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FORCE-VELOCITY CHARACTERISTICS AND METABOLISM OF CARP MUSCLE FIBRES FOLLOWING TEMPERATURE ACCLIMATION

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SUMMARY

Common carp (*Cyprinus carpio* L.), 1 kg body weight, were acclimated for 1–2 months to water temperatures of either 7–8 °C (cold-acclimated group) or 23–24 °C (warm-acclimated group). Single fast fibres and small bundles of slow fibres were isolated from the myotomal muscles and chemically skinned. Force-velocity (P-V) characteristics were determined at 7 °C and 23 °C.

The contractile properties of carp muscle fibres are dependent on acclimation temperature. In the warm-acclimated group maximum isometric tensions (P_0, kN m^{-2}) are 47 ± 6 and 64 ± 5 for slow muscle fibres and 76 ± 10 and 209 ± 21 for fast muscle fibres at 7 °C and 23 °C, respectively. Maximum contraction velocities (V_{max}, muscle $lengths^{-1}$), are 0·4 ± 0·05 and 1·5 ± 0·1 at 7 °C (slow fibres) and 0·6 ± 0·04 and 1·9 ± 0·4 at 23 °C (fast fibres). All values represent mean ± S.E.

P_0 and V_{max} at 7 °C are around 1·5–2·0 times higher for slow and fast muscle fibres isolated from the cold-acclimated group. Fibres from 7 °C-acclimated carp fail to relax completely following maximal activations at 23 °C. The resulting Ca-insensitive force component (50–70 % P_0) is associated with the development of abnormal crossbridge linkages and very slow contraction velocities.

Activities of enzymes associated with energy metabolism were determined at a common temperature of 15 °C. Marker enzymes of the electron transport system (cytochrome oxidase), citric acid cycle (citrate synthase), fatty acid metabolism (carnitine palmitoyl transferase, β-hydroxyacyl CoA dehydrogenase) and aerobic glucose utilization (hexokinase) have 30–60 % higher activities in slow muscle from cold-acclimated than from warm-acclimated fish. Activities of cytochrome oxidase and citrate synthase in fast muscle are also elevated following acclimation to low temperature. It is concluded that thermal compensation of mechanical power output by carp skeletal muscle is matched by a concomitant increase in the potential to supply aerobically-generated ATP at low temperatures.

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Key words: Carp, *Cyprinus carpio*, skeletal muscle, skinned fibres, temperature acclimation, force-velocity characteristics.

INTRODUCTION

Many freshwater fish are found in environments in which the water temperature may range from around 0–4 °C in winter to 25–30 °C in summer. These animals frequently exhibit a remarkable maintenance of activity patterns. The properties of Mg^{2+}, Ca^{2+}-ATPase of myofibrils from goldfish (*Carassius auratus*) are modified by a period of temperature acclimation lasting several weeks or months (Johnston, Davison & Goldspink, 1975; Sidell, 1980). For example, ATPase activity at 1 °C is around 3–4 times higher for preparations isolated from 1 °C-acclimated, compared with 26 °C-acclimated fish (Johnston *et al.* 1975). Moreover, temperature acclimation has also been reported to affect the potential for ATP production in fish skeletal muscle (see Hazel & Prosser, 1974; Sidell, 1983 for reviews). Cold acclimation results in an increase in myoglobin content and in the activities of mitochondrial enzymes, particularly for red skeletal muscles, whereas glycolytic enzyme activities remain unchanged or decline slightly (e.g. *Lepomis cyanellus*, Shaklee *et al.* 1977; *Carassius auratus*, Sidell, 1980). The relationship between alteration in enzyme systems involved in the utilization and supply of ATP contraction have yet to be studied in the same species. Furthermore, the physiological significance of the data on isolated proteins is unclear since the temperature dependence of myofibrillar ATPase activity has been shown to differ markedly from that for unloaded contraction speed for both intact and skinned muscle fibres (Bárány, 1967; Johnston & Sidell, 1984; Sidell & Johnston, 1985). The effects of temperature on contractile function in fish may conveniently be studied using isolated muscle fibres from which the membrane has been removed by either mechanical or chemical skinning (Altringham & Johnston, 1982; Johnston & Salamonski, 1984). The myofilament lattice structure of skinned fibres is preserved in a physiological state and ionic composition of the solutions surrounding the fibre can be manipulated.

