THE INFLUENCE OF TEMPERATURE ACCLIMATION ON ISOLATED MUSCLE PROPERTIES AND BURST SWIMMING PERFORMANCE OF THE SCULPIN (MYOXOCEPHALUS SCORPIUS L.)

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The influence of temperature acclimation on isolated muscle properties and burst swimming performance of the sculpin (*Myoxocephalus scorpius* L.).

A thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy.

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Declaration

I hereby declare that the research reported in this thesis was carried out by me and that the thesis is my own composition. No part of this work has previously been submitted for a higher degree.

The research was conducted in the School of Biological and Medical Sciences, United College of St. Salvator and St. Leonard, University of St. Andrews, under the direction of Professor I.A. Johnston.

Signed Date: 30.9.93

Certificate

I hereby certify that Toni A. Beddow has spent eleven terms engaged in research under my direction and that she has fulfilled the conditions of General Ordinance No. 2 (Resolution of the University Court No. 1, 1967) and is qualified to submit the accompanying thesis for the Degree of Doctor of Philosophy.

Signed: Date: 30. 9. 93

This thesis is dedicated to my parents Ann & Neil Beddow.

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Summary

Chapter 1

A brief history of fish biomechanical studies is given and a general introduction on the composition and functional properties of fish muscle, in relation to different swimming activities is presented. Thermal adaptations in teleost fish over both evolutionary and seasonal time scales are discussed at different levels of biological organisation.

Chapter 2

The isometric properties of live fast fibres, isolated from the abdominal myotomes of the short-horned sculpin (*Myoxocephalus scorpius*) were examined at temperatures of 5, 10 and 15°C. The properties of fibres isolated from laboratory-acclimated (5 and 15°C) and naturally-acclimatised, summer (July - September) and winter (January - March) fish were compared to assess the modulating effect of environmental factors other than temperature.

At the experimental temperature of 5°C, maximum isometric tension of the fibres was similar in all the acclimation groups studied. Fibres isolated from winter and 5°C-acclimated sculpin failed to maintain tension and to relax completely at 15°C. Maximum isometric tension of fibres from summer-acclimatised sculpin increased from 179 ± 18.6 kN m^{-2} to 266.7 ± 12.0 kN m^{-2} between 5°C and 15°C. Similarly, isometric tension of fibres isolated from 15°C-acclimated fish increased from 124.9 ± 20.4 kN m^{-2} at 5°C, to 282.0 ± 2.6 kN m^{-2} at

15°C. As a result, force generation of fast fibres isolated from warm-acclimated sculpin is 3.6-times greater than that of cold-acclimated fish, at 15°C.

Times for fibres to develop half maximum tetanic tension ($T_{0.5}a$) decreased with increasing temperature (5 - 15°C) with a Q_{10} of approximately 1.9. Tetanic half activation times were longer in 5°C-acclimated fish (26.0 ms), than in 15°C-acclimated fish (17.8 ms) at 15°C. Tetanic half activation times were 25 - 30% shorter in summer and 15°C-acclimated compared to winter and 5°C-acclimated sculpin. Fibres isolated from natural populations of winter and summer sculpin had shorter $T_{0.5}a$ than their laboratory acclimated counterparts.

Time to loss of 50% maximum force ($T_{0.5}r$) decreased with increasing temperature (5 - 15°C) with a Q_{10} of 2 for twitches and 1.6 for tetani. Rates of half loss of tetanic tension were relatively unaffected by acclimation regime. The exception was at 15°C where fibres from winter sculpin had significantly shorter $T_{0.5}r$ than summer fish. Twitch half relaxation rates varied more with temperature and acclimation regimes than those of tetani, though no underlying pattern was obvious.

Chapter 3

Live fast fibre bundles were isolated from the abdominal myotomes of naturally acclimatised (summer and winter) and laboratory acclimated (5°C and 15°C) sculpin.

Force-velocity (P-V) characteristics of the fibres were investigated using iso-velocity releases. Data below 0.8 P_o was

iteratively fitted to the hyperbolic-linear equation, $V = [B (1-P/Po)/ (A +P/Po)] + C (1-P/Po)$. P-V curves were constructed for each acclimation group at 5, 10 and 15°C.

The shape of the P-V curve is represented by the power ratio $Wmax/ Vmax Po$. The P-V relation of fibres isolated from 5°C-acclimated sculpin (0.19) is significantly flatter than that of 15°C-acclimated fish (0.12), at 5°C.

Maximum velocity of shortening (V_{max}) was measured using the Slack test and estimated by extrapolating P-V curves. Fibres isolated from summer and 15°C-acclimated fish have V_{max}'s that increase with a $Q_{10(5\text{-}15°C)}$ of 1.8 - 2.0. However, the Q_{10} for V_{max} in fibres from winter and 5°C-acclimated fish is close to 1.0. The net result of the different Q_{10}'s is that fibres from summer and 15°C-acclimated sculpin have values of $V_{max} \geq 1.6$ times faster than those of winter and 5°C-acclimated fish.

Power output declined by 74% from 131.1 W kg^{-1} at 5°C to 33.6 W kg^{-1} at 15°C in the 5°C-acclimated fish, as both V_{max} and Po fell. For 15°C-acclimated fish, power output of fast muscles increased from 55 W kg^{-1} at 5°C to 206 W kg^{-1} at 15°C. Similar results were found for summer acclimatised sculpin. Overall the muscle power output was six times greater in fibres from 15°C-acclimated relative to 5°C-acclimated fish at 15°C. At 5°C, power output of 5°C-acclimated fish was double that of 15°C-acclimated fish (though this was not statistically significantly). This is believed to be a result of the decreased curvature observed in the P-V relation.

The mechanisms behind the acclimatory increases in power output in summer and 15°C-acclimated fish, at 15°C are partly due to increases

in the Vmax of the fibres. More importantly force generating capacity shows a major acclimatory adaptation which greatly enhances power output at warm temperatures.

Chapter 4

Short-horned sculpin were acclimated to either 5 or 15°C (12 h light: 12 h dark) for 6 - 8 weeks. Prey-capture of *Crangon crangon* was filmed at 200 frames s^{-1}, using a high speed video.

The fish employed the three classic kinematic stages as described by Weihs (1973) in a typical "S" shaped fast start. The kinematics of the fast start were unaffected by temperature or acclimation state.

5°C-acclimated sculpin were tested at 5, 10 and 15°C. Rates of acceleration doubled in the 5°C-acclimated fish between 5 and 15°C. Tail-beat frequency also increased significantly from 5.5 Hz at 5°C to 8 Hz at 15°C. However, mean and maximum velocity and tail-beat amplitude were relatively independent of test temperature. The total distance covered by the end of stage 2 decreased with increasing experimental temperature.

At 15°C the burst speed swimming performance of 5°C- and 15°C-acclimated sculpin were compared. Tail-beat frequency was 31% higher (10.5 Hz) in the 15°C-acclimated compared to the 5°C-acclimated fish. Mean velocities increased by 52% from 0.59 m s^{-1} in the 5°C-acclimated fish to 0.90 m s^{-1} in the 15°C-acclimated sculpin. Rates of maximum velocity and acceleration followed similar trends. Increased speeds were a result of the increased thrust generated by the 24% larger tail-beat amplitude of the 15°C- relative to the 5°C-acclimated fish.

15°C-acclimated fish covered a distance on average 34% further, per attack sequence than the 5°C-acclimated fish.

Net differences in the fast start capabilities of the acclimation groups at 15°C, resulted in a 68% reduction in prey-capture success rate of 5°C-acclimated relative to 15°C-acclimated sculpin.

Chapter 5

Fast muscle fibres were isolated from rostral and caudal myotomes of summer-caught, short-horned sculpin. In collaboration with Dr. Johann van Leeuwan, Department of Physiology, University of Leiden, muscle strain patterns for three positions (0.31L, 0.52L, 0.77L) along the body were calculated from changes in body curvature, during fast-starts at 15°C. Isolated rostral and caudal muscle fibres were subjected to the *in vivo* strain fluctuations and stimulated with a similar duty cycle (25 - 32%) to that found *in vivo*. Work loops were generated by plotting force and length. The effect of varying muscle stimulation phase on power output and force generation was investigated.

At the anterior position in the body the rostral fibres produced significantly greater force (41.9 kN m^{-2}) compared to the caudal fibres (12.2 kN m^{-2}). Similarly, power output at position 1 was also significantly greater in rostral fibres (28.0 W kg^{-1}) compared to caudal fibres (3.7 W kg^{-1}). No difference was found in the properties of the fibres at positions 2 and 3. This suggests that the rostral fibres have adapted to their local mechanical environment to increase force and power generation, although the mechanisms responsible are unknown.

Chapter 6

The major findings of this thesis are discussed relating different aspects of muscle performance to locomotory capabilities of sculpin. Thermal adaptations over the natural environmental temperature range encountered by sculpin are considered and the possible mechanisms involved are discussed. Finally, suggestions for elucidating the molecular mechanisms behind adaptations in the shortening speed and force generation are presented.

CHAPTER 1

General Introduction

History of fish biomechanics

Locomotion in fish was studied as early as the fourth century B.C. when Aristotle observed and commented on swimming movements. He was the first scientist to note that forward motion depends on the resistance of the external media, "there will be no progress, that is, no walking unless the ground were to remain still, and flying or swimming unless the air or sea were to offer resistance". Aristotle however did not appear to understand the undulatory nature of swimming and believed that paired fins were the primary means of propulsion; the tail-fin, he believed was a steering mechanism. During the seventeenth century Giovanni Borelli (1680) made the first attempts to analyse the mechanics of fish swimming. Unlike Aristotle, Borelli observed that fish swam with their pectoral fins held against the body. Borelli was the first person to note that fish swam by sweeping the caudal fin from side to side in an arc. Relatively few studies were recorded in the eighteenth and early nineteenth centuries, though in 1809 the first theories of streamlining were being developed by Sir George Cayley. Cayley (1810) noticed that in the natural environment fast moving animals had very streamlined bodies *i.e.* in trout and the woodcock.

Prior to the 1890's swimming studies were difficult as measurements were made primarily by eye. However, with the advent of cinematography in the late nineteenth century, precise measurements of locomotion were made possible. Marey (1895) filmed tethered *Raia* and swimming *Hippocampus, Anguilla* and *Scyliorhinus*. Unfortunately, due to the slow framing rate (20 Hz) an optical illusion was produced whereby the waves of movement appeared to travel in

reverse (observed by Gray, 1933a). However, Marey's films provided an insight into how forward locomotion was achieved and Dean (1895) used them to observe that locomotion occurred via "pressure of the fishes body against the water enclosed in those incurved places which causes the forward movement". In 1926 the locomotion of fish was extensively observed and categorised by Breder. Breder (1926) stated that all fish movement (except exhalation) was of an undulatory nature, brought about "by the serial action of metameral muscles". Many of the terms coined by Breder (1926) are still widely used today *i.e.* Carangiform, Anguilliform and Ostraciform.

Around the turn of the twentieth century the first plane was flown by the Wright brothers. Wind tunnels were built which enabled aerodynamic theories such as lift, drag and stability to be tested on innumerable shapes. In the 1920 - 30's engineers inevitably became interested in locomotion through water and subsequently fish swimming (see Alexander, 1983). One of the most influential figures to study animal locomotion, having successfully combined film and mathematical analysis was Sir James Gray. Gray analysed animal movements on film to show how undulatory movements generated thrust (Gray, 1933a, b, c; summarised Gray, 1968). Gray (1933a) made one of his biggest breakthroughs by considering fish locomotion in aspects of transverse movements of each section of the body, rather than studying the overall propagated waves of contraction. Gray (1933a) estimated "that the relative velocity of the body and surrounding water is the resultant of the transverse velocity of the body and its forward velocity through the water". However, as Gray said himself the analysis did not take into account the fact that water passing posterior regions would have already been influenced by the action of the anterior segments.

Breder (1926) measured the swimming speeds of fish (*Scardineus*) after removal of the caudal fin. No difference in cruising speed was observed between fish with intact tail fins and those without, and it was suggested that the body surface itself produced most of the thrust (Breder, 1926). Gray (1933b) also filmed the swimming action of fish (*Merlangus*) before and after the caudal fin was removed. Cruising speed was not significantly altered by tail fin removal but the kinematics of the motion became much more unsteady (Gray, 1933b). A purpose built machine allowed the super imposition of the undulatory swimming movements on a dead whiting, immersed in water. Following the removal of the caudal fin from the dead whiting, thrust decreased by 40%. This reinforced the idea that it is the tail-fin that generates most of the thrust. It appears that following amputation of the tail fin body movements automatically compensate for the reduced thrust capacity, and cruising speed is maintained (Gray, 1933b).

The swimming speed and associated drag factor of dolphins was calculated and compared to the estimated power output of its muscles (Gray, 1936). Muscular power output was assumed to be the same as that found in man or dog, per unit mass. Following mathematical analysis, Gray concluded that for dolphins to swim at the reported speeds, cetacean muscle would need to produce seven times greater power than that of man or dog (Gray, 1936). Alternatively, turbulence of the boundary layer was lower than that predicted in the equations used and laminar flow was maintained during swimming (Gray, 1936). This discrepancy, "Gray's Paradox" has motivated much research to attempt to solve this difference. Early analysis of the energetics of fish swimming use drag values calculated from either measurements on

equivalent rigid straight shapes (Gray, 1936; Bainbridge, 1961) or on stretched out fish (Brett, 1963). Observations on flow around a swimming fish's body suggest that these values are unlikely to provide true estimate of swimming drag (Webb, 1970). Early measurements of thrust and power output were also unreliable and subject to large errors. Houssay (1912) forced fish to lift various weights while swimming and Gero (1952) measured the strain of a fish swimming on a line.

A distinguished mathematician Sir James Lighthill developed his own 'reactive' theory on undulatory swimming (1960). This theory was modified and applied to films of swimming dace (*Leuciscus*) made by Bainbridge in Grays Cambridge department (Lighthill, 1971). Lighthill (1971) showed that drag in swimming dace was approximately four times greater than that of a rigid body of the same size and shape. This was interpreted by Lighthill as a result of increased drag on an undulatory compared to a rigid body, possibly due to thinning of the boundary layer. More accurate measurements of muscle power output have been obtained (Hill, 1950; Weis-Fogh & Alexander, 1977) and more precise calculations of swimming speeds have been recorded (Bainbridge, 1958; Webb, 1975a; Harper & Blake, 1990). Using Lighthill's relatively easy to use equations other workers have utilised the principles to further the understanding of swimming power outputs and hydrodynamics (Webb, 1971; Weihs 1972, 1973; Videler & Wardle, 1978). Weihs (1974) used Lighthills theories to argue that kick and glide swimming, common in many fish species, saved energy compared to constant undulatory swimming, due to the increased drag in the latter. Recent estimates of power outputs and swimming speeds

now indicate that Gray's Paradox is only applicable to burst speed swimming in scombroid fishes (Blake, 1983).

The development of sophisticated swimming flumes and annular tanks have enabled fish to be swum long distances in the laboratory (Fry & Hart, 1948; Bainbridge, 1958; Brett, 1964). This has allowed direct measurements of oxygen consumption to be made during swimming (Brett, 1964; Farmer & Beamish, 1969). Electromyography (EMG) was first successfully used to examine muscle activity patterns in the human shoulder (Inman, Saunders & Abbotts, 1944). Since that time it has been used to examine muscle activity in fish during feeding (Osse, 1969), respiration (Hughes & Shelton, 1962) and swimming (Rome, Loughna & Goldspink, 1984, Rome, 1990). Using a combination of swimming flumes, cinematography and EMG techniques, detailed analysis of all aspects of fish swimming is now possible.

Fibre geometry

The locomotory musculature of elasmobranchs and teleosts is segmentally divided into myotomes (Nursall, 1956; Bone, 1966; Alexander, 1969). Red fibres have a simple arrangement, with fibres running in thin sheets parallel along the body axis. Myotomes of white fibres run between sheets of connective tissue, called myosepta. The myosepta forms cones of white fibres which are stacked up in specific orders, parallel to the body axis. Viewed from the side the myotomes appear as 'V' shapes in primitive fish species and become progressively more complex, appearing to be 'W' shaped in higher teleosts and elasmobranchs. Furthermore, white fibres are arranged in complex 3-dimensional helical patterns. The fibre orientation varies with both depth and position along the body length, forming angles up to 30° or

more with the long axis of the fish (Alexander, 1969). Two different white fibre arrangements were described, one for selachians (eg. *Scyliorhinus*) and primitive teleosts (eg. *Anguilla* and *Salmo*) and another for more advanced teleosts (Alexander, 1969). Alexander's (1969) analysis was notable because he drew several conclusions about the functional significance of the differing fibre geometry's. Mathematical analysis of the fibre orientation calculated that all the white fibres would contract to a similar extent for a given body flexure (Alexander, 1969). Therefore, the sarcomere length change during muscle shortening would be small and relatively constant over the whole body of the fish. The second conclusion of Alexander was that the helical muscle arrangement of teleosts would result in a faster rate of body bending for a given intrinsic muscle contraction velocity, than the selachian pattern would. The selachian arrangement is retained in the caudal peduncle of higher teleosts possibly to maintain a strong bending moment (reduced in the helical arrangement), in order to transmit force to the tail (Alexander, 1969). Recent examinations of the velocity at which the muscle shortens *in vivo* supports the theory that all fibres in the fishes body shorten at a similar V/ Vmax (Rome, Funke, Alexander, Lutz, Aldridge, Scott & Freadman, 1988). The helical orientation of white fibres enables fast powerful contractions to occur at velocities optimal for efficiency and power generation, at all positions along the fish (Rome *et al.*, 1988).

Fibre types

Most vertebrates have muscles which contain a mixture of fibre types, however fibre types in fish are arranged in anatomically discrete regions (Bone, 1966). Different fibre types can be identified using

histochemical, ultrastructural, biochemical and physiological criteria. In fish, fibre colour and muscle contraction speeds have become the most widely used and accepted terms for differentiating muscle fibre types. Two major muscle groups have been classified, these being red and white fibres. However, it should be noted that within these two main groups various sub-divisions of fibre types have been found to exist (Johnston, Patterson, Ward, & Goldspink, 1974). The number of fibre types has been found to vary with different species with only two in the brook trout, three in carp and three in the Antarctic *Notothenia rossii* (Johnston, Davison & Goldspink, 1977; Johnston & Moon, 1980; Walesby & Johnston, 1980). Bone (1978), found five different muscle fibre types in the dogfish: inner red, outer red, inner white, outer white fibres and superficial tonic fibres.

Red, slow fibres usually form a superficial layer or a small, discrete wedge along the lateral line of the fish, just below the skin (Johnston & Moon, 1980). Some scombroid fish and sharks however show a deep band of red muscle internalised as part of a counter-current heat exchange mechanism (see Graham, 1983). Red slow fibres are so called due to their coloration and low speed shortening velocities. Red fibres have high myoglobin and cytochrome concentrations, a high volume density of mitochondria and a dense capillary bed (Bone, 1966, 1978; Johnston, 1981). These factors are associated with a high aerobic capacity which enables repetitive contractions, necessary for sustained swimming. The proportion of red muscle in the locomotory musculature varies from 0.5 - 29% (Greer-Walker & Pull, 1975). The differing proportions of fibre types is thought to be related to the different behavioural activities and lifestyles of fish species. The highest proportion of red muscle is found in pelagic swimmers and the

lowest in sedentary, benthic species and fish that swim using their paired fins.

White muscle forms the majority of the locomotory musculature (up to 90%) and provides the power for burst speed swimming. White muscle has high myofibrillar densities, low mitochondrial volumes (0.5 - 4%) and a poor capillary network (Johnston, 1981). White fibres have a low aerobic capacity, mainly functioning anaerobically to rapidly synthesise ATP. White fibres have fast contraction velocities and readily go into oxygen debt (Flitney & Johnston, 1979).

So called 'pink' fibres have been found in a number of fish species (Johnston *et al.*, 1974). These fibres have properties intermediate to fast and slow fibres and utilise both oxidative and glycolytic metabolic pathways (Johnston *et al.*, 1977).

Fibre innervation

Slow fibre innervation seems to be relatively conserved between different fish species (Bone & Ono, 1982). All myotomal slow fibres are multi-terminally innervated by two or more axons, and nerves terminate in en-grappe endings (Bone, 1978). However, some physiological differences in the twitch activation capabilities are found between species. Tench (Barets, 1961) and *Tilipia* (Flitney & Johnston, 1979) have slow fibres that do not generate action potentials or mechanical twitches in response to junction potentials. However, twitch responses are found in red fibres of many species including, elasmobranchs (Stanfield, 1972), the cod and cuckoo ray (Johnston, 1982) and in the *M. hyoideus* muscle of carp (Granzier, Wiersma, Akster & Osse, 1983).

The pattern of fast fibre innervation shows much greater variation than that of red muscle. Most vertebrate fast fibres are focally innervated by one axon terminating at a single endplate (Hess, 1970). Elasmobranchs, agnathans and primitive teleosts are also focally innervated, but fibres are generally stimulated by two axons that fuse into a single endplate (Bone, 1964, 1972; Bone & Ono, 1982). Focal and dual innervation both result in action potentials in response to a single stimulus therefore, the functional significance behind dual innervation is as yet unclear (Hagiwara & Takahashi, 1967). The majority of advanced teleosts have multi-terminal/ polyneuronal innervation, with up to 23 endplates per fibre (Barets, 1961; Bone, 1964; Hudson, 1969; Altringham & Johnston, 1981). Polyneuronally innervated fibres are activated by overshooting action potentials (Altringham & Johnston, 1988b). Work on *Myoxocephalus scorpius* found 8 - 20 endplates present on each fibre (Altringham & Johnston, 1989). However, the fast fibres were generally innervated by only 4 - 6 axons, with pre terminal branching of the axons accounting for the high number of endplates present (Altringham & Johnston, 1989). Polyneuronal innervation differed in zebrafish compared to *M. scorpius* (Westerfield, McMurray & Eisen, 1986). Fibres of zebrafish are stimulated by one primary motor neurone and up to four secondary motor neurones. Each primary neurone on the side of each part of the body musculature, stimulates a specific set of fibres (Westerfield *et al.*, 1986). Evidence suggests that polyneuronal innervation in fish has evolved on at least eight separate occasions, which indicates a strong natural selection pressure (Ono, 1983). The reasons behind the parallel evolution of polyneuronal innervation in fast fibres of many different fish species is unknown, but is possibly associated with greater

versatility and control of swimming speeds. Further investigation of the whole neural network is required for greater understanding of the advantages incurred by polyneuronal innervation.

Contractile proteins

Myofibrils are composed of two major components; myosin (55%) and actin (25%). Structural elements such as the M-line and C proteins are associated with myosin, and regulatory proteins such as troponin and tropomyosin are associated with actin thin filaments. Myosin's of fish fast and slow skeletal muscles have similar molecular structures to mammalian and avian myosins (Watabe & Hashimoto, 1980). The basic structure of myosin consists of two heavy chain subunits (MHC) of 200 kDa and four light chain subunits (LC) of about 20 kDa (Huriaux & Focant, 1977; Focant, Jacob & Huriaux, 1981). The two heavy chains form a coil over their C-terminal ends and then separate to form a globular head. This S1 head consists of two different light chains, and is the site of ATPase activity and actin binding. Various isoforms of myofibrillar proteins exist, differing in amino acid sequences and functional properties. Isoforms are thought to represent multi-gene families or arise from differential RNA processing. Cloning studies indicate a minimum of 28 genes encoding for MHC isoforms in common carp (Gerlach, Turay, Mailik, Lida, Scutt & Goldspink 1990), which are expressed throughout development (Whalen, Sell, Butler-Browne, Schwarty, Bouveret & Pinset-Harstrom, 1981). Mechanical properties such as contraction speed and force production are thought to

be largely determined by the myosin heavy chain isoforms (Reiser, Moss, Giulian & Greaser, 1985; Lannergren, 1987) with the alkali light chain isoforms (LC1 and LC3) playing a modulatory role (Watabe, Ochiai & Hashimoto, 1982; Lowey, Waller & Trybus, 1993). Myosin isoforms differ between fast and slow fibre types being unique in slow, intermediate and fast fibre types (Scapolo & Rowlerson, 1987). As in avian and mammalian muscle, fish myosin slow fibres have two light chain isoforms $LC1_S$ and $LC2_S$, whereas fast fibres have three different light chain isoforms $LC1_f$, $LC2_f$ and $LC3_f$ (Focant, Huriaux & Johnston, 1976; Rowlerson, Scapolo, Mascarello, Carpene, & Vegetti, 1985; Yancey & Johnston, 1982). However, light chains show more variation between different fish species than between mammals and birds (Focant *et al.*, 1976; Rowlerson *et al.*, 1985). The large number of myosin isoforms and the possible combinations of different isoforms, underpins the plasticity of muscle proteins. Myosin expression varies with environmental conditions and appears to be related to adaptive changes in locomotory performance.

Fibre recruitment

The different fibre types power different levels of swimming activity. Electromyographical studies on carp, show that only slow red fibres are active at low sustainable speeds, with no activity being observed in fast or intermediate fibres (Davison, Goldspink & Johnston, 1976; Rome *et al.*, 1984). As speed increases, first intermediate and then fast fibres are recruited, with fast fibres providing the power for burst speed swimming (Rome *et al.*, 1984). However short bursts of activity in fast fibre regions were observed during prolonged swimming (Rome *et al.*, 1984). This could arise from activity of the intermediate

fibres which are believed to be recruited at transitionary speeds between steady and burst swimming, before the white fibres are fully recruited (Davison *et al.*, 1976). The same pattern of recruitment has been observed in other polyneuronally innervated species *i.e.* tuna (Brill & Dizon, 1979), striped bass (Freadman, 1979; Sisson & Sidell, 1987), blue fish (Freadman, 1979) and scup (Rome, Choi, Lutz & Sosnicki, 1992). In contrast, studies on focally innervated fibres of both Pacific Herring (*Clupea harengus pallasi*) and the dogfish, suggest a clear division of labour between fibre types. Only red slow fibres are used for swimming at low speeds with the fast fibres being recruited for short periods of burst speed swimming (1 - 2 min) (Bone, 1966; 1978).

Fibre action during swimming

Several workers have utilised the work loop technique first developed by Machin and Pringle (1959) and later adapted by Josephson (1985). The technique is used to imitate the fibre length changes that occur during steady swimming and impose them on isolated muscle fibres (Altringham & Johnston, 1990a & b; Johnson & Johnston, 1991a; Anderson & Johnston, 1992). Power output was maximised by adjusting the timing and number of stimuli at different cycle frequencies (Altringham & Johnston, 1990a). Maximum power output was produced following a small pre-stretch of the active muscle, and declined at higher and lower phases of stimulation. The pre-stretch acted as a mechanism to store potential energy which was released during the shortening part of the cycle (Altringham & Johnston, 1990a). During swimming, lateral body curvature travels as a wave down the body of the fish. The bending moment however functions as a standing wave (Hess & Videler, 1984). Simultaneous stimulation of both sides of

the body produces alternative contractions down the whole length of the body, and propels the fish through the water (Hess & Videler, 1984). Electromyographical (EMG) studies suggest that the mechanical wave passes caudally down the body of the fish, faster than the stimulation wave (Grillner & Kashin, 1976; Williams, Grillner, Smoljaninov, Wallen, Kashin & Rossignol, 1989; Leeuwen, Lankheet, Akster & Osse, 1990). This would mean that muscles are activated at different phases of the length cycle and would therefore produce different power outputs along the body. Leeuwen *et al.*, (1990) analysed the power output and recruitment of slow fibres of the carp at different swimming speeds and developed a model to estimate the strain fluctuations and power output along the body. The model took into account; a) changing sarcomere properties; b) modulation of crossbridge tension as a function of the force velocity curve; and c) changing rates of tension development and decline. The model predicted that the phase of stimulation was delayed along the length of the body and the speed of contraction also varied according to fibre position (Leeuwen *et al.*, 1990). During continuous swimming the anterior myotomes produced mainly positive power output, with net positive power output overall. In posterior myotomes the fibres were stimulated while almost fully stretched, and therefore produced net negative work (Leeuwen *et al.*, 1990). Work loop experiments on isolated muscle found that by changing the phase delay of the stimulus in relation to the length cycle, similar changes in net positive and negative power occurred (Johnson & Johnston, 1991a). During intermittent burst speed activity, peak positive power output of activated red fibres was predicted to occur in the anal region of the carp (Leeuwen *et al.*, 1990). During this kick and glide phase, the fast fibres were predicted to work more effectively than the slow fibres (Leeuwen

et al., 1990). Altringham, Wardle & Smith, (1993) found similar negative power output in caudal fibres of saithe (*Pollachius virens*). Altringham *et al.*, (1993) suggested that the caudal region of fish acted as a transmitter of power from the rostral myotomes to the tail fin. So although caudal fibres contribute no net power to the swimming stroke, they had enhanced force production and transmit power from rostral regions to the tail fin, thus maximising thrust (Altringham *et al.*, 1993).

Mechanical properties of fast muscle fibres have been found to vary down the length of some fish species. Wardle (1985) examined twitch contraction times of whole blocks of muscle and found them to increase from the head to the tail. Similar results were found for isolated fibre bundles of cod, (*Gadus morhua*) (Davies & Johnston, 1992) and saithe (Altringham *et al.*, 1993). The above fish species are mainly carangiform swimmers adapted for pelagic lifestyles. Examination of isolated muscle properties of a sedentary benthic species *Myoxocephalus scorpius*, found no difference between rostral and caudal regions (Johnston, Franklin & Johnson, 1993). Under optimal conditions rostral and caudal fibres both generated 25 - 30 W kg^{-1} maximum power output, at tail-beats relevant to burst speed swimming 4 - 9 Hz, (Johnston *et al.*, 1993). At higher cruising speeds carp use kick and glide swimming, with the white fibres powering the initial tail flip (Leeuwen *et al.*, 1990). The model proposed by Leeuwen *et al.*, (1990) predicted that the fast fibres of carp would produce net positive work along the whole length of the fish. Burst speed swimming in the sculpin is also powered by the white fast fibres (Johnston *et al.*, 1993). EMG swimming studies and work loop experiments reaffirm the model's prediction, that fast fibres appear to produce net positive work

along the whole length of the body, under most conditions (Johnston *et al.*, 1993).

Swimming activity

Swimming ability appears to provide an integrated measure of an animal's fitness to specific environmental conditions (Nelson, 1989). Three main swimming activity levels have been distinguished in fish: a) sustained or cruising, b) prolonged or steady, c) burst or sprint speeds. These modes of swimming involve using body and caudal fin propulsion (see Webb, 1975b). Sustained levels are those activities that can be maintained for more than 200 min (Brett, 1964; 1967). These include routine foraging, territorial and schooling behaviours. Sustained swimming is powered solely by the activation of aerobic red fibres (Bone, 1978; Freadman, 1979; Sisson & Sidell, 1987; Rome *et al.*, 1984; Rome *et al.*, 1992). Prolonged levels are defined as sustained and burst activities that can be maintained for 15 s to 200 min. Many physiological studies have observed prolonged activity levels with special reference to the critical swimming speed, U_{crit} (Brett, 1964). U_{crit} is the maximum sustainable steady swimming speed attained by the fish (Brett, 1964). Prolonged swimming levels are generally powered by red, aerobic fibres with short bursts of activity in the fast white fibres (Rome *et al.*, 1984). Burst speeds are periods of high activity maintain for less than 15 s, including sprint behaviour (Blaxter, 1969) and acceleration. Acceleration generally occurs during predator avoidance or prey-capture manoeuvres (Weihs, 1972, 1973; Webb, 1978a & b). Burst swimming speeds are generally powered by fast glycolytic muscle fibres (Bone, 1978; Freadman, 1979; Sisson & Sidell, 1987; Rome *et al.*, 1984; Rome *et al.*, 1992).

Swimming and changing environmental conditions

There have been relatively few studies on the effects of environmental conditions on burst speed swimming. Burst speed activities rely on energy stores present within the cell and are considered relatively independent from environmental conditions, unless the energy supplies or the properties of the muscle are affected. In contrast, the aerobic capabilities of fish are greatly affected by environmental conditions. The mechanisms affected depend on the type of environmental change encountered. The aerobic scope (prolonged and sustained swimming) of trout is reduced when swimming in acid and alkaline waters (Ye, Randall & He, 1991). Acid conditions cause a reduction in the oxygen carrying capacity of the blood (Bohr effect, where haemoglobin's affinity for O_2 is reduced), which lowers the scope for aerobic activity and contributes to a reduced U_{crit} (Ye *et al.*, 1991). Exposure of fish to alkaline conditions causes an increase in ammonia levels in the blood and blood alkalosis, which leads to ammonia accumulating in the muscles (Lin & Randall, 1990; Ye *et al.*, 1991). High concentrations of ammonia produce convulsions in fish and in other vertebrates (Hillaby & Randall, 1979). It therefore seems likely that swimming is impaired and U_{crit} is reduced due to neural rather than muscular disruption, when swimming in alkaline conditions.

