

Evolutionary adaptation of muscle power output to environmental temperature: force-velocity characteristics of skinned fibres isolated from antarctic, temperate and tropical marine fish

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Abstract. 1. Single fast fibres were isolated from the myotomal muscles of icefish (*Chaenocephalus aceratus* Lönnberg, Antarctica), North Sea Cod (*Gadus morhua* L.) and Pacific Blue Marlin (*Makaira nigricans* Wakiya, Hawaii). Fibres were chemically skinned with the non-ionic detergent Brij-58.

2. Maximum tensions (P_0 , kN m^{-2}) developed at the characteristic body temperature of each species are 231 for icefish (-1°C), 187 for cod (8°C) and 156 for marlin (20°C). At 0°C P_0 is 7 times higher for fibres from the icefish than from the marlin.

3. Fibres from icefish and cod failed to relax completely following activations at temperatures above approximately 12°C . The resultant post-contraction force is associated with a proportional increase in stiffness, suggesting the formation of a population of Ca-insensitive cross bridges.

4. At 0°C there is little interspecific variation in unloaded contraction velocity (V_{\max}) among the three species. V_{\max} (muscle lengths s^{-1}) at normal body temperatures are 0.9 for icefish (-1°C), 1.0 for cod (8°C) and 3.4 for marlin (20°C).

5. The force-velocity (P-V) relationship becomes progressively more curved with increasing temperature for all three species.

6. Maximum power output for the fast muscle fibres from the Antarctic species at -1°C is around 60% of that of the tropical fish at 20°C . Evolutionary temperature compensation of muscle power output appears largely to involve differences in the ability of cross bridges to generate force.

Key words: Muscle – Temperature – Mechanics – Force-velocity – Skinned fibres – Antarctic fish

Introduction

In contrast to reptiles and amphibians, which usually become torpid at temperatures approaching 0°C , many species of fish remain active. Cold-adaptation is associated with selective changes in myosin structure resulting in proteins which, on isolation, have labile ATPase activities, and are susceptible to aggregation (Connell 1961; Johnston et al. 1975; Johnston 1982). The mechanisms underlying compensation of mechanical performance in fish muscles at

low temperature are poorly understood. Recently we have utilised skinned fibre preparations to study the effects of temperature on the mechanical properties of the contractile proteins directly (Johnston and Brill 1984; Johnston and Salamonski 1984; Johnston and Harrison 1985; Johnston 1985a). Preliminary observations using such preparations suggest that at 0°C maximum tensions, but not unloaded contraction velocities, are consistently higher for muscle fibres isolated from cold than warm adapted fish species (Johnston and Brill 1984). Small adjustments in the curvature of the force-velocity (P-V) relationship could, in theory, provide a mechanism for increasing power output at low temperature. The effects of temperature on the P-V relationship of the muscles of ectotherms have been little studied. Lannergren (1978) found no change in the curvature of the P-V relationship of twitch fibres from the tropical toad, *Xenopus laevis* between $5-20^\circ\text{C}$.

Visits to Antarctica and Hawaii during 1984 presented us with the opportunity to study the effects of temperature on the force-velocity characteristics of muscles from fish adapted to widely different temperatures. Antarctic fish are of special interest in that they provide an extreme, since their body temperatures are below 0°C for most of the year, and a variety of special adaptations are present to prevent freezing (see Clarke 1983, for review).

In the course of our studies it was noticed that at temperatures above $10-12^\circ\text{C}$ skinned fibres from Antarctic fish failed to relax completely following even short activations. The nature of this residual, Ca-insensitive force was therefore also investigated, in a locally available temperate fish, the North Sea cod (*Gadus morhua*). A preliminary account of part of this work has been published (Johnston 1985b).