In the present study, we have investigated the effects of temperature acclimation on muscle contractile properties using skinned fibre preparations isolated from slow and fast myotomal muscles of the cyprinid fish, *Cyprinus carpio* L. The results show thermal compensation of maximal mechanical power output at low temperatures for both skeletal muscle fibre types from cold-acclimated carp. At least in red muscle, this is supported by an enhanced potential for fatty acid catabolism.

MATERIALS AND METHODS

Fish

Common carp (*Cyprinus carpio* L.) were obtained from commercial suppliers during February and maintained in tanks of recirculated, filtered fresh water at 7–8 °C, under a photoperiod of 12 h light: 12 h dark. A group of six fish (955 ± 43 g body weight, 34·3 ± 0·6 cm total length, mean ± S.E.) were gradually adjusted to 23–24 °C over a period of 2 weeks and held at that temperature for a further 1–2 months (warm-acclimated group). The remaining seven fish (992 ± 58 g body weight, 36·7 ± 0·9 cm total length, mean ± S.E.) were maintained at 7–8 °C for a similar period of time (cold-acclimated group). During this maintenance period fish were fed daily with a commercial fish food.

Isolation of muscle fibres

Fish were killed by a blow to the head followed by transection of the spinal cord. Small strips of fast muscle fibres were rapidly excized from myotomes number 3–5 counting from the head. Slow muscle strips were dissected from a region superficial to the lateral line nerve at a point corresponding to the mid-point of the dorsal fin. Small bundles of fibres from a single myotome were transferred to an ice-cooled dissection stage and single fibres were isolated under silicone oil (MS 550, BDH, Poole, England) using a binocular microscope (×40).

Experimental protocol

Single fast fibres (50–110 μm diameter) and bundles of 2–5 slow fibres (50–110 μm diameter) were transferred directly to the apparatus using jewellers' forceps and attached between two stainless steel hooks, using Plexiglas-acetone glue (Altringham & Johnston, 1982). Fibres were immersed in the first of three water-jacketed baths (temperature control ± 0·2 °C) and chemically skinned (15–20 min at 7 °C and 10 min at 23 °C) in 1 % (w/v) Brij 58 (polyoxyethylene 20 cetyl ether) in relaxing solution containing (in mmol l^{-1}) imidazole-HCl, 20; EGTA, 5; KCl, 100; $MgCl_2$, 3; ATP, 2·5; phosphocreatine, 10; and 20 units ml^{-1} creatine phosphokinase (pH value was adjusted to 7·2 at 20 °C with KOH). (All reagents from Sigma Chemical Company, Poole, England.) Fibres were subsequently transferred to a second bath containing relaxing solution without Brij 58 for 3–5 min. Sarcomere length was measured by laser diffraction and set to 2·3 μm. Fibre length (typically, 1200–3200 μm) and diameter were measured *in situ* using a high power microscope.

Activating solutions (contained in a third bath) were made by additions of $CaCl_2$ to a final concentration of 4·5–5·2 mmol l^{-1} (added as a 1 mol l^{-1} volumetric solution). Calcium concentrations required to give maximal forces at 7 and 23 °C were determined in a series of preliminary experiments. Free ion concentrations were calculated from apparent dissociation constants correcting for pH and temperature using an iterative computer programme as described previously (Johnston & Salamonski, 1984) and were as follows (in mmol l^{-1}): pCa, 4·63–4·36; pMg, 3·19–3·23; pMg ATP, 2·66–2·68; at an ionic strength of 181 mmol l^{-1}. The pH of solutions was allowed to vary freely with temperature (7·15 at 23 °C, and 7·43 at 7 °C) to conform with the with the $\Delta pH/\Delta T$ relationship established for physiological fluids (see Reeves, 1977).