Acute changes in temperature can have both direct and indirect influences on physiological processes. Indirect effects include a shift in the haemoglobin oxygen dissociation curve to the right with increasing temperature, thus reducing the oxygen carrying capacity of the blood. Increasing temperature also decreases the level of dissolved oxygen in the external environment and in the plasma. Therefore, at higher

temperatures reduced oxygen delivery to the tissues could reduce performance. Low temperatures however do not limit oxygen supplies, but do directly affect isolated muscle properties. Two to three-fold decreases in power output and maximum shortening speeds occur with a 10°C drop in temperature (Hill, 1938; Rome, 1983). For a 10°C drop in temperature the energetic cost for isolated muscle generating force was found to decrease threefold (Rome *et al.*, 1984). Comparisons with whole animal experiments however have found relative temperature independence of the energetic cost of running (Rome, 1982).

Adaptation

Fish operate over a wide range of environmental conditions. These conditions may change over a daily or seasonal basis and also over evolutionary time scales. In order to maintain function over these altered environmental conditions fish have developed both long and short term compensatory mechanisms. These biological changes can occur immediately or after days or weeks of acclimation/ acclimatisation. Acclimation refers to biological compensation that occurs in response to changes in controlled laboratory conditions. Acclimatisation refers to compensatory mechanisms that occur in response to seasonal changes in the natural environment including: food availability, photoperiod, salinity and temperature (Prosser, 1973). Evolutionary adaptations involve changes in the genome, which if selected for may persist in future generations. These various adaptations however, do not necessarily produce optimisation of the

different physiological processes, at each environmental condition. The idea of symmorphosis put forward by Taylor and Weibel (1981) states that "the formation of structural elements is regulated to satisfy but not exceed the requirements of the functional system". One of the problems with this concept is that most biological processes are interlinked and perform more than one function. Conflicting pressures may be exerted on each component of a particular function and therefore, adaptations that optimise one particular function may adversely affect another. Also, due to time and spatial variation in selective pressures the physiological performance required can also change. Randall and Brauner (1991), suggest that symmorphosis, especially in ectotherms, should be considered as a compromise, in that structures are designed to operate over a wide range of conditions rather than be optimised for a given set of conditions. This appears to be widely illustrated by the adaptations in physiology and performance of fish swimming.

Adaptation responses are classified as either resistance or capacity adaptations (Precht, 1958; Prosser, 1973). Resistance adaptations alter the temperature range over which functions are maintained by modifying the upper and lower limits of the species (Precht, 1958). One example of a resistance adaptation is the presence of antifreeze proteins in the plasma of Arctic and Antarctic fish species. This enables muscle function and survival at temperatures as low as -2.7°C (DeVries & Lin, 1977). Capacity adaptations alter rate processes to compensate for the effects of temperature over the thermal range of the species. The different levels of compensation during capacity adaptations are; 1) over compensation; 2) perfect compensation, when rates are the same despite different acclimation temperatures; 3) partial compensation, 4) no compensation, where the rates follow a Q_{10} relationship; 5) inverse

compensation, where the acclimated rate is lower than that following an acute change in temperature (Precht, 1958). Capacity adaptations commonly occur in the rate processes of metabolic enzymes.

Adaptation to temperature

Of all the environmental factors affecting biological processes, temperature is probably the most important (Alexandrov, 1977). Even so, life occurs throughout a wide range of temperatures; methanobacteria occur in oceanic thermal vents, in temperatures up to 250°C (Baross & Deming, 1984), also many invertebrates in polar regions survive winters below -60°C. Fish species exploit environments ranging in temperature from -1.9°C in the poles to about 45°C in geothermal hot springs, though no species can tolerate the entire thermal range. Low temperatures generally depress the physiological processes of poikilothermic animals. Therefore low temperatures would be expected to reduce muscle mechanical properties and thus locomotory capabilities of fish species. Decreased locomotory abilities would have important consequences on behavioural aspects such as migratory capacities, prey capture and predator avoidance, all of which may form intense natural selection pressures. The plasticity of the piscine genome has enabled the expression a wide range of muscle phenotypes. This wide phenotypic range has enabled fish species to maintain and adapt their locomotory performance throughout the temperature range observed in the natural environment.

Immediate responses to temperature

One immediate response to temperature was first recognised using synchronised electromyography and cinematography (Rome *et al.*,

1984). Neural mechanisms were found to compensate for reduced power generation of muscles at low temperatures in the carp (Rome *et al.*, 1984). The swimming speed at which white muscle was first recruited decreased from 46 cm/ s at 20°C, to 26 cm/ s at 10°C (Rome *et al.*, 1984). Thus more fibres and faster fibre types were recruited at lower swimming speeds to compensate for reduced power output with decreasing temperatures. This response was termed "compression of recruitment order", and is also seen in the activity of muscles from Savannah monitor lizards (*Varanus exanthematicus*) (Jayne, Bennett & Lauder, 1990) and in the scup (Rome *et al*, 1992).

Evolutionary adaptations to temperature

The metabolic rates of similar sedentary fish species show partial compensation to natural body temperature (NBT). The resting metabolic rate of tropical species approaches zero between 10 and 5°C (Johnston, Clarke & Ward, 1991). At their respective acclimation temperatures however, the maximum metabolic rate of tropical species was 2 - 4 fold higher than that of Antarctic species (Johnston *et al.*, 1991). This difference in metabolic rate suggests that the scope for sustained activity is much lower for cold water species than warm water fish (Johnston *et al.*, 1991).

Mechanical properties of muscle fibres are highly temperature dependent. Maximum tetanic tension shows a strong correlation with the natural environmental body temperature (NET) of each species. Evolutionary adaptations within the genome have enabled fish from polar, temperate and tropical regions to produce similar values of absolute tension at their NET, (Altringham & Johnston, 1985; Johnston, 1987; Johnson & Johnston, 1991b; Langfeld, Altringham & Johnston,

1989). Even small changes in preferred body temperature were sufficient to cause resistance type adaptations, in the maximum tension produced by fibres from closely related Australian skinks (John-Alder & Bennett, 1987).

Contractile processes of muscle involve many enzymatic steps which are highly dependent on temperature. The thermal dependence of these contractile properties is reflected throughout the natural environment. Rates of activation and relaxation are faster in tropical > temperate > polar species at their respective physiological temperatures (Johnson & Johnston 1991b). Even so, some evolutionary adaptations in contractile properties are apparent between fish species from tropical and polar regions. Twitch relaxation times are much longer in tropical species at low temperatures than in polar species (Johnson & Johnston 1991b). Evidence suggests that adaptations have occurred in the Ca^{2+} transport rates of the sarcoplasmic reticulum (SR) (McArdle & Johnston, 1980). At 0°C, the SR isolated from Antarctic species accumulated Ca^{2+} at significantly faster rates than SR from warm-adapted species. This is thought to be due in some part to changes in the activation enthalpies ($\Delta H^{\neq}$) of the SR ATPase, which showed a positive correlation with habitat temperature (McArdle & Johnston, 1980).

The maximum contraction velocity (V_{max}) is positively correlated to natural body temperature. V_{max} is about 1 fibre length/ s in Antarctic fish at 1°C, and 16 fibre length/ s in tropical species at 24°C (Johnson & Johnston 1991b). No evolutionary compensation appears to have occurred in V_{max}, as at 0°C V_{max} is approximately 1 fibre length/ s in Antarctic, temperate and tropical species (Johnston & Altringham, 1985). Curvature of the P-V relation increases with temperature, over

the normal body temperature range of each species (Johnston & Altringham, 1985; Johnson & Johnston, 1991b). Fibres from Antarctic fish however, have significantly less curved P-V relations than those of temperate and tropical species at normal body temperatures (Johnson & Johnston, 1991b). This decrease in curvature may be an evolutionary adaptation to increase power output capabilities at low temperatures. Decreased curvature represents an increase in velocity for a given load.

Adaptations that occur over evolutionary time scales are normally associated with changes in the myosin tertiary structure. The protein structure of myosin isolated from cold water species is thermally unstable compared to that of tropical fish, birds and mammals; It is also more susceptible to aggregation (Connell, 1958, 1961). The actomyosin ATPase activity of Antarctic fish is also 200 - 500 times more sensitive to thermal denaturation than the ATPase of tropical species (Johnston & Walesby, 1977).

Acclimation and acclimatisation

Enzyme activity

Measurements of enzymic adaptations are often utilised to investigate metabolic adaptations to temperature (Hazel & Prosser, 1974). Adaptation in the contractile properties of fish muscle was first demonstrated by Johnston, Davison and Goldspink, (1975). The Mg^{2+} - myofibrillar ATPase activity of fast muscle was nearly three times higher in 1°C-acclimated goldfish, (*Carassius auratus*) compared to 26°C-acclimatised fish, at 1°C (Johnston *et al.*, 1975). Also, the ATPase isolated from cold acclimated fish showed greater sensitivity to thermal denaturation than that of warm acclimated goldfish (Johnston *et al.*,

1975). Differences in the thermal sensitivity of the ATPase suggests that conformational changes occur in the region of the active site. Some enzymes are severely disrupted by temperature change and altering the type of enzyme present is the only way of ensuring effective enzyme function at differing temperatures. The expression of different isoenzymes is therefore one way of maintaining enzyme activity following temperature acclimation. The most common response is changes in enzyme sub-unit composition, e.g.: cold acclimation increased the relative rates of M to H subunits of lactate dehydrogenase in the goldfish (Hochachka, 1965). Also, some evidence is available for the existence of 'on-off' switches for the synthesis of different isoenzymes in accordance to the environmental temperature encountered. A classic example of this is trout acclimated to 2°C- and 18°C-, which possess distinct forms of acetylcholinesterase (Baldwin & Hochachka, 1970). Not all eurythermal fish species exhibit changes in isoenzymes, e.g.: the green sunfish (Shaklee, Christiansen & Sidell, 1977). Therefore the response is thought to be limited to certain species, mainly salmonids and cyprinids (Cossins & Bowler, 1987). Change in microenvironment also alters the rate of enzyme activity. Decreasing temperature causes an increase in the pH of the cytoplasm, which can alter enzyme conformation and hence rate (Somero, 1981). A wide variety of animals have been found to regulate changes in blood and cystolic pH in response to temperature acclimation (Reeves, 1977).

Wilson (1973) found the first definite evidence for a quantitative change in enzyme concentration in relation to temperature acclimation. Concentrations of cytochrome oxidase in goldfish were 66% greater in skeletal muscle of 5°C-acclimated fish, compared to 25°C-acclimated goldfish. Cytochrome C concentrations in the skeletal muscle of the

green sunfish (*Lepomis cyanellus* R.) were also found to increase with decreasing acclimation temperature, (5, 15 and 25°C) (Sidell, 1977). Evidence suggested that the increase in cytochrome C concentration, resulted from a relative decrease in degradation rate relative to that of synthesis with decreasing temperature (Sidell, 1977). Other enzyme concentrations are believed to be mediated by the activities of enzymes responsible for protein synthesis (Haschemeyer, 1969). The cytochromes are associated with aerobic capacities of fish which determine sustained swimming capabilities, but fast fibres use anaerobic pathways (i.e. glycogenolysis) to utilise energy stores. In contrast to enzymes from aerobic pathways, the activities of glycolytic enzymes of muscles do not appear to vary with temperature acclimation, e.g.: in green sunfish (Shaklee *et al.*, 1977), goldfish (Sidell, 1980), carp (Johnston, Sidell & Driedzic, 1985) and chain pickerel (Kleckner & Sidell, 1985).

Adaptations of the nervous system

Control of locomotory activity is co-ordinated by the central nervous system (CNS). Therefore the neural network of poikilotherms must also function adequately during temperature fluctuations, in order to maintain that control. Sharks have developed one method of maintaining neural control. Essential organs such as the brain and the eyes are held at a constant temperature through the use of heater organs and thus are independent of the external environment (Block & Carey, 1985). Other compensatory mechanisms involve the adaptation of various neural processes in response to temperature acclimation.

Thermal adaptation has been shown to increase the speed of some neural processes within the CNS. Electrical stimulation of trout retina produced an electrical response in the midbrain (Konishi & Hickman, 1964). These midbrain responses were 40% slower when 10°C-acclimated trout were exposed to 4°C. After three weeks of acclimation to 4°C however, the effects of acute temperature change were halved (Konishi & Hickman, 1964). Similarly, the duration of slow negative potentials in the facial lobes of the brain showed perfect compensation between 10°C and 20°C in the brown bullhead, (*Ictalurus nebulosus*) (Bass, 1971). Conduction velocities of peripheral nerves of the brown bullhead were not affected by temperature acclimation (Bass, 1971). From this and other studies it is thought that adaptive changes occur in synaptic transmission processes (Cossins & Bowler, 1987).
Evolutionary compensation in the speeds of nervous responses and the maintenance of function is also apparent in Antarctic fish. The velocities of saccadic eye movements in Antarctic fish species was much higher than that of temperate fish species, at 2°C. In fact, saccadic eye movement and function failed at 5°C in 14°C-acclimated fish. As eye movement is controlled by the burst frequencies of the motor neurones, this represents a major compensatory adaptation to low temperatures (Montgomery & Macdonald, 1984), (Montgomery, McVean & McCarthy, 1983). Cold acclimation in goldfish also produces changes in the morphology of the optic nerves that are associated with faster conduction velocities (Matheson & Roots, 1988a & b). Changes in the conduction properties of the axons does not however appear to be as important as changes in synaptic and neuromuscular junctions (Cossins & Bowler, 1987). Certain behavioural activities depend on the effective function of neuronal systems. Escape responses of fish are often

mediated by Mauthner cells. Mauthner cells are a pair of giant interneurones located in the hindbrain (Eaton, Bombardieri & Meyer, 1977). The latencies of startle response were 10 - 30 ms at 15°C, with a Q_{10} of 2 (Webb, 1980). Latencies of predator attack chases are 3 - 10 times longer, due to the additional "direction of attack" information processing time (Webb, 1984a). The reflex escape response would therefore convey an advantage to the prey and would be an important determinant of survival.

Properties of nerves are modified by changes in the myelin membrane. Alteration in the fluidity and permeability of the myelin membrane does occur in response to thermal acclimation (Selivonchick & Roots, 1976).

Membrane properties

Properties of lipid bilayer membranes are important as they control the flux of electrolytes and non-electrolytes into the cell. Complex changes in the composition of the phospholipids of the membrane occur in response to variations in temperature. The tendency is for the proportion of unsaturated relative to saturated fatty acids to increase following cold acclimation. Examination of the rotational properties of the membrane indicate that the more unsaturated fatty acids present, the greater the fluidity of the membrane (Hazel & Prosser, 1974; Cossins, 1983). This increase in fluidity is believed to offset "the ordering influence of cooling" and maintain the ion transport capabilities of the cell (Hazel & Prosser, 1974). Indeed, the permeability of artificial membranes and mitochondria was much greater when prepared from cold acclimated animals as opposed to warm acclimated animals (Cossins, 1983; Cossins & Bowler, 1987).

The mechanisms which regulate lipid composition in the membrane are relatively unknown. Cold acclimation in carp does induce a 30 - 40 fold increase in the activity levels of Δ^9 desaturase in liver microsomes (Schvenke & Wodtke, 1983). Δ^9 desaturase inserts double bonds into saturated fatty acids. Increased enzyme activity following cold acclimation suggests that protein synthesis or genomic expression, may control membrane fluidity. Regulation of membrane fluidity following thermal acclimation may also be brought about by changes in the cholesterol levels (Anderson, Minton, Li & Hahn, 1981). Acclimatory compensation in the production rates of hepatic cholesterol have been observed in carp (Teichert & Wodtke, 1987) and catfish (Hunter & Rodwell, 1980). Membrane properties do not change uniformly, e.g.: the inner and outer membranes of brain mitochondria differ in fluidity responses at temperature extremes. The fluidity of the membrane influences the activity of the membrane bound enzymes. The more fluid the membrane the greater the scope for conformational change in enzyme function with temperature (reviewed by Cossins & Bowler, 1987). For example, the activity of Na^+ -K^+ -ATPase in the intestinal mucosa of goldfish increases following cold acclimation. However, the number of active Na^+ -K^+ -ATPase molecules remains the same (Smith & Ellory, 1971). Also, reconstitution of lipid free extracts of succinic dehydrogenase from goldfish mitochondria was greatest with extracts from cold, rather than warm acclimated fish (Hazel, 1972).

Ultrastructural changes

Changes in swimming performance and contractile processes may involve modifications in structural elements of skeletal muscle systems. The average diameter of myofibrils was found to decrease in cold

relative to warm acclimated fish (Penny & Goldspink, 1980). Cold acclimated goldfish also had higher surface densities of sarcoplasmic reticulum (SR) throughout the muscle systems compared to warm acclimated fish (Penny & Goldspink, 1980). These two factors serve to reduce the diffusion pathways between the SR and contracting filaments. The reduced diffusion pathways would partly account for the observed increases in the rate of muscle relaxation at low temperatures, following cold acclimation (Johnston, Fleming & Crockford, 1990). Cold acclimation generally results in higher mitochondrial densities in cold compared to warm acclimated fish (Johnston & Maitland, 1980; Johnston & Dunn, 1987: Egginton & Sidell, 1989). This combined with increased capillary densities (Johnston, 1982) would increase the potential aerobic ATP production in cold relative to warm acclimated fish. Aerobic capacity is also raised by the increased activites of some mitochondrial enzymes following cold acclimation (Hazel, 1972). As the concentrations of these enzymes does not differ with thermal acclimation, raised activity is believed to result from changing membrane properties (Hazel, 1972; Wodtke, 1981; Tyler & Sidell, 1984). The overall changes in ultrastructure speed up diffusion processes and increase aerobic capacity of the muscle following acclimation to cold temperatures.

In vitro and *in vivo* performance

Mechanical performance of skeletal muscle is greatly dependent on temperature. Maximum capabilities of the muscle, sets the upper limits for locomotory performance of the whole animal. Most poikilotherms studied show no compensation in mechanical muscle properties, even after long periods at low temperatures; instead rates

follow simple Q_{10} relationships (Bennett, 1985). Thermal acclimation did not occur in the contractile kinetics or the force generating capacities of muscle from frogs and toads (Renaud & Stevens, 1981a & b, 1984; Rome, 1983), salamanders (Else & Bennett, 1987), or lizards (Putnam & Bennett, 1982). Due to the lack of plasticity in the muscle properties of the above species it is not surprising to find a similar lack of compensation in their locomotory performance capacities (Putnam & Bennett, 1981). Maximum running and swimming performances, and endurance capabilities did not change with acclimation to 10°C or 20°C, in the salamander (Else & Bennett, 1987). Behavioural capacities of the salamander had relatively low thermal dependencies with Q_{10}'s of 0.99 - 1.36 for burst speeds and Q_{10}'s of 1.58 - 1.66 for endurance (Else & Bennett, 1987). Isolated muscle properties of the salamander however, were much more dependent on temperature with Q_{10}'s of 1.89 - 2.33 (Else & Bennett, 1987). Other workers have also found that the thermal dependence of performance, is much lower than that of rate processes in isolated muscle (Marsh & Bennett, 1985; Putnam & Bennett, 1981; Rome, 1983). The reason for this difference in temperature dependence is not known, but could involve neurological differences and/ or storage of potential energy in elastic components during locomotion (Marsh & Bennett, 1985; Rome, 1986). The only vertebrate group known so far, to modify the properties of the muscle in response to temperature, are some fish species. Live fibre experiments on red muscles of common carp (*Cyprinus carpio* L.) showed an increase of 32% in Po and 17% in Vmax following cold acclimation (Langfeld, Crockford & Johnston, 1991). The contractile rates of force development and relaxation were also found to be highly dependent on acclimation temperature in both skinned and live fibre

preparations (Johnston *et al.*, 1985; Langfeld *et al.*, 1991). Maximum power output also increased by nearly 50% in 8°C-, compared to 20°C-acclimated fish, at 8°C (Langfeld *et al.*, 1991). The increased muscle capabilities at low temperatures is reflected in the swimming ability of cyprinids (Fry & Hart, 1948; Rome, Loughna & Goldspink, 1985). Compensatory adaptations in skeletal muscle properties are not universal in fish, as no acclimatory response is shown in other eurythermal fish, such as flounder (*Platichthys flesus*) (Johnston & Wokoma, 1986) or striped bass (*Morone saxatilis*) (Moerland & Sidell, 1986a & b). The first examples of adaptation in the contractile muscle properties of a marine teleost (*Myoxocephalus scorpius*) were demonstrated by Johnson and Johnston (1991a). The muscle modifications of *M. scorpius* occurred in response to warm temperatures. In contrast, freshwater cyprinids modify muscle properties and swimming performance over a whole range of environmental temperatures.

The present study focused on the fast muscle fibres of the marine teleost *M. scorpius*. Isometric and isotonic contractions were used to investigate which characteristics of the muscle change following temperature acclimation. The importance of extrinsic factors other than temperature were assessed by comparing the properties of fibres isolated from wild-caught and laboratory acclimated *M. scorpius*. Parameters such as maximum velocity of shortening and force generation of fibres from wild-caught and laboratory-acclimated fish were compared at 5, 10 and 15°C. Power output was estimated at each temperature and the shape of the force-velocity curve was ascertained for each acclimation group (Chapters 2 and 3).

Video analysis of *M. scorpius* performing fast-starts was used to determine if *in vitro* changes in the muscle properties are reflected in swimming capabilities of the fish (Chapter 4). Oscillatory work loop experiments were conducted on fast fibres to determine power output values relevant to burst speed swimming (Chapter 5). Values of muscle strain for three positions along the body of the fish were calculated from video analysis of sculpin prey-capture events by Johann van Leeuwan, University of Leiden, Holland. Changes in muscle properties along the length of the fish could then be assessed and the relative contribution of power from the anterior and posterior musculature to swimming, could be determined.

CHAPTER 2

Influence of thermal acclimation on the isometric contractile properties of muscle fibres in the short-horned sculpin

Introduction

Fry and Hart (1948) found that goldfish (*Carassius auratus* L.) were able to modify their swimming performance following a period of temperature acclimation lasting several weeks. Cold- and warm-temperature acclimation improved sustained cruising speeds over 2 min intervals at low and high temperatures respectively. The adaptations in the neuromuscular system of freshwater cyprinids underlying such improvements in swimming ability are complex and include changes in myosin ATPase activity (Johnston *et al.*, 1975; Heap, Watt & Goldspink, 1986), force production, maximum contraction velocity (Johnston *et al.*, 1985; Langfeld *et al.*, 1991) and relaxation rates (Fleming, Crockford Altringham & Johnston, 1990). Changes in the ionic properties of neuronal membranes have also been demonstrated (Harper, Shelton & Watt, 1989). The molecular mechanisms behind these changes include altered expression of myosin light and heavy chain genes (Crockford & Johnston, 1990; Gerlach *et al.*, 1990; Ushio & Watabe, 1993), changes in sarcoplasmic reticulum Ca^{2+} -ATPase activity (Fleming *et al.*, 1990) and modifications in the activities of enzymes involved in ATP generating pathways (Sidell, 1980; Johnston *et al.*, 1985).

Relatively few studies have examined the influence of thermal acclimation on muscle contractile properties in marine fish which are usually subject to smaller annual changes in temperature than freshwater species. A previous study on *Myoxocephalus scorpius* L. by Johnson and Johnston (1991a) employed the work loop technique to measure the power output of fast muscle fibres undergoing sinusoidal length changes which approximate steady swimming. The cycle frequency which produced the maximum work per cycle under optimal stimulation

conditions rose from 4 to 9 Hz as temperature increased from 5 to 15°C. At 15°C, the peak force generated per cycle and the average power output per kg of wet mass of muscle tissue were greater from summer than winter-acclimatised fish (Johnson & Johnston, 1991a).

The short-horned sculpin (*Myoxocephalus scorpius scorpius* L.) is a boreal species, widely distributed on both sides of the Atlantic Ocean. In the Eastern Atlantic sculpin range from Barents Sea down through to the Baltic Sea and south, to the north-west coast of France (Donovan, 1808; Ehrenbaum, 1932; Wheeler, 1969). Sculpin are common around the British Isles and Ireland (Jenkins, 1936; King, Fives & Dunne, 1983), and have been found in deeper waters off north-west Scotland (Günther, 1888).

In this study, isometric contractions have been used to determine which parameters (force generation, relaxation rate etc.) of fast fibres from sculpin are modified by temperature acclimation. Many enzymes of energy metabolism, including creatine phosphokinase, show different activities in naturally acclimatised populations compared to individuals acclimated to similar temperatures in the laboratory (Kleckner & Sidell, 1985). This reflects the importance of other factors such as day-length (Kolok, 1991), physical condition, seasonal migrations (Guderley & Blier, 1988) and reproductive state (Guderley & Foley, 1990) in modifying responses to temperature change. To investigate the importance of endogenous rhythms and extrinsic factors other than temperature on seasonal changes in the muscle properties of wild-caught fish, comparisons were made with laboratory-acclimated sculpin. Sculpin were acclimated to average winter and summer temperatures, under a constant photoperiodic regime of 12 h light: 12 h dark. The

results indicate that seasonal adaptations in muscle properties occur in peak force generation, activation and shortening speed, primarily associated with changes in water temperature alone.

Materials and methods

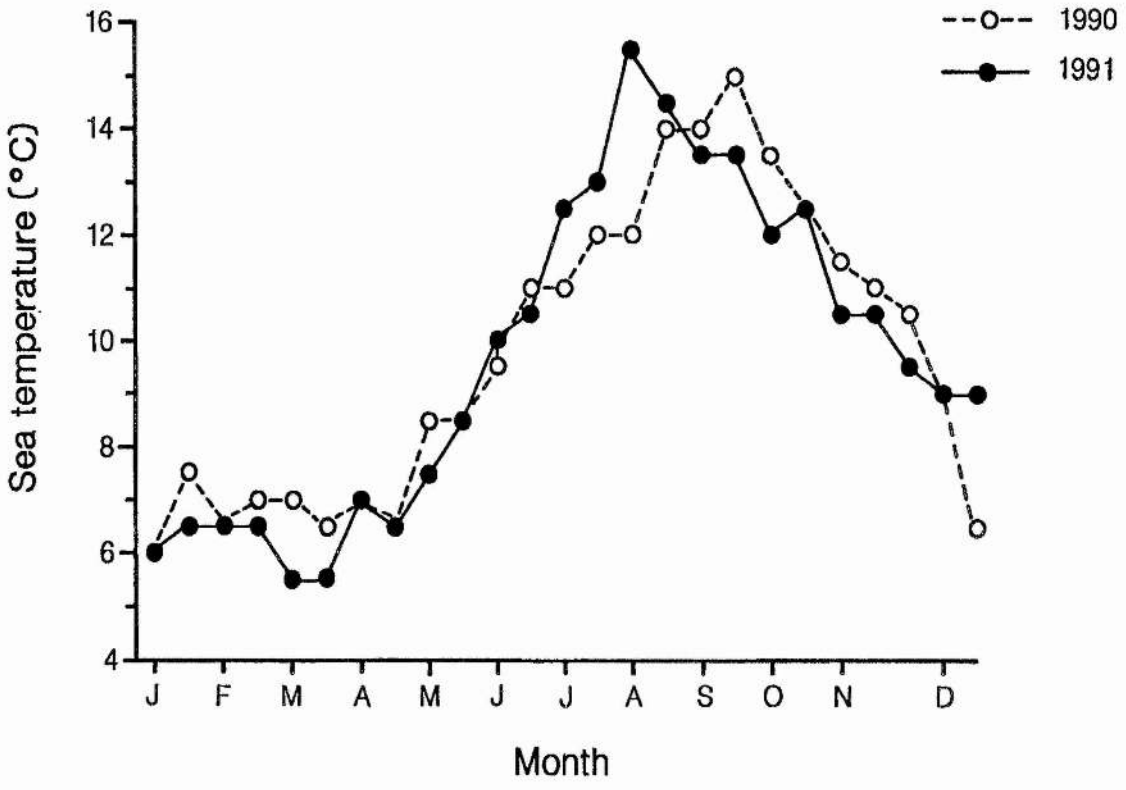
The fish

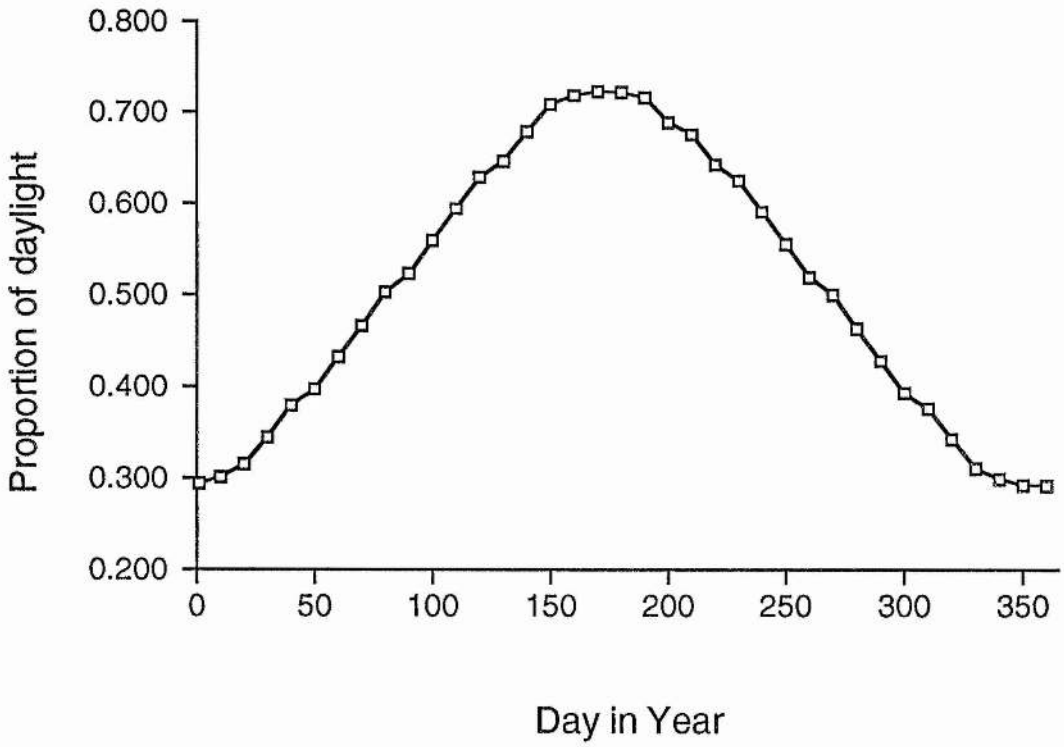
Experiments were conducted on short-horned sculpin, *Myoxocephalus scorpius* (L.) of standard length from 17 - 25.5 cm. Experimental animals were caught in the Firth of Forth or obtained from Millport University Marine Biological Station, throughout the year. The laboratory-acclimated fish were held at ambient temperature in flow-through 385 l circular tanks, for 1 week after capture. The temperature was subsequently adjusted by 1°C per day, until the required acclimation temperature of either 5°C or 15°C (± 0.5°C) was reached. Sculpin were acclimated to these temperatures for 6 - 8 weeks, under a constant photoperiodic regime of 12 h light:12 h dark. Fish were fed regularly on a diet of fish flesh, squid and crustaceans.

Fish naturally acclimatised to winter conditions were caught in the Firth of Forth between January and March 1990 and 1991, when the sea temperature was 5 - 6°C. Summer acclimatised fish were caught between July and September in 1991 and 1992 when the temperature reaches 14 - 15°C, (Fig. 1.1a). The annual photoperiod of the natural environment is illustrated in Figure 1.1b. Field-acclimatised fish were held at ambient temperature in flow-through sea water aquaria for 1 - 2 weeks prior to use.

Figure 1.1.

Figure 1.1 a) Sea surface temperature of St. Andrews bay in 1990 and 1991. Data obtained from the Meteorlogical Office, Aberdeen. **b)** Photoperiod of the St. Andrews region, shown as the proportion of sunlight per day.


