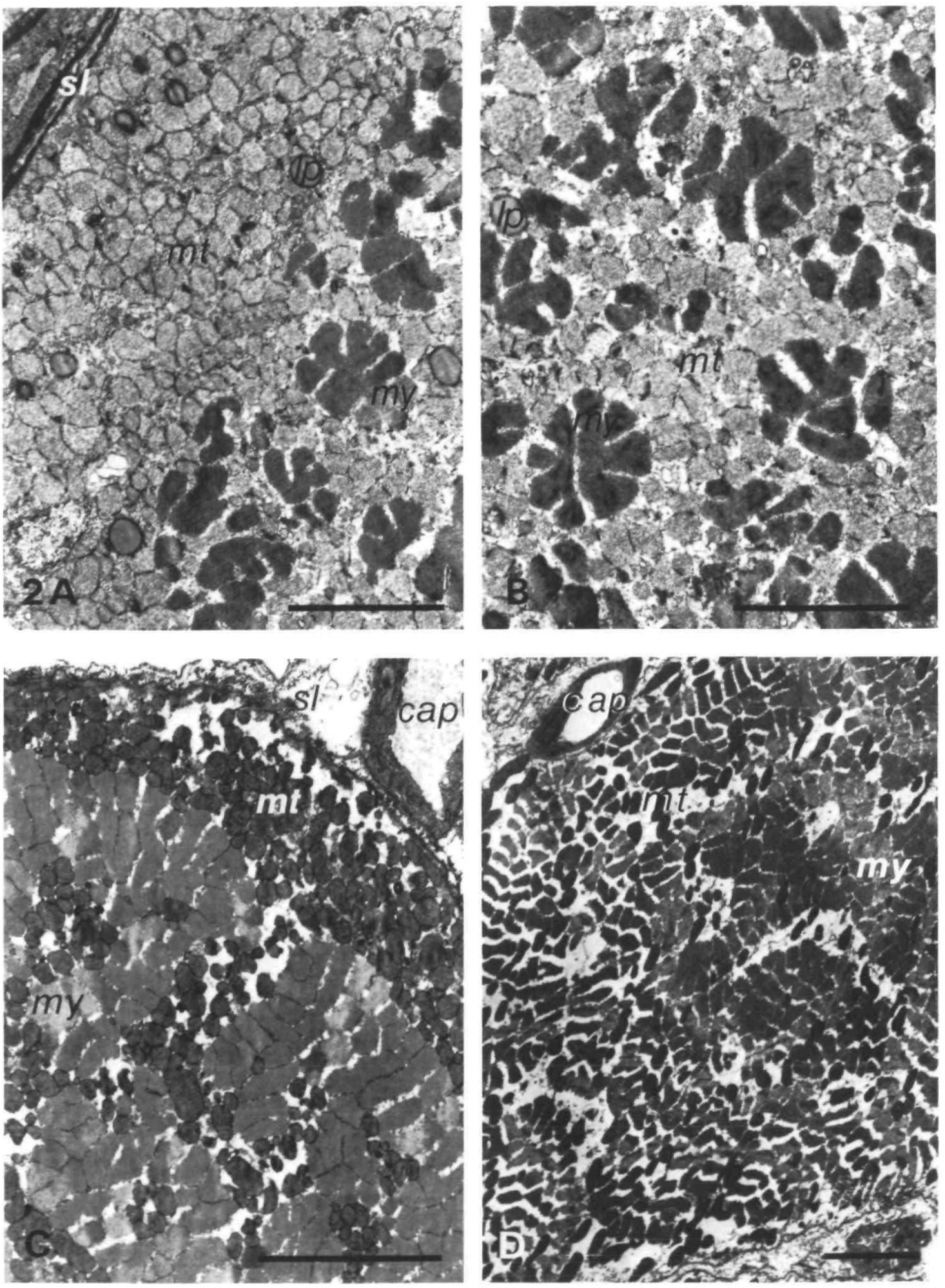

Fig. 2. Electron micrographs of transversely-sectioned slow muscle fibres isolated from various fish. (A) *Chaenocephalus aceratus*: note the sub-sarcolemmal zone is packed with mitochondria. (B) *C. aceratus*: the myofibrils are irregular in cross-section and surrounded by mitochondria. (C) *Notothenia gibberifrons*: the fibres contain fewer mitochondria and myofibrils are more regular in cross-section than in *C. aceratus*. (D) *Oreochromis niloticus*: details of the muscles sampled are given in the text. *mt*, mitochondria; *my*, myofibrils; *lp*, lipid droplets; *cap*, capillary; *sl*, sarcolemma. Scale bars, 5 μm.