Determination of force-velocity (P-V) characteristics

Force-velocity characteristics were determined on maximally Ca^{2+}-activated fibres using an isotonic lever as described in detail by Altringham & Johnston (1982). Typical isotonic transients obtained following step-tension releases against a series of pre-set loads are shown in Fig. 1. Contraction velocity was measured over the first 50 ms of steady isotonic shortening. Force-velocity (P-V) data from releases up to 0·6 P_0 could be fitted to a linear form of Hill's equation for muscle shortening:

$$V(P + a) = b(P_0 - P),$$

where V is velocity of shortening, P is force, P_0 is maximum isometric force and *a,b*

are constants (Hill, 1938). V_{max} and Hill's constants were calculated for individual fibres as shown in Fig. 1.

Determination of enzyme activities and myoglobin content

Tissue samples were homogenized in ground glass homogenizers with ice-cold extraction buffer (1:10 w/v) containing (in mmol l^{-1}) tris (hydroxymethyl) aminomethane-HCl (Tris-HC1), 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 15 °C. Substrate concentrations, cofactor concentrations and pH were set to yield optimal activites. Enzyme activities were monitored by following changes in absorbance at 340 nm for hexokinase, 6-phosphofructokinase and lactate dehydrogenase, at 412 nm for citrate synthase and carnitine palmitoyl transferase, and 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.

Hexokinase (E.C.2.7.1.1): (in mmol l^{-1}) Tris-HCl, 75; $MgCl_2$, 7·5; EDTA, 0·8; KCl, 1·5; $NADP^+$, 0·4; ATP, 2·5; glucose, 10; creatine phosphate, 10; 0·9 units ml^{-1} creatine phosphokinase; 0·7 units ml^{-1} glucose-6-phosphate dehydrogenase; pH 7·6.

6-Phosphofructokinase (E.C.2.7.1.11): (in mmol l^{-1}) triethanolamine, 75; $MgCl_2$, 7·5 KCl, 200; KCN, 1; AMP, 2; ATP, 1; NADH, 0·15; fructose-6-phosphate, 0·4; 2 units ml^{-1} aldolase; 10 units ml^{-1} triosephosphate isomerase; 2 units ml^{-1} glycerol-3-phosphate dehydrogenase; pH 8·2.

Pyruvate kinase (E.C.2.7.1.40): (in mol l^{-1}) imidazole, 50; KCN, 1; KCl, 150; $MgSO_4$, 10; NADH, 0·15; ADP, 5; phosphoenolpyruvate, 2·5; 10 units ml^{-1} lactate dehydrogenase; pH 6·9.

Lactate dehydrogenase (E.C.1.1.1.27): (in mmol l^{-1}) imidazole, 50; KCN, 2·5; NADH, 0·15; pyruvate, 10; pH 7·3.

Carnitine palmitoyltransferase (E.C.2.3.1.23): (in mmol l^{-1}) Tris, 75; EDTA, 1·5; DTNB, 0·25; carnitine, 1·25; palmitoyl CoA, 0·035; pH 8·0.

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

β-Hydroxyacyl CoA dehydrogenase (E.C.1.1.1.35): (in mmol l^{-1}) imidazole, 50; EDTA, 1; NADH, 0·15; acetoacetyl CoA, 0·1; pH 7·5.

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

Myoglobin was measured by a direct spectrophotometric technique (Sidell, 1980). Haemoglobin interference was eliminated by gel filtration chromatography of homogenates (Driedzic & Stewart, 1982). Absorption spectra were determined over 490–600 nm and the concentration of myoglobin was calculated from absorbance at 581 nm, utilizing a millimolar extinction coefficient of 12·8 (Hardman *et al.* 1961).

