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CONTRACTILE AND METABOLIC CHARACTERISTICS OF MUSCLE FIBRES FROM ANTARCTIC FISH

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SUMMARY

Contractile properties were determined for muscles from three species of Antarctic fish with broadly similar activity patterns: *Trematomus hansoni, Notothenia rossii* and *Chaenocephalus aceratus*. *C. aceratus* differs from the other species in that the genes for the respiratory pigments are not expressed.

Red and white fibres were isolated from the pectoral fin adductor and trunk muscles, respectively. Fibre segments were chemically skinned with the non-ionic detergent Brij-58. All experiments were carried out at 0 °C. Maximum isometric tensions (P_0) were 6·6–7·1 N cm^{-2} for red, and 21·4–25·1 N cm^{-2} for white muscle fibres. The force-velocity (P-V) characteristics of muscle fibres were determined by step isotonic releases. Unloaded contraction velocities (muscle lengths s^{-1}, L_0 s^{-1}) were 0·7 for red, and 0·9–1·1 for white fibres. Maximum mechanical power outputs (W kg^{-1} muscle for white muscle), calculated using Hill's equation for muscle shortening, were 26·7 (*T. hansoni*), 15·7 (*N. rossii*) and 22·7 (*C. aceratus*). Corresponding values for red pectoral muscle fibres were around 4·2 W kg^{-1} for all three species.

Maximum activities of enzymes of carbohydrate utilization (hexokinase, phosphofructokinase, lactate dehydrogenase), fatty acid metabolism (carnitine palmitoyl transferase, 3-OH acyl CoA dehydrogenase) and aerobic mitochondrial metabolism (cytochrome oxidase) were measured in muscle homogenates from *C. aceratus* and *N. rossii* at 0 °C. Red pectoral muscle fibres from *C. aceratus* and *N. rossii* had similar activities of cytochrome oxidase, carnitine palmitoyltransferase and glycolytic enzymes. Hexokinase activities were two times higher in the red fibres of *C. aceratus* than *N. rossii*, suggesting a greater capacity for aerobic glucose utilization in the former species.

In spite of the lack of respiratory pigments, the metabolic and mechanical characteristics of the swimming muscles in *C. aceratus* apppear to be similar to those of other Notothenioids. Power outputs and enzyme activities of Antarctic fish muscle measured at 0 °C are comparable to those for temperate species measured at 15 or 25 °C, indicating a high degree of cold-adaptation of both energy-producing and energy-utilizing pathways.

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Key words: Skeletal muscle, teleost fish, contractile properties.

INTRODUCTION

The Southern Ocean has low, relatively stable temperatures (−1·9 to 0·5 °C), and shows marked seasonal variations in the degree of ice-cover and plankton growth rates. Antarctic fish exhibit a number of characteristics which reflect these environmental constraints. These include the possession of glycoproteins, which lower serum and tissue freezing points (DeVries, 1971), reduced haematocrits (Hureau, Petit, Fine & Marruex, 1977), low resting metabolic rates (Holeton, 1970; Morris & North, 1984) and low growth rates (Clarke, 1983). The fish fauna is dominated by perciforms of the sub-order Notothenioidea (Norman, 1938). The family Channicthyidae whichcomprises seventeen species is of special scientific interest, since its members do not possess the respiratory pigments haemoglobin and myoglobin (Ruud, 1954; Walesby, Nicol & Johnston, 1982). The oxygen-carrying capacity of blood from *Chaenocephalus aceratus* (Channicthyidae) blood is only 0·5 vol % compared with 8 vol % for most other cold-water fish (Ruud, 1954; Grigg, 1967). In spite of the lack of respiratory pigments, channichthyids have similar growth rates (Olsen, 1955) and resting metabolic rates to other notothenioids (Clarke, 1983) and there is also evidence from analyses of stomach contents that some species prey on a wide range of fast-swimming fish and invertebrates, including Antarctic krill (*Euphausia superba*) (Permitin & Tarverdieva, 1978; Burchett, Sayers, North & White, 1983).

In order to determine to what extent muscle performance might be limited by (a) low temperatures and (b) the lack of respiratory pigments, we have compared the contractile and metabolic characteristics of muscle fibres from *Chaenocephalus aceratus* (Channicthyidae) with those of two other notothenioids which have haemoglobin (*Notothenia rossii* and *Trematomus hansoni*) and also with similar data on temperate and tropical species from the literature.

METHODS

Fish

Icefish (*Chaenocephalus aceratus* Lönnberg), 16 fish, 1303 ± 72 g body weight, 57·0 ± 1·2 cm total length; *Trematomus hansoni* Boulenger, 4 fish, 678 ± 53 g body weight, 38·2 ± 0·4 cm total length; juvenile *Notothenia rossii* Fischer, 5 fish, 460 ± 40 g body weight, 32·2 ± 0·3 cm total length (mean ± S.E. mean) were obtained from Signy Island, British Antarctic Territory, 60° 43′ S, 45° 36′ W during Austral summer, December 1983–January 1984. Fish were caught by trammel nets set in either 30–50 m (*N. rossii*) or 140–200 m of water (*C. aceratus* and *T. hansoni*). Mean water temperature was −1 °C. Fish were maintained for up to 5 days in a recirculated seawater aquarium at 0–2 °C.