Materials and methods

Fish. Icefish (*Chaenocephalus aceratus* Lönnberg), 6 fish, $1,035 \pm 72$ g bodyweight (all values are mean \pm SE), 53.4 ± 1.4 cm total length, were caught with trammel nets in Normana Strait, Signy Island, South Orkneys, British Antarctic Territories ($60^\circ 43' \text{S}$, $45^\circ 36' \text{W}$) during Austral summer, 1983/84. Mean sea temperature was -1°C . Fish were maintained for up to 5 days, without food in a recirculated seawater aquarium at $0-2^\circ\text{C}$.

Pacific Blue Marlin (*Makaira nigricans* Wakiya), 15 fish, 84.1 ± 8.3 g bodyweight, were obtained during the 26th

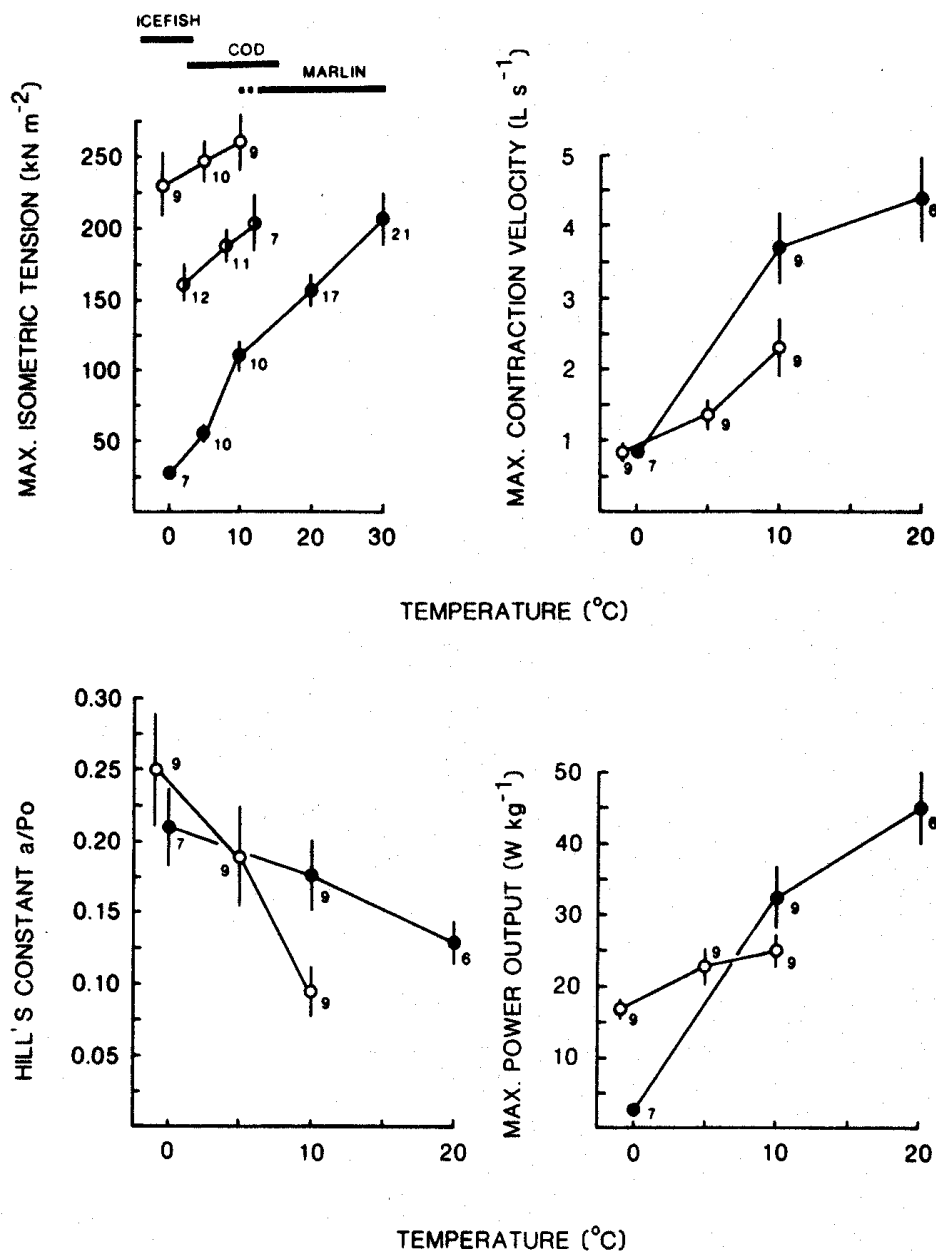


Fig. 1a-d

The effects of temperature on tension development, maximum contraction velocity, Hill's constant a/P_o and maximum power output, by skinned fibres isolated from fish fast muscles. Open circles: icefish; half-filled circles: cod; filled circles: marlin. Values are mean \pm SE; numbers: number of fibres. The normal range of body temperatures experienced by the animal are shown in a

Hawaiian International Billfish Angling Tournament, held at Kailua-Kona, Hawaii during August 1984. Muscle biopsies were taken from freshly caught specimens within 15–40 min of boating, and transferred on ice to the nearby Pacific Gamefish Foundation Research Laboratory.

North Sea Cod (*Gadus morhua* L.) were caught by local fishermen in the Firth of Forth, East Scotland (5 fish, 52.6 ± 6 cm, 1.86 ± 0.6 kg), and maintained in filtered sea water aquaria at 10°C for up to 5 days before use.

Muscles. Small bundles of fast fibres were isolated from the dorsal myotomal muscles, from a point approx. 0.25 of the trunk length from the head of cod and icefish, and 0.85 of the trunk length for marlin. Single fibres were isolated under silicone oil at $0-5^\circ\text{C}$.

Solutions. Relaxing solution contained 5 mM EGTA, 3.2 mM MgCl_2 , 2.5 mM ATP, 10 mM phosphocreatine, 110 mM KCl, 20 mM imidazole-HCl buffer pH 7.2 at 20°C . Activating solutions were made by the addition of CaCl_2 to produce maximal force (5–5.5 mM final concentration). All solutions were maximally activating over the entire