Statistical analyses

Results obtained for homologous fibre types from cold- and warm-acclimated populations were compared using Student's *t*-test.

RESULTS

Contractile properties of skinned muscle fibres

The contractile properties of skinned fibres isolated from carp myotomal muscle were found to be dependent on acclimation temperature (Tables 1, 2). At 7 °C, maximum isometric force (P_0) for muscle fibres from cold-acclimated carp was 1·5-fold (slow fibres) and 2-fold (fast fibres) higher than for fibres from warm-acclimated carp (Tables 1, 2) ($P < 0{\cdot}01$). For both acclimation groups, successive cycles of activation and relaxation at 7 °C resulted in no significant decrease in maximum Ca^{2+}-activated force for slow fibres and only a small decrease (5–10 % per activation) for fast fibres. All fibres relaxed completely following up to three activations at 7 °C.

Isotonic transients obtained following step tension releases showed some deviation from linearity, especially at high loads (Fig. 1). Maximum contraction velocities at 7 °C were 1·5-times (slow fibres) and 1·9-times (fast fibres) higher for muscles from cold- than warm-acclimated fish (Tables 1, 2). The degree of curvature of the P-V relationship at 7 °C (comparable a/P_0 values) was not affected by acclimation temperature (Tables 1, 2). Maximum power output, which according to Hill's (1938) equation is produced at a load corresponding to $[(a^2 + a/P_0)^{\frac{1}{2}} - a/P_0]$, was found to be 3·5 times higher at 7 °C for muscle fibres from cold- than warm-acclimated populations.

Experiments at 23 °C produced qualitatively different results for fibres from cold- and warm-acclimated fish. Muscle fibres from 7 °C-acclimated fish were unstable following maximal activations at 23 °C, and showed a rapid decrease in force with time. Furthermore, even after very short activations, both red and white fibres failed to relax completely after transfer to 'low' calcium (10^{-9} mol l^{-1}), resulting in 75 % residual (Ca-insensitive) tension. Successive isotonic releases at constant load were progressively slower, and highly curved transients were produced by these fibres, such that it was not possible to obtain force-velocity data from cold-acclimated fish at 23 °C. Development of residual tension was not reduced by inclusion of SH-protecting agents (5 mmol l^{-1} 2-mercaptoethanol or 0·5 mmol l^{-1} dithiothreitol to the incubation solution), and appears to involve the formation of 'abnormal' crossbridge linkages. In contrast, slow fibres from warm-acclimated fish relaxed completely following up to five maximal activations of 3 min duration at 23 °C. Although fast fibres from warm-acclimated carp also relaxed completely at 23 °C following relatively short activations (~1 min), they produced around 2–10 % residual tension for the longer activations needed to obtain a complete set of P-V data. Contraction velocities of fibres at near zero load ($< 0{\cdot}02\ P/P_0$) were therefore determined immediately following attainment of steady isometric force (Table 2).

For warm acclimated carp, maximum isometric force at 23 °C was 64 kN m^{-2} for slow and 209 kN m^{-2} for fast muscle fibres (Tables 1, 2). For these fish, P_0 had a

Table 1. *Force-velocity characteristics of skinned fibres isolated from the slow myotomal muscle of common carp* (Cyprinus carpio *L.*) *acclimated to either 7°C or 23°C*

Parameter (units)	Experimental temperature 7°C		Experimental temperature 23°C	
	Acclimation temperature 7°C	Acclimation temperature 23°C	Acclimation temperature 7°C	Acclimation temperature 23°C
Isometric force ($kN\ m^{-2}$)	76 ± 9 (9)	47 ± 6 (10)	unstable	63·6 ± 5 (8)
Unloaded contraction speed, V_{max} ($L_0\ s^{-1}$)	0·95 ± 0·08	0·42 ± 0·05 (10)	unstable	1·49 ± 0·11 (8)
a/P_0	0·36 ± 0·04	0·33 ± 0·04	unstable	0·24 ± 0·04
$b(L_0\ s^{-1})$	0·36 ± 0·05	0·15 ± 0·03	unstable	0·35 ± 0·06
Maximum power output ($W\ kg^{-1}$)	8·5 ± 0·8	2·4 ± 0·5	unstable	8·5 ± 1·0

Values represent mean ± S.E.
The number of fibres used is shown in brackets.
L_0, initial muscle length.

