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# EFFECTS OF TEMPERATURE AND THERMAL ACCLIMATION ON CONTRACTILE PROPERTIES AND METABOLISM OF SKELETAL MUSCLE IN THE FLOUNDER (*PLATICHTHYS FLESUS* L.)

BY IAN A. JOHNSTON AND ALELE WOKOMA

*Gatty Marine Laboratory* and *Department of Physiology, University of St Andrews, St Andrews, Fife, Scotland KY16 9TS*

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SUMMARY

Flounder (*Platichthys flesus* L.) were acclimated in sea water for 1–2 months to either 5 °C or 23 °C (12 h light: 12 h dark photoperiod).

Single fast muscle fibres were isolated from anterior ventral myotomes and skinned with detergent (Brij 58). Fibres were maximally activated and force-velocity (P-V) characteristics determined by step tension releases using an isotonic lever. Unloaded shortening speed was independently determined using the slack-test method.

The contractile properties of flounder skinned fibres are not altered by temperature acclimation. Maximum isometric tension development has a low thermal dependence, $Q_{10} = 1{\cdot}2$, increasing from 145 kN $m^{-2}$ at 0 °C to 200 kN $m^{-2}$ at 25 °C. The force-velocity relationship becomes progressively less curved with decreasing temperature (higher values of Hill's constant $a/P_0$) such that the thermal dependence of contraction velocity is significantly less at loads for optimum power output ($Q_{10} = 1{\cdot}3$) than at zero load ($Q_{10} = 2{\cdot}0$). Values for $a/P_0$ are 0·27 at 0 °C, 0·12 at 10 °C and 0·08 at 25 °C. Reductions in the curvature of the P-V relationship with decreasing temperature may represent an important mechanism for stabilizing muscle power output at low temperatures.

Longer term metabolic adjustments to temperature were studied by determining maximal enzyme activities in fast and slow muscles (at 15 °C). Activities of marker enzymes for mitochondrial metabolism (cytochrome oxidase), aerobic glucose utilization (hexokinase) and fatty acid oxidation (carnitine palmitoyl transferase) are 1·5–2·8 times higher in muscles of cold-acclimated compared to warm-acclimated flounders. Increases in the activities of these enzymes with cold acclimation may serve to offset the effects of low temperature on aerobic ATP supply. Glycolytic enzyme activities (phosphofructokinase, lactate dehydrogenase), however, are similar at both acclimation temperatures.

The results are briefly discussed in relation to the ecology of the flounder and evolutionary strategies of temperature adaptation in teleosts.

Key words: skeletal muscle, temperature, teleost fish, contractile properties, metabolism, flounder.

## INTRODUCTION

In some cyprinid fish the effects on swimming performance of an acute decrease in temperature are partially offset by several weeks' acclimation to low temperature (Hazel & Prosser, 1974; Smit, van den Berg & Kijn-den Hartog, 1974). The mechanisms underlying this are complex and include changes in excitation-contraction coupling (Penney & Goldspink, 1980), contractile properties (Johnston, Davison & Goldspink, 1975; Sidell, 1980; Johnston, Sidell & Driedzic, 1985) and metabolic characteristics (Sidell, 1980; Johnston, 1982; Jones & Sidell, 1982).

In common carp acclimation from 23 to 7°C results in a two-fold increase in muscle unloaded contraction velocity and maximum isometric tension development at low temperatures (Johnston *et al.* 1985). Measurements of enzyme activities (Sidell, 1980; Johnston *et al.* 1985) and mitochondrial volume densities (Johnston, 1982) in a carp and goldfish suggest that adaptations in contractile performance are matched by concomitant increases in the potential for aerobic ATP production and increases in the capacity to utilize fatty acids as fuels (Johnston *et al.* 1985).

Similar metabolic changes have been observed with other teleost fish following temperature acclimation experiments, including mummichogs (*Fundulus heteroclitus*), chain pickerel (*Esox niger*) and striped bass (*Morone saxatilis*) (Jones & Sidell, 1982; Kleckner & Sidell, 1985; Moerland & Sidell, 1985). However, it is something of a paradox that in none of these cases is there any evidence for changes in the properties of the contractile proteins with acclimation (Sidell, Johnston, Moerland & Goldspink, 1983; Sidell & Johnston, 1985).