1990
1991
Sea temperature (°C)
16
14
12
10
8
6
4
J
F
M
A
M
J
J
A
S
O
N
D
Month


Proportion of daylight
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0
50
100
150
200
250
300
350
Day in Year

Fibre bundle preparation

Fish were killed by a blow to the head followed by pithing and transection of the spinal cord. Standard length and body mass were recorded. The outer layer of skin was removed from the abdomen of the fish and a group of fast fibres excised. A thin section of muscle, 5 - 6 cm wide and 4 - 7 myotomes long was pinned out at resting length onto a silicone elastomer base (Sylgard 184, Dow Corning, Midland MI, USA). The tissue was immersed in fresh, cold Ringer (mmol l^{-1}: NaCl 132.2; Na Pyruvate 10; KCl 2.6; $MgCl_2$ 1; $CaCl_2$ 2.7; $NaHCO_3$ 18.5; $NaHPO_4$ 3.2; pH set to 7.4 using HCl/ NaOH (Hudson, 1969)), and the dissection was carried out on a cooled aluminium stage (< 5°C) under a binocular microscope. The under layer of skin was removed from the surface of the tissue. Then the peritoneum was removed from a central myotome and pared down to 10 - 20 undamaged muscle fibres. Muscle fibres were removed from adjacent myotomes leaving a thin strip of the peritoneum. Aluminium foil clips were attached to the remnants of the peritoneum, as close to the myoseptum as possible.

Experimental apparatus

Fibre bundles were transferred to a perspex chamber (5 x 1 x 1 cm) containing circulating Ringer at the desired temperature (± 0.1°C). A peristaltic pump (Watson - Marlow) circulated aerated Ringer through a coil immersed in a thermostatically controlled water bath (Grant LTD 6). Further temperature control was gained by insulating all the plastic tubing of the system. Temperature was regularly monitored using a digital probe (RS 650-419) situated in the chamber.

The Ringer was changed every 2 - 3 h to maintain a constant pH and prevent calcium precipitation. One end of the muscle fibre preparation was attached, via a stainless steel hook, to a force transducer (AE 801, AME Horten, Norway) held in a stainless steel tube, which was water-proofed with silicon grease. The transducer was positioned on a micromanipulator, enabling the fibre length to be adjusted. A bridge circuit and amplifier unit modified the transducer output for display. The other end of the preparation was attached to a servo-controlled motor (MFE model R4-077, Emerson Electronics, Bourne End, Bucks), that could perform isotonic length changes (see Chapter 3). The isotonic releases were controlled by a unit built in the laboratory and a LED-photodiode assembly system monitored fibre length. Length changes and tension were measured using a Gould 1602 digital oscilloscope which produced hardcopies of the traces.

Preparations were stimulated via two platinum electrodes (Goodfellow) placed parallel, on either side of the fibres. Stimulation pulses were 1.5 ms duration, at a voltage 1.2 times that used to produce maximum tension (Grass S48 stimulator). The length of the muscle fibre was adjusted to produce a maximum twitch, without residual resting tension. This corresponded to a sarcomere length of 2.2 μm, as measured by He-Ne laser diffraction (Barr and Stroud, Hughes). The maximum tension (Po) of muscle fibres tended to increase when first transferred to the chamber and was probably due to recovery from dissection. Preparations were therefore left for up to 60 min (until Po stabilised) before being stimulated. Rest intervals of 10 min were imposed between trains of stimuli and following a temperature change preparations were left for 40 - 60 min until Po stabilised. The effects of changing temperature were completely reversible over the range used

and reproducible results could be obtained from several cycles of heating and cooling. Data were only analysed from preparations which showed a decline of less than 10% in maximum tension, at each experimental temperature.

Isometric contractions

At each temperature the stimulatory conditions required to produce maximum isometric tetanus were determined. Maximum peak tension (P_o) and time from first stimulus to half peak tension ($T_{0.5a}$) and from the last stimulus to half peak tension ($T_{0.5r}$) were measured for isometric twitches and tetani (Fig. 1.3).

Pharmacological experiments

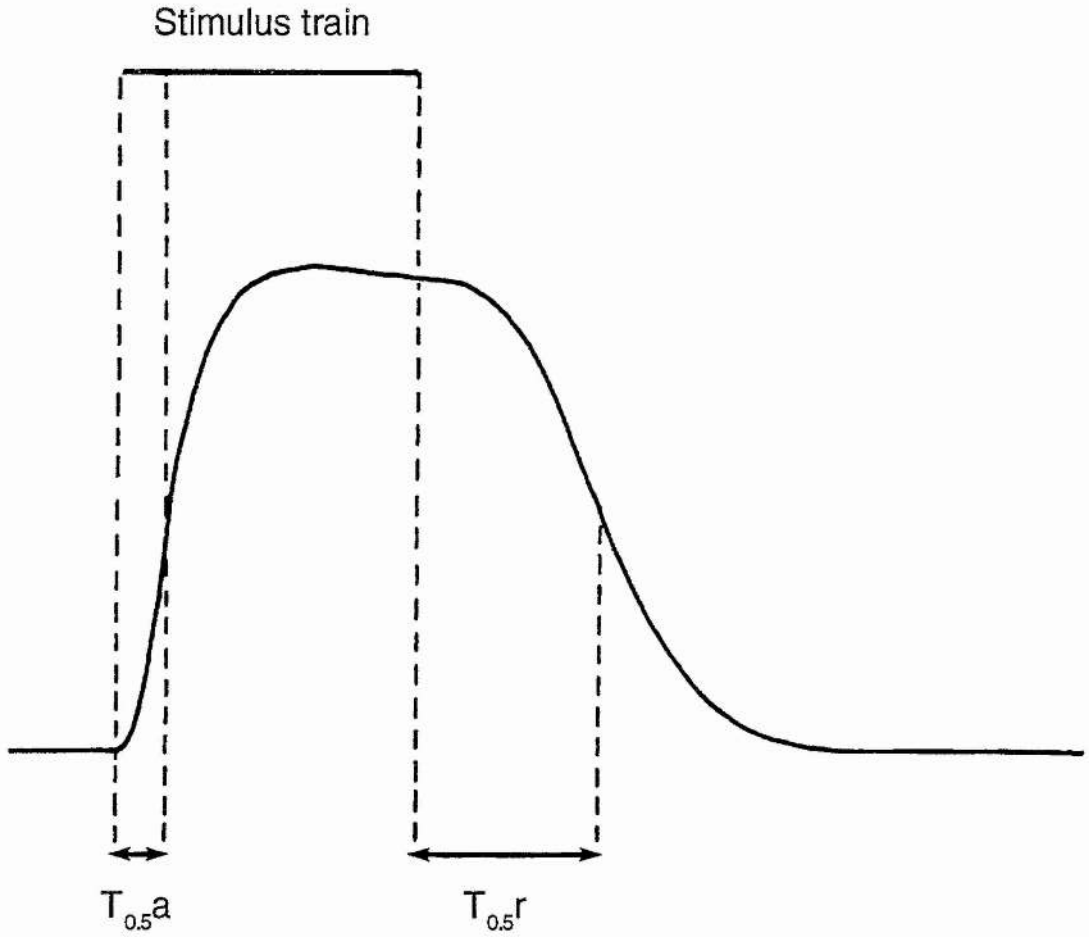
Fibres were exposed to 1 mM caffeine and 10^{-5} g.ml^{-1} eserine which was added to the normal Ringer, and circulated through the experimental chamber at the desired temperature. Potassium contractures were studied using a high-potassium solution (190 mM - K^+). The solution was made up by replacing the NaCl and KCl in the normal Ringer with 95 mM -K_2SO_4. A quick solution exchange was achieved by squirting the high-K^+ solution directly into the chamber for 5 s, while removing excess fluid by suction.

Measuring the cross-sectional area

In order to calculate the absolute power output produced by each fibre, the muscle lengths and cross-sectional areas were measured at the end of each experiment. The preparations were set to resting length and immersed in liquid isopentane which was cooled to near freezing point (-159°C), with liquid N_2. The fibres were quickly taken out of the

Figure 1.3.

Figure 1.3. Illustrative trace showing the isometric parameters measured for each tetanic stimulation.


Stimulus train
$T_{0.5}a$
$T_{0.5}r$

isopentane and the foil clips removed using a cooled scalpel blade and fine forceps. The muscle fibres were mounted vertically in a small piece of fish liver and placed on moist filter paper on a cryostat "chuck". Support medium, (Tissue-Tek. Miles scientific, Illinios, USA) was placed over the mounted preparation and immersed for 10 s in liquid isopentane. Transverse frozen sections 10-15 μm thick, were cut using a cryostat (Bright, Huntingdon, England) cooled to -20°C and mounted on cover slips. Sections were left to air dry for 30 - 45 min and then stained for myofibrillar ATPase activity (Johnston *et al*, 1974). The outlines of undamaged fibres were traced using a microscope drawing arm (X160) (Nikon, Labophot), digitised, and the cross-sectional areas measured (Sigmascan, Jandel Scientific).

Statistical Analysis

Results are presented as means ± S.E.M. Significant differences between acclimation groups and with acute temperature changes were examined using one-way analysis of variance test (Minitab Inc, Philadelphia, USA).

Results

Force generation

The maximum stress (P_o) of muscle fibres from 15°C-acclimated fish increased with an Q_{10} of 2.3, reaching 282 kN m^{-2} at 15°C (Table 1.1; Fig. 1.7a). Representative twitch and tetani traces for each acclimation group are shown in Figures 1.4 and 1.5. Tetanic force generation did not differ significantly between muscles isolated from

Table 1.1.

Table 1.1: Influence of temperature acclimation on the contractile properties of fast muscle fibres isolated from anterior myotomes of the short-horned sculpin. Values represent mean ± S.E. Significant differences between acclimation groups at equivalent temperatures indicated by *, **, *** ($P< 0.05, 0.01, 0.005$). P_o = maximum tension; $T_{0.5}a$ = time from first stimulus to 50% maximum tension; $T_{0.5}r$ = time from last stimulus to 50% maximum tension.

		15°C-acclimated fish			5°C-acclimated fish.		
		Experimental temperature (°C).					
Parameter	Units	5°C	10°C	15°C	5°C	10 °C	15°C
Twitches		n = 11	n = 8	n = 9	n = 12	n = 9	n = 11
Po	kN m^{-2}	80.6 ± 15.0	83.3 ± 10.2	127.4 ± 19.0	72.3 ± 13	54.5 ± 15.1	26.2 ± 10.0***
$T_{0.5a}$	ms	18.3 ± 1.2	12.2 ± 0.5	10.0 ± 0.6	18.0 ± 0.6	12.8 ± 0.7	9.4 ± 0.5
$T_{0.5r}$	ms	35.9 ± 5.0	16.7 ± 0.7	17.7 ± 2.1	35.0 ± 3.6	28.9 ± 3.3***	23.1 ± 3.2
Tetani		n = 10	n = 8	n = 8	n = 11	n = 9	n = 10
Po	kN m^{-2}	124.9 ± 20.4	194.7 ± 14.2	282.0 ± 2.6	139.4 ± 14.5	94.0 ± 14.6 ***	78.3 ± 15.8***
$T_{0.5a}$	ms	26.2 ± 3.1	25.0 ± 1.0	17.8 ± 1.2	31.1 ± 1.8	30.4 ± 2.2	26.0 ± 1.3***
$T_{0.5r}$	ms	93.1 ± 6.0	72.3 ± 4.1	57.8 ± 5.6	103.3 ± 3.4	78.5 ± 4.1	56.2 ± 4.2
Twitch: tetanus ratio		0.65	0.43	0.45	0.52	0.58	0.33

Table 1.2.

Table 1.2: Seasonal influences on the contractile properties of fast muscle fibres isolated from anterior myotomes of the short-horned sculpin. Values represent means ± S.E. Significant differences between seasons at equivalent temperatures indicated by *, **, *** ($P < 0.05$, 0.01, 0.005). P_o = maximum tension; $T_{0.5}a$ = time from first stimulus to 50% maximum tension; $T_{0.5}r$ = time from last stimulus to 50% maximum tension.

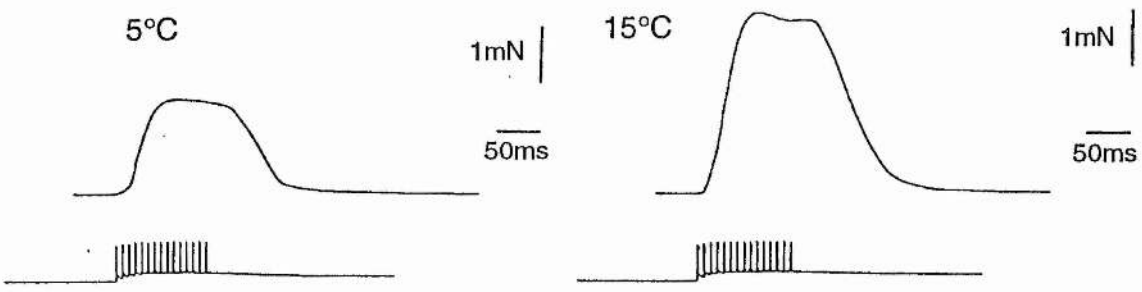
		Summer-acclimatised fish.			Winter-acclimatised fish.		
		Experimental temperature (°C).					
Parameter	Units	5°C	10°C	15°C	5°C	10 °C	15°C
Twitches		n = 7	n = 9	n = 8	n = 12	n = 13	n = 12
Po	kN m^{-2}	114.5 ± 9.2	154.2 ± 12.2	128.6 ± 21.2	82.8 ± 10.5	48.42 ± 7.7***	24.06 ± 4.3***
$T_{0.5}a$	ms	17.7 ± 0.9	12.8 ± 0.6	9.9 ± 0.5	16.4 ± 0.90	11.58 ± 0.36	8.58 ± 0.34*
$T_{0.5}r$	ms	39.7 ± 5.7	27.7 ± 2.7	18.8 ± 1.9	33.7 ± 3.44	20.95 ± 1.11*	16.69 ± 0.80
Tetani		n = 6	n = 6	n = 7	n = 12	n = 12	n = 9
Po	kN m^{-2}	179.4 ± 18.6	227.6 ± 26.4	266.7 ± 12.0	161.7 ± 14.2	111.5 ± 12.5***	74.2 ± 12.5***
$T_{0.5}a$	ms	23.5 ± 2.0	18.4 ± 2.0	16.96 ± 1.7	30.4 ± 1.1***	24.5 ± 0.9***	21.44 ± 1.57
$T_{0.5}r$	ms	94.8 ± 6.1	68.3 ± 3.0	66.21 ± 6.20	90.5 ± 5.6	68.8 ± 5.0	51.0 ± 3.50*
Twitch: tetanus ratio		0.64	0.68	0.48	0.51	0.43	0.32

15°C-acclimated fish or summer-acclimatised fish, at any experimental temperature (Tables 1.1 & 1.2). In both summer and 15°C-acclimated sculpin, the twitch:tetanus ratio of fibres declined by 25 - 30% when the temperature was raised from 5°C to 15°C (Tables 1.1 & 1.2). Peak twitch force however, was significantly greater in fibres isolated from summer-acclimatised fish, than in fibres from 15°C-acclimated fish at 10°C. This is reflected in the high twitch:tetanus ratio of 0.68 found in the summer-sculpin at 10°C (Table 1.2). Maximum Po values at 5°C were similar between all the acclimation groups studied (Tables 1.1 & 1.2). However, in contrast to the results from summer-acclimatised and 15°C-acclimated fish, tension was not maintained at the higher temperatures, in the winter or 5°C-acclimated fish (Fig. 1.7a & b). Fibres isolated from summer and 15°C-acclimated fish produced 3.6-times more tension than the winter and 5°C-acclimated fish at 15°C ($P < 0.001$; Tables 1.1 & 1.2). Similar differences occurred in twitch Po, with fibres isolated from summer and 15°C-acclimated fish producing 5-times more tension than either winter or 5°C-acclimated fish at 15°C (Tables 1.1 & 1.2). The addition of 1 mM caffeine and 10^{-5} g.ml^{-1} eserine to the Ringer did not increase twitch or tetanic tension at any temperature. However, the addition of the high-K^+ solution to fibres from the 5°C-acclimated sculpin resulted in a 2.2-fold increase in force production at 15°C and a 1.5-fold increase at 5°C (average values for 3 experiments). Maximum isometric tensions obtained in this study are similar to those of other fish species *i.e.* 315 ± 17.9 kN m^{-2} in winter/ late spring *M. scorpius* at 8°C (Langfeld *et al*, 1989), 222.55 ± 13.18 kN m^{-2} in the saddle wrasse (*Thalassoma duperryi*), at 24°C and 189 ± 21.4 kN m^{-2} in the icefish (*Chaenocephalus aceratus*), at 0°C (Johnston, 1987).

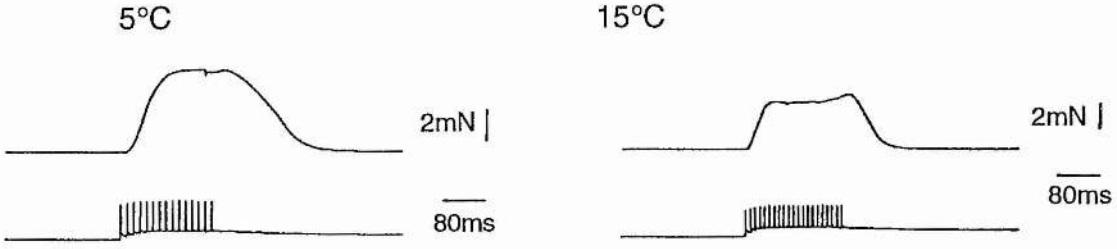
Figure 1.4.

Figure 1.4. Isometric tension records of fast fibres isolated from laboratory-acclimated sculpin at 5 and 15°C. Traces on the bottom line show tension of fibres from 5°C-acclimated sculpin at 15°C. Left hand side shows the tension records in normal Ringer (solid line) and following the addition of 1 mM caffeine plus 10^{-5} g.ml^{-1} eserine (dashed line). Right hand side shows tension trace following the addition of 190 mM K^+ solution.

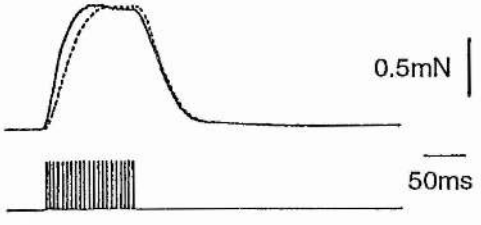
15°C-acclimated fish



5°C-acclimated fish



15°C 1mM caffeine and 10^{-5} g.ml^{-1} eserine



15°C 190 mM [K+]

Figure 1.5.

Figure 1.5. Isometric tension records of fast fibres isolated from naturally-acclimatised sculpin at 5, 10 and 15°C.

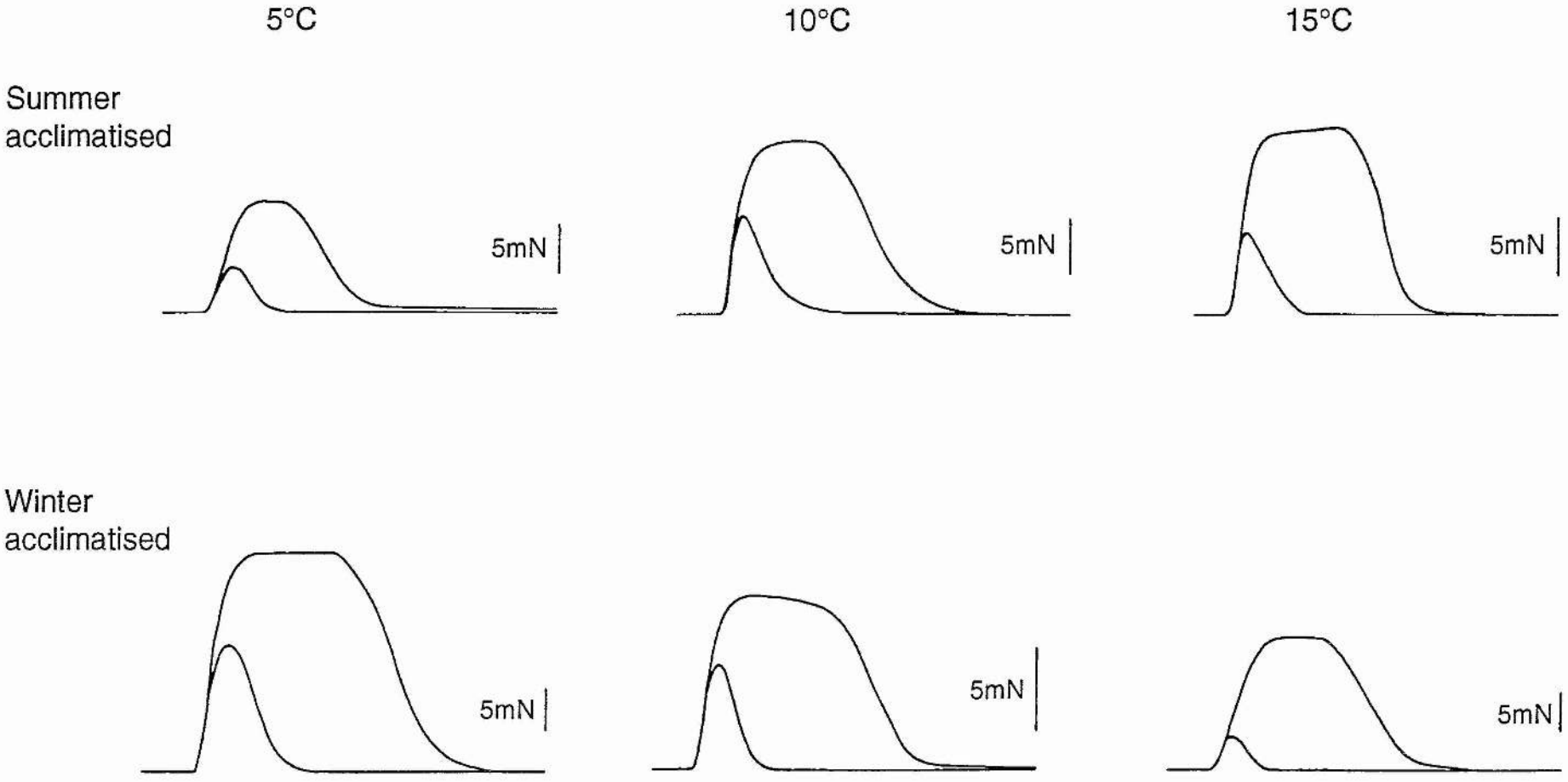
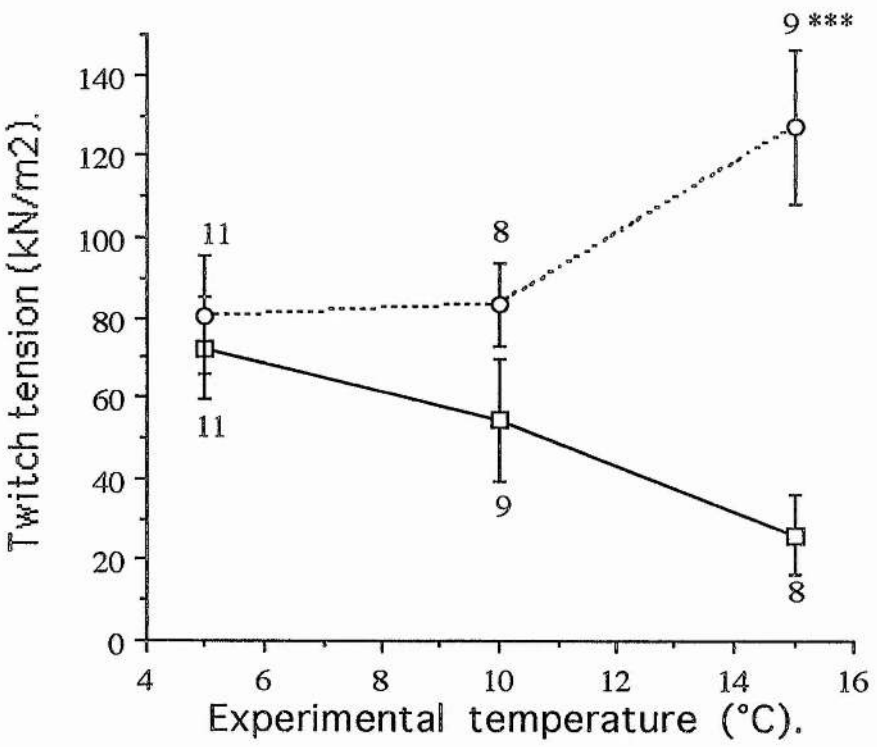

5°C
10°C
15°C
Summer acclimatised
5mN
5mN
5mN
Winter acclimatised
5mN
5mN
5mN

Figure 1.6.

Figure 1.6. The relationship between experimental temperature and isometric twitch tension (kN m^{-2}) of fast muscle fibres from **(a)** laboratory-acclimated sculpin **(b)** naturally-acclimatised sculpin. Values are means ± S.E.(*n*; refer to graph). $P < 0.005$.

Open squares = 5°C-acclimated, open circles = 15°C-acclimated, closed squares = winter-acclimatised, closed circles = summer-acclimatised sculpin.

a)


Twitch tension (kN/m2).
140
120
100
80
60
40
20
0
4
6
8
10
12
14
16
Experimental temperature (°C).
11
8
9 ***
11
9
8

b)

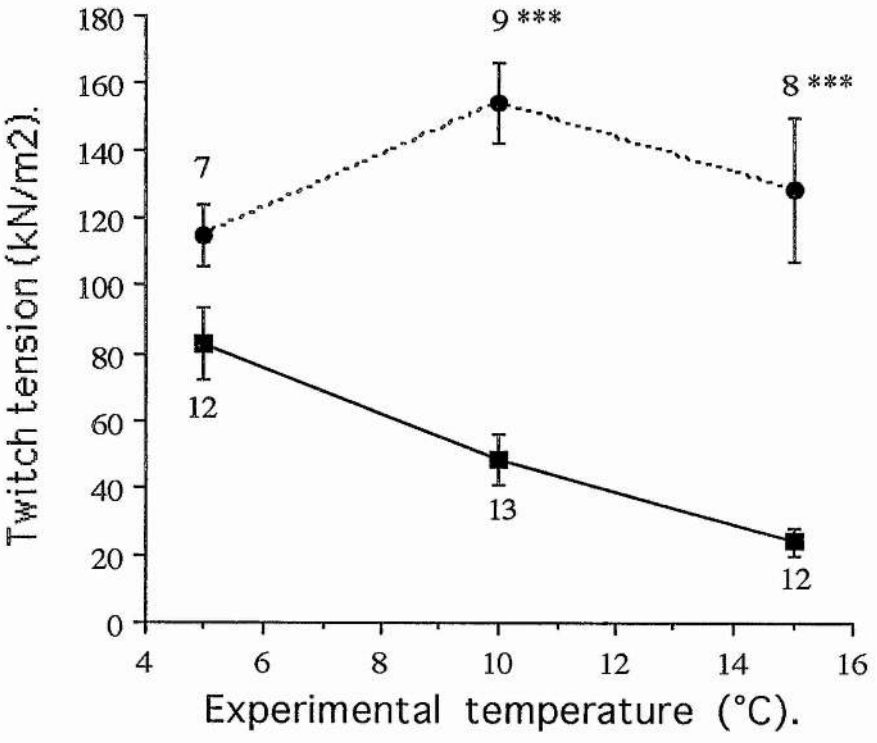
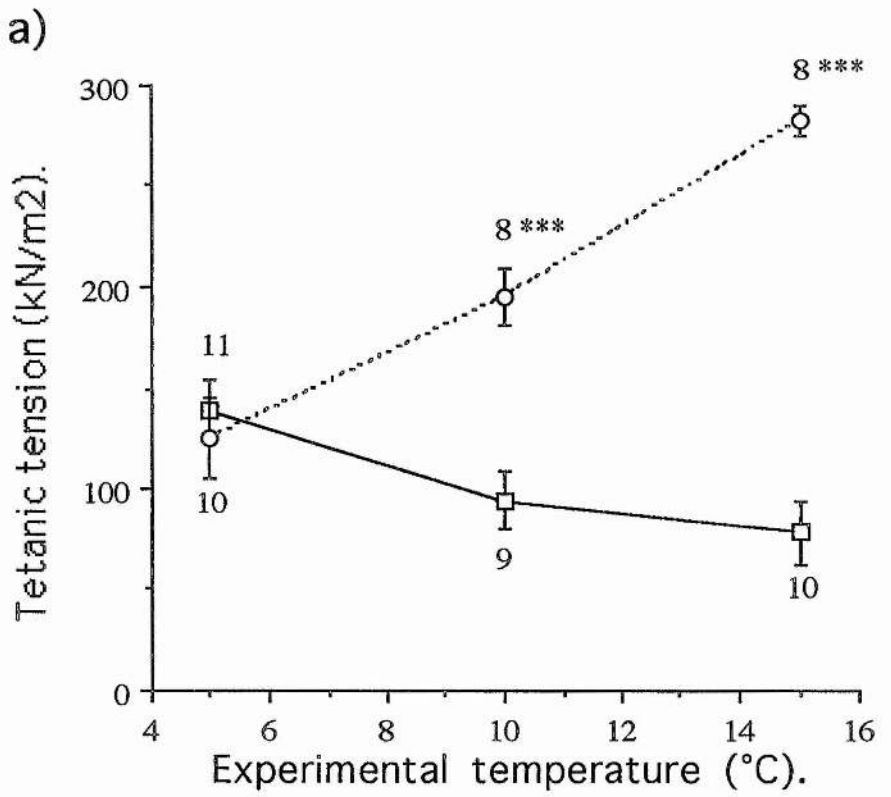
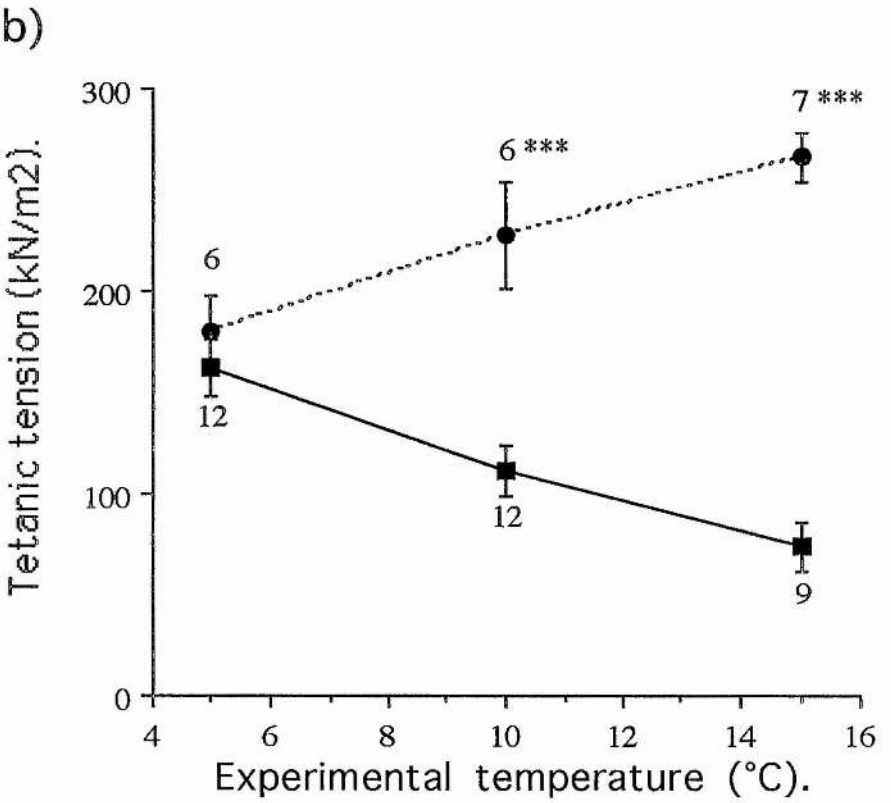

Twitch tension (kN/m2).
180
160
140
120
100
80
60
40
20
0
4
6
8
10
12
14
16
Experimental temperature (°C).
9 ***
8 ***
7
12
13
12

Figure 1.7.

Figure 1.7. The relationship between experimental temperature and maximum isometric tetanic tension (kN m^{-2}) of fast muscle fibres from **(a)** laboratory-acclimated sculpin **(b)** naturally-acclimatised sculpin. Values are means ± S.E.(*n*; refer to graph). $P < 0.005$.