Table 2. *Force-velocity characteristics of skinned fibres isolated from the fast myotomal muscle of common carp* (Cyprinus carpio *L.*) *acclimated to either 7°C or 23°C*

Parameter (units)	Experimental temperature 7°C		Experimental temperature 23°C	
	Acclimation temperature 7°C	Acclimation temperature 23°C	Acclimation temperature 7°C	Acclimation temperature 23°C
Isometric force ($kN\ m^{-2}$)	178 ± 15 (16)	76 ± 10 (10)	unstable	209 ± 21 (12)
Unloaded contraction speed, V_{max} ($L_0\ s^{-1}$)	1·18 ± 0·05 (16)	0·62 ± 0·04 (10)	unstable	1·85 ± 0·4 (16)*
a/P_0	0·30 ± 0·03	0·33 ± 0·04	unstable	–
$b(L_0\ s^{-1})$	0·35 ± 0·03	0·19 ± 0·02	unstable	–
Maximum power output ($W\ kg^{-1}$)	19·3 ± 2·2	6·9 ± 0·6	unstable	–

Values represent mean ± S.E.
The number of fibres used is shown in brackets.
*Determined for lightly loaded releases.
L_0, initial muscle length.

$Q_{10(7–23\,°C)}$ of 1·2 for slow and 1·9 for fast muscle fibres (Tables 1, 2). V_{max} for slow fibres from warm-acclimated carp had an apparent $Q_{10(7–23\,°C)}$ of 2·2 (Table 1) compared with 2·0 for fast fibres determined from releases at low loads at 23 °C. The P-V relationship of slow fibres from warm-acclimated fish was significantly more curved (lower a/P_0 ratio) at 7 °C than 23 °C (Table 1). For the warm-acclimated carp, maximum power output had an apparent Q_{10} of 2·2 over the range 7–23 °C (Table 1). However, when measured at their respective acclimation temperatures, maximum power output of slow muscle fibres (8.5 $W\ kg^{-1}$, Table 1) was similar for both the cold- and warm-acclimated populations.