The flounder (*Platichthys flesus* L.) is a common coastal European fish living from 55 m to the tide line, and also penetrating into rivers and lakes in communication with the sea. It is a relatively eurythermal species, tolerating temperatures from 0 to 25°C (Duthie & Houlihan, 1982). In the present study, acclimation of flounders to temperatures towards the extreme of their natural range did not alter contractile properties. Instead changes in the curvature of the force-velocity relationship are described which may serve to stabilize power output at lower temperatures.

## MATERIALS AND METHODS

### *Fish*

A group of 12 flounders (*Platichthys flesus* L.) (303 ± 38 g body weight, 25·9±1·2 standard length; mean ± S.E.) were acclimated in tanks of filtered sea water to either 5°C or 23°C for 5–9 weeks. They were subject to a 12 h light: 12 h dark photoperiod and fed daily on live ragworms and lugworms.

### *Isolation of muscle fibres*

Strips of white muscle were isolated from ventral myotomes 3–5 (counting from the head). Small bundles of fibres were transferred to a cooled (0°C) dissection stage

and single fibres isolated under silicone oil (BDH MS 550) using a binocular microscope (×35).

### *Experimental solutions*

Single fibre segments were chemically skinned after mounting on the apparatus (see below) in a 1 % solution of Brij 58 (polyoxyethylene 20 cetyl ether) dissolved in relaxing solution (in mmol $l^{-1}$): imidazole-HCl, 20; EGTA (ethyleneglycol-bis-($\beta$-aminoethyl ether) *N*, *N'*-tetraacetic acid), 10; phosphocreatine, 10; ATP, 5; $MgCl_2$, 6·8; KCl, 110; and 20 units $ml^{-1}$ crystalline creatine phosphokinase. The pH of solutions was adjusted to 7·2 at 20°C (with HCl) and allowed to vary freely with temperature such that it closely followed the $\Delta pH/\Delta T$ relationship of the imidazole buffer (pH was 7·58 at 0°C and 7·11 at 25°C).

Activating solution was made by addition of $CaCl_2$ (1 mol $l^{-1}$ volumetric solution) to relaxing solution to give a final concentration of 10–12 mmol $l^{-1}$. Free ion concentrations were calculated with the aid of an iterative computer programme (based on Fabiato & Fabiato, 1979) using values for the apparent dissociation constant $K_{Ca.EGTA}$ of $3{\cdot}865\times10^6$ (7·1 at 22°C). Corrections were made for changes in temperature as described by Godt & Lindley (1982). Activating solution had the following composition at pH 7·2 and 20°C; pMg 3·01, pMgATP 2·29, pCa 3·37, ionic strength 0·18 mol $l^{-1}$ at 0°C. The free $Ca^{2+}$ concentration of solutions was found to be maximally activating at all temperatures.

### *Determination of mechanical properties*

Single fibre segments (~2 mm length, 90 $\mu$m diameter) were mounted between stainless steel hooks using Plexiglas/acetone glue (Altringham & Johnston, 1982). Muscle fibres were immersed in the first of three water-jacketed baths (temperature control ± 0·2°C) and skinned for 15–20 min in detergent. Sarcomere length of relaxed fibres was determined by laser diffraction and set to 2·3 $\mu$m. Fibre length and diameter were measured *in situ* using a graticule and high-power microscope. Force was measured with a silicon beam strain gauge (AME 801, Horten, Norway) with a resonant frequency of 3 kHz including fibre and hook, sensitivity was 0·5 mN $V^{-1}$ and baseline stability was 5 mV $h^{-1}$.