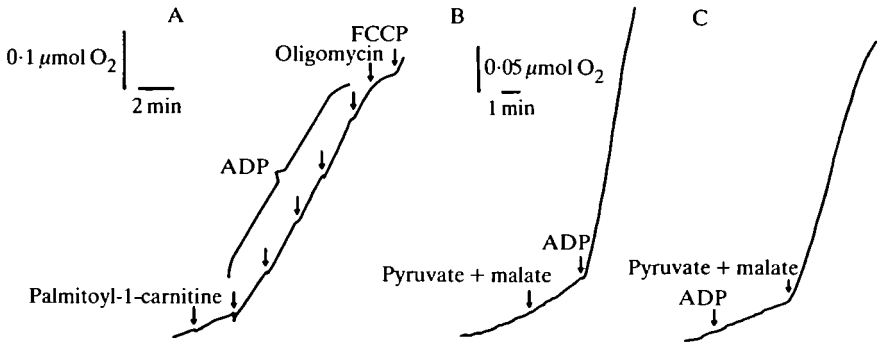

Fig. 3. Oxygen uptake by fibre segments isolated from fish slow muscles. Details of the muscles studied and assay conditions are given in the text. (A) *Notothenia gibberifrons*; wet mass of tissue, 0·048 g; experimental temperature, 0°C. On addition of 0·8 μmol samples of ADP the rate of oxygen utilization (μmol O_2 g^{-1} wet mass min^{-1}) was increased from 0·25 to a maximum of 1·40. The ADP-stimulated rate was 1·15 μmol g^{-1} wet mass min^{-1}. (B,C) *Oreochromis niloticus*; experimental temperature, 25°C; wet mass of tissue, 0·039 g (B) and 0·030 g (C). A similar rate of oxygen utilization was observed regardless of the order of addition of substrate pyruvate + malate (10 μmol) or ADP (2 μmol): 2·8 μmol g^{-1} wet mass min^{-1} in B and 2·5 μmol g^{-1} wet mass min^{-1} in C.

contain fewer mitochondria, the rate of oxygen utilization per unit volume of mitochondria was 46% higher than for the icefish (Fig. 4). The only previous measurement of tissue respiration in antarctic fish is for minced muscles incubated in Ringer's solution (Lin, Dobbs & DeVries, 1974). ADP-stimulated respiration rates of the isolated fibre segments in the present study are 10–20 times higher than these values (Table 4). The effects of temperature on the rate of oxidation of pyruvate + malate by slow fibres from four species of fish is shown in Fig. 4. Oxygen consumption rates ($\dot{V}_{O_2}$) were expressed both in terms of muscle wet mass and the mitochondrial content of the fibres. Over its normal temperature range (0–15°C), the temperate species, *Myoxocephalus scorpius*, oxidized pyruvate + malate with a Q_{10} of 1·6 (Fig. 4). At 0°C, the rate of oxygen utilization per gram muscle in

Table 4. *ADP-stimulated respiration rates (μmol O_2 utilized g^{-1} wet mass min^{-1}) of slow fibres isolated from the pectoral fin abductor muscles of antartic fish*

Substrate	*Chaenocephalus aceratus*	*Notothenia gibberifrons*
Pyruvate + malate	1·20 ± 0·28	1·07 ± 0·23
Palmitoyl-1-carnitine + malate	1·06 ± 0·12	0·91 ± 0·16
Glutamate	0·83 ± 0·12	ND
α-ketoglutarate	0·87 ± 0·07	ND
α-glycerophosphate	0·50 ± 0·06	ND

Interspecific comparisons were not significantly different at the $P = 0·05$ level.
All experiments were carried out at 0°C.
Values represent mean ± S.E. of determinations from six fish.
ND, not determined.
See text for assay conditions.

