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Thermal dependence of contractile properties of single skinned muscle fibres from Antarctic and various warm water marine fishes including Skipjack Tuna (*Katsuwonus pelamis*) and Kawakawa (*Euthynnus affinis*)

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Summary. Single fast fibres and small bundles of slow fibres were isolated from the trunk muscles of an Antarctic (*Notothenia neglecta*) and various warm water marine fishes (Blue Crevally, *Carangus melampygus*; Grey Mullet, *Mugil cephalus*; Dolphin Fish, *Coryphaena hippurus*; Skipjack-tuna, *Katsuwonus pelamis* and Kawakawa, *Euthynuus affinis*). Fibres were chemically skinned with the non-ionic detergent Brij 58.

For warm water species, maximum Ca^{2+}-activated tension (P_0) almost doubled between 5–20 °C with little further increase up to 30 °C. However, when measured at their normal body temperatures, P_0 values for fast fibres were similar for all species examined, 15.7–22.5 $N \cdot cm^{-2}$. Ca^{2+}-regulation of contraction was disrupted at temperatures above 15 °C in the Antarctic species, but was maintained at up to 30 °C for warm water fish.

Unloaded (maximum) contraction speeds (V_{max}) of fibres were determined by the "slack-test" method. In general, V_{max} was approximately two times higher in white than red muscles for all species studied, except Skipjack tuna. For Skipjack tuna, V_{max} of superficial red and white fibres was similar (15.7 muscle lengths $\cdot s^{-1}$ ($L_0 \cdot s^{-1}$)) but were 6.5 times faster than the V_{max} of internal red muscle fibres (2.4 ± 0.2 $L_0 \cdot s^{-1}$) (25 °C).

V_{max} for *N. neglecta* fast fibres at 0–5 °C (2–3 $L_0 \cdot s^{-1}$) were similar to that of warm water species measured at 10–20 °C. However, when measured at their normal muscle temperatures, the V_{max} for the fast muscle fibres of the warm water species were 2–3 times higher than that for *N. neglecta*.

In general, $Q_{10(15-30\,°C)}$ values for V_{max} were in the range 1.8–2.0 for all warm water species studied except Skipjack tuna. V_{max} for the internal red muscle fibres of Skipjack tuna were much more temperature dependent ($Q_{10(15-30\,°C)} = 3.1$) ($P < 0.01$) than for superficial red or white muscle fibres. The proportion of slower red muscle fibres in tuna (28% for 1 kg Skipjack) is 3–10 times higher than for most teleosts and is related to the tuna's need to sustain high cruising speeds. We suggest that the 8–10 °C temperature gradient that can exist in Skipjack tuna between internal red and white muscles allows both fibre types to contract at the same speed. Therefore, in tuna, both red and white muscle may contribute to power generation during high speed swimming.

Introduction

Myosins from cold-water fish are unstable and susceptible to aggregation on isolation compared to those prepared from tropical species (Connell 1961; Johnston et al. 1974). The greater susceptibility of myosin ATPase from Antarctic fish to thermal denaturation has been correlated with an increased enzyme activity at low temperatures and reduced free energy of activation relative to myosin ATPase from warmer water species (Johnston and Walesby 1977, 1979). Previous studies of temperature compensation of contraction have dealt with the properties of isolated proteins in solution (see Clarke 1983, for a review). The extent to which the mechanical properties of muscles of animals

from widely different thermal environments are similar is unknown. There is evidence to suggest that the thermal dependence of biochemical systems in vitro does not reflect that of more highly organised systems in vivo. For example, MgCa-ATPase activity of Sculpin (*Myoxocephalus scorpius*) fast muscle myofibrils increases 8-fold between 0.5 and 20 °C, compared with an increase of only 2.5-fold for unloaded (maximum) contraction speed (V_{max}) (Johnston and Sidell 1984).

The present study was undertaken to examine the thermal dependence of muscle contraction speeds at the two extremes of body temperature found among fishes. Notothenioid fishes are restricted to South Polar seas (DeWitt 1971), and have a number of adaptations, such as glycoprotein antifreeze molecules, which enable them to live at sub-zero temperatures (Clarke 1983). At the other extreme, tuna species are highly specialised tropical and sub-tropical scrombroids that are able to maintain elevated red muscle temperatures which are especially dramatic during periods of high speed swimming.