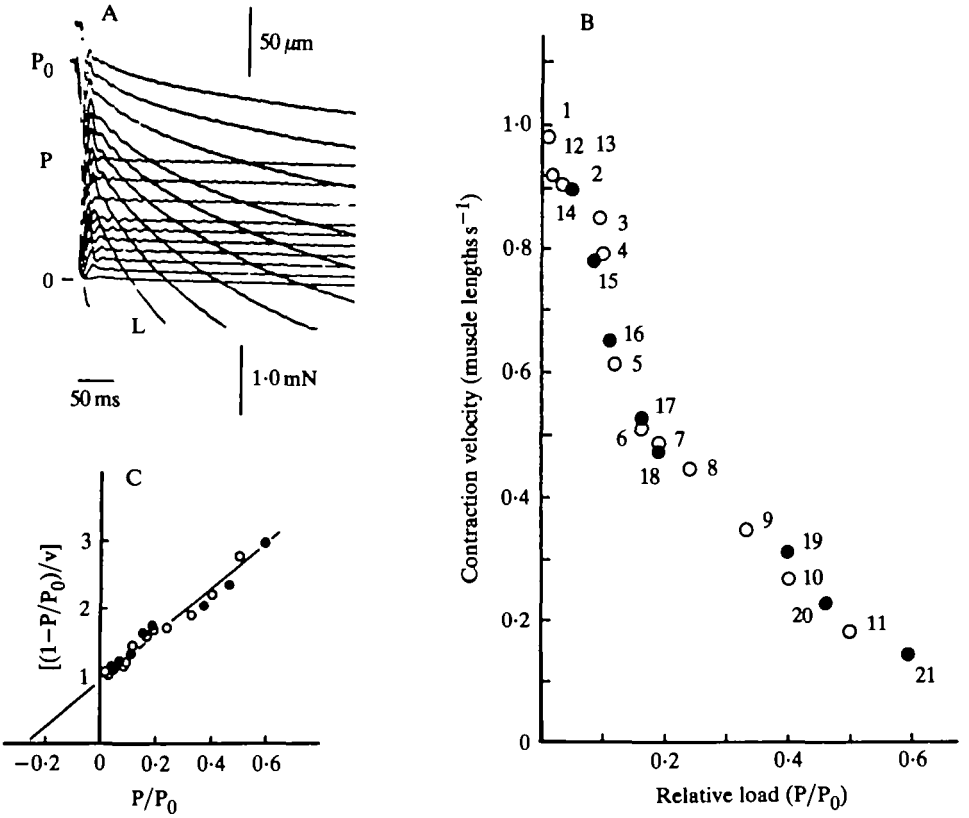

Fig. 1. Typical results from a single skinned fibre, isolated from the fast myotomal muscle of a 7 °C–acclimated carp, to illustrate the method for determining force-velocity characteristics. Experiment at 7 °C. (A) A series of isotonic velocity transients following step tension releases (note the transient at 'zero' applied load is off-screen). P_0, maximum isometric force; L, isotonic velocity transients; 0, zero force baseline. (B) Force-velocity curve of the data shown in A (solid circles). These results were obtained from the second of two maximal activations. Data from the first activation is also shown (open circles) and the numbers refer to the order that the releases were given. Similar values for contraction velocity at a given load were obtained for successive activations. (C) Data for individual fibres was subsequently analysed using a linear form of Hill's (1938) equation for muscle shortening. Hill's constant a/P_0 is obtained from the intercept with the x-axis; $1/V_{max}$ is given by the intercept with the y-axis and $1/b$ by the gradient. Linear regression analysis was performed using a least squares fit ($P<0{\cdot}001$).

Enzyme activities and myoglobin content

Maximal enzyme activities and myoglobin content in fast and slow muscles are summarized in Table 3. 6-Phosphofructokinase was found to be unstable in homogenates from skeletal muscles, and reliable estimates of its activity could not be obtained. In slow muscle, activities of cytochrome oxidase, citrate synthase, carnitine palmitoyl transferase, β-hydroxyacyl CoA dehydrogenase and hexokinase were 30–60 % higher in tissue from cold- compared with warm-acclimated carp. Myoglobin content, however, was lower in slow muscle of cold- compared with warm-acclimated fish. In fast muscle, cytochrome oxidase and citrate synthase were two- to three-fold higher in the cold acclimated group. The absolute activities of enzymes associated with aerobic metabolism were many times lower than in slow muscle.

Table 3. *Enzyme activity levels and myoglobin content in slow and fast muscles of carp acclimated to either 7°C or 23°C*