Muscles

Red and white muscle fibres were isolated from the pectoral fin adductor and trunk muscles, respectively (Fig. 1). Dissection was carried out initially in a cold-room (+4 °C) and subsequently on an ice-cooled plate.

Skinned fibre experiments

The experimental protocol and apparatus for isolation of fibre segments and determination of their force-velocity characteristics have been described in detail elsewhere (Altringham & Johnston, 1982; Johnston & Salamonski, 1984). Fibre

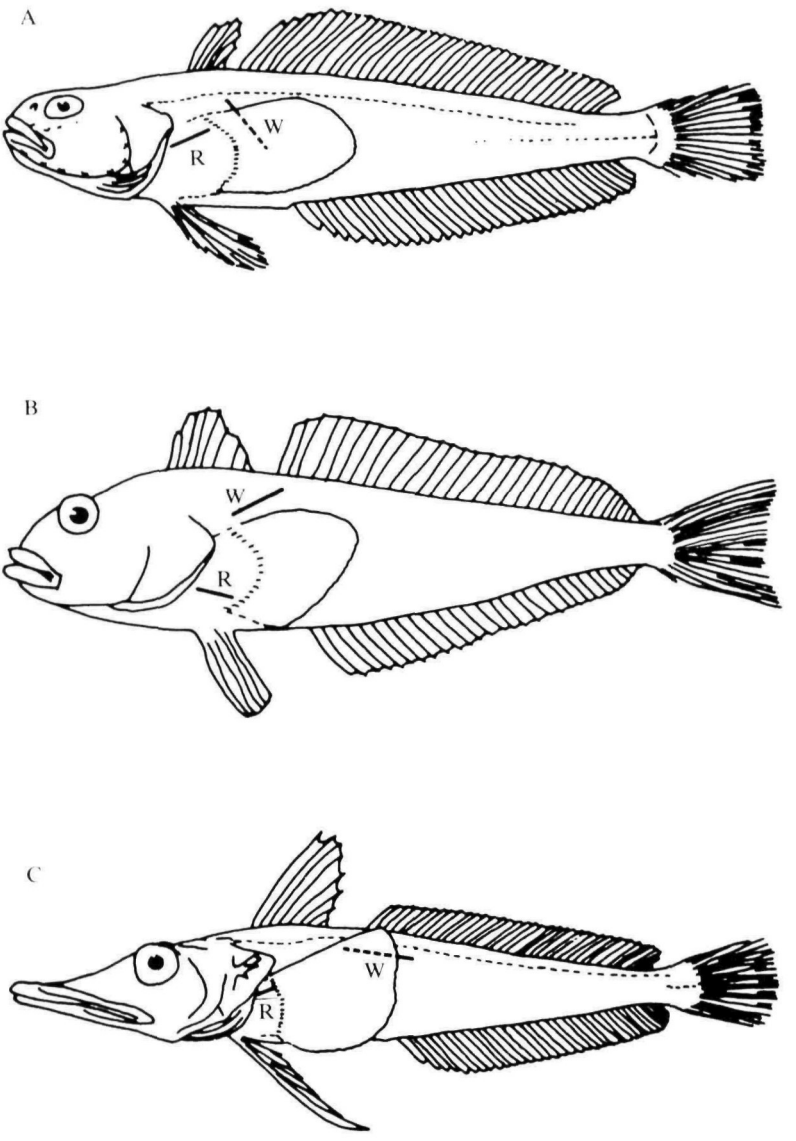

Fig. 1. Diagram showing the sites where muscle samples were taken for mechanical and biochemical experiments. (A) *Trematomus hansoni*, (B) *Notothenia rossii* and (C) *Chaenocephalus aceratus*. The solid line represents the longitudinal axis of white (W) and red (R) muscle fibres. Whereas white fibres from the trunk muscle of *C. aceratus* ran parallel to the skin at the chosen sample site, fibres from *N. rossii* and *T. hansoni* were inclined at angles of 35° and 45° respectively. Sample sites were chosen for the longest myotomes (7–12 mm).

segments (50–129 μm diameter and 1600–2500 μm length) were chemically skinned by a 10-min soak in 1 % Brij 58 dissolved in relaxing solution (in mmol l^{-1}): imidazole-HCl, 20; KCl, 110; $MgCl_2$, 3; EGTA (ethylene glycol bis-β-aminoethylether $N.N^1$ tetraacetic acid), 5; phosphocreatine, 10; ATP, 2·5; and 20 units ml^{-1} creatine phosphokinase, pH 7·56 at 0 °C). Following skinning, fibres were transferred to relaxing solution for 5 min and sarcomere length determined by laser diffraction and set to 2·3 μm. Muscle length and diameter were measured *in situ* using a high power microscope. Temperature was maintained at 0 °C by circulating refrigerated ethylene glycol through a water-jacket surrounding each muscle chamber. The Ca^{2+}-concentration required to give maximal activations (pCa 5·9) was determined from preliminary experiments. Standard activating solution contained 4·80 mmol l^{-1} added $CaCl_2$ (1 mol l^{-1} volumetric solution) and had the following ionic composition; pCa 5·90, pMg 3·19, MgATP 2·68, ionic strength 180 mmol l^{-1}, pH 7·56 at 0 °C.