Open squares = 5°C-acclimated, open circles = 15°C-acclimated, closed squares = winter-acclimatised, closed circles = summer-acclimatised sculpin.


a)
Tetanic tension (kN/m2).
300
200
100
0
4
6
8
10
12
14
16
Experimental temperature (°C).
8 ***
8 ***
11
10
9
10


b)
Tetanic tension (kN/m2).
300
200
100
0
4
6
8
10
12
14
16
Experimental temperature (°C).
7 ***
6 ***
6
12
12
9

Activation

In fibres of 15°C-laboratory acclimated fish the time required to develop half maximum tension ($T_{0.5}a$) decreased with Q_{10}'s of 1.8 for twitch and 1.5 for tetani, between 5 and 15°C (Tables 1.1 & 1.2). The Q_{10}'s for $T_{0.5}a$ were generally lower for tetanic than twitch contractions, indicating a relatively lower thermal dependence of tetanic contractile properties. Tetanic $T_{0.5}a$ were generally longer in winter than summer fish, and in 5°C-acclimated than 15°C-acclimated fish, at any given temperature (Tables 1.1 & 1.2). These differences were significant at 5°C and 10°C for winter *versus* summer fish, and at 15°C for cold-acclimated *versus* warm-acclimated fish ($P < 0.004$; Tables 1.1 & 1.2). At 15°C, fibres isolated from 5°C-acclimated fish took 46% longer to develop half maximum force than fibres from 15°C-acclimated fish ($P = 0.001$). Fibres isolated from winter and summer sculpin had shorter tetanic $T_{0.5}a$ than 5°C- and 15°C-laboratory acclimated sculpin respectively, at a given temperature. These differences were significant at 10°C and 15°C in the winter *versus* 5°C-acclimated fish, and at 10°C in summer *versus* 15°C-acclimated fish ($P < 0.035$) (Fig 1.8a & b).

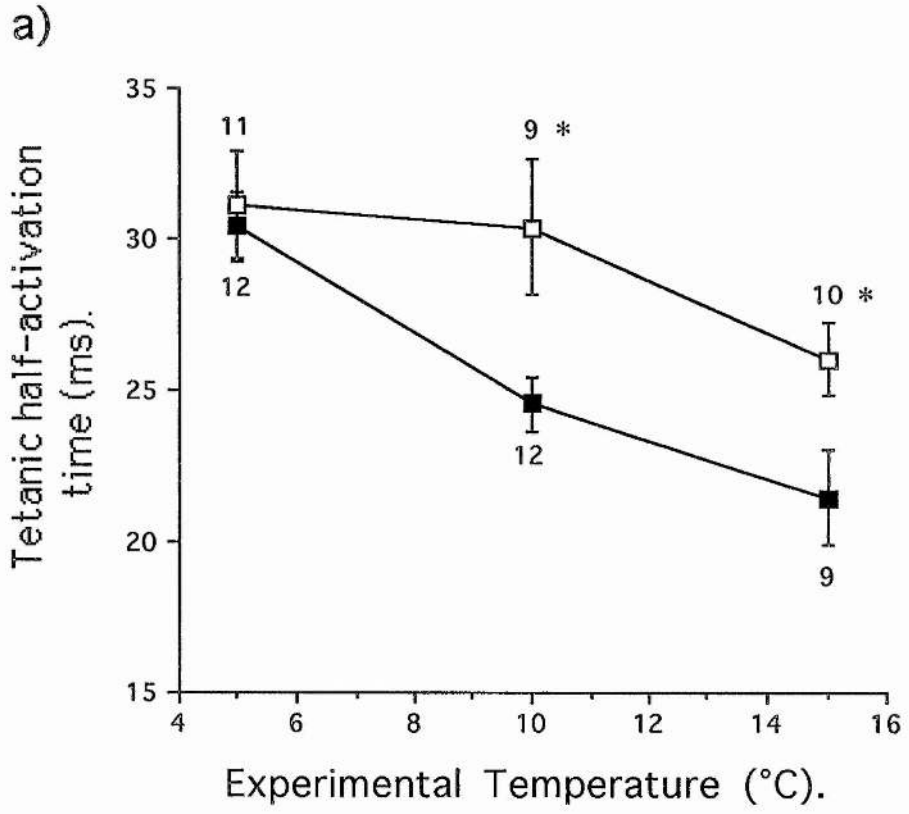
Relaxation

The time required for the loss of 50% maximum force ($T_{0.5}r$), measured from the last stimulus, increased with a $Q_{10(5\text{-}15°C)}$ of 2 for twitches and 1.6 for tetani in 15°C-acclimated fish (Table 1.1). Q_{10}'s for relaxation rate were similar between acclimation groups. Values for tetanic $T_{0.5}r$ were relatively independent of both acclimation and acclimatisation regime for a given temperature (Table 1.1 & 1.2; Fig.

Figure 1.8.

Figure 1.8. Comparison of the time taken to develop half maximum isometric force in naturally-acclimatised and laboratory-acclimated sculpin. **(a)** winter and 5°C-acclimated sculpin **(b)** summer and 15°C-acclimated sculpin. Values are means ± S.E. Significance levels are: * = $P < 0.05$, ** = $P < 0.01$.

Open squares = 5°C-acclimated, open circles = 15°C-acclimated, closed squares = winter-acclimatised, closed circles = summer-acclimatised sculpin.


a)
Tetanic half-activation time (ms).
35
30
25
20
15
11
9 *
10 *
12
12
9
4
6
8
10
12
14
16
Experimental Temperature (°C).

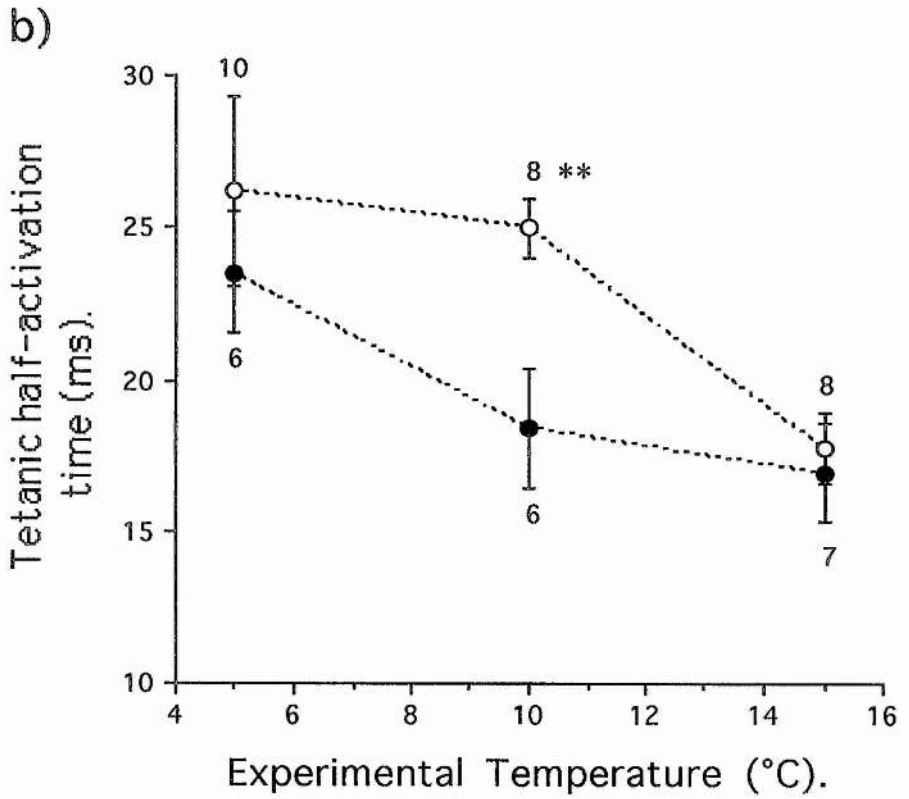
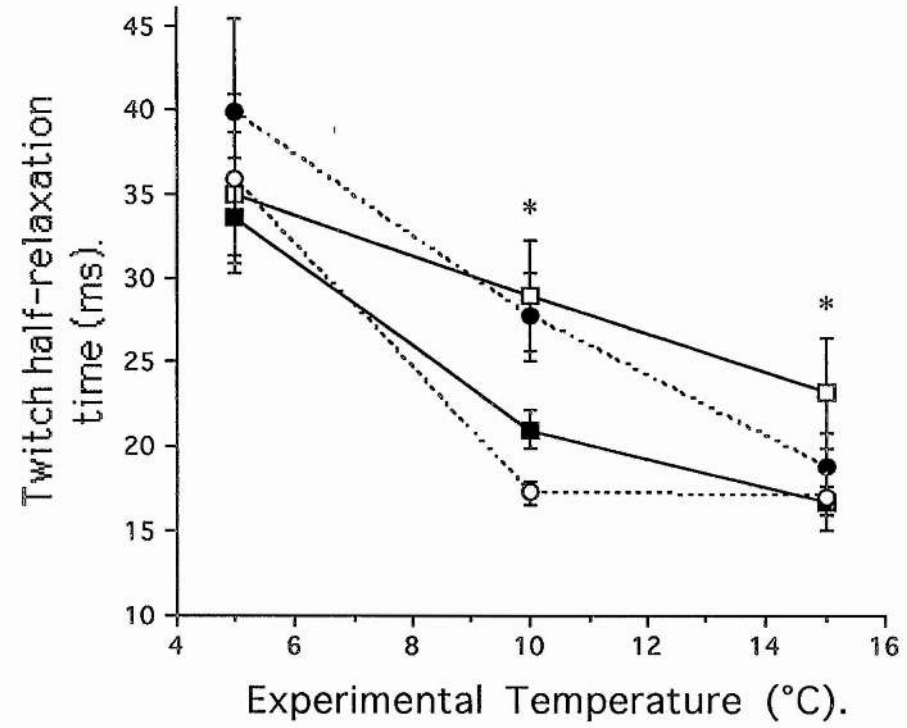

b)
Tetanic half-activation time (ms).
30
25
20
15
10
10
8 **
8
6
6
7
4
6
8
10
12
14
16
Experimental Temperature (°C).

Figure 1.9.

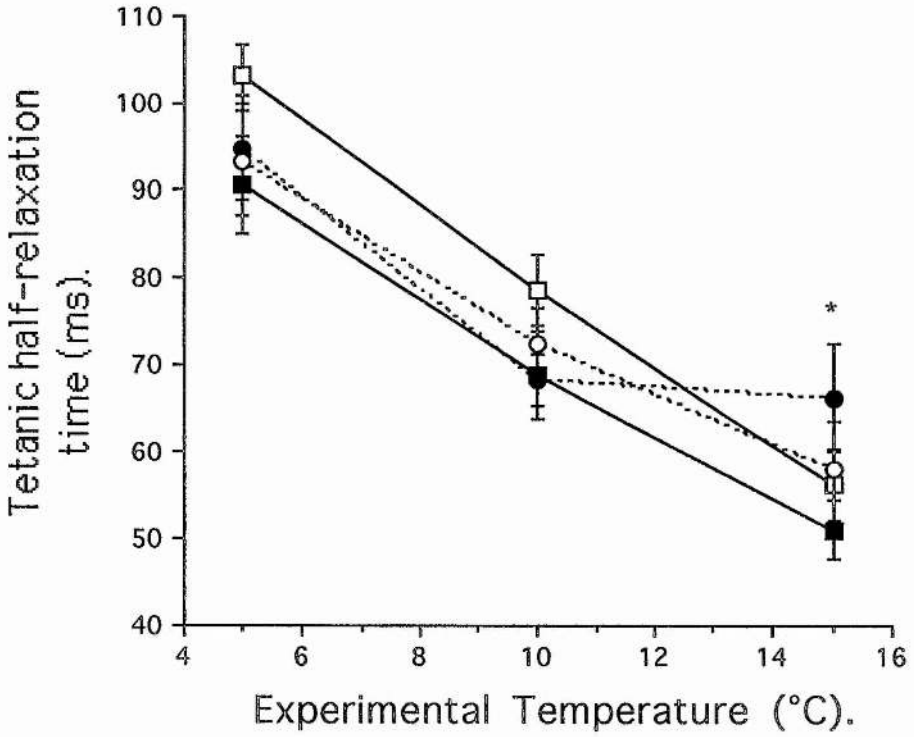
Figure 1.9. The relationship between experimental temperature and **(a)** twitch half-relaxation time (ms), **(b)** tetanic half-relaxation time (ms) for live fast muscle fibres of both laboratory-acclimated and naturally-acclimatised sculpin. Values are means ± S.E. (*n*, refer to Tables 1 & 2). * refers to $P < 0.05$ where fast muscles isolated from (a) winter sculpin have significantly shorter twitch half-relaxation times than summer sculpin, at 10°C, and 5°C-acclimated sculpin at 10°C and 15°C (b) winter sculpin have significantly shorter tetanic half-relaxation times than summer sculpin at 15°C.

Open squares = 5°C-acclimated sculpin, open circles = 15°C-acclimated, closed squares = winter-acclimatised, closed circles = summer-acclimatised sculpin.

a)


Twitch half-relaxation time (ms).
45
40
35
30
25
20
15
10
4
6
8
10
12
14
16
*
*
Experimental Temperature (°C).

b)


Tetanic half-relaxation time (ms).
110
100
90
80
70
60
50
40
4
6
8
10
12
14
16
*
Experimental Temperature (°C).

1.9b). The exception was at 15°C, where the winter fish had shorter tetanic $T_{0.5r}$ than summer fish (Table 1.2; $P < 0.039$). In contrast, twitch half relaxation times were highly dependant on temperature and were altered by thermal acclimation. At 10°C, fibres isolated from 15°C-acclimated fish have shorter twitch $T_{0.5r}$ than both 5°C-acclimated and summer fish ($P < 0.004$). In contrast, winter fish have shorter twitch $T_{0.5r}$ at 10°C than both summer and 5°C-acclimated fish, and shorter $T_{0.5r}$ than 5°C-acclimated fish at 15°C ($P < 0.035$; Fig 1.9a). The Q_{10} values for relaxation observed in this study are similar to those found in the same species by Langfeld *et al.*, (1989), though slightly lower than reported values of 2.5 in the rat and mouse (Stein, Gordon & Shriver, 1982) and in frog skeletal muscle (Edman & Flitney, 1982). Some variability in the relaxation rate could be due to differences in the duration of the tetanus (Hou & Rall, 1987), as the relaxation rate of frog skeletal muscle has been shown to slow exponentially with increasing stimulus duration (Abbott, 1951).

Discussion

Isometric tension

The most striking finding of the present study was that fast fibres isolated from cold-acclimated and winter-acclimatised sculpin failed to activate fully and to maintain steady tension on multiple stimulation at typical summer temperatures. At 15°C the maximum tetanic tension was around three times higher for muscle fibres from 15°C-acclimated compared to 5°C-acclimated fish (Table 1.1). The impairment of contractile performance in cold-acclimated animals at high temperatures

was completely reversible. Twitch and tetanic tension was not enhanced by caffeine which suggests that low force is not a result of incomplete calcium release from the internal stores of the sarcoplasmic reticulum (Fryer & Neering, 1989). Fast muscle fibres in this species typically have 8 to 20 endplates, innervated by 4 to 6 motor axons (Altringham & Johnston, 1989). Tension is abolished by treatment with α-bungarotoxin which suggests that the fibres are activated via channels associated with the neuromuscular junctions (Johnson & Johnston, 1991b). However, the addition of eserine, a potent acetylchlolinesterase inhibitor, to the Ringer failed to increase maximum force generation or the rates of activation and/ or relaxation at any temperature (Fig. 1.4). Potassium contractures in fibres from 5°C-acclimated fish elicited tensions 53% greater than tetanic Po at 15°C. These results are similar to those found in *Xenopus* live fibres following high-K^+ contractures following fatiguing exercise (Lannergren & Westerblad, 1989). Thus, it seems that crossbridges of fibres from 5°C-acclimated fish are able to produce the same or greater tension at 15°C as at 5°C, if fully activated. The action of caffeine and high-K^+ solutions differ in their action. Caffeine acts directly on the SR membrane to release Ca^{2+}, whereas K^+ causes step depolarisation of the surface membrane and by association, the T-tubular membrane (Constantin, 1971). These results suggest that it is failure of activation and the T-tubule system that causes the decrease in tetanic tension of fibres from cold-acclimated fish at 15°C. It should be noted however, that tensions obtained during high-K^+ contractures fall well below values obtained for 15°C-acclimated fish at 15°C.

Studies with skinned and live muscle fibres from teleosts adapted to diverse thermal environments suggest that maximum force

production (T_{max}) shows perfect temperature compensation i.e. at their normal habitat temperature fast muscle fibres produce similar tensions (Johnston & Brill, 1984; Johnston, 1985; Johnston & Altringham, 1985; Johnson & Johnston, 1991b). T_{max} is also found to be independent of body mass (Anderson & Johnston, 1992). Maximum force decreases at non-physiological temperatures much more rapidly in live than in skinned muscle fibre preparations. For example, in several species of tropical reef fish living at 25°C, force of skinned fibres was reduced to 25 - 30% of the maximum when tested at 5°C (Johnston & Brill, 1984). Live fibres of the same species however, were inexcitable at such low temperatures (Johnson & Johnston, 1991b). On average force production increased between 10 - 20°C with an R_{10} of around 3.2 for live fibres compared to 1.9 for skinned fibres. Force also declined more rapidly in live than skinned muscle fibre preparations at temperatures above the physiological range. It would appear that low force production at high temperatures in cold-acclimated fish reflects some failure of E-C coupling and/ or Ca^{2+} -activated force production relative to 10°C, and fail to relax completely following maximal activations (Johnston & Sidell, 1984). Johnston & Altringham (1985) found that the residual Ca^{2+} -independent tension produced at high temperatures was associated with a dramatic reduction in shortening speed, implying abnormal crossbridge function.

According to Huxley's (1957) sliding filament theory, force production is proportional to the number of crossbridges attached and the average force produced per crossbridge. Measurements of rigor in rat skinned fibres suggests that the number of crossbridges attached increases as the temperature rises (Stephenson & Williams, 1981). However, measurements of a fibres instantaneous stiffness, taken as an

indicator of the number of crossbridges, prove relatively temperature independent (Bressler, 1981; Goldman, McCray & Ranatunga, 1987; Kuhn, Guth, Drexler, Berberich & Ruegg, 1979). It is possible that changes occur in both the number of crossbridges formed and the force generated per crossbridge following temperature acclimation.

In common carp, cold acclimation results in an increase in force production and Vmax at low temperatures, particularly in fast fibre types (Johnston, Sidell & Dreidzic, 1985; Langfeld *et al.*, 1991). Skinned fibres isolated from 7°C-acclimated carp were unstable at 23°C producing low forces and failing to relax completely following transfer from maximally activating, to relaxing solution (Johnston *et al.*, 1985). In contrast, fast fibres from 23°C-acclimated fish had a maximum Ca^{2+} -activated force of 209 kN m^{-2} at 23°C and showed no build up of residual tension, which is a comparable result to that found for sculpin in the present study. Carp inhabit temperate freshwater regions subject to large seasonal and diurnal temperature fluctuations from 0°C to more than 30°C. Therefore, the temperatures at which most studies on carp were conducted, fall well within the natural environmental range of the species. At 15°C however, 5°C-acclimated and winter acclimatised sculpin are close to their upper lethal limit. As previously stated, the upper thermal limit could have a detrimental effect on muscle force production in sculpin, thereby accentuating the difference between warm and cold acclimated groups at 15°C. Cold acclimation in cyprinids is known to alter the expression of myosin heavy chains (Gerlach *et al.*, 1990) and myosin light chains (Crockford & Johnston, 1990). It appears that the expression of low temperature myosin isoforms in carp following cold acclimation are produced at the expense of muscle function at higher temperatures. Studies are underway to

investigate changes in myosin expression in the fast fibres of sculpin following temperature acclimation.

In amphibian muscle the twitch: tetanus ratio decreases as the temperature is raised (Putman & Bennett, 1982); this was also observed for sculpin fibres in the present study (Table 1.1 & 1.2). This may reflect competitive differences in the rates of Ca^{2+} re-sequestration by the sarcoplasmic reticulum (SR) and Ca^{2+} binding to troponin C, in twitch contractions as the temperature is raised (Josephson, 1981). The higher thermal dependence of the former means that at higher temperatures there is insufficient time available for the crossbridges to form and thereby produce maximal force, before Ca^{2+} is removed by the SR (Josephson, 1981).

Contractile rates

Tetanic half-activation times of fibres isolated from 15°C-acclimated and summer-acclimatised fish were 25 - 30% shorter than that of fibres isolated from 5°C-acclimated and winter sculpin ($P < 0.005$; Tables 1.1 & 1.2). Whole nerve-muscle preparations of carp fast fibres produced twitch $T_{0.5}$a 50% shorter in 8°C- compared to 20°C-acclimated fish, at 8°C (Fleming *et al.*, 1990). Rates of contraction and relaxation are highly sensitive to temperature due to the number of thermally dependent steps involved. In this study, twitch half activation and relaxation times had Q_{10} values around 1.9, similar to those found in other fish species (Langfeld *et al.*, 1989; Johnson & Johnston, 1991b) and in reptiles (Bennett, 1985; Else & Bennett, 1987). The mechanisms involved in increasing contraction rates could be higher myosin ATPase activity (Crockford & Johnston, 1990; Hwang, Watabe & Hashimoto, 1990) or the expression of faster myosin types (Crockford & Johnston,

1990; Gerlach *et al.*, 1990). The molecular structure of sarcoplasmic reticulum (SR) Ca^{2+} -ATPase and fluidity of the SR membrane, are also modified in response to low temperatures in carp fast muscle (Ushio & Watabe, 1993). Theses changes in SR enable carp to modulate intracellular [Ca^{2+}], and to compensate for effects of variations in temperature (Ushio & Watabe, 1993). Changes in myofibrillar architecture also occur during cold acclimation that shorten diffusion pathways between the sarcoplasmic reticulum (SR) and troponin C (Penney & Goldspink, 1980). Thus, a combination of faster Ca^{2+} and crossbridge cycling times speed up the contractile processes at low temperatures, following cold acclimation. Modest increases in the twitch relaxation rate occur in 15°C- relative to 5°C-acclimated sculpin, though this is only significant at 10°C ($P < 0.005$; Table 1.1). In sculpin, major changes in the SR volume density, parvalbumin concentration or SR Ca^{2+} ATPase activity are unlikely, as tetanic relaxation rate does not change with warm acclimation. Major alterations however, occur in the relaxation rate of cyprinid muscle following cold acclimation. At 8°C, the half-times for relaxation of isometric twitches in the common carp were 20% and 50% shorter for slow and fast muscle fibres respectively at an acclimation temperature of 8°C-, compared to 20°C (Fleming *et al.*, 1990; Langfeld *et al.*, 1991). Fleming *et al.*, (1990) found that faster twitch relaxation rates in common carp were associated with changes in the concentration and/ or kinetics of the SR Ca^{2+} ATPase. Goldfish however, contained a higher surface density of SR and a larger number of small fibres (Penney & Goldspink, 1980). These factors together with increased myofibrillar ATPase activity (Johnston *et al.*, 1975) could account for reductions in twitch duration following cold acclimation.

Acclimation *versus* acclimatisation

Factors such as photoperiod, reproductive cycle, oxygen concentration and food availability, may interact to modify the physiological responses to temperature acclimation. In the present study some minor differences in the muscle contractile properties were observed between natural and laboratory-acclimated sculpin. Tetanic half activation times were 36% higher in 15°C-acclimated than in summer-acclimatised sculpin, at 10°C ($P = 0.007$) (Fig. 1.8a & b). Twitch and tetanic Po of fast fibres were also consistently greater in summer, than in 15°C-acclimated fish, at 10°C; this was not however statistically significantly due to the variability of the data. There is some evidence that sculpin in the natural environment limit their exposure to temperature extremes, by migrating to cooler, deeper waters during the summer months (Günther, 1888); this could explain the improved contractile performance of muscle fibres from summer fish at 10°C. Winter-acclimatised fish had faster twitch half relaxation and tetanic half activation times at 10°C and 15°C, relative to cold-acclimated sculpin ($P < 0.035$; Tables 1.1 & 1.2). One study of sculpin stomach contents showed that food was eaten all year around, with feeding and growth rate being lowest during the summer months (King *et al.*, 1983). This suggests that food supply is not limited during the winter months and that the detrimental effects of starvation on protein synthesis are not a major concern (Watt, Marshall, Heap, Loughna & Goldspink, 1988). Observations of 5°C-laboratory acclimated sculpin suggest that they remain relatively inactive at this temperature. The locomotory activity of sculpin during the winter is unknown but they would need to catch food. It is therefore possible that differences in

locomotory activities of natural- and laboratory-acclimated sculpin could account for the altered contractile properties. Though relatively few studies have compared laboratory-acclimated to naturally-acclimatised fish, some experimenters have found that locomotory activity influences muscle properties. Kleckner & Sidell (1985) studied enzyme activity associated with aerobic pathways of natural and laboratory-acclimated chain pickerel (*Esox niger*), at an intermediate temperature of 15°C. Enzyme activities were higher in the cold-acclimated groups, than in warm-acclimated groups, but all enzyme activities were lower in natural compared to laboratory-acclimated pickerel (Kleckner & Sidell, 1985). The capacity for rapid ATP production indicated by creatine phosphokinase (CPK) activity, was also found to differ between muscle groups, depending on whether the fish was naturally or laboratory acclimated (Kleckner & Sidell, 1985). The differing CPK activities between fibre types and acclimatory regimes suggested that altered swimming behaviour could modify the metabolic response to temperature. In the cisco (*Coregonus artedii*) the difficulty of migratory journeys was also found to have a greater influence on muscle aerobic capacity than temperature (Guderley & Blier, 1988). Another factor possibly affecting the contractile processes of wild-caught, compared to laboratory-acclimated sculpin could be photoperiod. At low temperatures (5 - 10°C) seasonally inconsistent photoperiods alter U_{crit} speeds of juvenile Largemouth Bass (*Micropterus salmoides*) (Kolok, 1991). Possible interactions between food supply, reproductive cycle and temperature have also been studied in male three and nine spine sticklebacks (Guderley & Foley, 1990). Cold laboratory-acclimated sticklebacks had increased mitochondrial enzyme activities in the axial muscle, compared to warm-acclimated fish

(Guderley & Foley, 1990). Similar results were found for enzyme activities of field-acclimatised sticklebacks, but the physical condition of summer fish also declined markedly (Guderley & Foley, 1990). The decrease in physical condition occurred despite an abundant food supply (Ward & Fitzgerald, 1983) and was thought to be related to reproductive activities of the male favouring a restricted food intake. Decreased food intake generally leads to mobilisation of somatic reserves and reduced enzyme activity (Love, 1980; Moon & Johnston, 1980; Heap *et al.*, 1986) which was found in summer sticklebacks (Guderley & Foley, 1990).

The major modification in the contractile properties of muscle fibres in the short-horned sculpin occur at high temperatures. Sculpin are essentially an Arctic species and like many polar and subpolar fish, secrete antifreeze proteins to depress the freezing point of body fluids (Hew, Slaughter, Fletcher & Joshi, 1981; Fletcher, Addison, Slaughter & Hew, 1982). Arctic sculpin, which are constantly exposed to temperatures below 5°C, contain high levels of antifreeze all year around (Fletcher *et al.*, 1982). In contrast, sculpin populations off the coast of Newfoundland encounter temperatures of -1°C in winter and up to 16°C in summer when antifreeze production is suppressed (Hew *et al.*, 1981). Variations in antifreeze levels in the Newfoundland sculpin, represents another seasonal adaptation to temperature change. It should be noted that 15°C is close to the maximum temperature experienced by the short-horned sculpin in the Firth of Forth (Fig. 1.1a), and is within less than 5°C of it's upper lethal temperature (unpublished observations).

CHAPTER 3

Influence of thermal acclimation on force-velocity characteristics of fast muscle fibres from the short-horned sculpin

Introduction

The maximum mechanical power output of muscle is reduced 2 - 3 fold by each 10°C decrease in temperature (Edman, 1979; Rome, 1983). Therefore, in order for fish to locomote at the same velocity irrespective of temperature, an increase in total power output of the muscle must occur. The force-velocity (P-V) characteristics of fish muscle have been studied using both skinned (demembranated) and live fibre preparations. However, live fibres are considered more suitable for estimates of power output, as they produce greater tension and faster shortening velocities than skinned fibre preparations (Altringham & Johnston, 1988a; Curtin & Woledge, 1988). Work on different species from different habitats suggest that over an evolutionary time scale the P-V relation has become flatter with decreasing body temperature (Johnston & Altringham, 1985; Johnson & Johnston, 1991b). This flattening of the P-V curve is believed to be a mechanism to increase power output at low temperatures, as a less curved relation yields a greater velocity and therefore power, for a given load. Curvature of the P-V relation is found to decrease with decreasing temperature in several species, i.e. carp (Johnson & Altringham, 1985; Rome & Sosnicki, 1990), sculpin (Langfeld *et al.*, 1989) and live type 1, *Xenopus* fibres (Lannergren, Linblom & Johansson, 1982). Contractile properties of fast fibres are also highly temperature dependant. V_{max} and power output of fast fibres isolated from sculpin were two fold higher at 12°C, than at 1°C (Langfeld *et al.*, 1989).

The effects of cold acclimation on the force-velocity characteristics of carp, have been studied using both skinned (Johnston *et al.*, 1985) and live fibres (Langfeld *et al.*, 1991). At 7°C, maximum

shortening velocity (Vmax) and maximum tension (Po) of fast and slow skinned fibres isolated from 7°C-acclimated carp were 1.5 - 2 fold higher than those isolated from 23°C-acclimated fish (Johnston *et al.*, 1985). Live red fibres from the same species showed a smaller increase in Vmax (17%) and Po (32%) in 8°C-acclimated compared to 20°C-acclimated carp, at 8°C (Langfeld *et al.*, 1991). The curvature of the P-V relation was found to be independent of acclimation temperature (Johnston *et al.*, 1985; Langfeld *et al.*, 1991). Curvature represented by Wmax/(Vmax Po) was 0.11 in 8°C-acclimated carp and 0.12 in 20°C-acclimated carp, at 8°C (Langfeld *et al.*, 1991).

The aims of this study were to investigate the effects of acclimatory and acute temperature changes on the shape of the force-velocity curve using live fast fibres from the short-horned sculpin. Comparisons of laboratory-acclimated and wild-caught fish were made to assess the importance of endogenous rhythms and extrinsic factors other than temperature on the force-velocity relation. Maximum shortening velocity and power output are of particular interest in relation to the results obtained for whole animal swimming experiments in Chapter 4. The results indicate that water temperature is the prevailing factor influencing the force-velocity characteristics.

Materials and methods

The fish

Experiments were conducted on Short-horned sculpin, *Myoxocephalus scorpius* (L.) of standard length 17 - 25.5 cm. Experimental animals were caught in the Firth of Forth or obtained

from Millport University Marine Biological Station, throughout the year. The laboratory acclimated fish were held at ambient temperature, in flow-through 385 l circular tanks, for 1 week after capture. The temperature was subsequently adjusted by 1°C day^{-1}, until the required acclimation temperature of either 5°C or 15°C (± 0.5°C) was reached. Fish were acclimated to these temperatures for 6 - 8 weeks, under a constant photoperiodic regime of 12 h light:12 h dark. Fish were fed regularly on a diet of fish flesh, squid and crustaceans.

Fish naturally acclimatised to winter conditions were caught in the Firth of Forth, between January and March 1990 and 1991 when the sea temperature was 5 - 6°C. Summer acclimatised fish were caught between July and September in 1991 and 1992 when the temperature reaches 14 - 15°C, (Fig. 1.1a). Field-acclimatised fish were held at ambient temperature in flow-through sea water aquaria, for upto 1 - 2 weeks prior to use.

Fibre bundle preparation and apparatus

See Chapter 2.

Experimental protocol

Preparations were stimulated via two platinum electrodes (Goodfellow) placed parallel, on either side of the fibres. Stimulation pulses were 1.5 ms duration, at a voltage 1.2 times that used to produce maximum tension (Grass S48 stimulator). The length of the muscle fibre was adjusted to produce a maximum twitch, without residual resting tension. This corresponded to a sarcomere length of 2.2 μm, as measured by He-Ne laser diffraction (Barr and Stroud, Hughes). The maximum tension (Po) of muscle fibres tended to increase when first

transferred to the chamber and was probably due to recovery from dissection. Preparations were therefore left for up to 60 min (until Po stabilized) before being stimulated. Rest intervals of 10 min were imposed between trains of stimuli and following a temperature change preparations were left for 40 - 60 min until Po stabilized. The effects of changing temperature were completely reversible over the range used and reproducible results could be obtained from several cycles of heating and cooling. Data were only analysed from preparations which showed a decline of less than 10% in maximum tension, over the duration of the experiment.