Elevated muscle temperatures are achieved using retia mirabilia as counter current heat exchangers to conserve metabolic heat (Carey and Teal 1969; Stevens and Neill 1978). Stevens and Neill (1978) have reported that for Skipjack tuna, aerobic metabolism can produce internal red muscle temperatures 10 °C above ambient water temperatures during periods of burst swimming.

Tuna have substantially higher metabolic rates than other fish (Stevens and Neill 1978) and a number of special adaptations for high speed swimming including the adoption of ram ventilation and various morphological features to reduce drag, such as caudal peduncle finlets, eye fairings, and slots for the dorsal fins (Magnuson 1978; Sharp and Pirages 1978). Therefore, a second objective of this study was to determine if adaptations for high speed swimming were also reflected in higher muscle contraction velocities.

Materials and methods

Fish. Notothenia neglecta (Nybelin) (500–700 g) were obtained from Signy Island, South Orkneys, British Antarctic Territories (Lat. 60°43′S Lon. 45°36′W) during Austral summer, 1982. They were transported to the U.K. aboard the R.R.S. Bransfield and maintained at St Andrews in filtered seawater tanks at +2 °C. *Notothenia* were fed a diet of chopped squid and fish flesh. The remaining species studied were caught by commercial fishermen around the Hawaiian Island of Oahu. Fish were maintained at the National Marine Fisheries Service, Honolulu, Hawaii, (USA) Kewalo Research Facility in outdoor 6 m diameter circular tanks with direct flow-through sea-water supply. Water temperature was that of ambient surface seawater for June-July, 24–28 °C. All fish were fed a diet of sprats and chopped squid. Species studied were Skipjack tuna (*Katsuwonus pelamis*, 8 fish, 1.2 ± 0.2 kg, 39.8 ± 0.4 cm); Kawakawa (*Euthynnus affinis*, 5 fish, 3.2 ± 0.4 kg, 51.2 ± 1.1 cm); Dolphin fish (*Coryphaena hippurus*, 1 fish, 11 kg, 120 cm); Grey mullet (*Mugil cephalus* L., 6 fish, $1,137 \pm 164$ g, 41 ± 2 cm) and Blue Crevally (*Carangus melampygus* Wakiya, 5 fish, 304 ± 21 g, 25 ± 1 cm).

Choice of preparation. Both fast and slow muscle fibres are polyneuronally innervated in most teleosts often with as many as 15–20 motor-endplates per fibre (Bone 1978; Johnston 1983a, b). Dissection of small fibre bundles inevitably results in a variable proportion of damaged and inexcitable fibres. Such preparations produce relatively low tetanic tensions on electrical stimulation (Flitney and Johnston 1979). Single skinned fibre preparations from fish produce much higher maximum tensions in the range reported for skinned preparations from other vertebrate muscles (Altringham and Johnston 1982). For frog fibres, maximum isometric tensions are somewhat lower for skinned than intact preparations, whereas maximum contraction velocities are similar (Julian 1971; Edman and Hwang 1977). Skinned fibres have therefore been chosen as the most suitable preparation for comparative studies of muscle mechanics in fish.

Experimental protocol. Fish were stunned by a blow to the head and pithed. Strips of red and white muscle were dissected from 2–3 myomeres half way along the length of the trunk. In the case of the tuna species, both superficial and internal deep red muscle were sampled (Fig. 1). Subsequent dissection was carried out under silicon oil (BDH MS 550) in a 2 mm deep glass trough. Single red and white fibres were isolated from a 20–30 fibre bundle dissected from a single myotome. Fibre lengths in the region sampled were 1.8 cm for Grey mullet and 0.8–1.0 cm for all other species studied. Single fibre segments, 90–100 μm diameter and 1.3–3.2 mm length, were transferred directly to the apparatus using jewellers' forceps. A thin coating of oil remained on each fibre and helped prevent dehydration during the 15–30 s required to transfer and mount fibres.