Free concentrations of ionic species were determined using an iterative computer programme which calculated apparent dissociation constants from absolute stability constants, making corrections for pH and temperature (stability constant Ca^{2+}-EGTA/CaEGTA = $8{\cdot}84\times10^{10}$ at 22 °C) (Fabiato & Fabiato, 1979).

In a typical experiment, fibres were transferred to baths containing sequentially, skinning, relaxing, activating and relaxing solutions. Force was measured using a silicon beam strain gauge (AME 801, sensitivity 0·5 mN V^{-1}) (AME, Horton, Norway). Initially, fibres were allowed to contract isometrically. Once steady force levels were attained, fibres were given step tension releases at a series of different afterloads (Altringham & Johnston, 1982). Following each release, fibres were re-extended to their original lengths. Force-velocity curves were fitted to a linear form of Hill's equation (1938) for muscle shortening:

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

in which P is force, P_0 is maximum isometric force, V is the velocity of shortening and a and b are constants.

The load at which maximum power output (P_{max}) is achieved was determined directly from Hill's equation and is at a value of force corresponding to $(a^2 + aP_0)^{1/2} - a$ (Hill, 1938).

Preparation of muscle homogenates

Homogenization medium contained (in mmol l^{-1}): Tris-HCl, 75; $MgCl_2$, 3; EDTA, 5; dithiothreitol, 1; pH 7·5 at 0 °C. Around 1 g of muscle tissue was finely minced with scissors and homogenized on ice in 8 volumes of medium using a ground glass homogenizer.

Measurement of enzyme activities

Enzyme activities were measured at 0 °C in a spectrophotometer (Pye Unicam SP6) fitted with a water-jacketed cell holder. Reactions were usually initiated by addition of substrate to cuvettes preincubated for 3–10 min on ice. Concentrations of substrates

and cofactors used were based on an early study by Walesby & Johnston (1980*a*) on *N. rossii* and optimal pH values were determined by preliminary experiments on both *C. aceratus* and *N. rossii*. Enzyme activities were monitored by following the reduction of pyridine nucleotides at 340 nm wavelength unless otherwise stated. Activities were expressed as micromoles substrate utilized per minute per gram wet weight (μmol min^{-1} g^{-1} wet weight).

Cytochrome oxidase (EC 1.9.3.1)

Assay medium contained 10 mmol l^{-1} potassium phosphate, 0·065% reduced cytochrome c, pH 7·4. The reaction was monitored by following the decrease in absorbance of reduced cytochrome c at 550 nm.

Carnitine palmitoyltransferase (EC 2.3.1.23)

Assay medium contained (in mmol l^{-1}): Tris-HCl, 75; EDTA, 1·5; DTNB (5,5 dithiobis-2-nitrobenzoic acid), 0·25; carnitine, 1·25; palmitoyl CoA, 0·035; pH 8·0. The reaction was monitored at 412 nm and the background activity in the absence of L-carnitine subtracted.

3-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.36)

Assay medium contained (in mmol l^{-1}): imidazole, 50; KCN, 1; acetoacetyl CoA, 0·1; NADH, 0·15; pH 7·5.

3-Hydroxybutyrate dehydrogenase (EC 1.1.1.30)

Assay medium contained (in mmol l^{-1}): Tris-HCl, 75; NAD^+, 2; DL-3-hydroxybutyrate, 30; KCN, 0·2; pH 6·8–8·5.

Hexokinase (EC 2.7.1.1)

Glucose-6-phosphate production was linked to $NADP^+$ reduction with glucose-6-phosphate dehydrogenase. Assay medium contained (in mmol l^{-1}): Tris-HCl, 75; $MgCl_2$, 7·5; EDTA, 0·8; KCl, 1·5; $NADP^+$, 0·4; ATP, 2·5; D-glucose, 1; phosphocreatine, 10; 0·9 units ml^{-1} creatine phosphokinase; 0·7 units ml^{-1} glucose-6-phosphate dehydrogenase; pH 7·6.

6-Phosphofructokinase (EC 2.7.1.11)

Assay medium contained (in mmol l^{-1}): Tris-HCl, 75; $MgCl_2$, 7; KCl, 200; KCN, 1; AMP, 2; ATP, 1; NADH, 0·15; fructose-6-phosphate, 4; 2 units ml^{-1} aldolase; 10 units ml^{-1} triosephosphate isomerase; 2 units ml^{-1} glycerol-3-phosphate dehydrogenase; pH 7·5.

Lactate dehydrogenase (EC 1.1.1.27)

Assay medium contained (in mmol l^{-1}): imidazole, 50; NADH, 0·15; pyruvate, 1; pH 7·6.

Statistical analyses

Enzyme activities and contractile parameters for homologous muscles were tested for equality of mean, using Students' *t*-test.