temperature range. The ionic composition of the solutions was determined using an iterative computer programme based on that of Fabiato and Fabiato (1979), with corrections for temperature effects on stability constants (Godt and Lindley 1982). The concentrations of important ionic species were as follows: pCa 4.60–4.11, pMgATP 2.66, pMg 3.08, ionic strength 0.18 M. Creatine phosphokinase (>40 units ml^{-1}) was added to all solutions immediately prior to experimentation. The pH of the solutions was allowed to follow the dissociation constant of the imidazole buffer, and varied from 7.58 at -1°C to 7.02 at 30°C . This conforms to the $\Delta\text{pH}/\Delta\text{T}$ relationship established for physiological fluids (Reeves 1977). However preliminary experiments established that at saturating calcium concentrations P_o and V_{max} are not significantly affected by pH over the range 7–8.

Force velocity measurements. Full details of the methods have been published previously (Altringham and Johnston 1982). Single fibre segments (1.5–3.5 mm in length, 50–120 μm in diameter) were mounted between two stainless steel hooks, one attached to a strain gauge (AME 801;

sensitivity 0.5 mN V^{-1} , resonant frequency 2 kHz with hook attached), the other to an isotonic lever. Sarcomere length, measured by laser diffraction, was set to $2.3 \mu\text{m}$, and fibre length and diameter measured in situ. In calculating cross-sectional area, fibres were assumed to be circular; fibres deviating visibly from circularity were not attached to the apparatus. Fibres were skinned by a 20 min incubation in relaxing solution containing 1% Brij 58 (polyoxyethylene 20 cetyl ether), before being transferred to relaxing solution at the experimental temperature. During each of 2–3 maximal activations, 8–10 isotonic releases were given. Force-velocity data were fitted to a linear form of Hill's (1938) equation: $v(P+a) = b(P_0 - P)$, in which P = force, P_0 = maximum isometric force, v = velocity of shortening, and a and b are constants.