Measurement of contractile properties. Muscle fibres were mounted with the aid of a binocular microscope and then chemically skinned with 1% Brij 58 (polyoxyethylene 20-cetyl ether) dissolved in relaxing solution. Relaxing solution contained 20 mM imidazole – HCl, 110 mM KCl, 3.0 mM $MgCl_2$, 5 mM EGTA (ethyleneglycolbis β-aminoethylether N,N′-tetraacetic acid), 10 mM phosphocreatine, 2.5 mM ATP, pH 7.2 at 20 °C. Crystalline creatine phosphokinase (>20 units·ml^{-1}, Sigma Chemical Co, Poole, England) was added to all incubation solutions just prior to experiments. In all experiments, pH was allowed to vary freely with temperature. Imidazole was chosen as the buffer since it has a $\Delta pH/\Delta T$ curve similar to that reported for blood and tissue from many fish species (Reeves 1977). Activating solutions were prepared by addition of a 1 M volumetric solution of $CaCl_2$ to yield a final concentration of 4.0–5.0 mM. Ionic compositions were determined using an iterative computer programme written in BBC Basic, based on that described by Fabiato and Fabiato (1979). The programme was modified to correct for changes in ionic composition with alterations in temperature and pH (stability constant CaEGTA/Ca. EGTA $= 8.89 \times 10^{10}$ at 22 °C). Total ionic strength of the activating solution was 0.17 M, free concentrations of Ca^{2+}, Mg^{2+} and MgATP were 5–15 μM, 0.50–0.54 mM and 2.31–2.52 mM, respectively. Ca^{2+}-concentrations required to give maximally activated concentrations at each temperature were determined in a series of preliminary experiments.

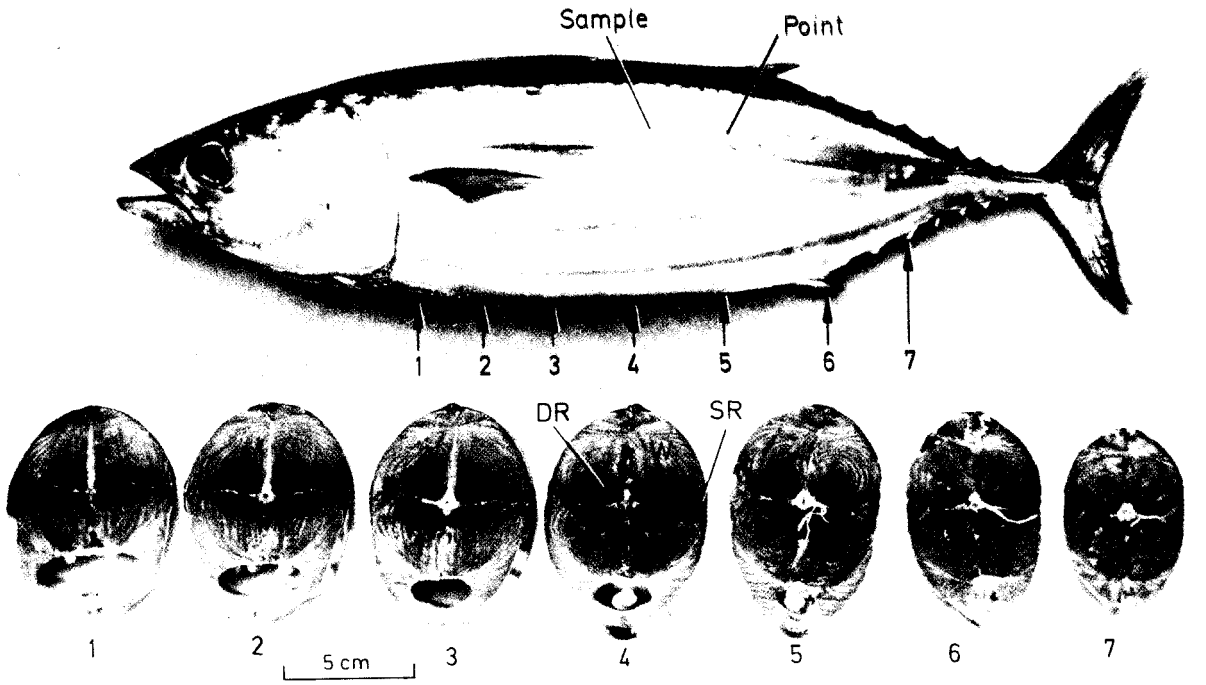

Fig. 1. Sample points for isolation of the different muscle fibre types in Skipjack tuna (*Katsuwonus pelamis*): *SR* superficial red muscle; *DR* deep internalised red muscle; *W* white muscle. Deep red muscle has a much higher myoglobin concentration than superficial red muscle