	Slow muscle fibres		Fast muscle fibres	
	7°C	23°C	7°C	23°C
Hexokinase	0·98 ± 0·15 (4)	0·55 ± 0·06 (5)*	0·14 ± 0·02 (5)	0·03 ± 0·01 (5)
6-Phosphofructokinase	–	–	–	–
Pyruvate kinase	46·3 ± 7·79 (7)	45·9 ± 6·64 (6)	116 ± 13·1 (7)	126 ± 11·8 (6)
Lactate dehydrogenase	74·6 ± 10·0 (7)	74·7 ± 8·69 (6)	208 ± 27 (7)	211 ± 20 (6)
Carnitine palmitoyl transferase	0·41 ± 0·08 (7)	0·13 ± 0·01 (6)*	–	–
β-hydroxyacyl CoA dehydrogenase	3·62 ± 0·52 (7)	1·89 ± 0·26 (6)*	0·36 ± 0·02 (6)	0·28 ± 0·05 (5)
Citrate synthase	11·34 ± 1·48 (6)	5·03 ± 1·22 (6)†	0·34 ± 0·07 (6)	0·12 ± 0·01 (5)*
Cytochrome oxidase	24·3 ± 2·89 (5)	15·4 ± 1·67 (5)*	3·68 ± 0·44 (5)	1·81 ± 0·25 (5)†
Myoglobin	136 ± 15 (5)	230 ± 20 (5)	–	–

Enzyme activities are expressed as μmol substrate converted to product min^{-1} g^{-1} wet weight at 15°C.
Myoglobin is expressed as nmol g^{-1} wet weight.
All values represent mean ± S.E.M.
Sample sizes are shown in parentheses, * $P < 0·02$, † $P < 0·01$.

Pyruvate kinase and lactate dehydrogenase activities were higher in fast muscle than slow muscle and did not vary significantly with acclimation temperature.

DISCUSSION

Measured at normal body temperatures, skinned fibres isolated from the fast myotomal muscles of fish from polar, temperate and tropical oceans generate broadly similar maximum power outputs (Johnston & Altringham, 1985). Such evolutionary modifications in contractile performance largely involve changes in the ability of crossbridges to generate force at different temperatures (Johnston & Brill, 1984; Johnston, 1985; Johnston & Altringham, 1985), and are correlated with structural changes in myosin (Connell, 1961; Johnston *et al.* 1975). However, for most species including carp (Table 1), maximum contraction velocity (V_{max}) has a Q_{10} of around 2·0 (Johnston & Brill, 1984). Thus, an acute drop of temperature will result in a significant decrease in muscle performance.

In carp, these acute effects of temperature on mechanical power output are largely offset by a period of acclimation to low temperature (Tables 1, 2). This is presumably an advantage for a freshwater fish which experiences a large seasonal drop in water temperature. Acclimation-induced changes in muscle V_{max} observed for carp in the present study parallel those documented for myofibrillar ATPase activity in goldfish muscle but are of a somewhat smaller magnitude (see Johnston *et al.* 1975; Penney & Goldspink, 1981; Sidell, 1980).

Little is known about molecular mechanisms underlying changes in mechanical performance of muscle with temperature acclimation. Studies of purified actomyosins have shown that acclimation-induced changes in ATPase activity are evident only for preparations containing thin-filament Ca^{2+}-regulatory proteins, suggesting the cooperative involvement of a number of myofibrillar proteins (Johnston, 1979). It is

not known whether changes in gene expression or post-translational modifications of proteins are responsible. Johnston & Maitland (1980) found that the fraction of fibre volume occupied by myofibrils did not alter in *Carassius* following 12 months acclimation to either 1 °C or 26 °C. Changes in myofibrillar density are therefore unlikely to account for observed increases in maximum isometric force production by muscles of cold-acclimated fishes.

A striking feature of skinned fibres from cold-acclimated carp is the development of residual tension following short activations at 23 °C. Similar phenomena have been observed for muscle fibres isolated from Antarctic and cold-temperate teleosts (Altringham & Johnston, 1982). In contrast, muscle fibres isolated from skipjack tuna (*Katsuwonus pelamis*), a tropical species, show no development of residual tension at temperatures up to 30 °C (Johnston & Brill, 1984). Studies with cod (*Gadus morhua*) skinned fibres have shown that increases in post-contraction tension at high temperatures are associated with a proportional increase in stiffness, suggesting the formation of Ca-insensitive crossbridges (Johnston & Altringham, 1985). Ca-insensitive crossbridges would appear to be abnormal since fibres showing residual tension development contract relatively slowly. While the origin of this force remains obscure, generation of high isometric tensions at low temperatures would appear to correlate with a loss of flexibility to operate over a broad temperature range and is probably related to structural changes in the contractile proteins.