Stiffness measurements. Rapid length changes of 1% muscle length in $300 \mu\text{s}$ were given, using a servo driven loudspeaker coil. The "instantaneous" force at the end of the length change is thought to be a measure of the proportion of cross bridges bound to actin, and the early recovery a measure of the reorientation of bound bridges (Huxley and Simmons 1971).

Results

Effects of temperature on maximum isometric tension

The effects of temperature on maximum tension in the three species studied are summarised in Fig. 1a. At 0°C P_0 is 7 times higher for fibres from the icefish than from the marlin ($P < 0.001$, standard t -test), and values for cod are intermediate (Fig. 1a). Maximum tensions for fast muscle fibres decrease with increasing characteristic body temperature: 240 kN m^{-2} for icefish, *C. aceratus* (0°C), 204 kN m^{-2} for cod, *G. morhua* (12°C), and 180 kN m^{-2} for marlin, *M. nigricans* (25°C). Below 10°C , fibres from *M. nigricans* were significantly more temperature sensitive ($Q_{10} = 3.3$) than those from *G. morhua* ($Q_{10} = 1.3$) and *C. aceratus* ($Q_{10} = 1.1$), but over the physiological range for the marlin, temperature sensitivity was similar ($Q_{10} = 1.3$) to that of the other two species.

Effects of temperature on Ca-insensitive, post-activation force

The temperature dependence of the Ca-insensitive force is illustrated in Fig. 2a, which shows data from a cod fast muscle fibre. At 2°C , the fibre relaxes fully after each activation, but at 12°C the fibre relaxes to a lesser extent after successive activations. The magnitude of the Ca-insensitive force, although variable from fibre to fibre (Fig. 2b), was related to the total time in the activated state. Between 17 – 21°C , cod fibres often underwent spontaneous contraction in relaxing solution, and did not relax after activation. These effects are all irreversible. A similar response was seen in icefish fast fibres, but at somewhat lower temperatures. Marlin fast fibres developed Ca-insensitive, post contraction forces only at temperatures above 30°C .

It is interesting to note that slow fibres in all three species did not show Ca-insensitive force until the temperature was raised approximately 10°C above that at which it was first seen in fast fibres (unpublished observations). Rapid stretches and releases of 1% muscle length, during experiments similar to that illustrated in Fig. 2a, demonstrated

that the decline in Ca-activated force at 12°C is associated with a proportional decline in active (total) stiffness, and the increase in Ca-insensitive force by a proportional increase in resting stiffness. The $t_{1/2}$ for recovery of active force after stretch increased with increasing levels of Ca-insensitive force. These results are illustrated for a typical fibre in Fig. 2c.

Effects of temperature on the P-V relationship

The results are summarised in Fig. 1b–d, and P-V curves for all three species at 0°C are shown in Fig. 3. At around 0°C , maximum contraction velocities are similar for the three species, at around one muscle length s^{-1} (L s^{-1}). The Q_{10} 's for V_{max} over the physiological ranges are 2.0 for the icefish (-1 to 5°C), 1.8 for cod [$V_{\text{max}} = 0.68 \pm 0.06 \text{ L s}^{-1}$ at 2°C (mean \pm SE, Fig. 3) and 1 L s^{-1} at 8°C (Altringham and Johnston 1982)] and 1.2 for the marlin (20 – 30°C). Between 0 – 10°C , the Q_{10} rises to 4.3 in the marlin. In both marlin and icefish, a/P_0 decreases with increasing temperature. A similar trend can be seen in cod, where an a/P_0 value of 0.34 ± 0.04 (mean \pm SE) was obtained at 2°C , compared to 0.21 at 8°C in a previous study (Altringham and Johnston 1982). Measured in the physiological range, the P-V relationship becomes less curved (higher a/P_0) as the characteristic body temperature decreases, i.e. in the order marlin > cod > icefish.