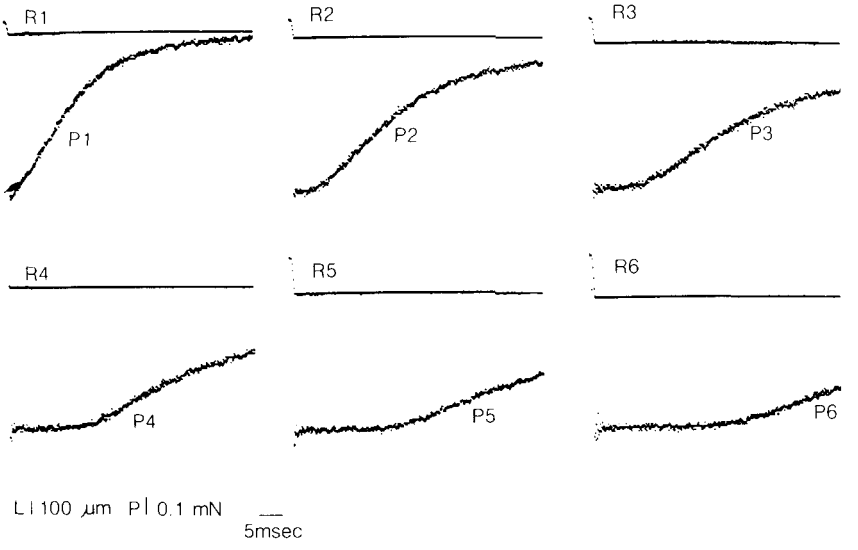

Fig. 2. A typical series of records (R1–R6) illustrating the 'slack-test' method for determining unloaded contraction velocity. White fibre segment, 2,300 μm in length and 90 μm in diameter, isolated from Skipjack tuna muscle. P indicates tension and L muscle length. Note the direction of shortening is down and tension redevelopment is up

Unloaded contraction velocity (V_{max}) was determined by the slack test method (Hill 1970). The apparatus and experimental protocol used have been described in detail elsewhere (Johnston and Sidell 1984; Johnston and Gleeson 1984). Force was measured using a silicon beam tension transducer (Akers AE801, AME, Horton, Norway) attached via a small glass hook to one end of the fibre. The other end of the fibre was attached to a second hook glued to a servo-controlled length transducer (Johnston and Sidell 1984). Fibres were attached to the glass hooks using plexiglass acetone glue (Altringham and Johnston 1982). In a typical experiment, fibres were transferred sequentially to baths containing skinning solution, relaxing solution, activating solution and relaxing solution. The baths were water-jacketed and temperature control (in the range of 0–30 °C) was maintained using a circulating constant temperature water bath (±0.1 °C). The sarcomere length of fibres was determined prior to releases by laser diffraction using a 2 mW Helium-Neon laser and adjusted to 2.3 μm. Once steady

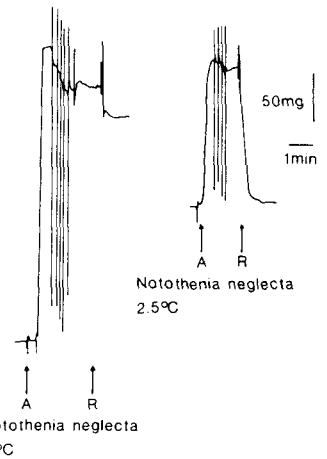

Fig. 3. Records showing isometric contractions of white fibres isolated from *Notothenia neglecta* at 2.5 °C and 18 °C. Arrows indicate transfer from relaxing to activating solutions (*A*) and vice versa (*R*). Fibres from Antarctic fish failed to relax following transfer from activating to relaxing solution at temperatures above 14–15 °C

Table 1. Maximum Ca^{2+}-activated tension (P_0) generation by red and white myotomal muscle fibres of fish measured at their normal body temperature. Means ±SEM, numbers of fibres used in brackets

Species and environmental temperature range	Temperature (°C)	Maximal Ca^{2+}-activated force ($N \cdot cm^{-2}$)	
		Red fibres	White fibres
Notothenia neglecta Antarctica (−2 to +1 °C)	0	–	22.5±2.2 (6)
Gadus morhua[a] (North Sea, 2–15 °C)	8	8.3±1.0 (11)	18.7±1.8 (13)
Mugil cephalus (Pacific reefs, 20–28 °C)	20	5.2±0.6 (11)	21.0±2.8 (8)
Carangus melampygus (Pacific reefs, 20–28 °C)	25	4.3±0.6 (4)	18.3±1.7 (6)
Katsuwonus pelamis[b] (Pacific, open ocean, 15–28 °C)	25	2.4±0.2 (6)	15.7±1.3 (11)
Euthynnus affinis[b] (Pacific, open ocean, 15–28 °C)	30	2.5±0.3 (10)	18.8±0.8 (6)