Acclimatory changes in muscle metabolic potential with temperature acclimation appear to be more commonplace than changes in mechanical properties which, so far, have only been reported for cyprinid fish. No differences in muscle myofibrillar ATPase activities are found for populations of *Fundulus heteroclitus* (Sidell, Johnston, Moerland & Goldspink, 1983), chain pickerel (Sidell & Johnston, 1985) or striped bass (T.S.Moerland & B.D.Sidell, unpublished results) acclimated to either 5 or 25 °C for several months. For some of these species which remain active at low water temperatures, muscle mechanical power output has a relatively low Q_{10}, e.g. chain pickerel (Sidell & Johnston, 1985). Low thermal sensitivity of muscle power output, together with changes in the relative proportions (Johnston & Lucking, 1978; Sidell, 1980) or recruitment patterns of different fibre types, may obviate the need for acclimatory changes in contractile properties in these species.

There is evidence for compensatory increases in pathways of aerobic energy production in the muscles of cold-acclimated fishes, and also for changes in the utilization of particular substrates in a number of different species (see Hazel & Prosser, 1974; Sidell, 1983 for reviews). These responses are widespread among fishes and presumably represent an adaptation to increase the maximum rate of ATP supply at low temperatures. For example, cold-acclimation (1–5 °C) in the genus *Carassius* is associated with an increase in the volume density and number of mitochondria per muscle fibre together with corresponding increases in capillary surface and volume densities (Johnston, 1982; Sidell, 1983). There is also evidence from isotopic labelling experiments with temperature acclimated striped bass, and from measurement of maximal enzyme activities *in vitro* (Jones & Sidell, 1982) that cold-acclimation is associated with increases in the utilization of fatty acids. A similar interpretation can be placed on the higher activities of enzymes associated with aerobic energy metabolism in the slow muscle tissue of cold- compared with

warm-acclimated *Cyprinnus carpio* in the present study (Table 3). Proportionately greater increases are observed for slow than for fast muscles because of their much higher mitochondrial volume densities, Johnston & Maitland, 1980; Tyler & Sidell, 1984.

The decrease in myoglobin content in slow (red) skeletal muscle in association with a decrease in acclimation temperature was unexpected in light of the alterations in the enzymes of aerobic energy metabolism and is in contrast to findings with goldfish (Sidell, 1980). Although the role of this protein in facilitating oxygen diffusion has been known for many years (Wittenberg, 1970) the precise conditions under which this process is expressed are yet to be fully elucidated (Driedzic, 1983). The relationships among myoglobin content, oxygen consumption and oxygen demand are currently under investigation.

ATP supply to fast muscle fibres is largely dependent upon energy sources already present within the fibres themselves. Initially, ATP levels are maintained constant by the breakdown of phosphoryl creatine. However, if contraction is prolonged for more than a few seconds, anaerobic glycogenolysis is activated, resulting in the accumulation of lactic acid. Rome, Loughna & Goldspink (1984) have shown that the threshold speed for recruitment of fast motor units in carp is decreased as water temperature is lowered and that the pattern of fibre recruitment may be modified by a period of acclimation to low temperature (unpublished results). One explanation for the lack of change of glycolytic enzyme activities in the fast muscle of cold-acclimated carp (Table 3) is that there are corresponding changes in the frequency and/or duration of burst swimming activity. For example, although peak power output is increased following acclimation to low temperatures a reduction in the average duration of burst swimming activity would result in an increased importance of phosphocreatine breakdown relative to glycogenolysis as an energy source for contraction. The enhanced potential of fast fibres to generate ATP aerobically following cold-acclimation may be related to the recruitment of fast motor units at sustained swimming speeds (see Johnston *et al.* 1977; Bone, Kicenuik & Jones, 1978).

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