Unloaded contraction velocity ($V_{slack}$) was determined for maximally activated fibres using the slack-test method (Edman, 1979). Rapid (~1 ms) releases to abolish tension were given using a servo-system based on a loud-speaker coil (Johnston & Sidell, 1984). The time to take up the slack was recorded using a storage oscilloscope. Between each release the fibre was re-extended (~20 ms) to its original length. Unloaded contraction velocity was determined from a least-squares fit of a plot of the applied length change *versus* the time taken to redevelop tension (Fig. 1).

The force-velocity (P-V) relationship of fibres was determined by step tension releases using an isotonic lever (Fig. 2) (see also Altringham & Johnston, 1982). The balsa wood lever was pivoted about a moving coil galvanometer which was used to provide a series of after-loads. Contraction velocity was determined over the first

50 ms of release and the P-V relationship of individual fibres analysed using Hill's (1938) equation for muscle shortening:

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

where $P_0$ = maximum isometric tension, P = load, V = contraction velocity and $a$ and $b$ are constants.

*Determination of enzyme activities*

The entire red muscle strip from the ventral myotomes was dissected, minced and approximately 0·5 g taken for enzyme analyses. White muscle was sampled from an adjacent site to that used for mechanics experiments.

Tissue was homogenized in a ground glass homogenizer with ice-cold extraction buffer (1:10 w/v) containing 100 mmol $l^{-1}$ Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), 1 mmol $l^{-1}$ EDTA, 0·5 mmol $l^{-1}$ dithioerythritol (DTE), pH 7·4. DTE was omitted for determinations of carnitine palmitoyl transferase. Maximal activities of the following enzymes: hexokinase, (EC 2.7.1.1.); 6-phosphofructokinase, (EC 2.7.1.11); lactate dehydrogenase, (EC 1.1.1.27); cytochrome c oxidase (EC 1.9.3.1.) and carnitine palmitoyl transferase (EC 2.3.1.23) were determined spectrophotometrically (15 ± 0·2 °C) at saturating substrate and cofactor concentrations. Controls were performed in the absence of substrate and any 'background' activity subtracted from the measured reaction rate. Details of the assay conditions used are given in Johnston *et al.* (1985).

*Statistical analyses*

Measurements of $P_0$, $V_{slack}$ and $V_{max}$ were made on 2–3 fibres per fish at each of the temperatures studied. Results obtained for fish acclimated to either 5 °C or 23 °C were compared using a one-way analysis of variance.

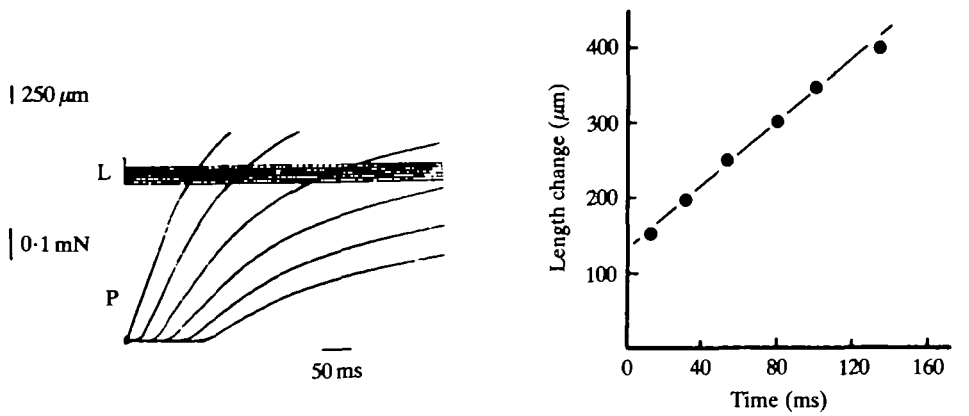


Fig. 1. Slack-test determination of unloaded contraction velocity ($V_{slack}$). A typical record from a single fibre segment, maximally activated at 0 °C, is shown on the right. $V_{slack}$ was determined from a plot of the applied length change (L) *versus* the time required to take up the slack (left). P = redevelopment of tension (up).