RESULTS

Muscles

In all three species, slow-speed swimming is achieved primarily by the use of enlarged pectoral fins (see Twelves, 1972; Burchett *et al.* 1983). Pectoral muscle is composed of around 80 % red fibres, and constitutes 2·8 ± 0·3 % of body weight for *C. aceratus* (16 fish) and 2·7 ± 0·1 % for *N. rossii* (6 fish). In contrast, red fibres only constitute a very small proportion of the trunk muscles (~2 %). The white trunk musculature of *C. aceratus* (28·0 ± 0·8 % body weight; 16 fish) is greatly reduced compared with that of *N. rossii* and *T. hansoni* (44–55 % body weight).

Skinned fibre experiments

The contractile properties of red and white muscle fibres are summarized in Tables 1 and 2. Maximum isometric tension development showed little inter-specific variation and was around 3–3·5 times higher for white than red fibres. A typical

Table 1. *Mechanical properties and maximum power outputs for 'red' pectoral muscle fibres isolated from the haemoglobinless icefish* Chaenocephalus aceratus *and from two species of Antarctic fish possessing respiratory pigments:* Notothenia rossii *and* Trematomus hansoni

Parameter	Species		
	C. aceratus	*N. rossii*	*T. hansoni*
Maximum isometric tension (P_0, N cm^{-2})	6·6 ± 0·7 (11)	6·7 ± 0·8 (6)	7·1 ± 0·5 (6)
Extrapolated maximum contraction velocity from P-V relationship (V_{max}, muscle lengths s^{-1}, L_0 s^{-1})	0·69 ± 0·07	0·73 ± 0·71	0·68 ± 0·10
Hill's constants			
a/P_0	0·27 ± 0·03	0·22 ± 0·02	0·26 ± 0·04
$b(L_0 s^{-1})$	0·17 ± 0·02	0·16 ± 0·02	0·17 ± 0·03
P_{max} (load for maximum power output)	0·30 ± 0·01P_0	0·30 ± 0·01P_0	0·29 ± 0·01P_0
Maximum power output (W kg^{-1})	4·2 ± 0·5	4·2 ± 0·5	4·3 ± 0·5

All experiments were carried out at 0 °C.
Number of muscle fibres used is shown in brackets.

isotonic step tension release is shown in Fig. 2. Contraction velocities for loads below $0{\cdot}6\,P_0$ could be fitted to a linear form of the Hill equation. Extrapolated maximum

Table 2. *Mechanical properties and maximum power outputs for white trunk muscle fibres isolated from the haemoglobinless icefish* Chaenocephalus aceratus *and from two species of Antarctic fish possessing respiratory pigments:* Notothenia rossii *and* Trematomus hansoni

	Species		
Parameter	*C. aceratus*	*N. rossii*	*T. hansoni*
Maximum isometric tension (P_0, N cm^{-2})	21·4 ± 1·4 (14)	26·0 ± 2·2 (9)	26·1 ± 2·1 (12)
Extrapolated maximum contraction velocity from P-V relationship (V_{max}, $L_0\,s^{-1}$)	1·1 ± 0·09	0·9 ± 0·09	0·9 ± 0·05
Hill's constants			
a/P_0	0·24 ± 0·02	0·17 ± 0·04	0·30 ± 0·03
$b(L_0\,s^{-1})$	0·26 ± 0·03	0·15 ± 0·03	0·26 ± 0·03
P_{max} (load for maximum power output)	0·30 ± 0·01P_0	0·27 ± 0·02P_0	0·33 ± 0·02P_0
Maximum power output (W kg^{-1})	22·7 ± 2·6	15·6 ± 1·1	26·7 ± 1·0

All experiments were carried out at 0 °C.
Number of fibres used is shown in brackets.

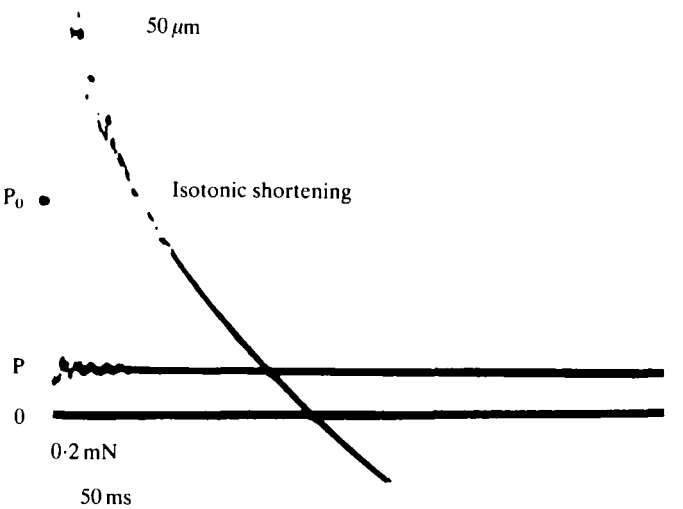

Fig. 2. Typical isotonic step tension release for a single white fibre isolated from the trunk muscle of *Trematomus hansoni*; P_0, maximum isometric tension; P, muscle fibre load. Note the initial rapid length change due to the shortening of the series elastic component. Contraction velocity was measured over the first 50 ms of steady isotonic shortening.