Discussion

Interspecific differences in the temperature dependence of contractile properties are clearly related to the characteristic body temperature of each species (Fig. 1). Stephenson and Williams (1981) investigated maximal force production by fast and slow skinned fibres from the rat, over a wide range of temperature. Whilst P_0 was relatively independent of temperature between 20 – 35°C , it declined rapidly to zero at 0°C . Similarly, maximum tetanic tension for whole muscles from several species of sub-tropical lizard is approximately constant between 15 – 35°C , but declines with a Q_{10} of 2–3 below 15°C (Putnam and Bennett 1982). In contrast, P_0 for skinned fibres from the Northern Leopard Frog (*Rana pipiens*) (Godt and Lindley 1982) and various cold water fish (Johnston and Brill 1984; Johnston and Sidell 1984), decrease very little as the temperature is lowered to 0°C .

At 0°C , maximum mechanical power output by skinned fibres isolated from marlin fast muscle is only 10% of that of the Antarctic species (Fig. 1). However, when compared at the characteristic body temperature of each species, power outputs are more similar. For example, power output by icefish fibres at -1°C is around 50%–60% of that for marlin fibres at 20°C (Fig. 1d). We interpret these results in terms of evolutionary adaptation in the structure of the contractile proteins which compensate for the direct effects of temperature on one or more steps of the cross bridge cycle. In teleost fish such evolutionary adjustment in mechanical power output at low temperatures largely involves changes in the ability of cross bridges to generate force (Fig. 1, Johnston and Brill 1984).

Differences in the temperature dependence of tension, contraction velocity and ATPase activity have been reported for a variety of muscles (Fig. 1; Kuhn et al. 1979; Johnston and Gleeson 1984). This implies that rate constants for the

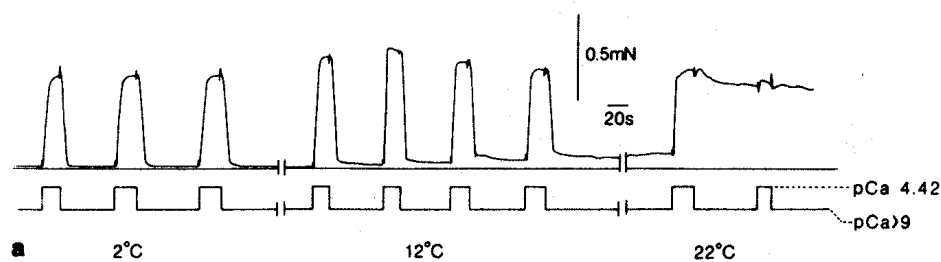


Fig. 2a-c

The effects of temperature on resting tension in cod fibres. **a** Isometric force record from a single fibre (diameter = 63 μm) at three temperatures, showing the progressive development of Ca-insensitive force at 12°C, and irreversible activation at 22°C. **b** Pooled data from six experiments, maximum isometric tension (closed circles) and Ca-insensitive tension (open circles), expressed relative to the initial tension for each fibre. Data are means \pm SE ($n = 6$). **c** Relative tension (\bullet), relative stiffness after stretch (\blacktriangle) and release (\blacktriangledown) and $t_{1/2}$ for recovery from stretch (\blacksquare) during maximum activation from four activations at 12°C in a cod fibre. After each contraction resting tension (\circ) and stiffness (Δ , ∇) were measured, and plotted with the data from the previous activation