Isotonic contractions

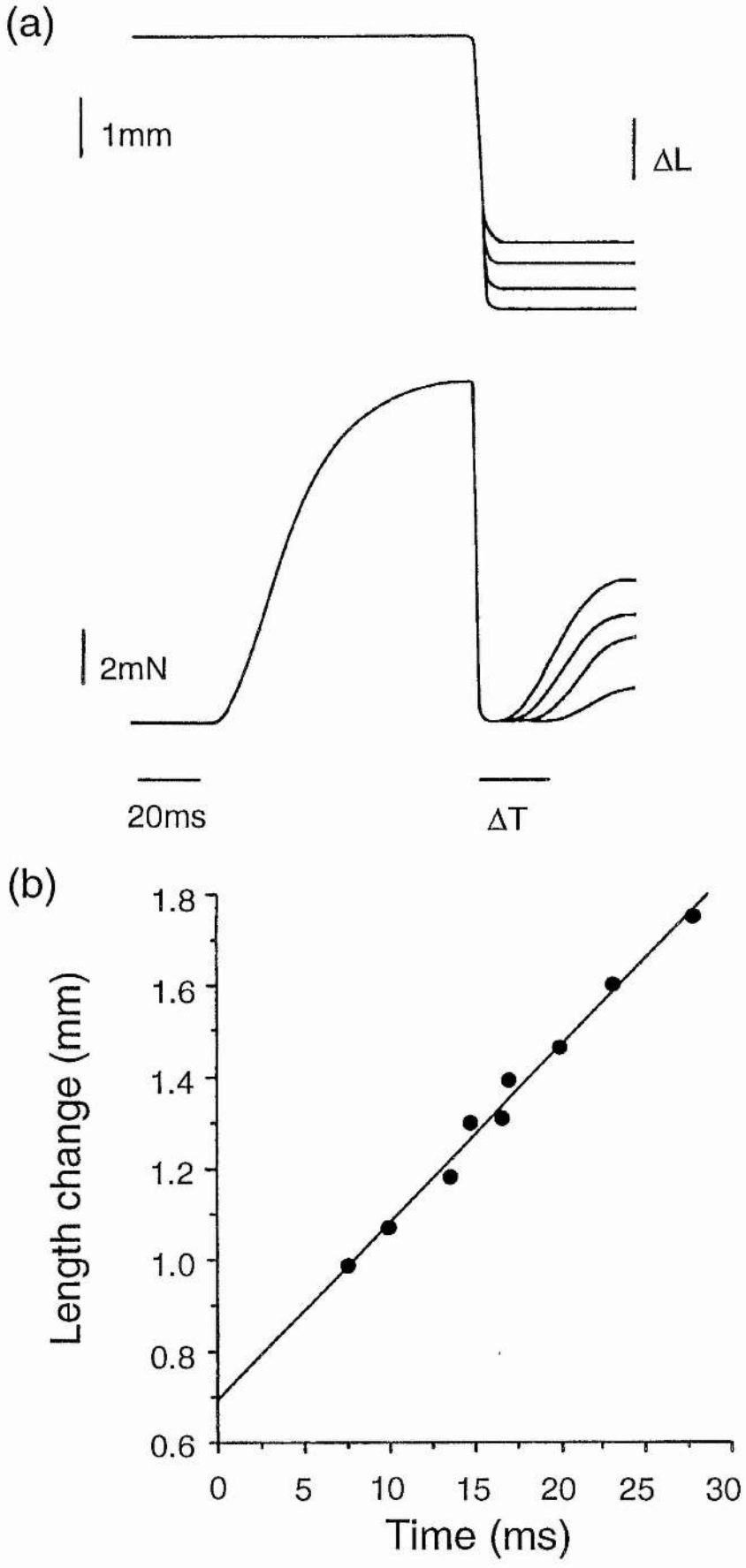
The maximum contraction velocity (Vu) was measured using the Slack-test method (Edman, 1979). During the plateau phase of isometric tetani, the muscle fibres were rapidly released (1 - 2 ms) which caused the tension to fall to zero. The fibre shortened at Vmax until the slack had been taken up, after which a phase of tension re-development occurred (Fig. 2.1a). Slack time was measured to 0.5% recovery of maximum tension if the redevelopment trace was noisy. There is a linear relationship between the length change imposed on the muscle fibre (ΔL) and the time spent shortening and taking up the slack (ΔT). Vmax was determined from the slope of the relationship between ΔL *versus* ΔT determined by least squares regression (Fig. 2.1b).

Force-velocity relationship

The force-velocity relationship was studied using a series of iso-velocity length releases. During the plateau phase of an isometric tetanus, an initial 2 ms length release was given to decrease the tension

Figure 2.1.

Figure 2.1. a) A set of representative traces from a slack test performed on a bundle of isolated fast muscle fibres from sculpin. A series of length changes (ΔL) are imposed on the muscle during the plateau phase of an isometric tetanus to drop the tension to zero. **B)** ΔL was plotted against the time for slack to be taken up (ΔT). Maximum unloaded contraction velocity was determined from a linear regression on the slope of ΔL against ΔT.


(a)
1mm
ΔL
2mN
20ms
ΔT
(b)
Length change (mm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0
5
10
15
20
25
30
Time (ms)

of the fibre to a new value (P). This was immediately followed by a second release, the speed of which was adjusted to hold the tension constant for 10 - 20 ms during the step (Fig. 2.2). The velocity of the second step was plotted against relative tension (P/ Po) in order to construct the force-velocity (P-V) curve. Typically, up to 20 iso-velocity releases were used to construct each curve.

Fitting of P-V data

Force-velocity data for individual preparations were analysed using a nonlinear curve-fitting program (Regression, Blackwell Scientific Software, Oxford, England), and fitted to the hyperbolic-linear (hyp-lin) equation developed by Marsh & Bennett, (1986):

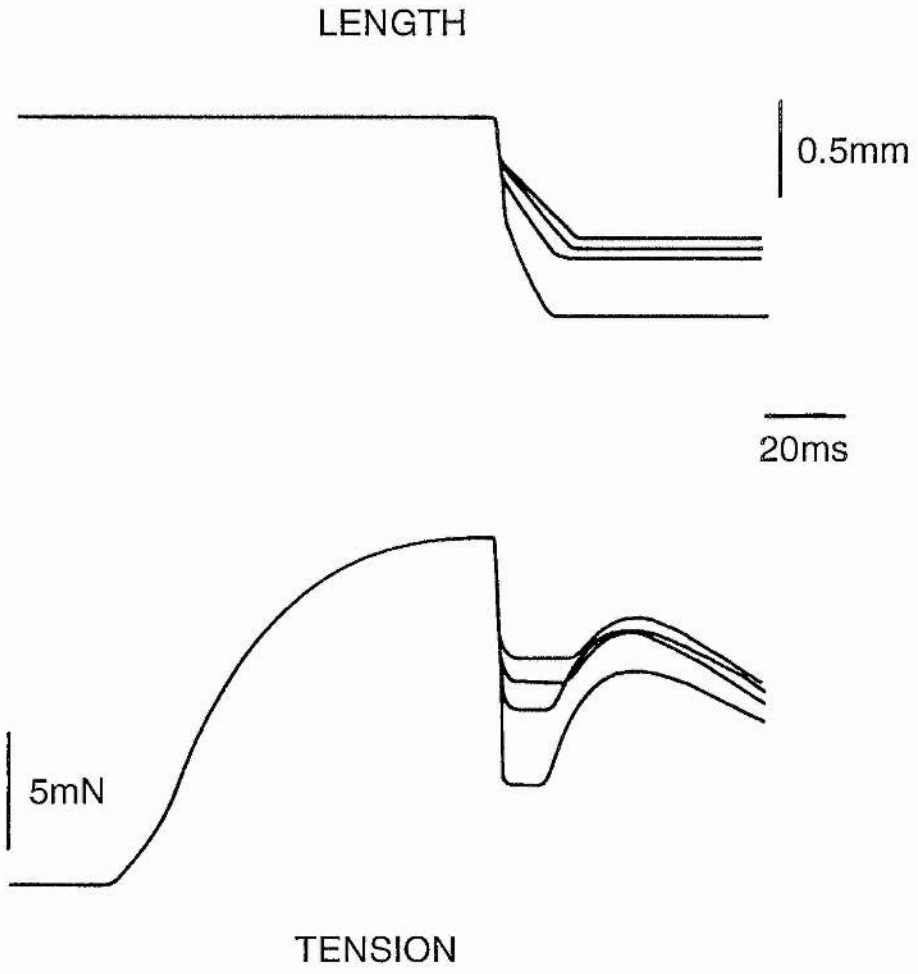
$$V= [B (1-P/Po)/ (A +P/Po)] + C (1-P/Po),$$

where A is dimensionless, B and C have dimensions of velocity. A least squares regression was iteratively fitted using the Apple MacIntosh LC II computer. The curve was not forced to go through Po. Initially, a wide range of values for each constant was used which were made progressively narrower, with increasing numbers of increments, until the minimum mean squared difference between observed and predicted data was obtained (Johnson, 1990).

Values for Vmax were extrapolated from the curve at the point of zero load. The curvature of the P-V relation is described by the power ratio Wmax/ Vmax Po, where Wmax is the maximum power output derived from the curve of V x P plotted against P. Values for normalised power output (W Kg^{-1}) can be calculated for each muscle by multiplying the force (N) by the contraction velocity (m s^{-1}) and dividing by the mass of the preparation.

Figure 2.2.

Figure 2.2. An illustrative series of isovelocity releases on fast fibres isolated from the abdomen of sculpin.


LENGTH
0.5mm
20ms
5mN
TENSION

Measuring the cross-sectional area

In order to calculate the absolute power output produced by each fibre, the muscle lengths and cross-sectional area were measured at the end of each experiment as described in Chapter 2.

Statistical Analysis

Standard error of the estimates were calculated for each curve from the equation:

$$S.E.E. = \sqrt{RSS/(n-2)}$$

where, RSS is the residual sums of squares, n-2 is the degrees of freedom, which is the number of data points, minus the number of constants in the equation. The effects of temperature and acclimation regime on the P-V relation were compared using two sample *t*-tests. Vmax's obtained from the Slack test were compared using a one-way analysis of variance (Minitab Inc, Philadelphia).

Results

Maximum velocity (Vu) from the Slack test

Between 5°C and 15°C the maximum unloaded contraction velocity (Vu) increased with a Q_{10} of 1.76 in 15°C-acclimated and of 1.98 in summer-acclimatised sculpin (Table 2.1). For a given temperature, absolute values of Vu were generally higher for 15°C-acclimated than summer fish, whereas results for winter and 5°C-

Table 2.1.

Table 2.1. The maximum velocity of shortening of fast fibres using the slack test method (V_u) and extrapolation of the P-V curve (V_{iso}) for **a)** laboratory-acclimated and **b)** naturally-acclimatised. T-test significance levels are * = $P < 0.05$, *** = $P < 0.005$. Differences are between 5°C-acclimated and 15°C-acclimated sculpin at their respective temperatures and also between summer- and winter-sculpin at their respective temperatures.

a)

Experimental temperature.	5°C	10°C	15°C	5°C	10°C	15°C
Vmax	15°C-Acclimated			5°C-Acclimated		
Viso (L s^{-1})	3.59 ± 0.5	5.77 ± 1.02	7.12 ± 0.9	3.67 ± 0.18	3.55 ± 0.35	2.95*** ± 0.67
Hyp-lin equation.	n = 5	n = 6	n = 5	n = 8	n = 5	n = 5
Vu (L s^{-1})	4.66 ± 0.32	4.79 ± 0.35	8.2 ± 1.14	4.70 ± 0.32	4.44 ± 0.47	5.15* ± 0.62
Slack test.	n = 9	n = 10	n = 11	n = 12	n = 8	n = 10

b)

Experimental temperature.	5°C	10°C	15°C	5°C	10°C	15°C
Vmax	Summer-acclimatised			Winter-acclimatised		
Viso (L s^{-1})	4.55 ± 0.48	4.84 ± 0.38	7.95 ± 1.4	4.91 ± 0.28	_	5.00
Hyp-lin equation.	n = 6	n = 5	n = 5	n = 6		n = 1
Vu (L s^{-1})	3.20 ± 0.73	4.54 ± 1.04	6.35 ± 0.45	4.43 ± 0.35	4.85 ± 0.43	4.04* ± 0.27
Slack test.	n = 7	n = 6	n = 7	n = 10	n = 10	n = 7

acclimated fish were similar (Table 2.1). The Q_{10}'s for Vu in 5°C-acclimated (1.10) and winter fish (0.91) showed low thermal dependencies, with Vu of winter fish decreasing slightly between 5°C and 15°C (Table 2.1). As a result Vu was almost 60% higher at 15°C, for fast muscles isolated from warm than cold-acclimated fish ($P < 0.05$).

Force-velocity characteristics

Force-velocity curves fitted using the hyperbolic-linear equation are represented in Fig 2.3 and 2.4. The hyp-lin equation gave a good fit to the data as illustrated by the regression coefficients (r^2) and the standard errors of the estimates (SEE) (Tables 2.2 & 2.3). The hyp-lin equation has also been found to produce a good curve fit to data from live fibre preparations of sculpin (Altringham & Johnston, 1988a; Langfeld *et al.*, 1989) and of reptiles (Marsh & Bennett, 1986). Data deviated at both high and low loads from the Hill's (1938) fitted line therefore, only the hyp-lin equation was used (summarised in Tables 2.2 and 2.3). Data were difficult to obtain from winter fish at 10°C and 15°C, because the fibre preparations deteriorated irreversibly after a short period of time. The maximum isometric tension of winter-caught fish declined and was not maintained constant during tetani at higher temperatures, which accounts for the low n number.

Maximum velocity Viso extrapolated from P-V curves.

For 15°C-acclimated sculpins, extrapolated values of Viso in muscle fibre lengths/ s, increased from 3.6 at 5°C, to 7.1, at 15°C (Q_{10} = 2) (Table 2.1). Values for Viso were similar between 15°C-acclimated fish and summer fish at a given temperature. At 5°C, Viso

Table 2.2.

Table 2.2. Influence of temperature acclimation on the force-velocity characteristics of fast muscle fibres isolated from anterior myotomes of the short-horned sculpin. Values represent Means ± S.E. *, **, *** indicate significant difference at the $P < 0.05$, 0.01, 0.005 levels. Acclimation groups were compared at their respective temperatures.

Hyp-lin equation	15°C-Acclimated			5°C-Acclimated		
Experimental temperature.	5°C	10°C	15°C	5°C	10°C	15°C
n.	5	6	5	8	5	5
Wmax (W kg^{-1})	55.3 ± 20.0	146.0 ± 30.5	206.3 ± 17.5	121.1 ± 33.3	92.0 ± 22.8	33.62 ± 8.48 ***
A	0.037 ± 0.01	0.013 ± 0.003	0.023 ± 0.005	0.045 ± 0.013	0.106 ± 0.033 ***	0.068 ± 0.022
B (Ls^{-1})	0.07 ± 0.02	0.05 ± 0.02	0.10 ± 0.03	0.07 ± 0.03	0.18 ± 0.07	0.10 ± 0.03
C (Ls^{-1})	1.60 ± 0.47	2.52 ± 0.16	2.56 ± 0.21	2.48 ± 0.29	1.79 ± 0.44	1.27 ± 0.19 ***
Wmax/ Vmax Po	0.122 ± 0.015	0.133 ± 0.012	0.113 ± 0.013	0.187 ± 0.014 *	0.152 ± 0.017	0.147 ± 0.022
r^2	0.980	0.983	0.974	0.985	0.984	0.979
S.E.E.	0.113 ± 0.032	0.141 ± 0.02	0.194 ± 0.015	0.098 ± 0.012	0.116 ± 0.007	0.089 ± 0.021

Table 2.3.

Table 2.3. Influence of season on the force-velocity characteristics of fast muscle fibres isolated from anterior myotomes of the short-horned sculpin. Values represent Mean ± S.E. *, **, *** indicate significant difference at the $P < 0.05$, 0.01, 0.005 levels. Acclimation groups were compared at their respective temperatures.

Hyp-lin equation	Summer-Acclimatised			Winter-Acclimatised		
Experimental temperature.	5°C	10°C	15°C	5°C	10°C	15°C
n	6	5	5	6	2	1
Wmax (W kg^{-1})	101.9 ± 16.9	208.0 ± 31.1	235.1 ± 73.5	139.1 ± 18.1	103.2 ± 41.5	82.9
A	0.041 ± 0.014	0.244 ± 0.185	0.058 ± 0.027	0.065 ± 0.027		0.004
B (Ls^{-1})	0.31 ± 0.24	0.70 ± 0.52	0.20 ± 0.08	0.13 ± 0.06		
C (Ls^{-1})	1.92 ± 0.21	2.77 ± 0.72	3.39 ± 0.90	3.01 ± 0.18 ***		3.20
Wmax/ Vmax Po	0.133 ± 0.018	0.173 ± 0.02	0.121 ± 0.021	0.171 ± 0.004		0.179
r^2	0.985	0.990	0.986	0.979		0.977
S.E.E.	0.113 ± 0.018	0.103 ± 0.024	0.187 ± 0.04	0.132 ± 0.028		0.169

of fast fibres was similar between 5°C-acclimated and 15°C-acclimated fish, and also between naturally acclimatised fish. However, maximum shortening velocity declined slightly in 5°C-acclimated fish, between 5°C and 15°C (Q_{10} = 0.8). Due to the difference in thermal dependency of shortening velocity between the acclimation groups, Viso was 2.4-times higher in the 15°C-acclimated fish, compared to the 5°C-acclimated fish at 15°C ($P < 0.001$; Table 2.1). Vmax extrapolated from P-V curves gives similar results to Vu obtained using the slack test method (Table 2.1).

The shape of the P-V curve

The power ratio (Wmax/ Vmax Po) provides a measure of the curvature of the force-velocity relation. At 5°C, this ratio was significantly higher, indicating a less curved P-V relation in muscle fibres from 5°C-acclimated (0.19) than 15°C-acclimated (0.12) sculpin ($P < 0.05$) (Fig. 2.3; Tables 2.2 & 2.3). When normalised for Vmax and Po the difference in curvature alone are sufficient to increase relative power output by nearly 40% (Fig. 2.7). At 10°C and 15°C, the values for Wmax/ Vmax Po within the range of 0.11 - 0.15, at both acclimation temperatures. The lowest curvature (0.17) of fast fibres from summer sculpin occurred at 10°C (Tables 2.2 & 2.3; Fig. 2.4). No difference was found in the relative load at which maximum power output was obtained (0.51 Po), with either temperature or acclimation regime.

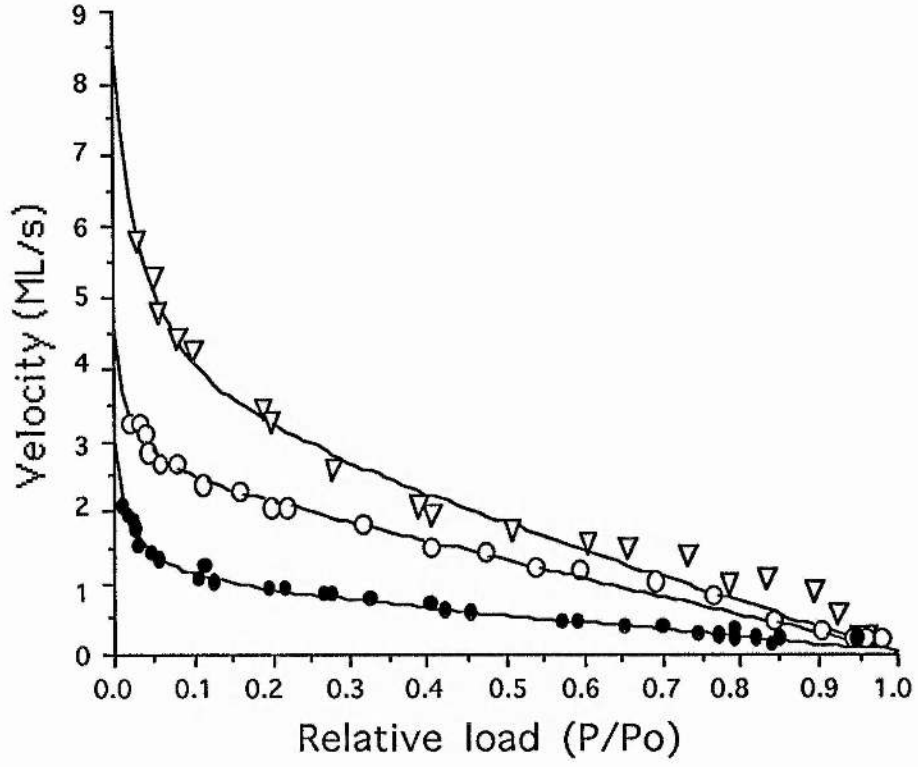
Power output

For 15°C-acclimated fish the power output of fast muscle increased from 55 W kg^{-1} of wet muscle mass at 5°C, to 206 W kg^{-1} at 15°C (R_{10} = 3.73) (Table 2.2; Fig. 2.5a). Similar increases in Wmax

Figure 2.3.

Figure 2.3. The effect of temperature on the force-velocity curve of fast fibres isolated from **a)** 15°C-acclimated sculpin **b)** 5°C-acclimated sculpin. Experimental temperatures are 5°C (closed circles), 10°C (open circles) and 15°C (open triangle).

a) 15°C-acclimated sculpin.



b) 5°C-acclimated sculpin

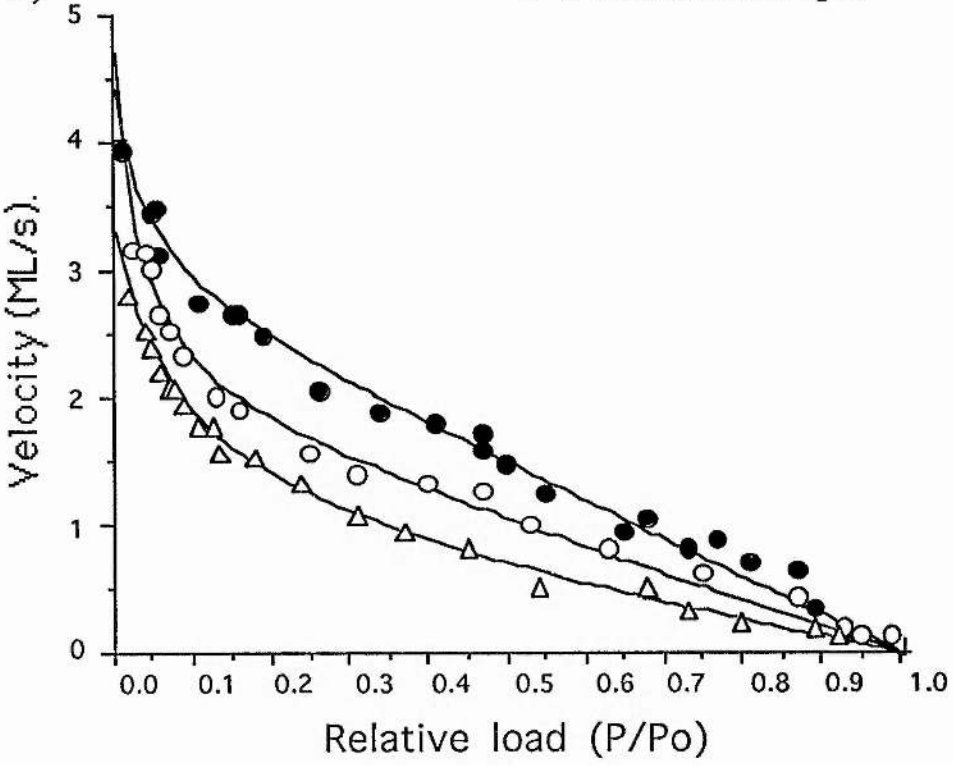
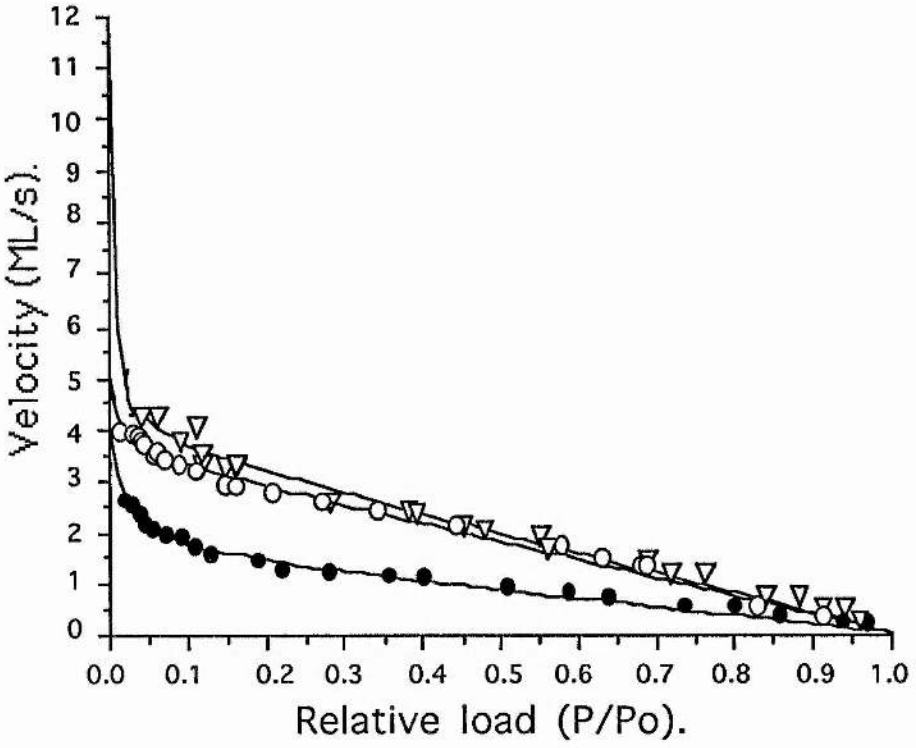


Figure 2.4.

Figure 2.4. The effect of temperature on the force-velocity curve of fast fibres isolated from **a)** summer-acclimatised sculpin **b)** winter-acclimatised sculpin. Experimental temperatures are 5°C (closed circles), 10°C (open circles) and 15°C (open triangle).

a) Summer-acclimatised sculpin.



b) Winter-acclimatised sculpin.

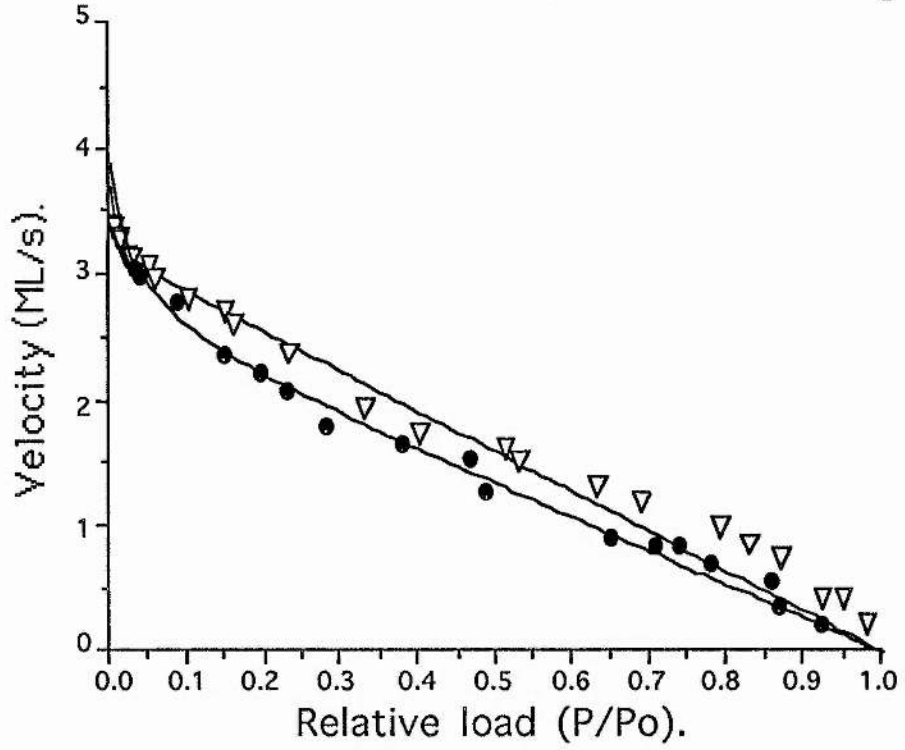
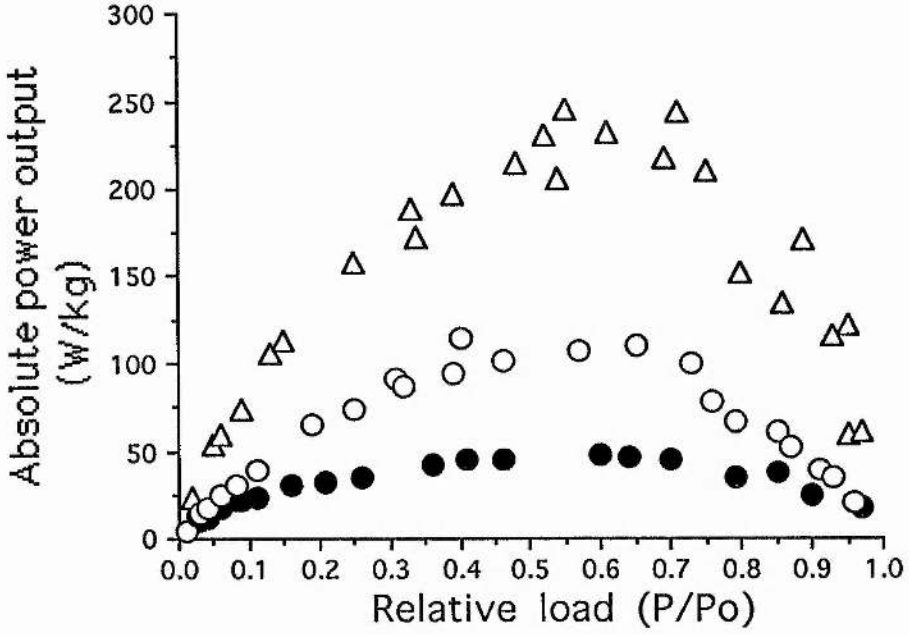


Figure 2.5.

Figure 2.5. Effect of laboratory acclimation on the power output of individual preparations **a)** 15°C-acclimated sculpin and **b)** 5°C-acclimated sculpin. Power output was calculated from parameters derived from the hyp-lin equation plotted against relative load at 5°C (closed circles), 10°C (open circles) and 15°C (triangles).

a)


Absolute power output (W/kg)
300
250
200
150
100
50
0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
Relative load (P/Po)

b)

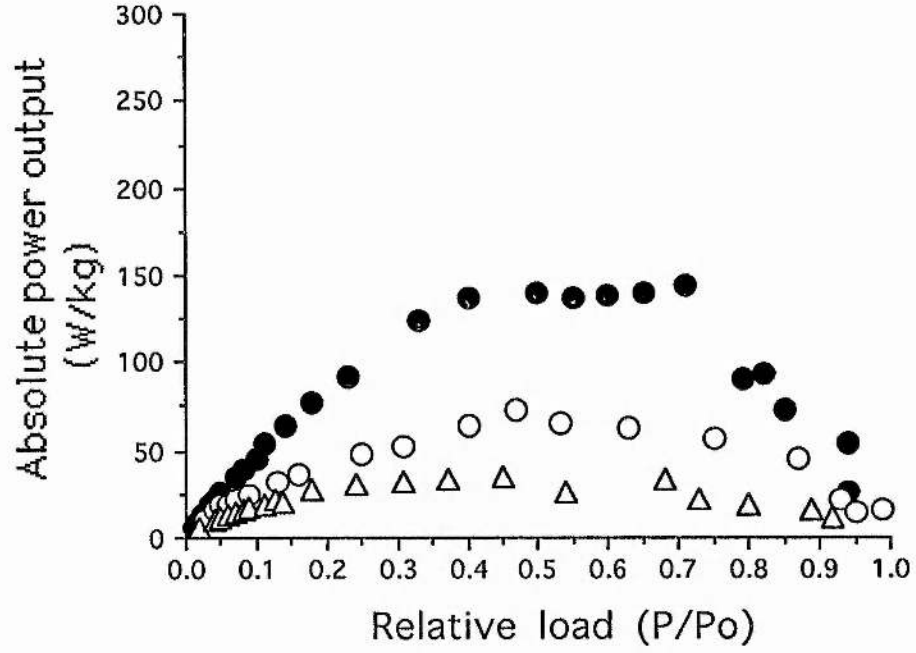
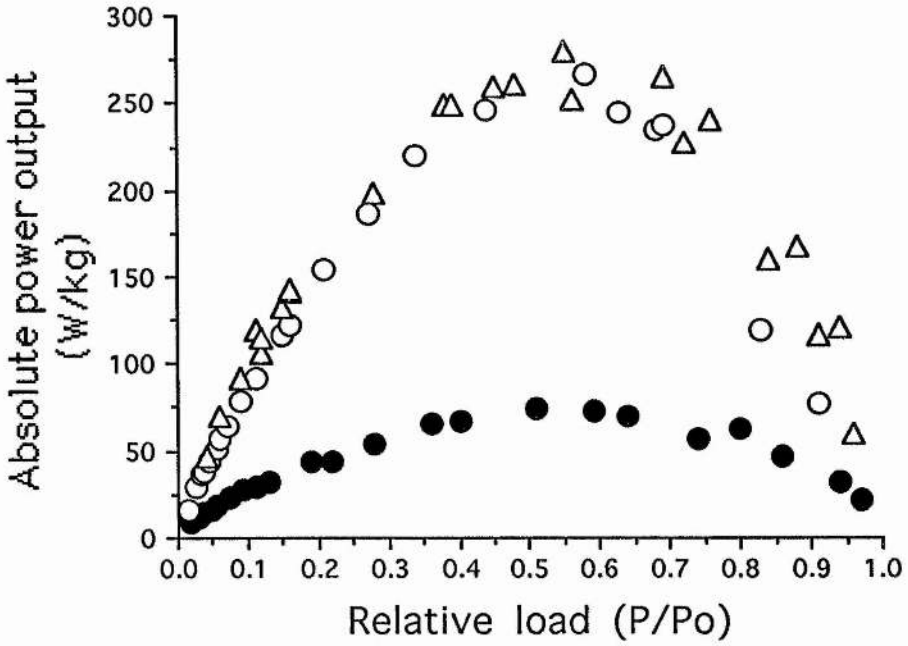

Absolute power output (W/kg)
300
250
200
150
100
50
0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
Relative load (P/Po)

Figure 2.6.