[a] Data from Altringham and Johnston (1982)
[b] Values are from deep-red muscle for the tuna species

tension was developed on transference to activating solution (30–60 s), a series of quick releases (≤1 ms) of sufficient magnitude to abolish tension, were given. Usually 4 or 5 lengthsteps of increasing magnitude (50–250 μm) were imposed on the fibre during each series of releases. Transients were recorded on a storage oscilloscope. Between each release (ΔL) fibres were re-extended to their original length. Unloaded contraction velocity (V_{max}) was determined from an average of the time taken to redevelop tension (Δt) following each release. A typical record illustrating the method of determining V_{max} is shown in Fig. 2. Muscle length and diameter were measured in situ using a high power microscope.

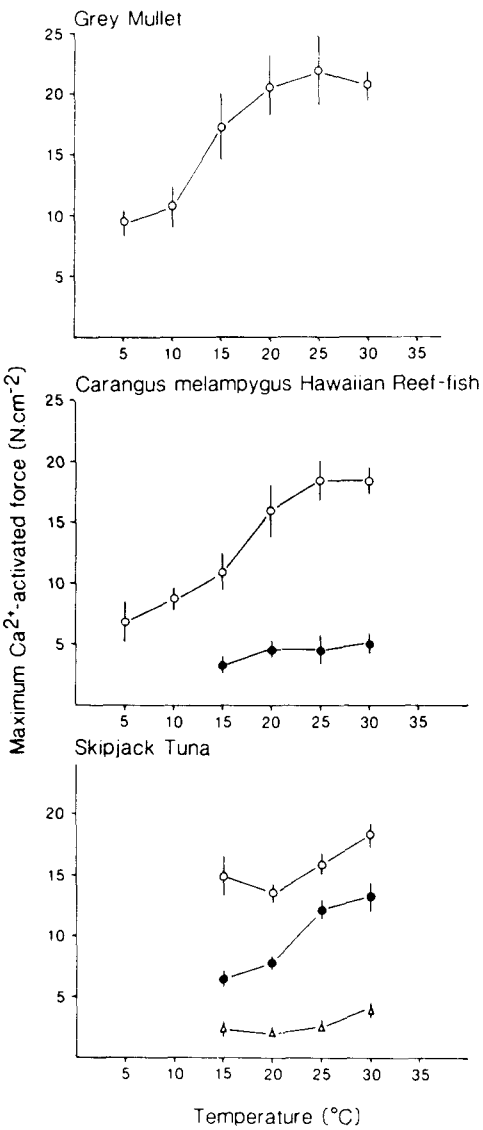

Fig. 4. Effect of temperature on maximum Ca^{2+}-activated force generation of muscle fibres isolated from (top) Grey mullet (white fibres), (centre) Blue Crevally (open circles, white fibres; closed circles, superficial red fibres) and (bottom) Skipjack tuna (open circles, white fibres; closed circles, superficial red fibres; open triangles deep internal red muscle fibres)

Results

At an initial sarcomere length of 2.3 μm, skinned muscle fibres from the warm water species showed

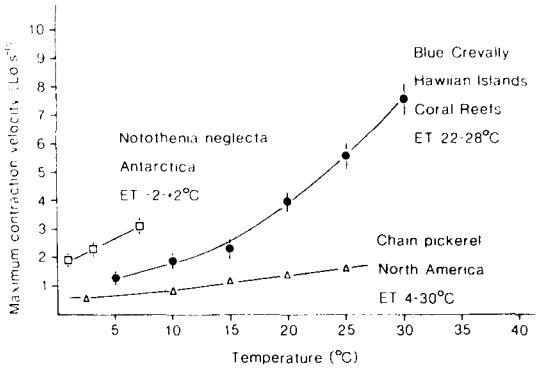

Fig. 5. Effect of temperature on unloaded contraction velocity of skinned fibres isolated from fish white muscle. Data on Chain Pickeral *Esox niger* is taken from Sidell and Johnston (submitted for publication). *ET* indicates the species' thermal habitat