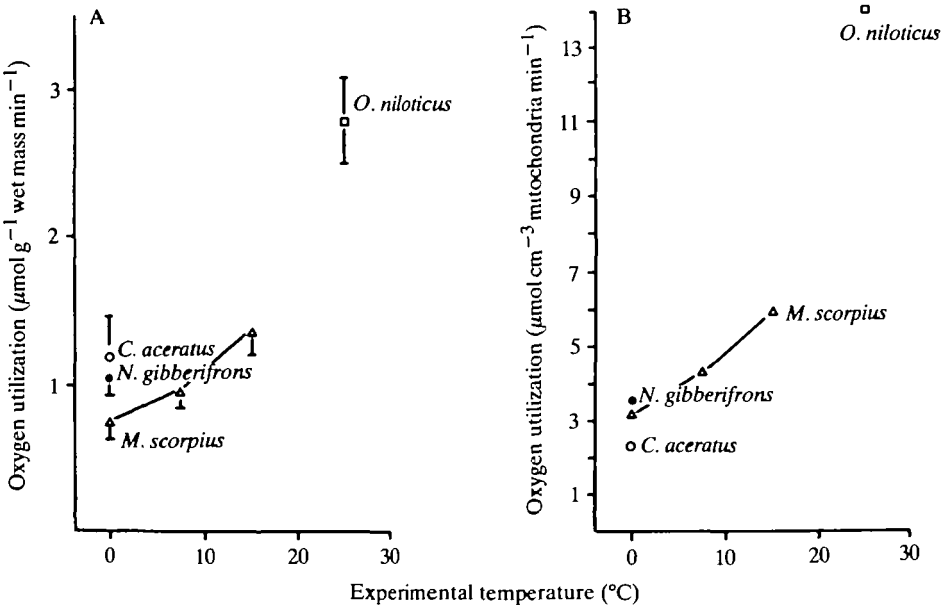

Fig. 4. Effects of temperature on maximal ADP-stimulated respiration rates of slow muscle fibres with 10 mmol l^{-1} pyruvate + malate as substrate. (A) μmol O_2 utilized g^{-1} wet mass min^{-1}. (B) μmol O_2 utilized cm^{-3} mitochondria min^{-1}. The data in B were calculated using the mean values for the volume of the mitochondrial compartment presented in Table 3, assuming equal densities of the various intracellular compartments.

N. gibberifrons was similar to that for *M. scorpius* at 15 °C but less than that for *O. niloticus* at 25 °C (Fig. 4). This difference was accentuated when values for oxygen consumption were corrected for the volume of the mitochondrial compartment in each fibre (Fig. 4).

The respiration rate of fast fibres in *C. aceratus* (with pyruvate + malate as substrate) was only 3 % of that for slow fibres ($\dot{V}_{O_2} = 0{\cdot}034 \pm 0{\cdot}008$ μmol O_2 g^{-1} wet mass min^{-1} at 0 °C).

DISCUSSION

Sustained cruising in *Chaenocephalus aceratus* is achieved by the rhythmic sculling action (1–2 Hz) of large fan-shaped pectoral fins (labriform swimming) (Twelves, 1972; unpublished observations). The pectoral fins are adducted during short bursts of high-speed swimming and thrust is provided by vigorous contractions of the myotomal muscles (sub-carangiform swimming). This pattern of swimming is typical of both demersal and cryopelagic notothenioids (Montgomery & Macdonald, 1984). Aerobic muscles in channichthyids lack myoglobin and are pale yellow, presumably due to the presence of cytochromes (Hamoir, 1978). Histochemical studies reveal that the pectoral fin adductor muscles are predominantly composed of relatively narrow diameter (20–40 μm) fibres which stain intensely for glycogen and aerobic enzymes (Davison & Macdonald, 1985; Harrison, Nicol & Johnston, 1987).

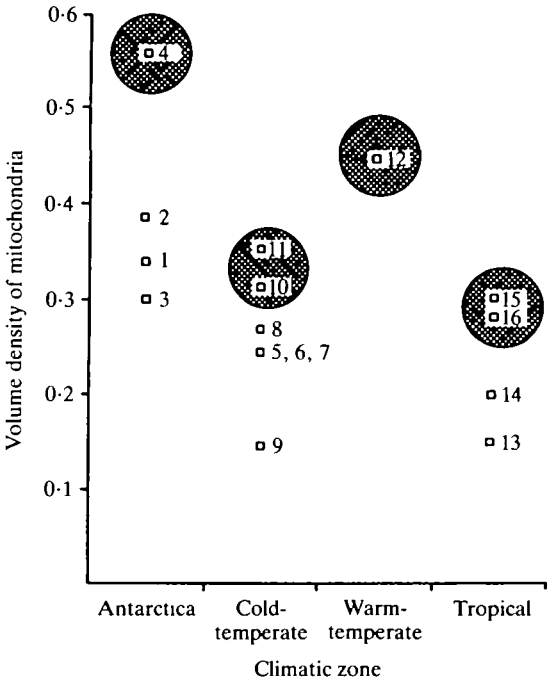

Fig. 5. Volume density of mitochondria in slow muscle fibres isolated from: (1) *Notothenia neglecta*, (Johnston & Camm, 1987); (2) *Notothenia rossii*, (Walesby & Johnston, 1980); (3) *Notothenia gibberifrons*, (present study); (4) *Pleuragramma antarcticum*, (I. A. Johnston, J. P. Camm & M. G. White, unpublished results); (5) *Conger conger* (conger eel), Egginton & Johnston, (1983); (6) *Myoxocephalus scorpius* (bullrout), (present study); (7) *Pleuronectes platessa* (plaice), (Johnston, 1981); (8) *Tinca tinca* (tench), (Johnston & Bernard, 1982); (9) *Carassius carassius* (crucian carp), (Johnston & Bernard, 1984); (10) *Salvelinus fontinalis* (brook trout), (Johnston & Moon, 1980); (11) *Pollachius virens* (saithe), (Beardall & Johnston, 1983); (12) *Engraulis encrasicolus* (anchovy), (Johnston, 1982); (13) *Clarius mossambicus* (catfish), (Johnston, Bernard & Maloiy, 1983); (14) *Oreochromis niloticus* (tilapia), (present study); (15) *Acanthocybium solanderi* (skipjack tuna), (I. A. Johnston & J. Salamonski, unpublished results). Species enclosed in shaded areas are all streamlined fish (mostly pelagic) with high maximum sustained swimming speeds. The remaining species are either sedentary (1–3,5,6,13) or are only moderately active (7–9,14). Values for $V_{V(mt,f)}$ in slow muscle are generally higher for active than sedentary species and for cold- than warm-water species.