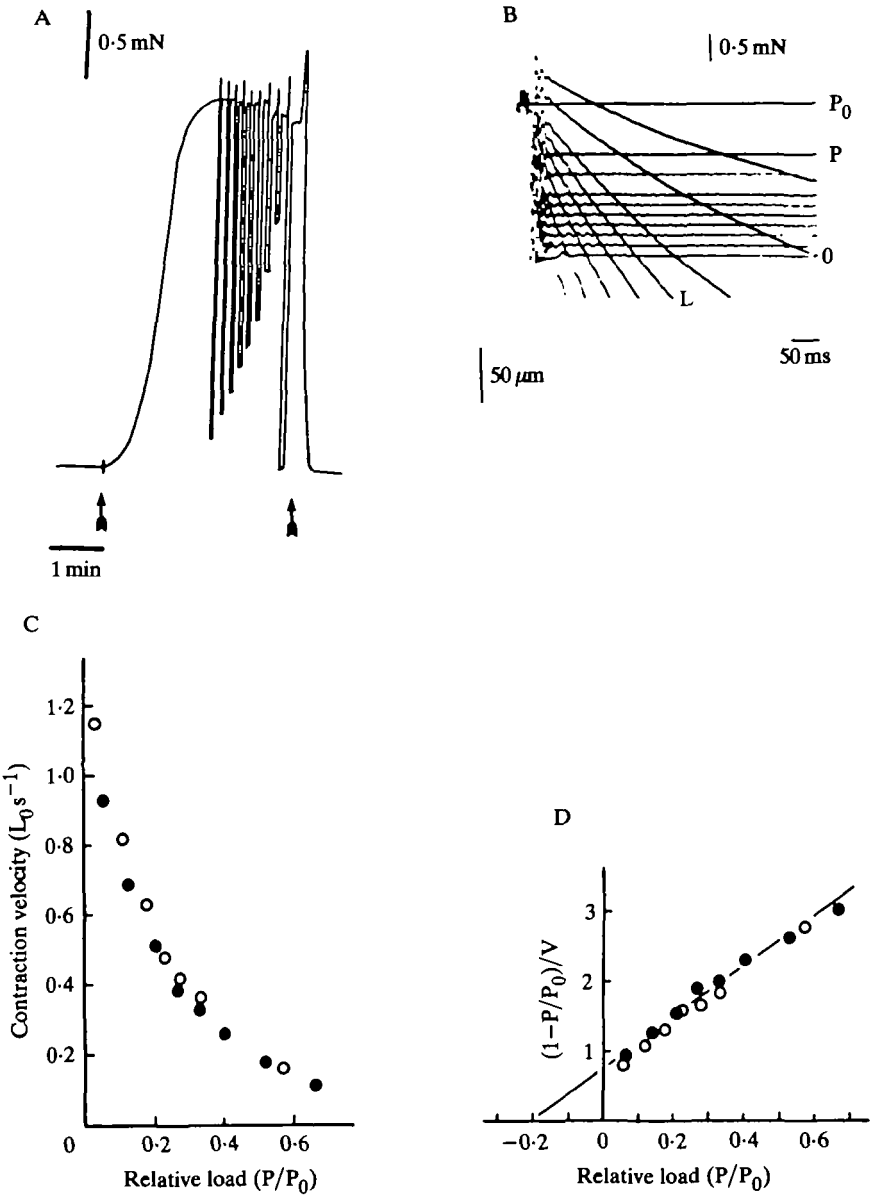


Fig. 2. Typical results from a single fast fibre segment at 0°C illustrating the method for determining force-velocity characteristics. (A) Record of an isometric contraction showing nine isotonic releases against a series of different after-loads. Arrows indicate transference between relaxing and activating solutions and *vice versa*. (B) Isotonic velocity transients following a series of step tension releases (same activation as shown in A but on a faster time-base, note the transient at zero load is off-screen). $P_0$ = maximum isometric force; L = isotonic velocity transients; 0 = zero force baseline. (C) Force-velocity curve of the data obtained in record B (solid circles). Points are also included from a second activation of the same fibre segment (open circles). (D) Data were 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. $L_0$ = initial muscle length.