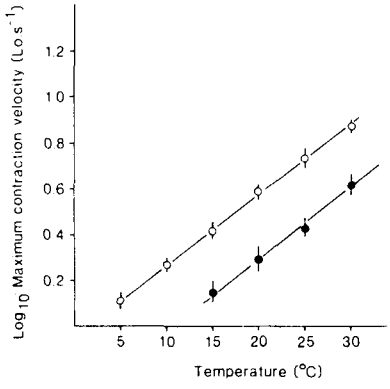

Fig. 6. Effect of temperature on unloaded contraction velocity of red (closed circles) and white (open circles) muscle fibres isolated from the Blue Crevally. The plot shows $\log_{10} V_{max}$ (muscle length·s^{-1}) versus temperature

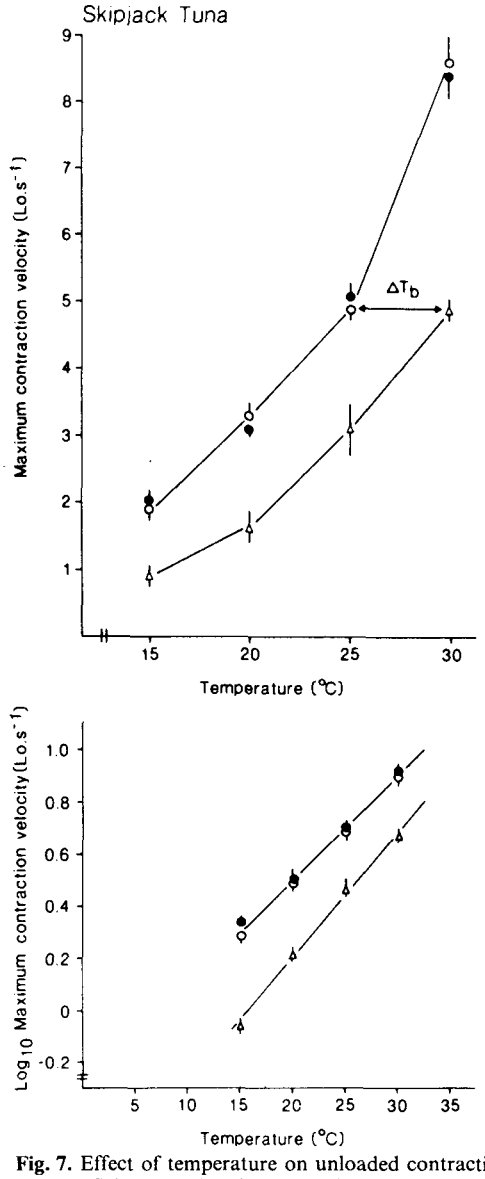

Fig. 7. Effect of temperature on unloaded contraction velocity of superficial red (closed circles), deep, internalised red (open triangles) and white muscle fibres (open circles) isolated from the Skipjack tuna. ΔT_b shows the maximum temperature gradient between internal red and white muscle reported by Stevens and Neill (1978). Note similar contraction speeds for red and white muscle fibres for an 8–12 °C temperature difference. A log $L_0 \cdot s^{-1}$ vs T plot gives straight lines in each case

no development of resting tension and complete relaxation of tension following activation for temperatures up to 30 °C. In contrast, fast fibres isolated from *Notothenia neglecta* failed to relax completely following activations above 15 °C (Fig. 3). At temperatures above 22 °C, *N. neglecta* fibres contracted in relaxing solution (pCa 7.4) and contractions were independent of Ca^{2+} concentrations.

For all species studied, maximal Ca^{2+}-activated tensions (P_0) were 2–3 times higher for white than superficial red fibres. This difference is somewhat greater than can be accounted for by the higher relative volume densities of myofibrils in white than red fibres (Johnston 1981). P_0 for the internal deep red muscle of the two tuna species was only 15% that of white fibres and less than that for the superficial red muscle of any other species (Table 1). In spite of a 30 °C difference in temperature, P_0 of white fibres were similar when measured at the normal body temperature of each species (15.7–22.5 $N \cdot cm^{-2}$: Table 1). For warm water species, P_0 almost doubled between 5–20 °C with little further increase over the range 20–30 °C (Fig. 4).