Figure 2.6. Effect of natural-acclimatisation on the power output of individual preparations **a)** summer-acclimatised sculpin and **b)** winter-acclimatised sculpin. Power output was calculated from parameters derived from the hyp-lin equation plotted against relative load at 5°C (closed circles), 10°C (open circles) and 15°C (triangles).

a)


Absolute power output (W/kg)
300
250
200
150
100
50
0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Relative load (P/Po)

b)

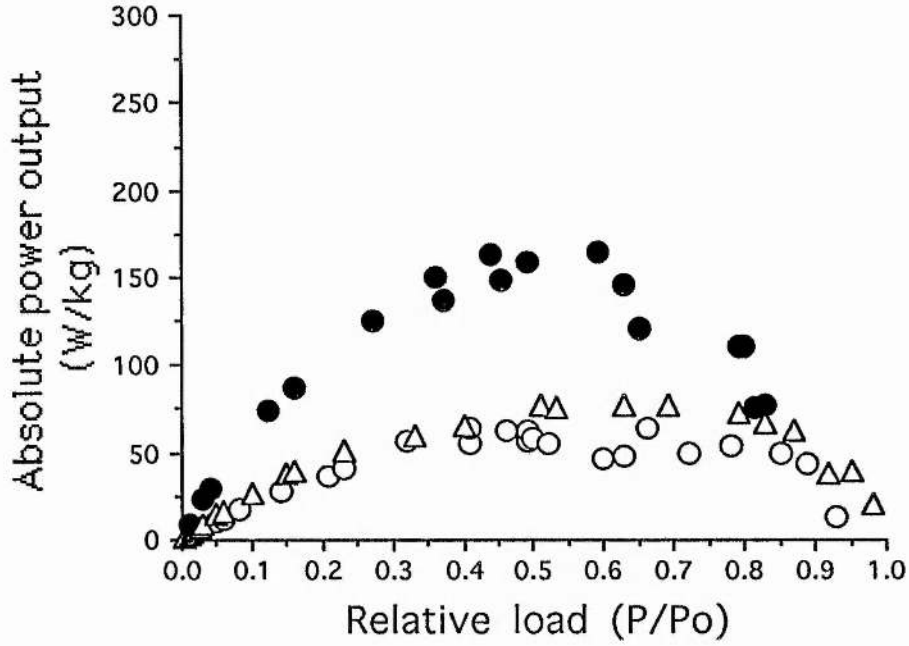
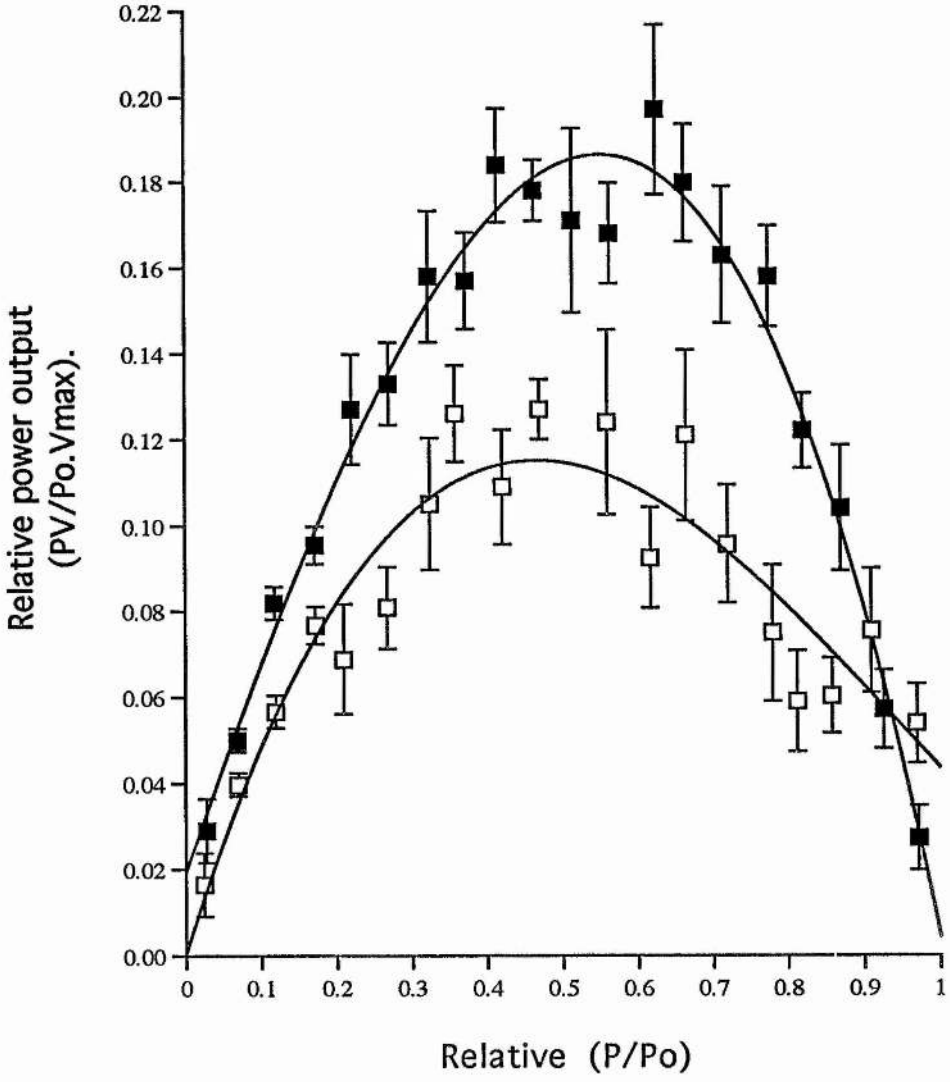

Absolute power output (W/kg)
300
250
200
150
100
50
0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Relative load (P/Po)

Figure 2.7.

Figure 2.7. Power output at 5°C normalised for changes in force and velocity plotted against relative load, (P.V/ Po.Vmax) versus P/ Po. Symbols represent 5°C-acclimated sculpin (solid squares) and 15°C-acclimated sculpin (open squares). Values represent Means ± S.E. $n = 8$ and 5 respectively.


Relative power output
(PV/Po.Vmax).
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Relative (P/Po)

were found in muscle isolated from summer sculpin, with the biggest increase occurring between 5°C and 10°C (Fig. 2.6a). In contrast, Wmax fell by 72% in the 5°C-acclimated sculpin, as both Vmax and Po declined between 5°C and 15°C. The net result of these differences was Wmax from 15°C-acclimated sculpin was six-times higher than Wmax of 5°C-acclimated fish, at 15°C ($P = 0.001$) (Table 2.2). However, at 5°C power output was around two-times higher for fibres from cold-, than warm-acclimated fish; although the results were not significantly different, they possibly reflect the decreased curvature in the P-V relation ($P < 0.05$) (Fig. 2.5 & 2.6). Wmax showed greater thermal sensitivity at the lower temperatures in 15°C-acclimated and summer sculpin, both increasing with R_{10}'s of around 4, between 5°C and 10°C ($P < 0.037$) compared to R_{10}'s around 2.5, between 10°C and 15°C.

Discussion

Maximum velocity of shortening Vu and Viso

Muscle fibres are believed to have unique maximum speeds of shortening, therefore values of Vu and Vmax extrapolated from the P-V curve are expected to be the same for a given fibre (Julian, Rome, Stephenson & Striz, 1986). Vmax values obtained using slack tests and P-V extrapolation were similar for each group, at each experimental temperature, except for the 5°C-acclimated fish. In fibres from 5°C-acclimated sculpin, estimates of Vmax from the P-V curve were 43% lower at 15°C, and 22% lower at 5°C, compared to Vu measurements; this suggests that hyp-lin equation may underestimate Vmax, possibly due to a discontinuity of the force-velocity relationship of 5°C-

acclimated fibres at low loads. When comparing all of the Vmax data, Vu/ Viso = 1.1 ± 0.33 (mean ± S.D.) which suggests that Viso extrapolated from hyp-lin curves is on average slightly lower than Vu values obtained from slack tests. Previous studies with live fibres have also found differences in Vmax using the two experimental methods, with Vu exceeding Viso by 5 - 30% (Edman, 1979; Lannergren *et al.*, 1982). Discrepancies between the two methods of estimating Vmax could possibly arise from, (a) inaccuracies in determining the exact time when all slack was taken up, during the slack test or, (b) as previously stated, discontinuity of the force-velocity relationship at low loads (see Julian *et al.*, 1986).

Vu and Viso were both highly temperature dependant in fibres isolated from summer and 15°C-acclimated sculpin. Vu increased with a Q_{10} of 1.98 in the summer-fish and 1.76 in the 15°C-acclimated sculpin between 5 and 15°C. Similarly, Viso of fibres isolated from 15°C-acclimated sculpin increased with a Q_{10} of 1.98, compared to a Q_{10} of 1.75 in summer-caught fish. Most live fibres studied exhibit similar thermal dependencies for Vmax with Q_{10}'s of around 2 i.e. *Rana* (Edman, 1979), *Xenopus* (Lannergren *et al.*, 1982) and sculpin (Langfeld *et al.*, 1989). The Vu and Viso of 5°C-acclimated and winter-acclimatised sculpin did not increase with temperature and even declined at 15°C. As a result of acclimation to warm temperatures Vu and Viso are 1.6-times faster in summer-, than winter-caught sculpin at 15°C ($P < 0.001$; Table 2.1). Vu was also 1.6-times faster ($P < 0.05$) and Viso was 2.4-times faster in warm- than cold laboratory-acclimated sculpin, at 15°C ($P < 0.005$; Table 2.1). The thermal dependency of skinned sculpin fibres was similar to that of live fibres with a Q_{10} of 1.8 (Johnston & Sidell, 1984). However, the maximum shortening speeds

Table 2.4.

Table 2.4. Summary of Vmax data taken from the literature. References: 1 = Johnson & Johnston (1991); 2 = Langfeld *et al*, (1989); 3 = Rome, Sosnicki & Choi (1992); 4 = Julian *et al*, (1986); 5 = Lannergren, (1978); 6 = Marsh & Bennet, (1985); 7 = Greaser, Moss & Reiser, (1988).

	V_{max} (L s^{-1}).	Experimental temp. (°C).	Experimental details.
FISH			
Notothenia neglecta[1]	1.92 ± 0.1	1	Live fast myotomal & pectoral muscle (Hill equation).
Callionymus lyra[1]	4.62 ± 0.1	8	Live fast myotomal muscle (Hill equation).
Thalassoma duperreyi[1]	16.36 ± 0.7	24	Live fast myotomal & pectoral muscle (slack test).
M. scorpius[2]	4.27 ± 0.1	1	Live fast myotomal & pectoral muscle (hyp-
	8.14 ± 0.2	8	lin equation).
	9.46 ± 0.2	12	
Stenotomus chrysops L.[3]	3.32	10	Live red muscle (Hill equation).
	5.55	20	
AMPHIBIANS			
Rana temporaia[4]	4.05	4	Intact anterior tibialis (Hill equation & slack test).
Xenopus laevis[5]	6.34	22	Intact single fibres of iliofibularis.
REPTILES			
Desert iguana[6]	18.7	40	Intact bundle of iliofibularis.
MAMMALS			
Rabbit[7]	2.3	15	EDL fast twitch muscle skinned fibres.

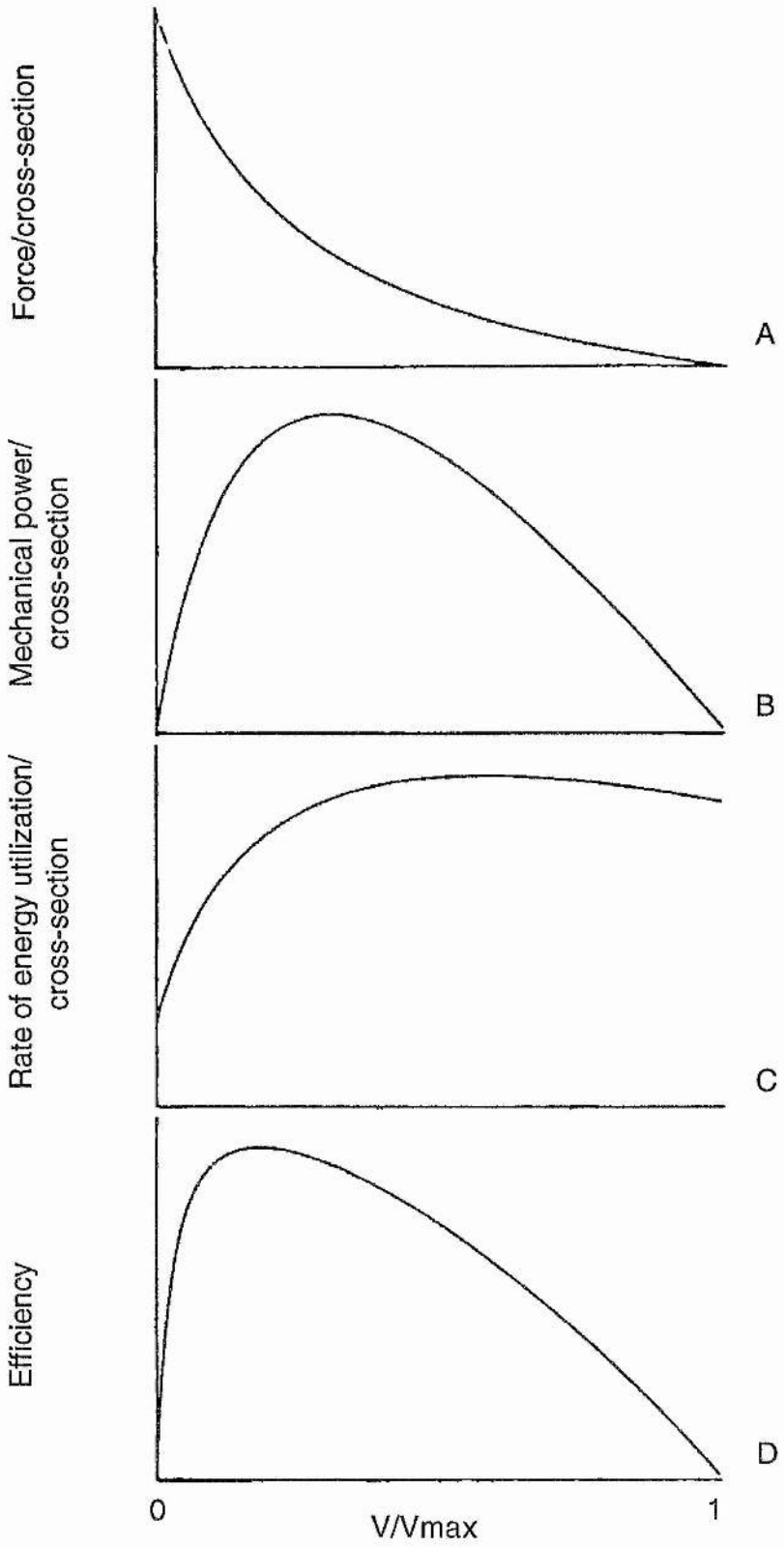
were much lower using skinned fibres, V_u being 0.41 L s^{-1} at 1°C, increasing to 0.75 L s^{-1} at 10°C (Johnston & Sidell, 1984). The lower V_{max}'s of skinned fibres may reflect limitations in metabolic energy supply (Curtin & Woledge, 1988). Johnston and Altringham (1985) found that residual Ca^{2+} -independent tension produced at high temperatures in skinned fibres was associated with a dramatic reduction in shortening speed, implying abnormal crossbridge function. In the present study with live fibres, V_{max} failed to increase at high temperatures in cold-adapted individuals. This observation is also consistent with an impairment of crossbridge function at high temperatures in the winter fish since V_{max} would be expected to be relatively independent of the degree of activation of the fibres.

Following cold acclimation the V_{max} of carp fibres also increases at low temperatures (Langfeld *et al.*, 1991; Johnston *et al.*, 1985). Unlike isometric tension, V_{max} shows no capacity adaptations between fish from different geographical regions (Johnston & Brill, 1984; Johnson & Johnston, 1991b). Also, closely related lizards with different preferred body temperatures show no compensation in maximum shortening velocity (John-Alder & Bennett, 1987).

Rome (1990) proposed that the velocity over which a muscle is used (V/ V_{max}) is an effective design constraint on muscle (Figure 2.8). Studies on carp and scup (*Stenotomus chrysops*), indicate that muscle fibres are used over a narrow range of V/ V_{max}; V being the velocity at which the muscle shortens (Rome *et al.*, 1988; 1990; 1992; Rome & Sosnicki, 1990, 1991; Sosnicki, Loesser & Rome, 1991). Carp, like most fish species use their red muscle to locomote aerobically, at low and sustained swimming speeds (Rome *et al.*, 1984). In order to

Figure 2.8.

Figure 2.8. Illustrating the importance of V/ Vmax. A-D: force, power, rate of energy utilisation, and efficiency as a function of V/ Vmax, respectively. Values derived from heat and mechanics measurements made on frog muscle (Hill, 1938; Hill, 1964). Taken from Rome (1990).


Force/cross-section
A
Mechanical power/
cross-section
B
Rate of energy utilization/
cross-section
C
Efficiency
D
0
V/Vmax
1

compensate for decreased mechanical power output at low temperatures, carp recruit more muscle fibres and faster fibres types (Rome *et al*, 1984). As the faster fibre types generate ATP using anaerobic pathways, the efficiency of swimming is vastly reduced. However, after a period of cold-acclimation the Vmax of muscle fibres increased enabling carp to swim at a faster velocity, while maintaining V/ Vmax (Rome *et al.*, 1985). Following acclimation to cold temperatures higher sustained swimming speeds can be maintained using the more economical aerobic red fibres, due to an increase in Vmax. Increased Vmax of sculpin fast fibres following warm-acclimation/ acclimatisation possibly enables the fish to swim at a faster velocity, again by maintaining V/ Vmax. Burst swimming speed of sculpin does increase by 30% at 15°C, following acclimation to 15°C (see Chapter 4). Johnson and Johnston (1991a) examined the economy of fast fibres from summer-acclimatised sculpin performing oscillatory work. The economy of muscle isolated from summer sculpin was found to decrease with a $Q_{10(4\text{-}15°C)}$ of about 1.5, with increasing cycle frequency. As tail-beat frequency was found to be 30% higher in warm, than cold-acclimated sculpin at 15°C ($P < 0.05$) (see Chapter 4), it is possible that warm acclimation causes a sacrifice in economy in order to increase Vmax and tension.

The mechanisms underlying changes in the Vmax of muscle fibres are probably complex. Cold acclimation in the carp increases enzyme activities associated with the potential to supply aerobically generated ATP in fast and slow muscles (Johnston *et al.*, 1985). Major changes also occur in the protein composition of skeletal muscles after adaptation to low temperatures. Cold acclimation changes the regulatory proteins in goldfish (Johnston, 1979) and the myosin light

chains in carp (Crockford & Johnston, 1990). Faster contraction velocities and greater force production are highly correlated with faster myosin heavy chain isoforms, as opposed to changes in light chain composition (Reiser *et al.*, 1985; Lannergren, 1987). Carp have a wide scope for different myosin heavy chain composition, with a minimum of 28 genes coding for myosin heavy chains characterised by Gerlach *et al.*, (1990). Changes also occur at protein structural levels, indicated by myosin subfragment-1 (S1) producing different peptide maps and having a lower thermal stability when isolated from 10°C-acclimated, as opposed to 30°C-acclimated carp (Hwang *et al.*, 1990; Hwang, Ochiai, Watabe & Hashimoto, 1991; Watabe, Hwang, Nakaya & Okamoto, 1992). Similar changes in enzyme activities, together with possible variations in myofibrillar composition are likely to be the mechanisms behind the faster Vmax's and tetanic activation times (Chapter 2) of sculpin fibres following warm acclimation.

The P-V relation

There was a tendency for the force-velocity curve to become less curved at low temperatures, indicated by higher values of Wmax/ Vmax. Po (Tables 2.2 & 2.3) this has previously been reported in this species (Langfeld *et al.*, 1989). Langfeld *et al.* (1989) normalised the P-V curve for Po and Vmax at each temperature and found that the change in curvature was sufficient to increase the relative power output by around 15% on decreasing the temperature from 8°C to 1°C. Wmax/ Vmax. Po was also significantly higher at 5°C for muscles from 5°C- than 15°C-acclimated fish (Tables 2.2 & 2.3). The calculated difference in curvature was sufficient to increase the relative power output by nearly 40% at low temperatures in the cold-adapted fish (Fig. 2.7). The

change in curvature could contribute to the increased (though statistically insignificant) power output of the 5°C- relative to the 15°C-acclimated sculpin at 5°C. The P-V curvature is also less in the winter- than summer-acclimatised sculpin, though not significantly different at 5°C. As previously stated it is possible that by using behavioural mechanisms, sculpin limit the environmental temperature extremes to which they are exposed.

Power output

Maximum power outputs of muscle fibres from 15°C-acclimated (206 W kg^{-1}) and summer- sculpin (235 W kg^{-1}) were 60 - 70% greater than those of 5°C-acclimated (131 W kg^{-1}) and winter sculpin (139 W kg^{-1}). Similar maximum muscle power outputs were found for live fibres by other workers; 292 W kg^{-1} for sculpin (Langfeld *et al.*, 1989), 134 W kg^{-1} for scup (Rome *et al.*, 1992), and 132 W kg^{-1} for the salamander (Else & Bennett, 1987). Thermal acclimation in sculpin produced capacity adaptations in the ability of the muscle to generate maximum power at specific temperatures. Power output values increased significantly, with increasing temperature in the 15°C-acclimated and summer fish, and decreased with increasing temperature in the 5°C-acclimated and winter-acclimatised fish. Capacity adaptations in V_{max} and more importantly in the maximum isometric tension of sculpin fast fibres, combine to increase power output with acclimation to summer temperatures (Johnston & Altringham, 1985, Altringham & Johnston, 1986; Johnson, 1990; see Chapter 2.). Similarly, maximum power outputs of fibres from the scup increased by 90% compared to only 60% in the carp, when the temperature was raised from 10°C to 20°C (Rome & Sosnicki, 1990; Rome *et al.*, 1992). Unloaded shortening velocity (V_{max}) of red muscle fibres was similar and the

increased power output was due to the larger force-generating capacity of scup fibres at 20°C, compared to the carp fibres (Rome *et al.*, 1992).

Values of muscle power output that are more relevant to fish swimming can be obtained from oscillatory work loop experiments (Josephson, 1985). Work loop experiments mimic the sinusoidal fibre length changes observed in continuous swimming and calculate the power produced during each length change cycle (Altringham & Johnston, 1990a & b). The timing and number of stimuli can be adjusted to maximise work during each length change cycle (Altringham & Johnston, 1990a & b). Power output values of the sculpin performing oscillatory work (Johnson & Johnston, 1991a) are lower than those calculated from the force-velocity curves in this study. This is due to the different nature of the experiments as during isotonic experiments power is calculated from muscles shortening under a constant load. In contrast, muscles performing oscillatory work are only stimulated for part of the length change cycle. However, using both experimental methods maximum power output was three times higher at 15°C in the summer- compared to winter-acclimatised sculpin. The acclimatory increase in power output of isolated muscle fibres of sculpin is reflected in whole animal swimming performance at 15°C (see Chapter 4). During prey-attack sequences at 15°C, the mean velocity attained by 15°C-acclimated fish was approximately 4 body lengths/ s (BL/ s), compared to 2.5 BL/ s in the 5°C-acclimated sculpin ($P < 0.02$). Increased temperature caused an increase in tail-beat frequency (TBF) during fast-starts (Webb, 1978a). In this study cold-acclimated sculpin had a TBF of ~5.5 Hz at 5°C, compared to ~8 Hz at 15°C (Chapter 4). The increased TBF was paralleled by the results from isolated muscle fibres of summer-acclimatised sculpin undergoing oscillatory work.

Johnson & Johnston (1991a) found that the optimum cycle frequency (which represents TBF) required to produce maximum power output, increased from 5 - 7 Hz at 4°C, to 9 - 13 Hz at 15°C (Johnson & Johnston, 1991a). Acclimation of sculpin to 15°C caused a significant increase in the TBF at 15°C, to ~10.5 Hz ($P < 0.05$). The value of 10.5 Hz is within the optimum cycle frequency observed during oscillatory experiments for summer-sculpin at 15°C. Generally, V_{max} and power output showed little difference between laboratory and naturally acclimatised sculpin, suggesting temperature was the major factor influencing these muscle properties.

CHAPTER 4

The effects of temperature acclimation on the fast-start capabilities of laboratory-acclimated sculpin

Introduction

Burst swimming speeds are short periods of high acceleration, powered by the fast, glycolytic muscle fibres (Rome *et al.*, 1984). Fast-start manoeuvres form the basis of two major locomotory activities, prey-capture and predator avoidance. Hydrodynamic theory predicts that large thrust is required for an effective fast-start, which is achieved by maximising depth along the body profile (Weihs, 1973). Experiments on trout, supporting this theory were carried out by Webb (1977). The area of the body profile was reduced by amputating various fin ray combinations, which led to a decrease in fast-start performance (Webb, 1977). Conflicting views on the effect of body form on maximum performance during fast-starts have been expressed by different workers. Fast-starts were found to be relatively independent of body form in seven species of teleosts, possibly because fish with large lateral profiles that are optimal for thrust, generally had a lower % muscle mass and therefore generated less power (Webb, 1978a). Fish with radically different body forms were however found to have altered performances (Webb, 1975a). Webb (1984a, 1986) examined the effects of body form on fast-starts of predators as opposed to prey. Predator fast-starts were independent of body form whereas prey fast-start performance varied according to body shape. Prey with greater acceleration rates tended to escape predators, as did prey with large spines which tended to misdirect attacks away from the prey's centre of mass (Webb, 1986). Harper and Blake (1990) used a combination of accelerometry and cinematography to study fast-starts, which produced more accurate results and higher swimming speed values than video analysis alone. Pike (*Esox lucius*) were found to have

higher velocities and accelerations during fast-starts than trout (Harper & Blake, 1990) and this was thought to be related to body form as pike are specialised predators highly adapted for fast-starts. Body form is also related to foraging behaviour and the lifestyle of fish species (see Webb, 1984b). The short-horned sculpin is a typical example of a fish that uses body/ caudal fin, transient propulsion, where whole body depth is increased by fins to maximise thrust generation. Sculpin have a large head and reduced axial musculature, a body form typical of sedentary sit-and-wait predators. These sedentary predators sit stationary on the sea bed and lunge at any prey item that comes within reach. As the prey will probably try to escape, predators need to have maximal acceleration capacities to ensure a successful catch (Webb & Skadsen, 1980; Rand & Lauder, 1981).

Results in Chapters 2 and 3 have shown increases in force generation and power output of muscle fibres isolated from 15°C-acclimated sculpin relative to 5°C-acclimated sculpin, at 15°C. Some isometric contractile properties such as tetanic half-activation times are also faster at 15°C, in 15°C- compared to the 5°C-acclimated fish. The aims of this study were to see if the acclimatory changes that occur in isolated fast muscle fibres of sculpin are reflected in the fast-start capabilities of the whole animal. Also, the fast-start performances of sculpin was compared to that of other fish species with similar body forms and lifestyles.

Materials and methods

The Fish

Short-horned sculpin, *Myoxocephalus scorpius* (L), were caught in the Firth of Forth between May and June 1991, some specimens were also obtained from Millport University Marine Biological Station. Sixteen fish (mean total length = 24 ± 2 cm) were held in a flow-through sea water aquarium, in 385 l circular tanks at 10 - 12°C. The ambient water temperature was adjusted by 1 - 2°C per day until the required acclimation temperature was reached. The fish were acclimated either to 5°C or 15°C ± 0.5°C for 6 - 8 weeks under a constant photoperiodic regime (12 hr light: 12 hr dark). Ten days prior to filming the fish were transported to the Dunstaffnage Marine Research Laboratory, Oban, at their respective acclimation temperatures. On arrival the fish were transferred to flow-through sea water tanks at 5°C and 15°C as appropriate and starved for 7 days before the experiment began.

Measurement of whole animal swimming performance

All experiments were carried out in constant temperature rooms with a similar photoperiod to that of the holding facilities. Fish were filmed in a static tank (150 cm long, 70 cm wide and 60 cm deep) via a 45° mirror using a NAC-incorporated VHS High Speed Video-400 unit at 200 frames s^{-1}. Illumination was provided by a strobe light and sharp silhouettes of the fish were obtained by placing a reflective Scotchlite background on the bottom of the tank. The fish were

restricted to an area 55 cm long, 70 cm wide using a plastic mesh, in a depth of water of 20 - 30 cm. Grid markings, 20 cm apart were placed on the Scotchlite board to provide a reference point for analysis.

Fish were placed in the observation tank, at their respective acclimation temperatures, in small groups of four or five in order to encourage competition. Burst speed swimming sequences were elicited by introducing invertebrate prey into the chamber. This study set out to directly compare the fast-start performances of the acclimated fish as opposed to investigating predator-prey relationships. No account was therefore taken of the acclimation state of the prey which was either *Crangon crangon* (3 - 5 cm) or the common shore crab, *Carcinus meanus* (3 - 4 cm wide). The fish were fed sparingly to avoid satiation at a single temperature. The 5°C-acclimated fish were filmed at 5, 10 and 15°C, whereas 15°C-acclimated fish were only filmed at 15°C. Fish remained in the observation tank while the temperature was slowly raised by ~1°C h^{-1}. At each experimental temperature the fish were left for at least 1 h before filming commenced.

Kinematic Analysis

The video tape from the NAC-incorporated VHS High Speed Video-400 unit was transferred onto a Sony U-matic VHS video tape. Still frames of the attack sequences were analysed at 10 ms intervals using a JVC CR-6600E video recorder and Barco CS1634-monitor. The outlines of the fish were traced onto acetate sheets. Only those sequences in which the fish travelled in a straight line were analysed.

Calculations

The fish were identified by length and distinctive features, and the fastest strike sequence from each fish was analysed. The distance travelled and duration of each kinematic stage was measured (Fig. 3.1). The velocity was calculated per 10 ms interval, and the mean velocity of each stage was calculated using regression analysis on distance-time plots (Fig. 3.2). The maximum velocity over any 10 ms interval was recorded for each strike sequence and the velocity of the glide was calculated by measuring the distance travelled over a uniform time period. Acceleration was calculated using regression analysis of velocity against time plots. The tail-beat frequency was measured during the propulsive stroke, as was the stride length and amplitude of the tail-beat (Fig. 3.2).