contraction velocities were around 1·4 times faster for white than red fibres (Tables 1, 2). The degree of curvature of the force-velocity relationship was similar for red and white fibres (Figs 3–5). The range of values for a/P_0 was 0·22–0·27 for red and 0·17–0·30 for white muscle fibres (Tables 1, 2). Maximum power output was at a load corresponding to $0{\cdot}3P_0$ for red fibres and was around 4 W kg^{-1} muscle for all three species (Table 1). Maximum power output for white fibres ranged from 15·6 W kg^{-1} for juvenile *N. rossii* to 26·7 W kg^{-1} for *T. hansoni* (Table 2).

Enzyme activity measurements

The activities of some key enzymes of energy metabolism in red and white muscles are summarized in Table 3. No significant difference was found in red muscle cytochrome oxidase activities between the two species. White muscle cytochrome oxidase activities were, however, significantly higher for *N. rossii* (5·9 % compared

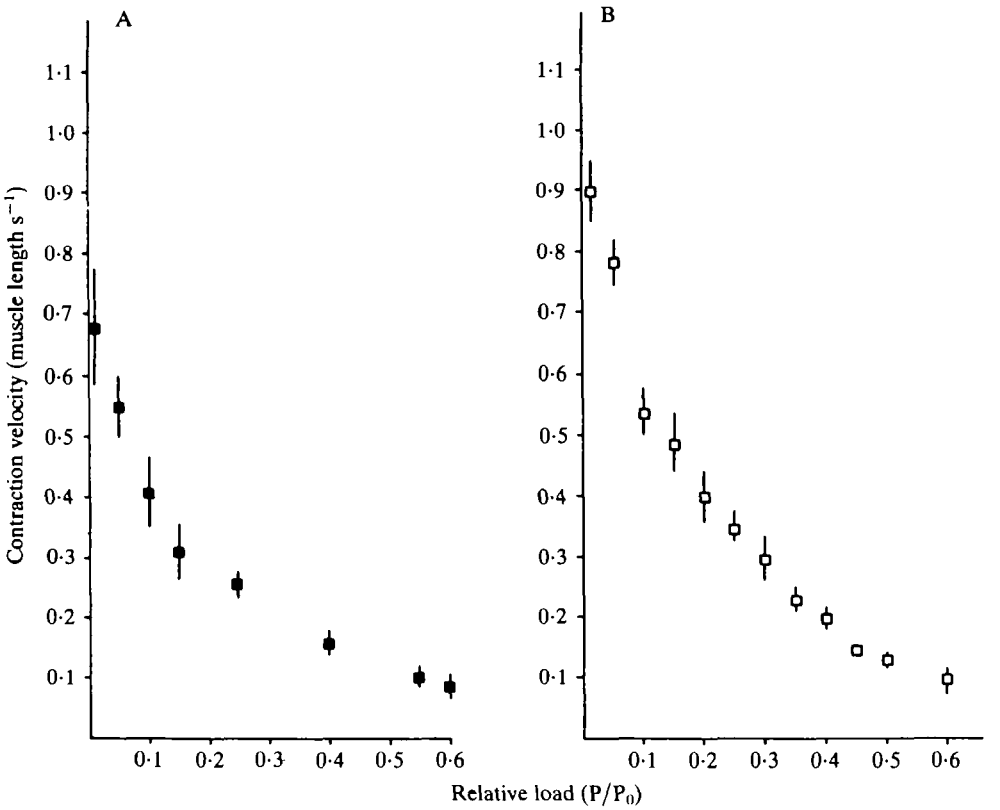

Fig. 3. Force-velocity relationship for 'red' pectoral (A) and 'white' myotomal (B) muscle fibres isolated from *Trematomus hansoni* (at 0 °C). Points represent mean ± S.E. for 12 white and 6 red fibres isolated from three fish.

with 11·6% of red muscle values) ($P < 0{\cdot}01$). Non-equilibrium enzymes of aerobic glucose and fatty acid oxidation (hexokinase and carnitine palmitoyltransferase, respectively) had high activities in red fibres and were almost undetectable in white fibres (Table 3). Hexokinase activities were two times higher in *C. aceratus* than in *N. rossii* red pectoral fibres ($P < 0{\cdot}01$, Table 3). Hydroxybutyrate dehydrogenase activity could not be detected in any of the tissues examined, suggesting a negligible contribution of ketone body oxidation (Table 3). Glycolytic enzymes had relatively low activities and were around two times higher in white than in red fibres (Table 3).