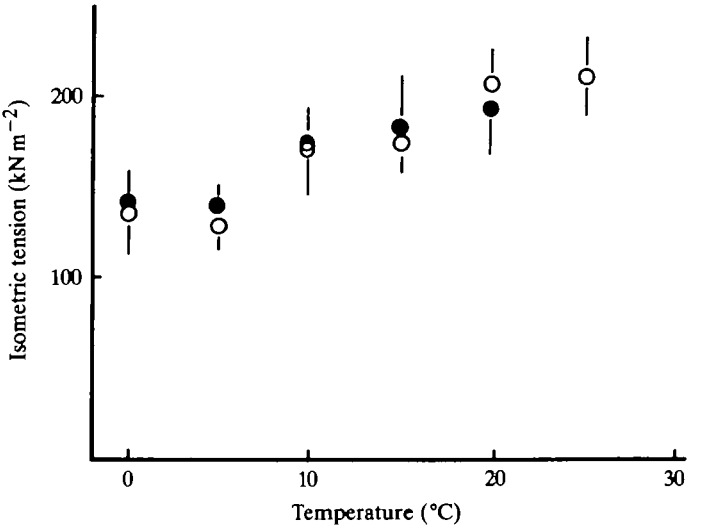


Fig. 3. Effects of temperature on maximum isometric tension development (kN m$^{-2}$) by single skinned fast fibre segments isolated from the myotomal muscles of 5°C-acclimated (open circles) and 23°C-acclimated (closed circles) flounders. Points represent mean ± S.E. of between 8 and 12 fibres isolated from 4–6 fish.

## RESULTS

### *Contractile properties*

No significant difference was found in the mechanical properties (maximum isometric tension, unloaded contraction velocity, force-velocity characteristics) of muscle fibres isolated from flounders acclimated to either 5°C or 23°C (Figs 1, 2). Muscle fibres in the flounder relaxed completely following 2–3 min activations at temperatures up to 25°C.

Maximum isometric tension development ($P_0$) increased from around 140 kN m$^{-2}$ at 0°C to 210 kN m$^{-2}$ at 25°C ($Q_{10} = 1{\cdot}2$; Fig. 3). Measurements of unloaded contraction velocity extrapolated from the force-velocity relationship were found to be in close agreement with those determined by the slack-test method (Fig. 4). $V_{max}$ (muscle lengths s$^{-1}$; $L_0$ s$^{-1}$) increased from 1·6 at 0°C to 5·8 at 25°C. A plot of log $V_{max}$ *versus* temperature (not shown) was found to be linear over the entire range, equivalent to a $Q_{10}$ of around 2·0.

The force-velocity relationship becomes progressively more curved with decreasing temperature (higher values of $a/P_0$) (Fig. 5). Hill's constant $a/P_0$ is 0·27 at 0°C, 0·12 at 10°C and 0·08 at 25°C ($P<0{\cdot}01$). For a given load power output increases as the P-V curve becomes less curved. The net result is that for flounder changes in the curvature of the P-V relationship partially compensate for the reduction in contraction speed as temperature is decreased. Thus contraction velocity at loads for maximum power output is much less temperature dependent ($Q_{10} = 1{\cdot}3$) than for $V_{max}$, being equivalent to 0·4 $L_0$ s$^{-1}$ at 0°C, 0·54 $L_0$ s$^{-1}$ at 10°C and 0·63 $L_0$ s$^{-1}$ at 25°C. Maximum power output has a similar low thermal dependence ($Q_{10} = 1{\cdot}2$) (Fig. 5).