Results are presented as means ± S.E.M. Statistical significance was determined using a one-way analysis of variance test (Minitab Inc. Philadelphia).

Predator-prey interaction

The strike distance was measured, as the initial distance of the fishes' snout from the prey item, at the start of an attack sequence. The proportion of successful prey captures, out of the total number of attacks made (% catch) was also calculated.

Figure 3.1.

Figure 3.1. Body outlines of sculpin during a fast-start manouevre. Each outline shows **A**, the start position **B**, the end of the preparatory stroke **C**, the end of the first tail-beat of the propulsive stroke **D**, the end of the propulsive stroke **E**, the glide position. The line drawing shows the mid-line of the fish during the fast-start.

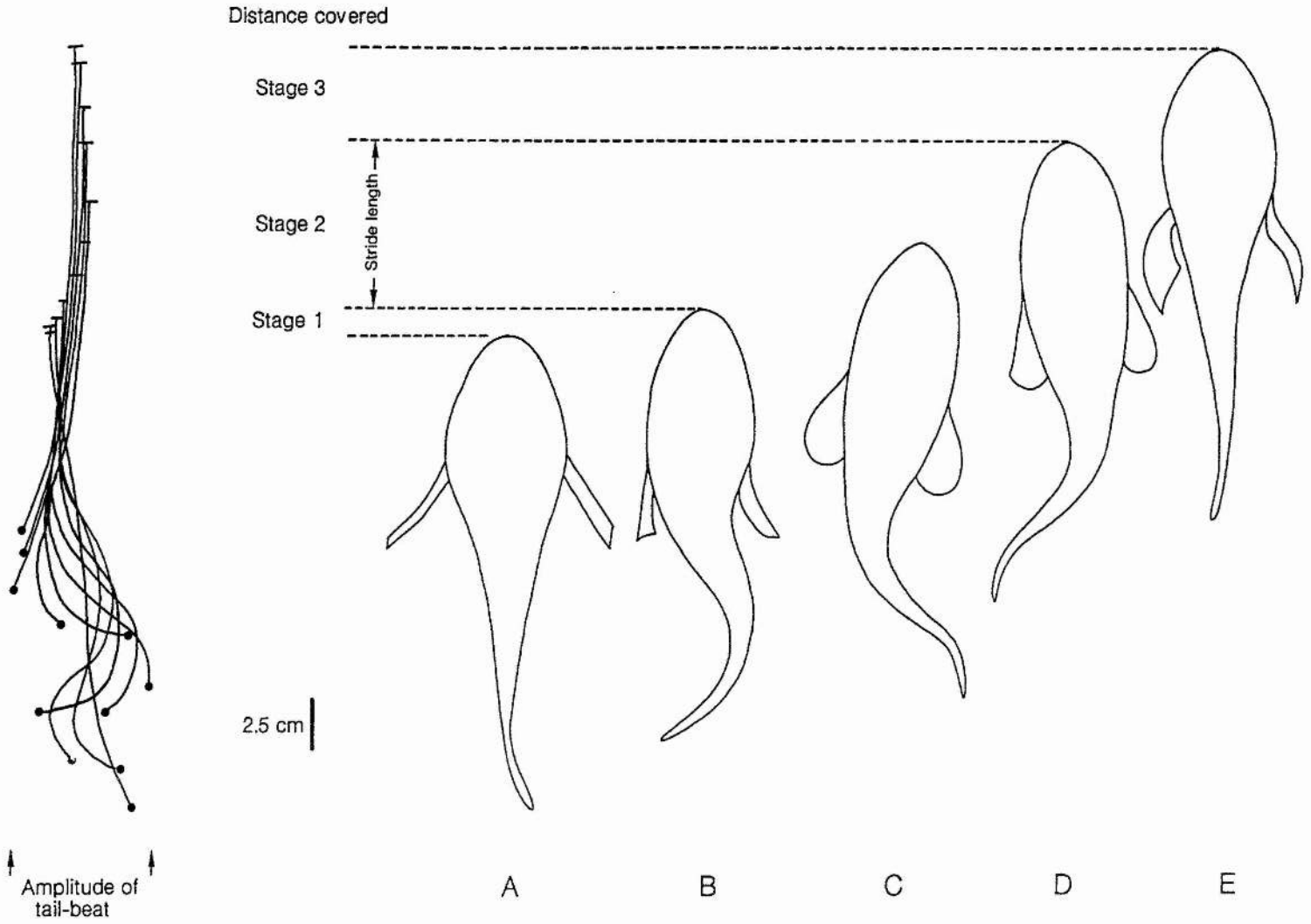
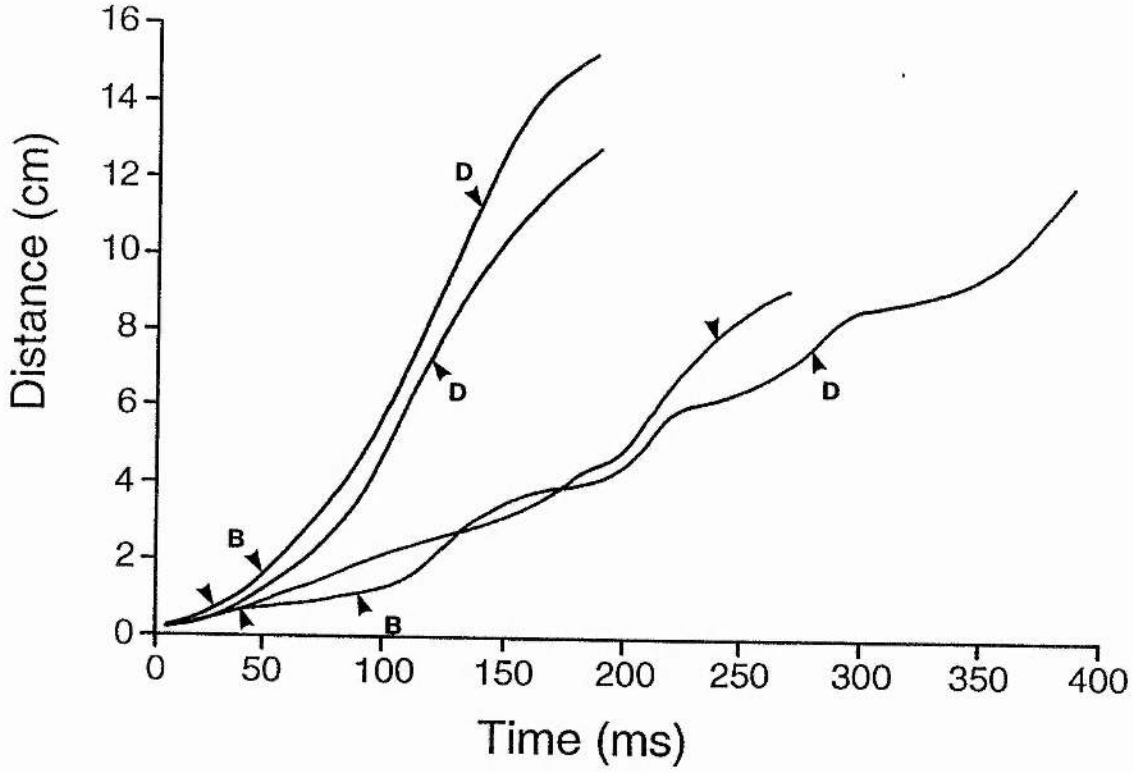

Distance covered
Stage 3
Stage 2
Stage 1
Stride length
2.5 cm
Amplitude of tail-beat
A
B
C
D
E

Figure 3.2.

Figure 3.2. The distance travelled over 10 ms intervals plotted as a cumulative distance. Data for 4 individuals tested at 15°C were shown, the two top lines being 15°C-acclimated sculpin, the two lower lines being 5°C-acclimated sculpin. Arrows show the transition points for each kinematic stage.


Distance (cm)
16
14
12
10
8
6
4
2
0
0
50
100
150
200
250
300
350
400
Time (ms)
B
B
D
D
D

Results

Kinematics

The fish stalked the prey by creeping forward propelled by their pectoral and pelvic fins. During a fast-start, sculpin employed the three kinematic stages similar to those described by Weihs (1973) and Webb (1978a & b) (see Fig. 3.1). Stage 1 represents a preparatory stroke, during which the tail was moved laterally to the body axis into the 'S' shaped start position. This was accompanied by rapid adduction of the pectoral fins and partial erection of the dorsal and ventral fins. Stage 2 was the propulsive stroke, where the tail was moved through one complete tail-beat cycle and where most of the thrust was generated. Stage 3 the final stage, was an unpowered glide, the duration of which depended on the behaviour of the fish. The glide can continue into steady swimming, or the fish can change direction with a turning manoeuvre, or abruptly stop using the large pectoral fins. Towards the end of stage 2 the jaws are protruded and fish try to suck the prey item into its mouth.

During fast-starts in sculpin, the mean velocity increased to a maximum during stage 2, being more than 2.5-times faster than stage 1. Also, the mean acceleration rate was greatest during the propulsive stroke (Table 3.1) with deceleration occurring during the glide.

Effects of acute temperature change on 5°C-acclimated sculpin

Raising the experimental temperature caused the tail-beat frequency to increase by 45% from 5.5 Hz at 5°C, to 8.0 Hz at 15°C ($P < 0.001$). Also, increasing the water temperature produced a small rise

Table 3.1.

Table 3.1. Relationship between acceleration rate, experimental and acclimation temperature. Acceleration was calculated from regression of velocity against time graphs, over each stage. Values are means ± S.E. n = 5,8,6,5 respectively. Significance levels are * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.005$.

Stage 2.

15°C-acclimated (15°C) > 5°C-acclimated (15, 10, 5°C) (P = 0.016, 0.000, 0.004)

5°C-acclimated (15°C) > 5°C-acclimated (10°C) (P = 0.012)

Stage 1 & 2.

15°C-acclimated (15°C) > 5°C-acclimated (15, 10, 5°C) (P = 0.004, 0.000, 0.000)

5°C-acclimated (15°C) > 5°C-acclimated (10, 5°C) (P = 0.034, 0.038)

Acceleration rate $m.s^{-2}$ of the fastest sequence of each fish

Acclimation group	5°C (Cold)	5°C (Cold)	5°C (Cold)	15°C (Warm)
Experimental temperature (°C).	5°C	10°C	15°C	15°C
Stage 1. (Preparatory stroke)	1.96 ± 0.64	1.52 ± 0.73	4.80 ± 1.35	4.83 ± 2.30
Stage 2. (Propulsive stroke)	2.81 ± 0.69	1.97 ± 0.42	4.98 ± 0.82	9.38 ± 1.48
Stage 1 & 2.	2.35 ± 0.48	2.53 ± 0.39	4.68 ± 0.71	8.84 ± 0.90

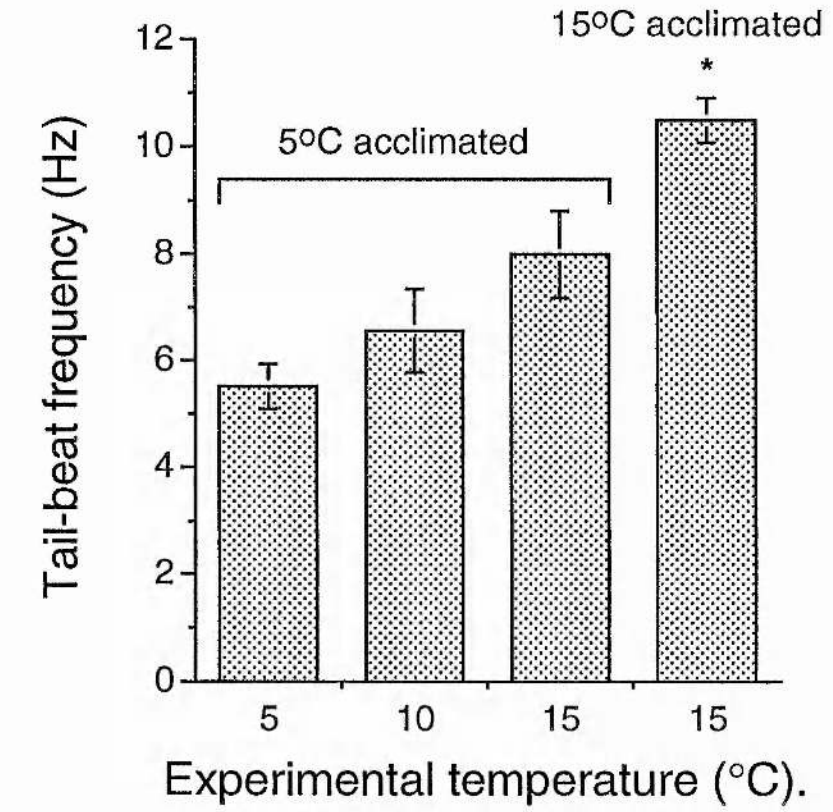
(though insignificant) in mean velocity and a two-fold increase in the acceleration rate attained, over stages 1 and 2 (Table 3.1) ($P < 0.038$). The maximum velocity attained did not increase between 5°C and 15°C, and the distance covered during stages 1 and 2, decreased with increasing experimental temperature (Fig. 3.6). The amplitude of the tail-beat (Fig. 3.3b) and the stride length both decreased slightly when 5°C-acclimated sculpin swam at 10°C and 15°C.

Comparison of laboratory-acclimated fish at 15°C

The tail-beat frequency of 15°C-acclimated sculpin at 15°C was 31% higher (10.5 Hz) than in 5°C-acclimated sculpin at 15°C (8 Hz) ($P < 0.05$; Fig. 3.3a). The mean velocity at which 15°C-acclimated sculpin attacked prey was 21% faster during stage 1 and 52% faster, during stage 2 ($P < 0.02$) than 5°C-acclimated fish, at 15°C (Fig. 3.4). The maximum velocity attained during the prey-capture event was 31% greater in 15°C-, than 5°C-acclimated fish at 15°C, and 57% greater than 5°C-acclimated fish at 5°C ($P < 0.02$; Fig. 3.5). Acclimation to 15°C also brought about a 1.9-fold increase in acceleration rate during the preparatory and propulsive strokes, compared to 5°C-acclimated sculpin ($P < 0.005$; Table 3.1). As a result of the greater acceleration rate and velocity attained by 15°C-acclimated sculpin, the mean total distance covered was 11 cm which was 34% further than that covered by 5°C-acclimated sculpin at 15°C ($P < 0.03$; Fig. 3.6). The major fast-start characteristic that was altered by warm acclimation was the tail-beat amplitude (Fig. 3.3b). The tail-beat amplitude increased by 24% from 0.26 BL in 5°C-acclimated sculpin, to 0.32 BL in 15°C-acclimated fish, at 15°C ($P < 0.01$). Correspondingly, the stride length at 15°C was

Figure 3.3.

Figure 3.3. **(a)** The tail-beat frequency **(b)** the trailing edge amplitude of the tail-beat attained during stage 2 (the propulsive stroke), of the prey-attack sequences of 5°C- and 15°C-acclimated sculpin. Values are means ± S.E. n = 6, 6, 8, 5, respectively. (a) $P<0.05$. (b) $P<0.01$.


15ºC acclimated
*
5ºC acclimated
Tail-beat frequency (Hz)
12
10
8
6
4
2
0
5
10
15
15
Experimental temperature (°C).

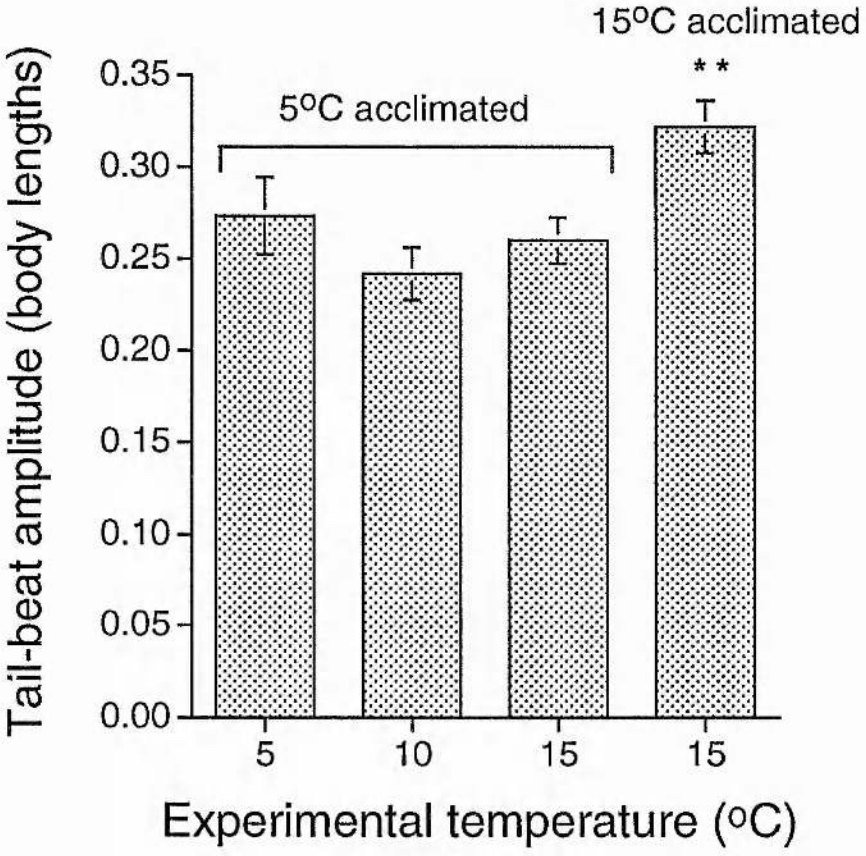
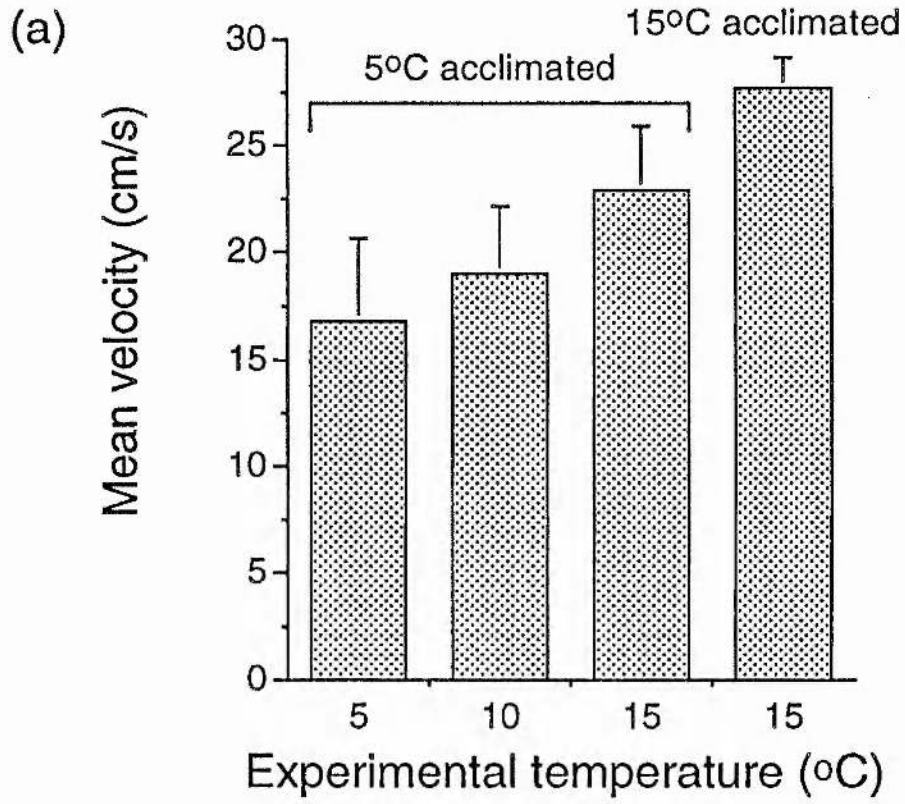

15ºC acclimated
* *
5ºC acclimated
Tail-beat amplitude (body lengths)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
5
10
15
15
Experimental temperature (ºC)

Figure 3.4.

Figure 3.4. The mean velocity (cm s^{-1}) of the prey-attack sequences of 5°C- and 15°C-acclimated sculpin. (**a**) measured over stage 1 (the preparatory stroke). (**b**) measured over stage 2 (the propulsive stroke). Values are means ± S.E. n = 5, 6, 8, 5, respectively. $P < 0.05$.


(a)
15°C acclimated
5°C acclimated
Mean velocity (cm/s)
30
25
20
15
10
5
0
5
10
15
15
Experimental temperature (°C)

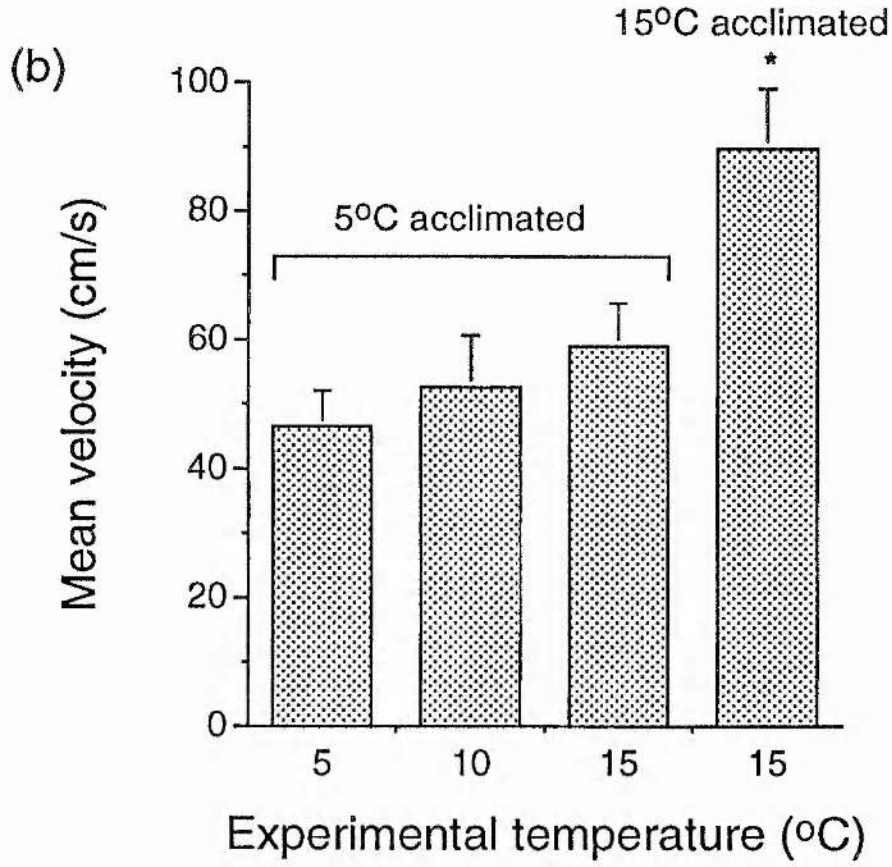

(b)
15°C acclimated
*
5°C acclimated
Mean velocity (cm/s)
100
80
60
40
20
0
5
10
15
15
Experimental temperature (°C)

Figure 3.5.

Figure 3.5. The maximum velocity (cm s^{-1}) attained during stage 1 or 2 of the prey-attack sequences of laboratory-acclimated sculpin. Values are means ± S.E. n = 6, 6, 8, 5, respectively. $P < 0.05$.

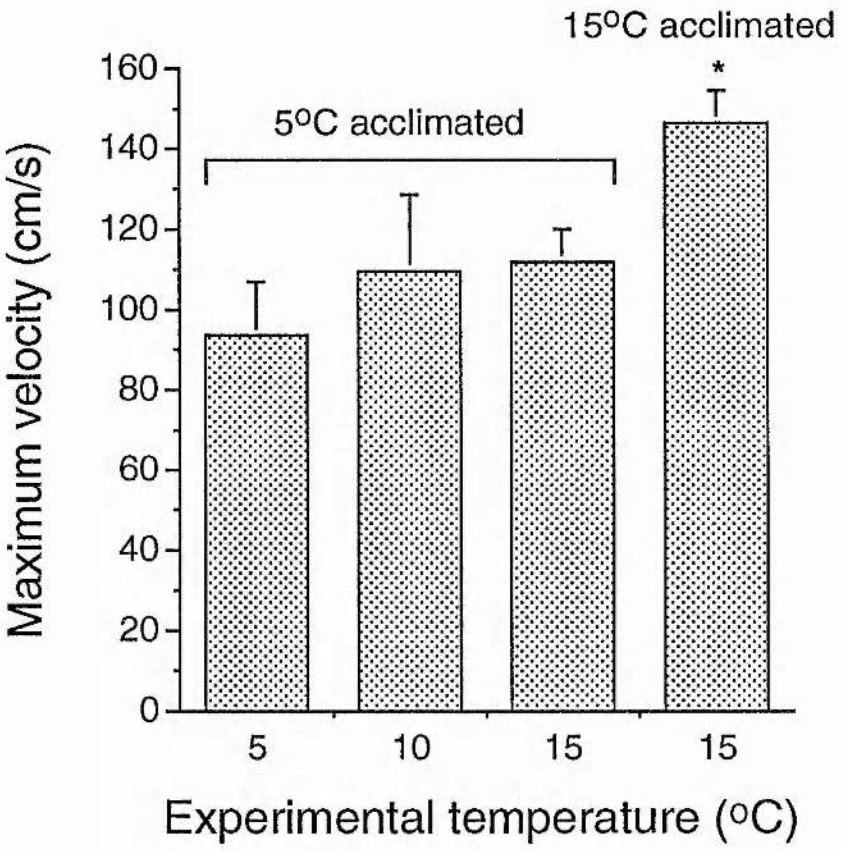
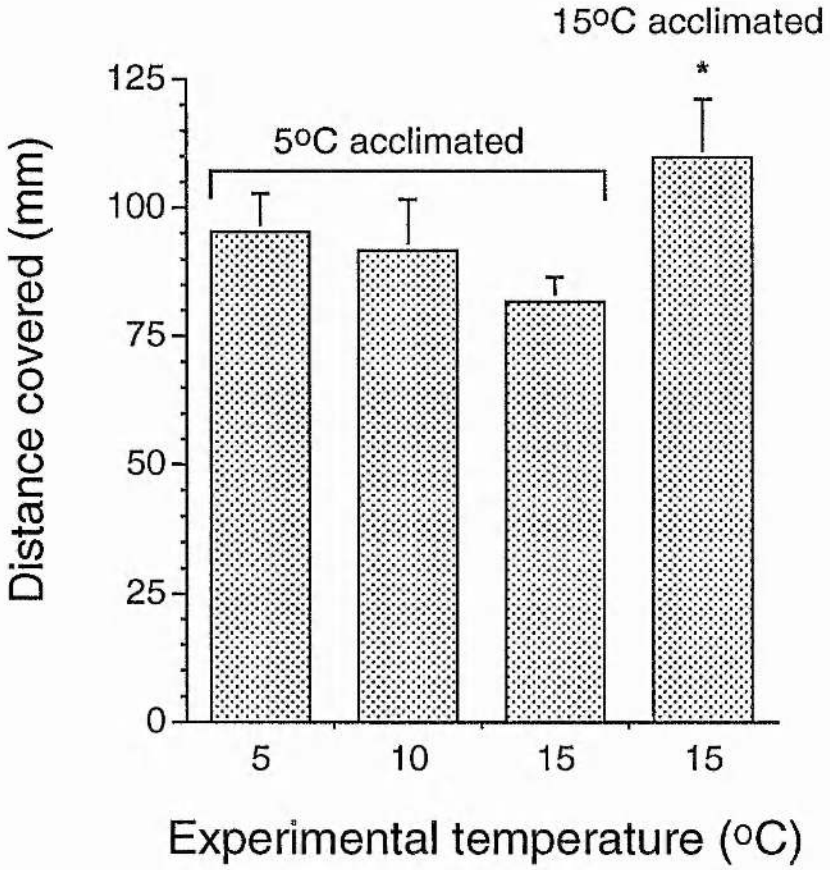

15°C acclimated
*
5°C acclimated
160
140
120
100
80
60
40
20
0
Maximum velocity (cm/s)
5
10
15
15
Experimental temperature (°C)

Figure 3.6.

Figure 3.6. The distance covered over stages 1 and 2, during prey-attack sequences of 5°C- and 15°C-acclimated sculpin. Values are means ± S.E. n = 6, 6, 8, 5, respectively. $P < 0.05$.


15°C acclimated
*
125
5°C acclimated
100
75
50
25
0
Distance covered (mm)
5
10
15
15
Experimental temperature (°C)

also significantly increased by 27% to 0.37 BL following warm acclimation ($P < 0.031$).

Prey-capture success

Warm and cold-acclimated groups of sculpin differed in their ability to catch prey at 15°C. The initial strike distance of the 5°C-acclimated sculpin was 5.5 ± 1.0 cm regardless of temperature, whereas 15°C-acclimated sculpin, successfully attacked prey from more than 10 cm away ($P < 0.047$; Fig. 3.7). The percentage catch data illustrates differences in the performance capabilities of 15°C- compared to 5°C-acclimated sculpin at 15°C. 15°C-acclimated sculpin had a success rate of 73.4% compared to 23.2% in the 5°C-acclimated sculpin at 15°C.

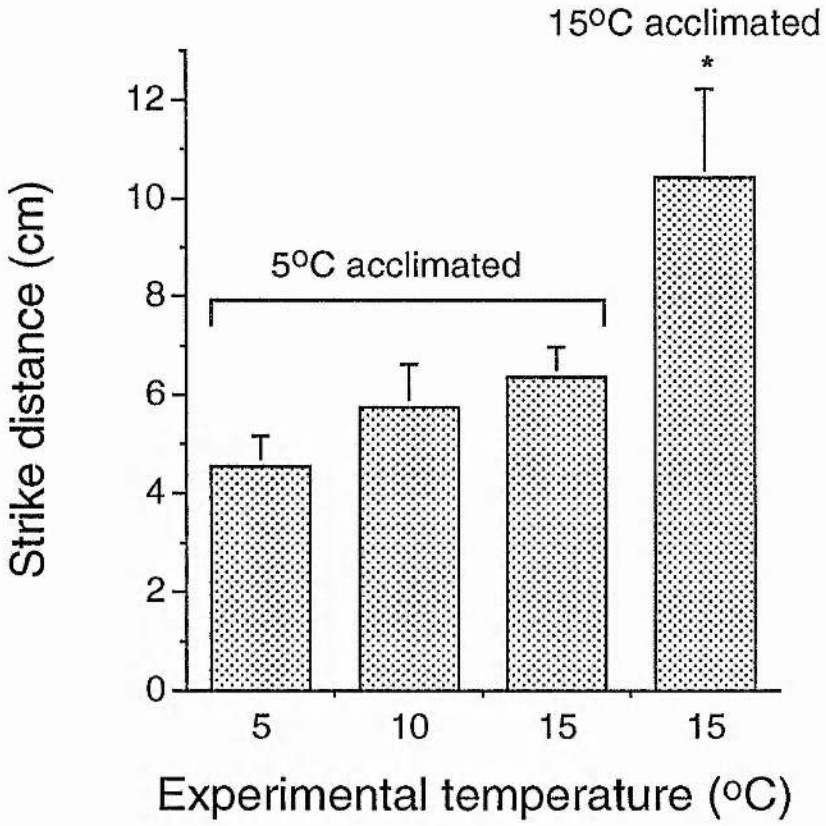
Discussion

Kinematics

The basic components of the fast-start in sculpin were little affected by acute temperature change or acclimation. In the fast-starts of sculpin, the maximum velocity was attained at the end of kinematic stage 2 (the propulsive stroke), and the maximum rate of acceleration occurred during stage 1, as has been reported for a number of other species (Webb, 1978a). Sculpin appear to suck in their prey when they get close enough. During suction feeding, forward velocity of a fish has been shown to increase the distance over which a prey item can be sucked by 60% (Weihs, 1980). Protrusion of the jaw is believed to add to the velocity of a fast-start and therefore to increase the efficiency of suction feeding (Alexander, 1967).

Figure 3.7.

Figure 3.7. The initial strike distance from the snout of the sculpin to the prey at the start of an attack sequence. Values are means ± S.E. n = 6, 6, 8, 5, respectively. $P < 0.05$.