DISCUSSION

The present study has shown that the mechanical power output and metabolic characteristics of muscles from *C. aceratus* are no different to those of notothenioids

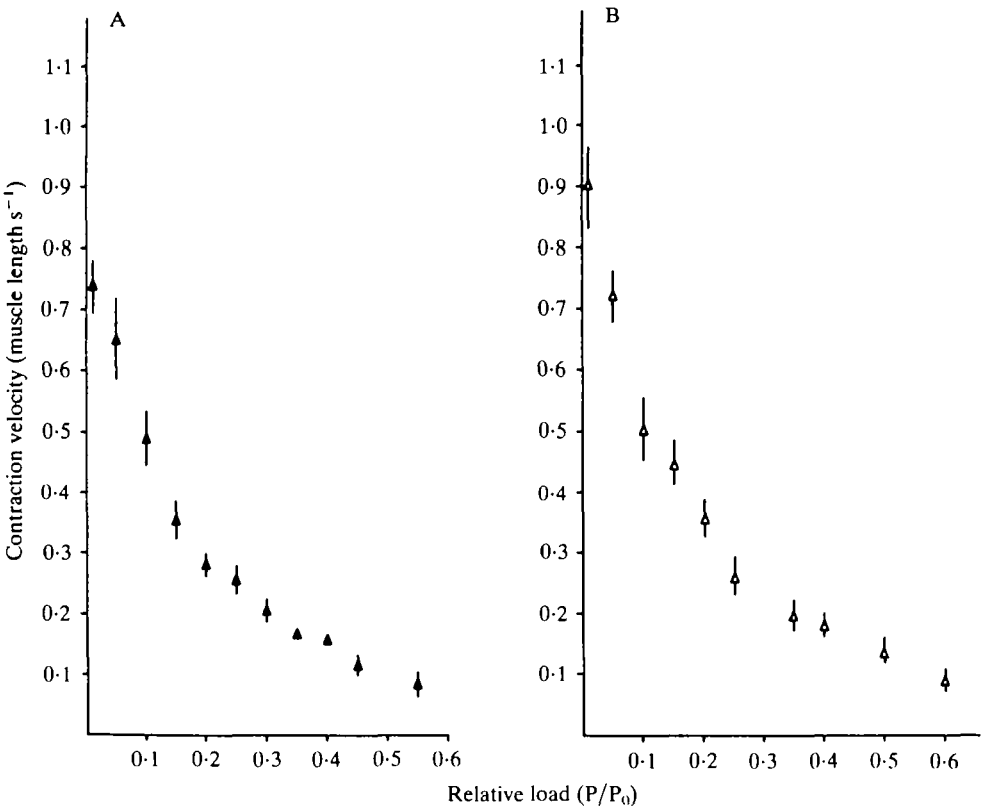

Fig. 4. Force-velocity relationship for 'red' pectoral (A) and 'white' myotomal (B) muscle fibres isolated from *Notothenia rossii* (at 0 °C). Points represent mean ± S.E. for 9 white and 4 red fibres from five fish.

which possess respiratory pigments. A number of adaptations in the circulatory system of *C. aceratus* have been described which may compensate for the reduced oxygen-carrying capacity of the blood and absence of myoglobin-mediated O_2 flux in the tissues. These include a large blood volume (~9% body weight) (Twelves, 1972), a high resting cardiac output (66–104 ml kg^{-1} min^{-1}) (Hemmingsen & Douglas, 1977), low resting ventral aortic blood pressures (12–20 mmHg) (Hemmingsen & Douglas, 1977) and a large ventricle (Johnston *et al.* 1983) relative to other teleost fish. Capillaries supplying the red muscles of channicthyiid fish occur at relatively low densities but are of large bore (Fitch & Johnston, 1983). This enables high blood flow rates to be maintained at low pressures such that high P_{O_2} values are found at the venous end of the capillary bed (Holeton, 1970). Together with the relatively high volume density of mitochondria (0·34%) reported for aerobic muscles (Fitch & Johnston, 1983), this may serve to maximize the O_2 gradient between exchange vessels and mitochondria, thus ensuring an adequate supply of oxygen.