*Enzyme activity measurements*

The activities of some enzymes of intermediary metabolism in red and white myotomal muscles are shown in Fig. 6. Marker enzyme activities of mitochondrial

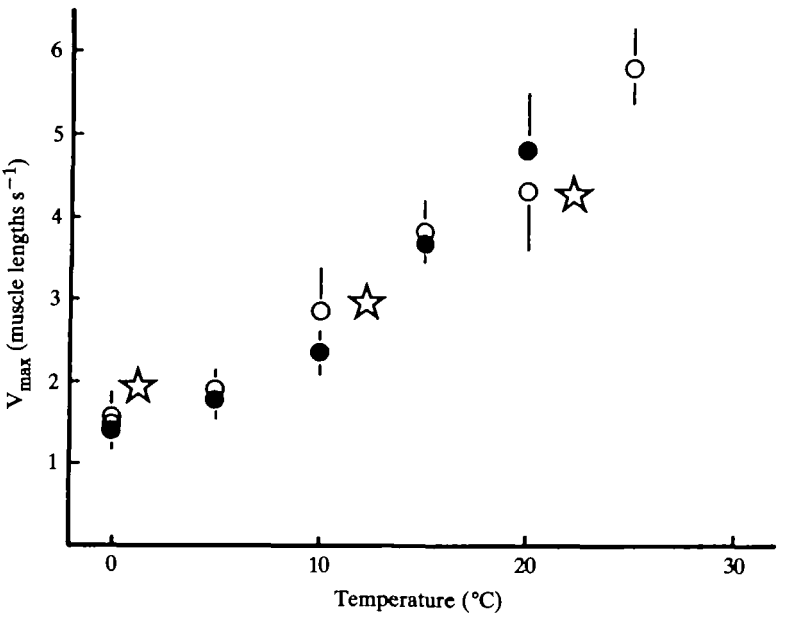


Fig. 4. Effects of temperature on unloaded contraction velocity ($V_{max}$) of single skinned fibres isolated from 5°C-acclimated (open circles) and 23°C-acclimated (closed circles) flounders. Points marked with stars were extrapolated from the force-velocity relationship. All other data were obtained using the slack-test method illustrated in Fig. 1. Points represent mean ± S.E. of between 8 and 12 fibres isolated from 4–6 fish.

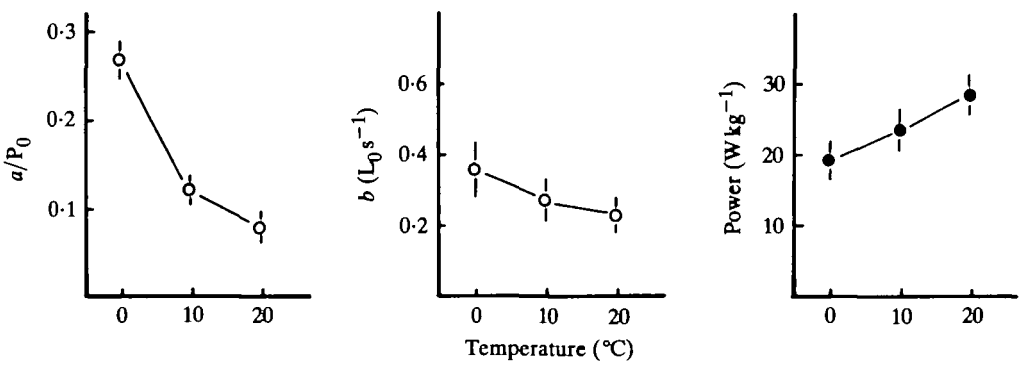


Fig. 5. Effect of temperature on force-velocity characteristics of single skinned fibres isolated from fast myotomal muscle of the flounder. Graphs show Hill's constants $a/P_0$ and $b$ together with power development at optimal load which correspond to $[a^2+a/P_0]^{1/2}-a$ (obtained from Hill's equation). Data illustrated represent 6–8 fibres isolated from four cold-acclimated flounders; similar results were obtained for warm-acclimated fish. Typically transformed data from individual fibres had at least 14 degrees of freedom.

metabolism (cytochrome oxidase), aerobic glucose utilization (hexokinase) and fatty acid oxidation (carnitine palmitoyl transferase) are 1·6 to 2·8 times higher in muscles of cold-acclimated than warm-acclimated fish. Glycolytic enzyme activities (6-phosphofructokinase, lactate dehydrogenase) are not significantly altered by temperature acclimation in either muscle type.