Table 2. Thermal dependence of unloaded contraction velocity for red and white muscles isolated from tropical marine fish. Means ±SEM (*n*)

Species	Muscle type	V_{max}, 20 °C ($L_0 \cdot s^{-1}$)	V_{max}, 30 °C ($L_0 \cdot s^{-1}$)	Q_{10}
Grey mullet	Red	2.9±0.2(11)	5.0±0.2(8)	1.7
	White	4.6±0.3(10)	8.4±0.5(5)	1.8
Blue Crevally	Red	2.0±0.2(6)	4.3±0.3(6)	2.2
	White	4.0±0.3(8)	7.6±0.4(5)	1.9
Dolphin fish	Red[a]	3.1±0.2(6)		
	White[a]	8.2±0.3(13)		
Skipjack tuna	Deep red	1.7±0.2(6)	4.9±0.4(11)	2.9
	White	3.3±0.2(10)	8.1±0.7(10)	2.5

[a] measured at 25 °C

Unloaded contraction velocity (V_{max}) for *N. neglecta* white fibres at 0–5 °C is similar to the warm water species measured at 10–20 °C (Fig. 5). Over the range 20–30 °C, V_{max} for white fibres from the tropical species were 2–3 times faster than those from the Antarctic fish measured at their normal body temperatures (≤2 °C) (Fig. 5).

The temperature dependence of V_{max} was similar for red and white muscle fibres of the Blue Crevally. Plots of $\log_{10} V_{max}$ vs $1/T$ were linear over the range 5–30 °C (Fig. 6). In contrast, V_{max} for the internal red muscle of Skipjack tuna had a higher $Q_{10(20-30°C)}$ than for either white or superficial red fibres (Fig. 7). Q_{10} for V_{max} for equivalent fibre types were somewhat higher for Skipjack tuna and Kawakawa than for the other warm water species studied that do not maintain elevated muscle temperatures (Table 2).

Discussion

A number of studies have measured the contractile properties of myotomal strips (Wardle 1975; Brill and Dizon 1979). These are useful in determining myotomal contraction time which can be used together with a knowledge of stride length to predict maximum swimming speeds (Wardle 1975). However, the arrangement of muscle fibres within fish myotomes is complex. White fibres make varying angles (from 0 to >40°) with the longitudinal axis of the body (Alexander 1969). Strips of myotomal muscle therefore make unsuitable preparations for determining muscle mechanical properties since the orientation of fibres is undefined, the percentage of cut or damaged fibres high, and the centres of strips anoxic. In the present study, single fast fibres have been utilised which avoids these problems. Furthermore, the use of skinned preparations allows the properties of the contractile proteins to be studied in isolation from nerve and membrane effects.

Maximum Ca^{2+}-activated tension (P_0) of fish fast fibres are similar when measured at the normal body temperature of each species (Table 1) and within the range found for other twitch fibres from ectotherms (Hellam and Podolsky 1969). P_0 also shows only a moderate temperature dependence within the physiological range for at least the warm water species studied (Fig. 4).

Measured at 0–5 °C, unloaded contraction speeds (V_{max}) of *Notothenia* fast fibres are similar or higher than those of many temperate and tropical species at 10–20 °C (Fig. 5). However, an assessment of the degree of temperature compensation between species is complicated by their different life-styles and modes of locomotion. For example, *N. neglecta* is a relatively inactive benthic species whereas the tunas are fast swimming pelagic fishes. All available data on V_{max} of skinned muscle fibres at physiological body temperatures are shown in Fig. 8. Although within each temperature range, there is considerable variation in muscle V_{max}, the highest values are generally found for tropical species. Comparisons of V_{max} at low temperatures (< 5 °C) indicates relatively modest compensations between Antarctic and tropical species (Fig. 5). However, values for P_0 at low temperature are generally much higher for Antarctic and cold-temperate fish (Fig. 4, Table 1 and Altringham and Johnston 1982). Thus it appears that the degree of temperature compensation for maximum power output (force × velocity) is more substantial.

It has recently been shown that for the desert iguana *Dipsosaurus dorsalis* muscle, V_{max} has a similar temperature dependence to that of burst running speed measured in the laboratory (Bennett 1980; Johnston and Gleeson 1984). However, fish with the highest V_{max} may not necessarily have the fastest burst locomotory speeds since any such correlation may be complicated by the segmental arrangement of the myomeres. For example, fibre geometry and myotome shape are likely to be important factors in determining the rate of deformation of each segment. It is noteworthy that at comparable temperatures, fast (white) muscle V_{max} for chain pickeral *Esox niger* is only 20–25% of that for most tropical species and yet the pickeral has a highly streamlined body shape and presumably a high maximum burst speed (Sidell and Johnston, submitted for publication).