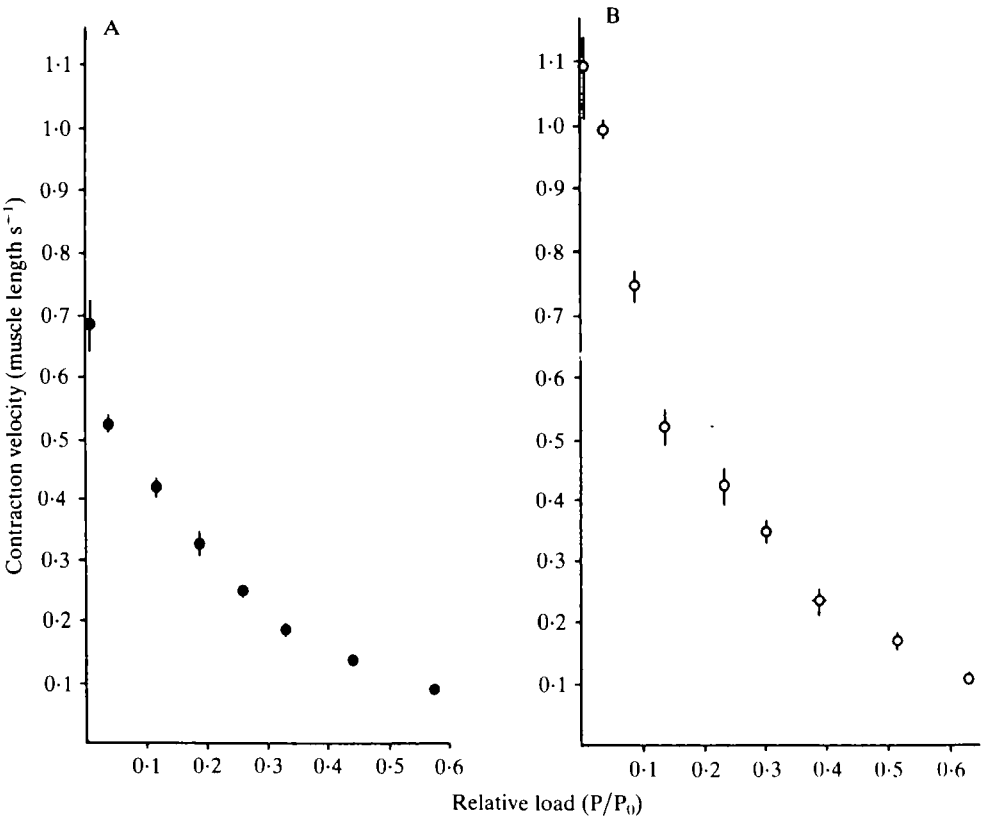

Fig. 5. Force-velocity relationship for 'red' pectoral (A) and 'white' myotomal (B) muscle fibres isolated from *Chaenocephalus aceratus* (at 0 °C). Points represent mean ± S.E. of 14 white and 11 fibres from six fish.

The data on the force-velocity and metabolic characteristics of red and white fibres from notothenioids presented in Tables 1–3 allow some insight into the degree of temperature compensation of contractile function exhibited by Antarctic fish. Direct comparisons with other species are, however, complicated by different muscle power requirements associated with variations in body-form, mode of swimming and activity patterns. The maximum power outputs for skinned fibres from white muscles of Antarctic fish (15–27 W kg^{-1} at 0 °C; Table 2) are similar to those obtained for temperate species (e.g. 16 W kg^{-1} for cod, *Gadus morhua* L. at 8 °C) (Altringham & Johnston, 1982) and are 40–70 % of those reported for tropical species when measured at their normal body temperatures (e.g. 37 and 55 W kg^{-1} for Pacific blue marlin (*Makaira nigricans*) at 15 and 25 °C, respectively) (Johnston & Salamonski, 1984).

Measurements of the maximal activities of non-equilibrium (usually flux-generating) enzymes can provide information on the potential carbon flow through different metabolic pathways, and also insights into the principal fuels supporting activity (Newsholme, Zammit & Crabtree, 1978). For example, hexokinase and carnitine palmitoyltransferase activities are thought to provide quantitative measures of glucose and fatty acid oxidation, respectively (Newsholme & Paul, 1983). Red muscle activities of cytochrome oxidase (an electron transport enzyme), hexokinase and carnitine palmitoyltransferase for *N. rossii* and *C. aceratus* at 0 °C are comparable to those for tench and trout measured at 15 °C (Johnston & Bernard, 1982; Newsholme & Paul, 1983) and skipjack tuna at 25 °C (Guppy, Hulbert & Hochachka,

Table 3. *Maximal activities of some key enzymes of energy metabolism for 'red' pectoral and 'white' trunk muscles isolated from* Chaenocephalus aceratus *and* Notothenia rossii

	C. aceratus		*N. rossii*	
Enzyme	'Red' pectoral muscle	'White' muscle	'Red' pectoral muscle	'White' muscle
CARBOHYDRATE METABOLISM				
Hexokinase	3·4 ± 0·4	<0·01	1·7 ± 0·2	<0·01
Phosphofructokinase	1·2 ± 0·1	2·1 ± 0·3	0·9 ± 0·2	1·9 ± 0·3
Lactate dehydrogenase	57 ± 11	130 ± 23	49 ± 12	72 ± 13
FATTY ACID METABOLISM				
Carnitine palmitoyltransferase	0·58 ± 0·06	<0·005	0·44 ± 0·09	0·015 ± 0·007
3-Hydroxyacyl-CoA dehydrogenase	7·4 ± 1·2	1·4 ± 0·3	3·3 ± 0·5	0·73 ± 0·13
KETONE BODY METABOLISM				
3-Hydroxybutyrate dehydrogenase	ND	ND	ND	ND
AEROBIC MITOCHONDRIAL METABOLISM				
Cytochrome oxidase	15·1 ± 1·8	1·3 ± 0·1	19·9 ± 2·4	3·4 ± 0·4

All activities are expressed as μmol substrate utilized per g wet weight of tissue per minute at 0 °C.
Values represent mean ± S.E. of determinations from 6–10 fish.
ND = not detectable.

1979). These results suggest a high degree of temperature compensation of both mechanical power output and aerobic energy producing pathways in Antarctic fish muscles.