## DISCUSSION

Temperature has a direct effect on a number of the extrinsic (fibre recruitment patterns, hormone titres) and intrinsic factors determining muscle performance (properties of myosin crossbridges, excitation-contraction coupling mechanisms, metabolism etc.). The extent to which the temperature dependence of individual parameters is important in establishing activity patterns is unclear. Skinned muscle fibres provide a useful preparation for investigating the effects of temperature on the mechanical properties of contractile proteins in isolation from nerve and membrane effects (see Johnston, 1985*a*).

Myosins from polar fishes are able to generate relatively high isometric tensions at low temperatures (Johnston & Harrison, 1985) but are unstable at high temperatures

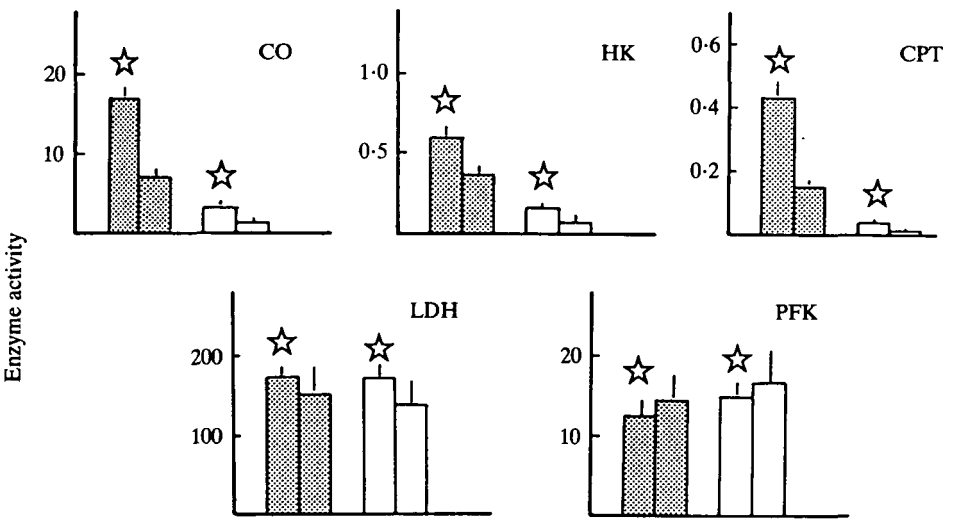


Fig. 6. Maximal activities (determined at 15 °C) of enzymes of energy metabolism in slow (red) (stippled bars) and fast (white) (clear bars) myotomal muscles of the flounder. Results from 5 °C- (stars) and 23 °C-acclimated flounders (no stars) represent data from at least six fish at each acclimation temperature. Abbreviations: CO, cytochrome c oxidase; HK, hexokinase; CPT, carnitine palmitoyl transferase; PFK, 6-phosphofructokinase; LDH, lactate dehydrogenase. Activities are expressed as $\mu$mol substrate utilized $g^{-1}$ wet weight tissue $min^{-1}$ and represent mean ± S.E. Activities of CO, HK and CPT in slow muscle and HK and CPT in fast muscle are significantly greater for cold- than for warm-acclimated fish ($P < 0{\cdot}01$). The remaining enzyme activities were not significantly different ($P > 0{\cdot}05$) between the two groups of fish.