It would appear that temperature related adjustments in V_{max}, P_0 and regulatory properties involve substantial changes in protein chemistry.

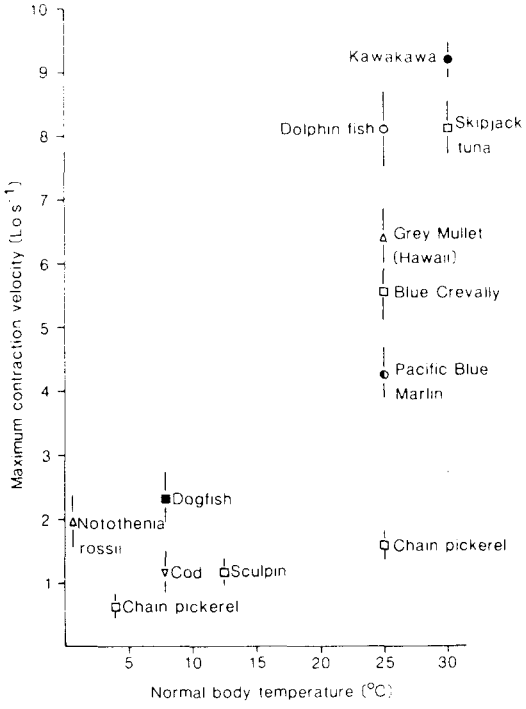

Fig. 8. Comparison of unloaded contraction speeds of various fish white muscle measured at their normal body temperatures. Data not from the present study was from the following sources: Chain pickeral (*Esox niger*, freshwater lakes, Maine, USA 4–30 °C) (Sidell and Johnston, to be published); Dogfish (*Scyliorhinus canicula*, North Sea, 2–15 °C) (Altringham and Johnston 1982); Cod (*Gadus morhua*, North Sea, 2–15 °C) (Altringham and Johnston 1982); Sculpin (*Myoxocephalus scorpius*, North Sea, 2–15 °C) (Johnston and Sidell 1984); Pacific Blue Marlin (*Maikaira nigrans*, Pacific Ocean) (Johnston and Salamonski, unpublished results)

For example, the temperature at which the Ca^{2+}-regulation of contraction becomes disrupted varies from 15 °C for Antarctic to 25 °C for cold-temperate species, whilst warm water fish and the desert iguana show full Ca^{2+}-control up to 30 and 45 °C, respectively (Fig. 3; Johnston and Sidell 1984; Johnston and Gleeson 1984). The half-life of thermal inactivation of myofibrillar ATPase activity under comparable conditions varies from around 1–2 min for Antarctic fish to several hours for tropical species (Johnston and Walesby 1977).

V_{max} of tuna fast muscles is not particularly different from that of other tropical species that do not maintain elevated muscle temperatures (Figs. 7 and 8). Similar results have been obtained for burst swimming speeds. For example, Walters and Fiersteine (1964) measured maximum speeds of 21 bodylengths·s^{-1} for yellowfin tuna and 19 bodylengths·s^{-1} for Wahoo (*Acanthocybium solandri*). The Wahoo lacks the specialised circulatory system necessary to maintain warm muscles (Collette 1978). Therefore, at first glance, it would appear that the advantage of warm muscles is not so much with increased power development per se. However, the effects of elevated muscle temperatures on high speed swimming abilities may be more complex. Only 5–7% of the total muscle is composed of red fibres in Wahoo (unpublished observations) compared to 25–33% for tuna. In most fish, the red fibres will be contracting passively when the fish is swimming at burst speeds. This would be a disadvantage for species such as tuna in which a high proportion of the tissue is red muscle. However, it appears that the high Q_{10} for V_{max} and the temperature gradient between internal red muscle and white muscle may allow both fibre types to contract at similar speeds. Therefore, both white and internal red muscle fibres may contribute to power generation at high speeds. Data on the temperature gradient between internal red and white muscle in free swimming fish at different water temperatures and activity levels would be needed to test this hypothesis. However, other advantages have been suggested for the evolution of warm muscles which include a faster catabolism of lactic acid and enhanced myoglobin mediated oxygen flux from capillary to mitochondrion (Stevens and Carey 1981).

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