The abductor muscles which are responsible for 'feathering' and repositioning the fin following the power stroke contain discrete regions of red and white muscle fibres (Harrison *et al.* 1987).

The myotomal muscles are almost exclusively composed of very large diameter (90–450 μm) white fibres which contain relatively few mitochondria and are poorly supplied with capillaries (Smialowska & Kilarski, 1981; Fitch, Johnston & Wood, 1984). Red and white muscle fibres in antarctic fish are not differentiated by histochemical methods for myosin ATPase activity (Davison & Macdonald, 1985; Harrison *et al.* 1987). Both these fibre types are multiply innervated in perciform fishes (Bone, 1964). The present study has shown that in *C. aceratus* red and white

muscle fibres correspond to slow and fast twitch fibre types, respectively (Fig. 1; Table 1). Red twitch fibres have also been described in the hyohyoideus muscle of the common carp (Granzier, Wiersma, Akster & Osse, 1983).

The enzyme activity profiles of fast and slow muscles in *C. aceratus* (Table 2) are very similar to those for other demersal antarctic fish which possess respiratory pigments, e.g. *Trematomus hansoni* (Johnston & Harrison, 1985), *Notothenia neglecta* (Dunn & Johnston, 1986). Activities of glycolytic enzymes in the fast muscle of *C. aceratus* are relatively modest, whereas those associated with immediate energy supply pathways (creatine phosphokinase, adenylate kinase) are similar to or higher than those for homologous muscles in temperate and tropical species assayed at 15–25°C (Dunn, Hochachka, Davison & Guppy, 1983; Johnston & Moon, 1980; Kleckner & Sidell, 1985). Thus the activities of individual enzymes would appear to vary more with the activity patterns of different species than with adaptation temperature. Direct evidence that phosphocreatine is the main fuel for contraction in the fast muscle of demersal notothenioids is provided by swimming experiments with *Notothenia neglecta*. Complete exhaustion in this species was found to occur after only 50–80 tailbeats of burst activity and was associated with the depletion of phosphocreatine, a large increase in IMP, but little change in concentrations of most glycolytic intermediates including glycogen, glucose and lactate (Dunn & Johnston, 1986).

The volume density of muscle mitochondria $Vv_{(mt,f)}$ in temperate fish is relatively plastic and is altered by several weeks' acclimation to a new temperature regime (Johnston & Maitland, 1980; Tyler & Sidell, 1984). For example, $Vv_{(mt,f)}$ in the slow muscle of crucian carp is 0·25 in 2°C-acclimated fish, compared with only 0·14 in 28°C-acclimated individuals (Johnston & Maitland, 1980). The proliferation of mitochondria in cold-acclimated fish is thought to represent a homeostatic response which partially compensates for the adverse effects of low temperature on aerobic ATP production and on the rate of diffusion of oxygen and metabolites (Sidell, 1983). There is evidence for a similar trend for higher mitochondrial densities in the muscles of fish habitually adapted to low temperature (Fig. 5). Slow fibres in *C. aceratus* contain a higher proportion of mitochondria than homologous fibres from *N. gibberifrons* (Fig. 2; Table 3) and yet have similar respiration rates (Table 4) and activities of aerobic enzymes per wet mass muscle (Table 2). This suggests that the trend for a high density of muscle mitochondria in antarctic fish is related in part to diffusion limitations. One possibility is that the very high values for $Vv_{(mt,f)}$ in icefish muscle serve to minimize the average diffusion path length for oxygen and thereby compensate for the absence of myoglobin.

This work was supported by a Special Topics Grant (GST/02/86) for Antarctic Research from the Natural Environment Research Council and was carried out in collaboration with the British Antarctic Survey. I am grateful to BAS staff for their support and encouragement, particularly to Nigel Bonner and Martin White (Life Sciences Division) and Ian Lovegrove (Base Commander, Signy Station). The

Oreochromis niloticus used in this study were kindly supplied by Dr Brendan McAndrew, Institute of Aquaculture, University of Stirling.

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