(Johnston *et al.* 1975). For example, skinned fibres isolated from the Antarctic icefish, *Chaenocephalus aceratus*, contract spontaneously in relaxing solution ($[Ca^{2+}] = 10^{-9}$ mol $l^{-1}$) when the temperature is raised to 22°C. At temperatures above 10°C they fail to relax completely following short activations at maximal $[Ca^{2+}]$ resulting in a $Ca^{2+}$-insensitive force component (Johnston, 1985*b*). The development of residual tension is associated with proportional increases in instantaneous stiffness and very low contraction velocities suggesting the formation of abnormal crossbridge linkages (Johnston & Altringham, 1985). In contrast, skinned fibres from tropical fish develop very low maximal tensions at 0°C but do not show the development of residual tension at temperatures up to 30°C (Johnston & Brill, 1984; Johnston & Altringham, 1985). In carp, contractile properties are modified by temperature acclimation: residual tension development is pronounced at high temperatures in fibres isolated from 7°C- but not 23°C-acclimated individuals (Johnston *et al.* 1985). The contractile properties of flounder skinned fibres are independent of acclimation temperature (Figs 3, 4). Maximum isometric tension at 0°C is 2–3 times that for homologous fibre types in tropical species but only 55% of those for Antarctic fish (Johnston & Harrison, 1985). In contrast to fibres from many cold-water teleosts (Johnston & Altringham, 1985) those from flounder relax completely following short activations at temperatures up to 25°C. The contractile proteins have properties that appear to represent a compromise between those optimal for the extremes of its thermal range. This would obviate the need for changes in the proteins with acclimation and may represent a general evolutionary strategy for fish that can tolerate sudden changes in body temperatures. Support for this idea comes from studies of the ATPase activity and $Ca^{+}$-sensitivity of myofibrillar suspensions from muscles of the mummichog, *Fundulus heteroclitus*. This species inhabits coastal saltmarshes and during summer tidal cycles may experience rapid temperature changes between 15°C and 30°C. In common with flounder, there are no changes in the characteristics of myofibrillar proteins with temperature acclimation and the ATPase has a low thermal dependence over the range 12–35°C (Sidell *et al.* 1983).

The changes in the curvature of the P-V relationship with temperature found for flounder muscle fibres (Fig. 5) may provide an intrinsic mechanism which serves to stabilize power output at different temperatures. A decrease in temperature from 20 to 0°C would result in a 75% decrease in unloaded contraction velocity but only a 31% fall in maximum power output (Figs 3, 5). A decrease in Hill's constant $a/P_0$ with increasing temperature has also been observed for skinned fibres from icefish and marlin but over a narrower range of temperatures (Johnston & Altringham, 1985). Interspecific variations in the shape of the force-velocity curve should therefore be considered along with maximum tension development and contraction speed in assessing temperature adaptation of fish myosins.

It seems likely that changes in central patterns of muscle fibre recruitment may also play a role in stabilizing locomotory performance following acute changes in body temperature. For example, in carp the threshold speed at which fast motor units are recruited decreases as the temperature is lowered from 20 to 10°C (Rome,

Loughna & Goldspink, 1984). This suggests that more muscle fibres are recruited in order to swim at the same speed following a step decrease in temperature.

In flounder, following a period of cold-acclimation, the activities of enzymes associated with aerobic ATP turnover and fatty acid catabolism increase (Fig. 6). There is evidence from a variety of other species that the mechanism underlying this metabolic restructuring is an increase in the volume density of mitochondria (Johnston, 1982; Sidell, 1983; Tyler & Sidell, 1984). Sidell (1977) investigated the rate of turnover of cytochrome c in the green sunfish *Lepomis cyanellus* and found that a reduction in body temperature resulted in a smaller decrease in its rate of degradation than synthesis, leading to a net increase in protein concentration, presumably reflecting changes in mitochondrial turnover. Expansion of aerobic metabolism by such a mechanism may require several weeks (Sidell, Wilson, Hazel & Prosser, 1973). Determinations of optimal enzyme activities provide information on maximal metabolic potentials of tissues but do not tell us anything about the fluxes of substrates under different physiological states. The flux of carbon through metabolic pathways is thought to be integrated and controlled *via* modulation of the activity of regulatory enzymes by $H^+$, adenylates, substrates, products and other metabolites (Newsholme & Start, 1973). One possibility is that the concentrations of these modulators change following an acute drop in body temperature in such a way as partially to offset the direct effects of temperature on enzyme reaction rate (Walesby & Johnston, 1980; Walsh & Somero, 1982). This may serve to reduce the temperature dependence of the ATP-generating pathways providing an immediate temperature compensation mechanism to parallel that described for the contractile proteins (Fig. 5).

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