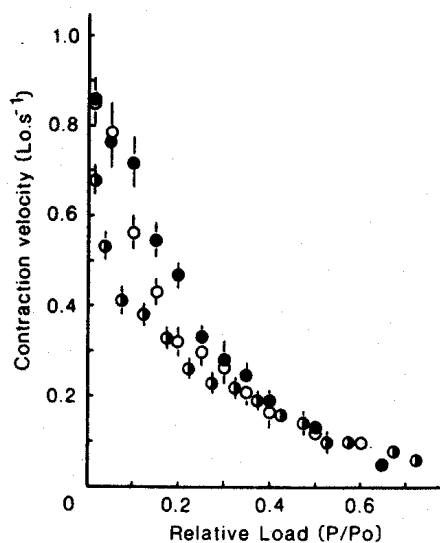


Fig. 3. Force-velocity curves of maximally activated skinned muscle fibres at 0°C. *Open circles*: icefish (-1°C , $n = 9$); *half-filled circles*: cod (2°C , $n = 6$); *filled circles*: marlin (0°C , $n = 9$). Data are mean \pm SE

various steps in the cross bridge cycle do not have the same dependence on temperature. Differences in the absolute force produced at a given temperature, and in the temperature dependence of P_0 , can be explained by differences in the number of bound cross bridges and the relative rates of cross bridge attachment and detachment respectively. Indirect evidence for the first point has been obtained by Stephenson and Williams (1981) for rat skinned fibres. They found that rigor force was 15–20 times lower at 5°C than

at 20°C, irrespective of whether rigor was induced independently at each temperature, or induced at 5°C and the temperature raised. This result is consistent with the number of bound cross bridges increasing with temperature. If the number of bound cross bridges is to increase, it follows from the cross bridge model that the rate of attachment must be more temperature dependent than that of detachment.

For homologous fibre types V_{max} at 0°C is rather similar for fish with different characteristic body temperatures (Fig. 1). Thus, despite a wide range of body shapes and sizes, locomotory habits and ecologies, fast muscles from fish appear to have very similar unloaded cross bridge cycle times at 0°C. However, the results from the three species in the present study are consistent with adjustment in the curvature of the P-V relation being a contributing factor to interspecific adjustment of power output, though quantitatively less important than changes in P_0 . Increasing a/P_0 with decreasing temperature would result in an increase in velocity and hence power output at a given load. At the characteristic body temperature of each species, the magnitude of a/P_0 increases in the order marlin > cod > icefish. In contrast, in the rat the P-V relationship of intact soleus muscle increases in curvature as temperature is decreased from 35°C to 20°C ($a/P_0 = 0.21$ and 0.14 respectively, Ranatunga 1982), and in the extensor digitorum longus muscle a/P_0 decreases only slightly between 35°C ($a/P_0 = 0.42$) and 25°C ($a/P_0 = 0.39$) before declining to 0.28 at 20°C. It is of interest to note that although the direction of the change of curvature is different with temperature change, in both cases a/P_0 is high at the characteristic body temperature of the animal, i.e. at 37°C for the mammal, and at lower temperatures for the fish, thus maximising power output.

In the present study, Ca-insensitive, post-contraction force was observed at lower temperatures in the Antarctic and North Sea fish than in the tropical marlin. Ca-insensitive force was not found in fibres from the iliofibularis muscle of the desert iguana at temperatures up to 45°C (Johnston and Gleeson 1984). The decrease in P_0 with successive activations is associated with a proportional decrease in active stiffness, and presumably results from a decrease in the number of active cross bridges. Above around 10°C the decrease in active force is also associated with an increase in Ca-insensitive force and increased passive stiffness (Fig. 2) due to the cycling of cross bridges in the absence of Ca. The decrease in $t_{1/2}$ for force recovery after stretch with increasing Ca-insensitive force suggests that these cross bridges are in some way abnormal. Whilst the origin of this abnormal cross bridge formation is obscure, and its magnitude variable, it does appear to be correlated with characteristic body temperature and fibre type, and may reflect the interspecific differences in myosin structure reported by Johnston et al. (1975).

Evolutionary adaptations in contractile performance for function at low temperature are clearly complex, and will depend upon many factors extrinsic to the contractile proteins themselves. However, the results presented provide some insight into the importance of temperature as a selective influence on the evolution of vertebrate myosins. By exploiting these evolutionary differences, temperature may be a useful probe of contractile mechanisms.

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