There is evidence that temperature adaptation of mechanical performance by fish muscle largely involves variations in isometric force development. Maximum Ca^{2+}-activated force (P_0) is relatively temperature-independent ($Q_{10} = 1{\cdot}2–1{\cdot}4$) over the usual range of temperatures experienced by a particular species, but may decline dramatically at higher or lower temperatures (Johnston & Brill, 1984; Johnston & Sidell, 1984). Thus, when compared at 0 °C, P_0 is 5–7 times higher for muscles from Antarctic than tropical fish species (Tables 2, 3; Johnston & Brill, 1984). Indeed, P_0 values for white muscle fibres from notothenioids are towards the upper range reported for teleosts when measurements are made at the usual body temperature of each species (Altringham & Johnston, 1982; Johnston & Brill, 1984). For example, P_0 for Pacific blue marlin white fibres at 25 °C is $17{\cdot}6\ N\ cm^{-2}$ compared with $21{\cdot}4–26{\cdot}1\ N\ cm^{-2}$ for Antarctic fish at 0 °C (Table 2). In contrast, unloaded contraction velocities at low temperatures are not significantly higher for cold- than for warm-water fish. For example, at 0 °C, white muscle V_{max} is about $1\ L_0\ s^{-1}$ for both *C. aceratus* (Table 2) and for various Hawaiian Reef fish (environmental temperature 15–28 °C) (Johnston & Brill, 1984).

Woledge (1968) studied the thermal and mechanical properties of tortoise rectus femoris muscle and obtained evidence that a more curved P-V relationship was associated with a more efficient conversion of chemical energy into work. Values of a/P_0 for white fibres from Antarctic fish (Table 2) are similar to that reported for cod ($0{\cdot}21P_0$) at 8 °C (Altringham & Johnston, 1982) but are somewhat higher than for Pacific blue marlin at 25 °C ($a = 0{\cdot}14P_0$) (Johnston & Salamonski, 1984). For muscles with identical values of V_{max} and P_0, a higher a/P_0 value (less curved P-V relationship) might be expected to result in an increased power output at the expense of a decreased isotonic efficiency.

It would appear that relatively high levels of contractile performance at temperatures near freezing have been achieved at the expense of the flexibility to operate over a wider range of temperatures. For example, skinned muscle fibres isolated from *Notothenia neglecta* (South Orkneys, water temperature −1 °C) fail to relax completely following activations in excess of 12–15 °C and contract spontaneously in relaxing solution (pCa 7·4) at temperatures above 22 °C (Gleeson, Johnston, Sidell & Stephens, 1983). In contrast, skinned fibres from skipjack tuna (normal muscle temperature 15–32 °C) show stable Ca^{2+}-regulation up to 35 °C (Johnston & Brill, 1984). Similarly, myosins from Antarctic and other cold-water species are unstable on isolation, readily undergoing an aggregation reaction with concomitant loss of ATPase activity (Connell, 1961; Johnston, Walesby, Davison & Goldspink, 1974).

Activities of phosphofructokinase (PFK) obtained for red muscles were less than for hexokinase and are unlikely to reflect maximal activities *in vivo*. Both PFK and lactate dehydrogenase activities for white muscles (Table 3) are towards the lower end of the range reported for teleost fish (Johnston & Moon, 1981). This, together with the low concentrations of lactic acid found in the blood of *C. aceratus* at rest

(0·22–0·66 mmol l^{-1}) and after severe hypoxic stress (2·7–4·0 mmol l^{-1}) (Hemmingsen & Douglas, 1977), suggest a relatively modest capacity for anaerobic glycolysis in white muscle. Since maximum mechanical power outputs for this tissue are similar to other fish (Table 2), this suggests that the fuel for sprint activity is likely to be phosphagen based. Typically, the concentrations of phosphocreatine in teleost muscle are around 20 mmol kg^{-1} (Guppy *et al.* 1979; Walesby & Johnston, 1980*b*). Assuming a $\Delta G°$ for ATP hydrolysis equivalent to 60 kJ mol^{-1}, phosphocreatine stores in notothenioid white muscle would be sufficient for 50–100 s activity at maximum power output. This would permit a large number of sprints of short duration without having to switch over to anaerobic glycolysis. Observations on the swimming behaviour of Antarctic fish of similar body-form and habits to those in the present study are consistent with this pattern of white fibre recruitment. For example, when swimming at top speed, *Pagothenia borchgrevinki* adducts its pectoral fins and adopts a subcarangiform mode of locomotion. Typically, this type of swimming only lasts for a few tailbeat cycles followed by a glide, giving way to either labriform locomotion or another period of subcarangiform swimming (Montgomery & Macdonald, 1984). It is also of interest that the maximum swimming speed recorded for 22·5-cm *P. borchgrevinki* at −1 °C was 4·9 body lengths s^{-1} at a tailbeat frequency of 5·7 Hz (Montgomery & Macdonald, 1984), which is comparable to the maximum swimming speeds of temperate fish employing a labriform mode of locomotion (Webb, 1973). The reduction of axial musculature found for *C. aceratus* (28% body weight) probably reflects its particular mode of prey capture and/or predator avoidance. *C. aceratus* held in tanks are observed to adopt a characteristic defensive posture on disturbance, lying motionless with their mouths fully gaped.

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