15°C acclimated
*
12
10
8
6
4
2
0
5°C acclimated
Strike distance (cm)
5
10
15
15
Experimental temperature (°C)

Kinematic analysis has revealed two major types of fast-start that show little variation between species, size classes or with temperature (Hertel, 1966; Weihs, 1973; Eaton *et al.*, 1977; Webb, 1975a & b, 1976, 1978b). Sculpin were found to perform 'S' shaped fast-starts characterised by the body bending into an 'S' shape. The shape is analogous to that found during steady swimming, though the amplitude of the tail-beat is much larger and acceleration occurs (Webb, 1976). 'S' shaped curvature balances the recoil forces along the body, enabling specific multidirectional movements to be made. For this reason, 'S' shaped fast-starts are often associated with prey capture events as in this study, and also for several other fish species including: *Esox lucius*, (Hoogland, Morris & Tinbergen, 1956; Harper & Blake, 1990; Webb, 1976), *Esox* sp., (Webb & Skadsen, 1980), *Salmo gairdneri*, (Harper & Blake, 1990; Webb, 1976). The second type of fast-starts are termed 'C' shaped starts, and generally occur during escape or startle manoeuvres (Webb, 1978a & b; Frith & Blake, 1991), and are thought to be mediated by Mauthner cells (Eaton *et al.*, 1977). The head and tail of the fish rotate in the same direction away from the centre of mass, into a 'C' shape during each tail-beat (Webb, 1976). Within the two major types of fast-start there is a range of kinematic behaviours that depend on factors such as the position of the fish at the start of an attack and the distance to the prey (Harper & Blake, 1990, 1991; Webb, 1976; Webb & Skadsen, 1980).

Effects of temperature on the swimming performance of 5°C-acclimated sculpin

Acute changes in experimental temperature from 5 - 15°C nearly doubled the rate of acceleration in the 5°C-acclimated fish. Tail-beat

frequencies were also increased with increasing temperature, with a $Q_{10(5\text{-}15°C)}$ of 1.45. However, the actual velocities reached were relatively independent of test temperature, as were the stride lengths. These results suggest that the time dependant contractile properties of the muscle *in vivo* are highly sensitive to temperature during fast-starts. This corresponds to data from fast muscle fibres isolated from 5°C-acclimated sculpin which had Q_{10}'s of 1.9 for twitch half-activation times. The maximum mechanical properties of isolated muscle fibres probably sets the upper limits for locomotory performance (Rome, 1990). Therefore, as the power output and V_{max} of muscle fibres isolated from 5°C-acclimated sculpin decreases when acutely exposed to 10 and 15°C, the locomotory performance would also be expected to decrease. Lack of increases in mean and maximum velocity, stride length and the overall distance travelled during fast-starts, is a reflection of the decreased power output and V_{max} of isolated muscle fibres at 10 and 15°C. A few other studies have also examined the effects of temperature on fast-start characteristics. Webb (1978a) studied escape fast-starts of trout (*Salmo gairdneri*) and found an increase in maximum velocity from 0.99 m s^{-1} at 5°C, to 1.7 m s^{-1} at 15°C, but relative temperature independence from 15°C - 25°C. The rate of acceleration followed similar trends. The distance travelled by 13.6 cm trout over 100 ms increased from 3.5 cm at 5°C to 11.3 cm at 25°C (Webb, 1978a). In contrast, the distance travelled by 24.2 cm sculpin in this study decreased from 9.5 cm at 5°C to 8.2 cm at 15°C to the end of stage 2 (Fig. 3.6). The differing effects of temperature on the fast-start properties of the two species is probably related to the different life histories. Sculpin are essentially an Arctic species, near their upper thermal limit when acutely exposed to 15°C. Trout however migrate

between sea and freshwater. In freshwater, temperatures are higher and fluctuate to a greater extent thus, the thermal range over which the trout muscles function is probably greater than that of the sculpin.

Comparison of laboratory-acclimated sculpin at 15°C

Fish acclimated to 15°C, had improved fast-start performances compared to 5°C-acclimated sculpin. The maximum velocity attained was 1.3 m s^{-1} in the 15°C-acclimated sculpin compared to 0.8 m s^{-1} in 5°C-acclimated fish at 15°C ($P < 0.01$: Fig. 3.5). Tail-beat frequency (TBF), mean velocity and acceleration rate, stride length and the distance travelled were all significantly higher in 15°C- sculpin, than in 5°C-acclimated sculpin at 15°C. Thrust is a major component of acceleration. Increased thrust was brought about by increased power output (see Chapter 3) and greater tail-beat amplitudes in the 15°C-, relative to the 5°C-acclimated sculpin at 15°C. Therefore, *in vivo* performance was improved in terms of both contractile speeds (i.e. increased TBF) and increased power output following warm-acclimation. Increased contractile speeds are reflected in the tetanic half-activation times of isolated muscle, these being significantly faster in 15°C- relative to 5°C-acclimated sculpin at 15°C ($P < 0.004$; Chapt 2). Also, power output values of fast fibres isolated from 15°C-acclimated sculpin increased with a $Q_{10(5\text{-}15°C)}$ of 3.73, which in theory should produce an increase in swimming speed (power output = swimming $speed^{2.5}$). Even though the sculpin are the same size and have similar % muscle mass, swimming abilities are vastly improved following warm-acclimation, due to increased tail-beat amplitudes and force generating capacities of the fast muscle. Therefore, studies

relating % muscle mass to swim speed should also consider the force produced per cross-sectional area of muscle.

Many more studies have concentrated on the effects of temperature on sustained swimming. Acclimation to higher temperatures caused a decrease in tail-beat frequencies in both trout and bass (Stevens, 1979). It was suggested that when fish swam at temperatures above acclimation temperatures the muscle operates high on the force-velocity curve *i.e* the fish swims at a higher tail-beat frequency (TBF) to compensate for reduced force production per tail-beat. Trout and bass acclimated to higher temperatures have lower TBF and larger stride lengths for a given velocity *i.e.* they operate lower on the force-velocity curve and develop more force per contraction than fish acclimated to lower temperatures (Stevens, 1979). Force-velocity curves of sculpin at 15°C, suggest that 15°C-acclimated fish produced six-times greater power output than 5°C-acclimated sculpin (Chapter 3). It is possible that differences in sustained swimming in bass and trout could be related to acclimatory effects on the force generating capacities rather than changes in the V at which the muscle shortens. According to Rome's theory that V/ Vmax is a design constraint on muscle systems, the V at which the muscle operates efficiently should stay approximately the same (Rome *et al.*, 1990). Calculation of muscle strains indicated that the V at which the anterior myotomes (0.31L) shorten was 2.23 muscle lengths/ s. Vmax of isolated fast muscle fibres was found to be 8.2 ML/ s and therefore the V/ Vmax at which the fast fibres operate during fast-starts was 0.27. This is within the range of V/ Vmax values reported for red carp muscle 0.17 - 0.36 (Rome *et al.*, 1990) and for scup, 0.17 - 0.37 between 10 - 20°C (Rome *et al.*, 1992). Increased Vmax of sculpin fast fibres following adaptation to summer temperatures

appears to enable fish to swim at a faster velocity while keeping V/ Vmax in the range of maximum power. This supports the theory that V/ Vmax is a design constraint.

Comparisons with studies on fish with similar body forms

Data on maximum burst swimming capacities of fish with similar body forms to that of sculpin are limited. Comparisons are complicated due to the different methods used to elicit fast-starts, different analytical procedures and also due to the high individual variability of fish swimming behaviour. The fast-start data obtained for other sedentary, benthic species with similar body forms and analysed using videotape are summarised in Table 3.2. Velocities and acceleration rates attained by these benthic species are similar despite differences between natural environmental temperature and size, possibly providing a measure of the optimum performance required for survival. This emphasises the fact that swimming is a dynamic process, involving a combination of factors including: muscle properties, neural mechanisms, respiratory pathways, elastic components and endurance capabilities. Examination of length specific swimming speeds suggests that scaling is an important determinant of fast-start capabilities, with smaller fish having relatively faster speeds (Table 3.2). Length specific swim speed increases with decreasing size, due to the faster tail-beat frequencies and greater tail-beat amplitudes of smaller fish (Bainbridge, 1958; Hunter & Zweifel, 1971; Webb, Kostecki & Stevens, 1984). *Sebastes mystinus* (Dorn, Johnson & Darby, 1979) *Etheostoma caeruleum* and *Cottus cognatus* (Webb, 1978b) could also have higher values of acceleration and velocity as they were performing escape fast-starts. Escape fast-starts are reflex responses, that are generally faster than feeding fast-starts

Table 3.2.

Table 3.2. Comparative data of burst speed swimming from fish with similar body forms from antarctic and temperate regions. Data taken from 1 = Dorn *et al*, (1979); 2 = Webb, (1984); 3 = Webb, (1978); 4 = This study; 5 = Archer & Johnston, (1989).

Species	Total length (cm).	Mean velocity ($m\ s^{-1}$)	Mean velocity ($BL\ s^{-1}$)	Maximum velocity ($m\ s^{-1}$)	Maximum velocity ($BL\ s^{-1}$)	Mean acceleration ($m\ s^{-2}$)	Mean acceleration ($BL\ s^{-2}$)	Environmental temperature (°C).
Warm/ Temperate.								
Sebastes mystinus[1]	15.1	1.06	7.0	–	–	–	–	15 - 20
Temperate.								
Ambloplites rupestris[2] (Freshwater).	15.1	0.43	2.87	–	–	–	–	15
Etheostoma caeruleum.[3] (Freshwater).	6.2	–	–	0.89	14.4	10.3	166.1	15
Cottus cognatus.[3] (Freshwater).	8.2	–	–	0.77	9.4	6.1	74.4	15
Temperate/ Arctic.								
Myoxocephalus scorpius.								
15°C-acclimated[4]	24.2	0.9	3.7	1.46	6.0	8.84	36.5	15
5°C-acclimated[4]	24.2	0.46	1.9	0.94	3.9	4.68	19.3	5
Antarctic								
Notothenia neglecta (adult).[5]	29.3	1.26	4.3	1.58	5.4	–	–	2

due to the life threatening nature of a fish failing to avoid a predator (Webb, 1976; Eaton *et al.*, 1977). The effects of scaling and the type of fast-start manoeuvre performed could have important consequences on the overall speed attained.

In the natural environment other extrinsic factors could mediate whole animal swimming performance. Seasonally inconsistent photoperiods alters the U_{crit} of juvenile largemouth bass (Kolok, 1991). The reproductive state of the fish is also known to affect the swimming speeds of fish, with gravid females generally having reduced swimming capabilities compared to non-gravid fish (Blaxter, 1969; Dorn *et al*, 1979).

Relation of *in vivo* and *in vitro* muscle performance

The reduced prey capture capabilities of sculpin acutely exposed to 15°C also has obvious ecological and survival implications. The phenotypic plasticity of the fast muscle of sculpin has enabled fast-start capacities to be maximised at summer temperatures, which would therefore increase the chances of survival. The major finding of this study, is that muscle changes are modified by thermal acclimation *in vivo*, although perhaps to a lesser extent than is observed for isolated muscle fibres *in vitro*.

CHAPTER 5

Power output of fast muscle fibres in the short-horned sculpin under the constraints operating during feeding

Introduction

Estimates of power output from force-velocity curves are not directly representative of the power output generated during swimming (Chapter 3). During isotonic contractions, power output is calculated when the muscles are shortening under a constant load, an event unlikely to occur during locomotion. Muscle fibres are also only active for part of the length change cycle *in vivo* (Rome *et al.*, 1984; Johnston *et al.*, 1993). Investigations of swimming at steady speeds suggests that muscle fibres undergo sinusoidal length-change cycles, increasing in amplitude from head to tail (Hess & Videler, 1984; Leeuwan *et al.*, 1990). Previous studies have utilised the work loop technique developed by Josephson (1985) and sinusoidal length changes, to measure the power output of muscle fibres under optimal conditions of strain and stimulation (Altringham & Johnston, 1990a & b; Johnson & Johnston, 1991a; Johnston *et al.*, 1993).

During balistic movements such as prey capture and sprint starts, kinematic analysis has revealed that fibre length changes are not sinusoidal. Therefore, values of power output obtained from fibres subject to sinusoidal waveforms may not be appropriate. In the present study *in vivo* strain fluctuations were calculated for three positions along the body during prey-capture (see Chapter 4). Isolated muscle fibres from the sculpin were used to measure force and work under the constraints operating *in vivo*. Information on the duration and timing of muscle activation were obtained from a previous study (Johnston *et al.*, 1993).

Materials and methods

The fish

Short-horned sculpin, *Myoxocephalus scorpius* (L), used for the swimming experiments, were caught in the Firth of Forth between May and June 1991, some specimens were also obtained from Millport University Marine Biological Station. Fish (total length, 24 ± 2 cm; mean ± S.E.) were held in a flow-through seawater aquarium, in 385 l circular tanks at 10 - 12°C. The ambient water temperature was raised by 1 - 2°C per day until the required acclimation temperature of 15°C was reached, the fish were then acclimated for 6 - 8 weeks. Sculpin used in the isolated muscle experiments, were caught in the Firth of Forth during July/ August 1993. Fish were held at ambient temperature in sea water aquaria for 2 - 15 days prior to use, under a constant photo periodic regime of 12 h light:12 h dark. All fish were fed regularly on a diet of fish flesh, squid and crustaceans.

Kinematic analysis

Sculpin performing attack sequences were filmed via a 45° mirror using a NAC-incorporated VHS High Speed Video-400 unit at 200 frames s^{-1} (Chapter 4). The film was transferred onto Sony U-matic VHS video tape. Still frames of the attack sequences were analysed at 5 ms intervals using a JVC CR-6600E video recorder and Barco CS1634-monitor. Successive outlines (5 ms apart) of the fish were traced onto separate acetate sheets. The outlines were enlarged and then carefully digitised (Sigmascan, Jandel Scientific).

Fibre Orientation

Four specimens of sculpin were sacrificed and the skin was carefully removed from the body to reveal the musculature. At fixed points along the body (0.31L, 0.52L, 0.77L from snout) a section of fibres was carefully removed and the fibre trajectories were traced onto acetate sheets. Fibre angle was then calculated in relation to the median and horizontal planes of the fish.

Strain calculation

Curvature and strain values were calculated by Johann van Leeuwan, of the University of Leiden using methodology described in (Leeuwan *et al.*, 1990). Briefly, the longitudinal axis of the sculpin was calculated from the midline of the digitised outlines assuming the fish was symmetrical down its length. Certain assumptions were made about swimming fish when calculating the strain values: a) in each infinitesimal segment the volume remains constant, b) the axis of the fish remains a constant length and bends as a smooth curve, c) muscle tissue is not displaced dorso-ventrally along the body, d) no buckling occurs in the muscle fibres on the concave side of the body.

Mechanical experiments

Fish (standard length 21.3 ± 1.5 cm, mass 292.1 ± 76.9 g; means ± S.E.; $n = 7$) were killed by a blow to the head, followed by transection of the spinal cord. Live preparations were dissected from the ventral abdominal myotomes 0.33L (rostral preparations) and from a point 0.8L down the body of the fish (caudal preparations) (see Chapter 2).

The muscle was pinned out at resting length onto a silicone elastomer base (Sylgard 184, Dow Corning, Midland MI, USA) and the tissue was immersed in fresh, cold Ringer (mmol l^{-1}: NaCl 132.2; Na Pyruvate 10; KCl 2.6; $MgCl_2$ 1; $CaCl_2$ 2.7; $NaHCO_3$ 18.5; $NaHPO_4$ 3.2; pH set to 7.4 using HCl/ NaOH (Hudson, 1969)). The dissection was carried out on a cooled aluminium stage (< 5°C) under a binocular microscope.

Experimental apparatus and protocol

The preparation was transferred to a flow-through perspex chamber containing Ringer held at a constant temperature of 15°C. A peristaltic pump (Watson - Marlow) circulated aerated Ringer through a coil immersed in a thermostatically controlled water bath (Grant LTD 6). Temperature was additionally monitored and controlled by a Peltier cooling unit that surrounded the chamber. One end of the preparation was attached to a force transducer (AE 801, AME Horten, Norway) which was held in a stainless steel tube and water-proofed with silicon grease. The transducer was positioned on a micromanipulator, that enabled the fibre length to be adjusted. The other end of the preparation was attached to a servo-controlled motor (MFE model R4-155, Emerson Electronics, Bourne End, Bucks), that performed the length changes. The unit controlling muscle length changes and stimulation patterns was fully programmable. The fibre was set to the sarcomere length that produced maximum twitch tension without any residual tension (see Chapter 2).

The waveforms of fibre length-changes at three positions along the body of the fish was calculated and digitised (Sigmascan, Jandel

Scientific). Muscle fibres were then subjected to these specified length changes around their *in situ* resting length, and stimulated at a selected phase during the strain cycle (full cycle = 360°). The timing (phase) and number of stimuli were systematically adjusted to produce the maximum work per cycle, which encompassed the range of values for E.M.G. duration and muscle activity phase reported in Johnston *et al.*, (1993). Work done was calculated from plots of force against muscle length. Cross-sectional area was measured at the end of the experiment (see Chapter 2) to obtain absolute measurements of power output and tension.

Statistical analysis

Results presented are means ± S.E.M. Peak force and power output of rostral and caudal fibres were compared at each body position, using one-way analysis of variance (Minitab Inc, Philadelphia, USA).

Results

A major finding of this study was that the strain patterns at specific points along the body differed considerably (Fig. 4.1). For example, muscle fibres at the front of the fish initially shortened during stimulation, whilst fibres at 0.52L and 0.77L were stretched prior to shortening (Fig. 4.1).

Properties of the fibres along the body of the fish.

Another important finding was that the properties of rostral and caudal muscle fibres appear to be adapted to their local mechanical

Figure 4.1.

Figure 4.1. Muscle strain patterns during prey- capture at points 0.31L, 0.52L and 0.77L along the body. The drawing of the sculpin shows the region of the fish from where the rostral and caudal muscle fibres were isolated.

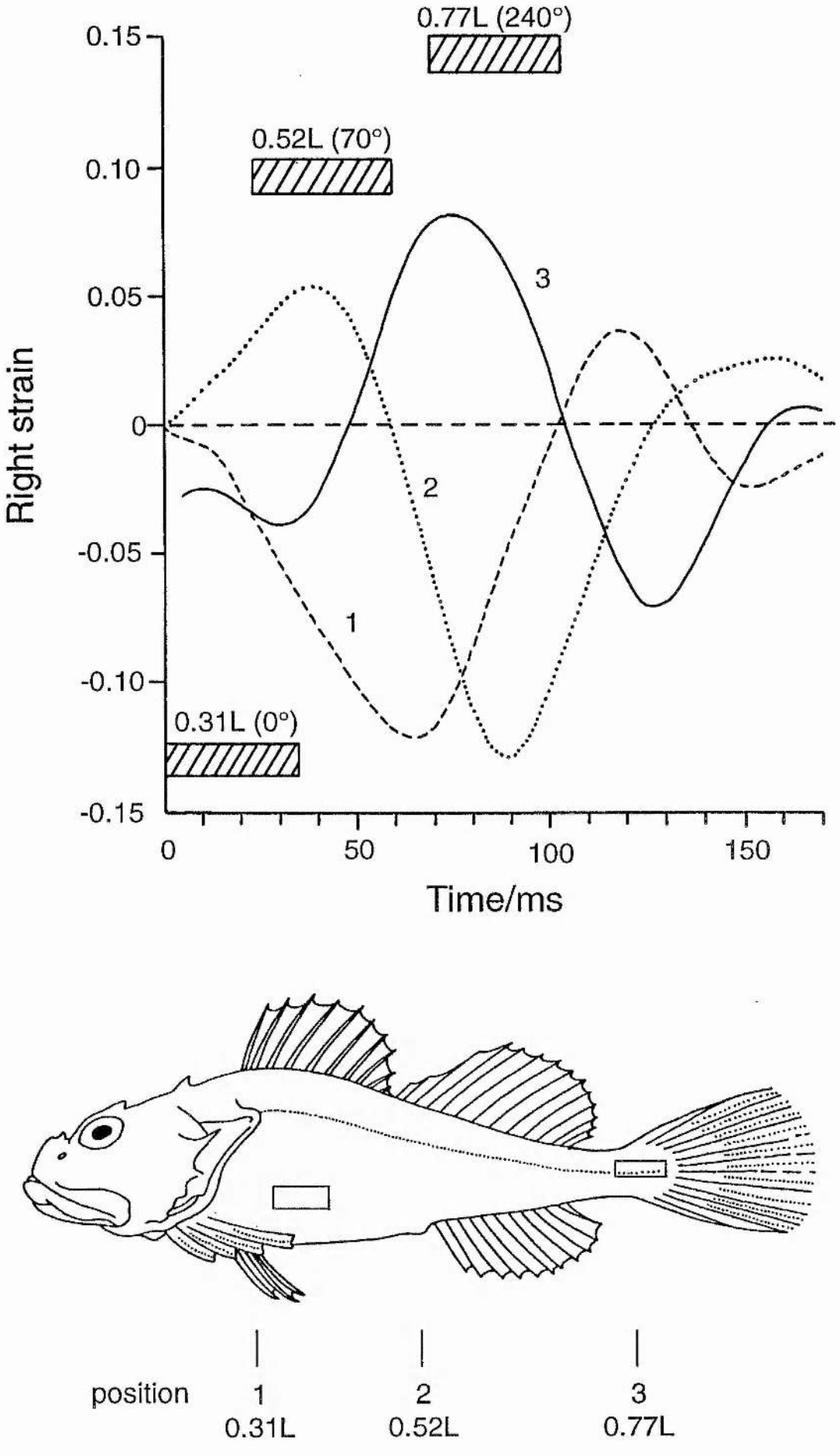

0.77L (240°)
0.52L (70°)
0.31L (0°)
0.15
0.10
0.05
0
-0.05
-0.10
-0.15
Right strain
1
2
3
0
50
100
150
Time/ms
position
1
0.31L
2
0.52L
3
0.77L

Figure 4.2.

Figure 4.2. The shape of the work loops at different positions along the body. a - d muscle fibres isolated from rostral myotomes (0.33L) with the strain fluctuations at 0.31L (a, b) and 0.77L (c, d). Stimulation phases were 10° (a), 40° (b), 40° (c) and 70° (d). e - f muscle fibres isolated from caudal myotomes (0.8L) with strain fluctuations at 0.31L (e) and 0.77L (f). The stimulation phase was 70° for (e) and 5° for (f). In all cases the duty cycle was between 25 - 32% shown as the slightly thicker line on the right hand side of the work loop.

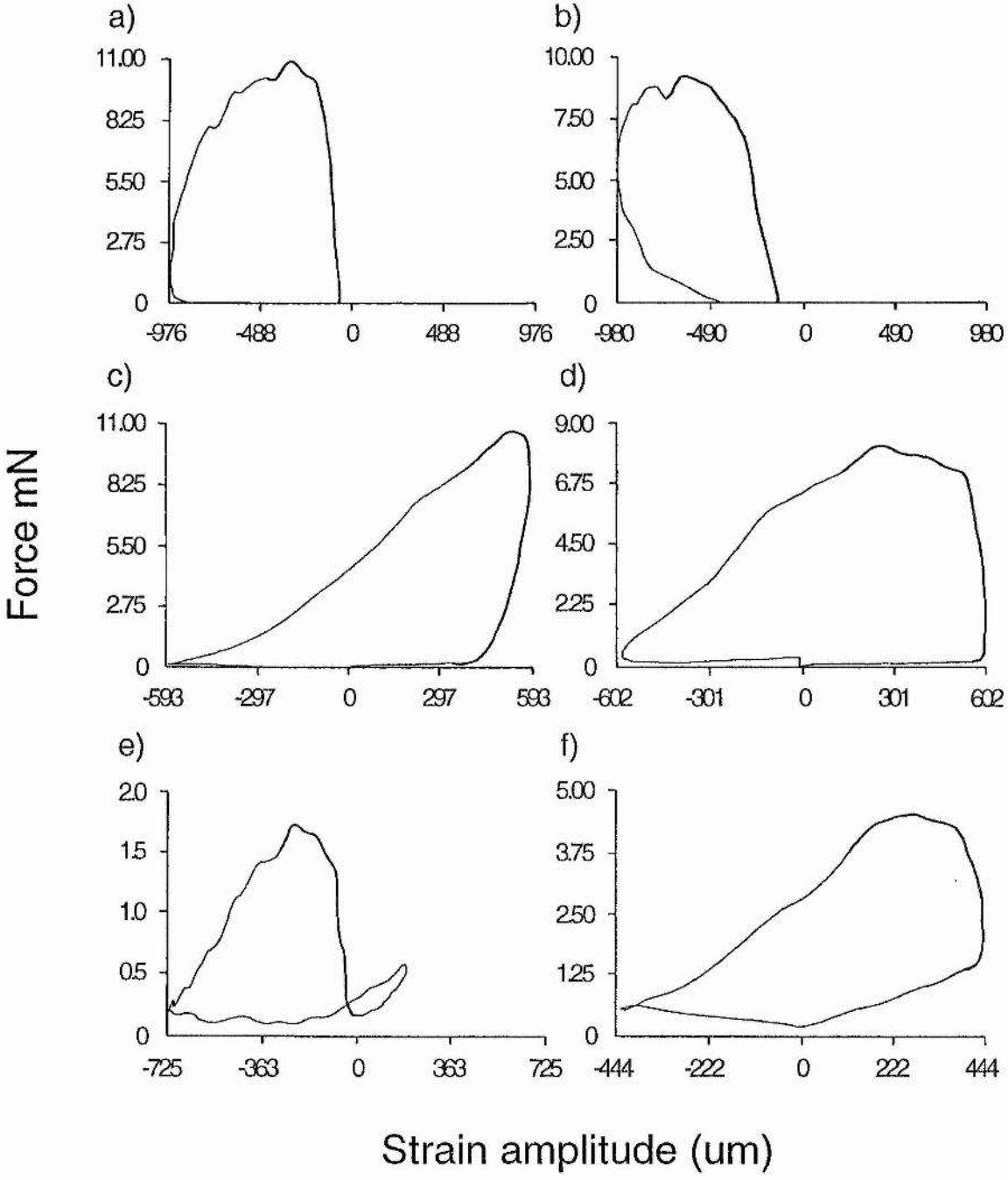
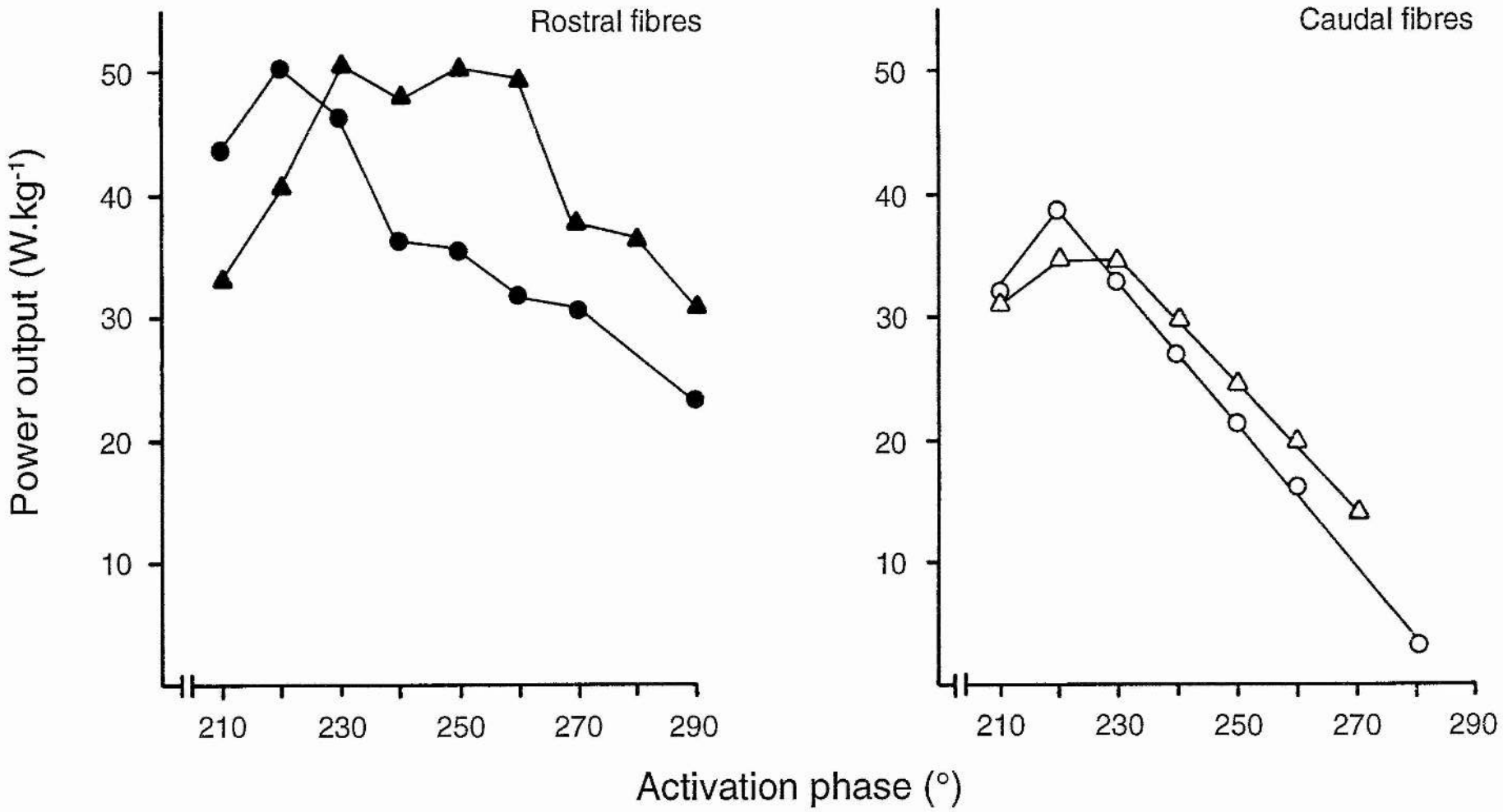

a)
11.00
8.25
5.50
2.75
0
-976
-488
0
488
976
b)
10.00
7.50
5.00
2.50
0
-980
-490
0
490
980
c)
11.00
8.25
5.50
2.75
0
-593
-297
0
297
593
d)
9.00
6.75
4.50
2.25
0
-602
-301
0
301
602
e)
2.0
1.5
1.0
0.5
0
-725
-363
0
363
725
f)
5.00
3.75
2.50
1.25
0
-444
-222
0
222
444
Force mN
Strain amplitude (um)

Figure 4.3.

Figure 4.3. Power output versus activation phase for a) rostral and b) caudal muscle fibres subject to the *in vivo* strain patterns at position 3 (triangles) and sinusoidal length changes (circles).


Rostral fibres
Caudal fibres
Power output (W.kg-1)
50
40
30
20
10
210
230
250
270
290
Activation phase (°)

environment. In particular muscle fibres from caudal myotomes did little net work per cycle, compared to the rostral muscle fibres under the strain conditions pertaining to the front of the fish ($P < 0.01$; Figs. 4.6 & 4.7).

The peak force per cycle for caudal fibres (12.2 kN m^{-2}) at position 1 on the fish was significantly lower than that of rostral fibres (41.9 kN m^{-2}) at position 1 ($P < 0.05$; Figs. 4.4 & 4.5). Similar results were found for values of power output at position 1, with rostral fibres producing 28.0 W kg^{-1}, compared to 3.7 W kg^{-1} in the caudal fibres ($P < 0.01$; Figs. 4.6 & 4.7). No difference was found in either peak force or power generation of caudal compared to rostral fibres at positions 2 and 3 (Figs. 4.4 - 4.7). In all cases, the average power output per cycle was in the region of 30 - 40 W kg^{-1}.

Twitch and tetanic half-activation and half-relaxation times were slightly shorter in rostral compared to caudal fibres. Tetanic half-activation times were 16.1 ms (± 1.3) in the rostral compared to 19.1 ms (± 2.0) in the caudal fibres. Similarly, twitch half-relaxation times were 15.3 ms (± 1.3) in the rostral compared to 19.1 ms (± 2.0) in the caudal fibres. However, these results were not statistically different.

Shape of the work loop

At each position along the length of the fish the fibres produced work loops with a characteristic shape (see Fig. 4.2). Loops (a), (d), (e) and (f) represent the optimum work obtained per cycle. Rostral fibres produce optimum work loops at 0.31L at an activation phase of around 10° (a). Increasing activation phase (b) causes the force to develop at a slower rate (loop slopes to the left) and also leads to the loop rising off

Figure 4.4.

Figure 4.4. Peak force (kN m^{-2}) of rostral fibres performing oscillatory work with *in vivo* strain patterns at all three positions along the body of the fish, against changing activation phase. A duty cycle of 25 - 32% was found to be optimal in most circumstances. $n = 6$ or 7. Values are means ± S.E.

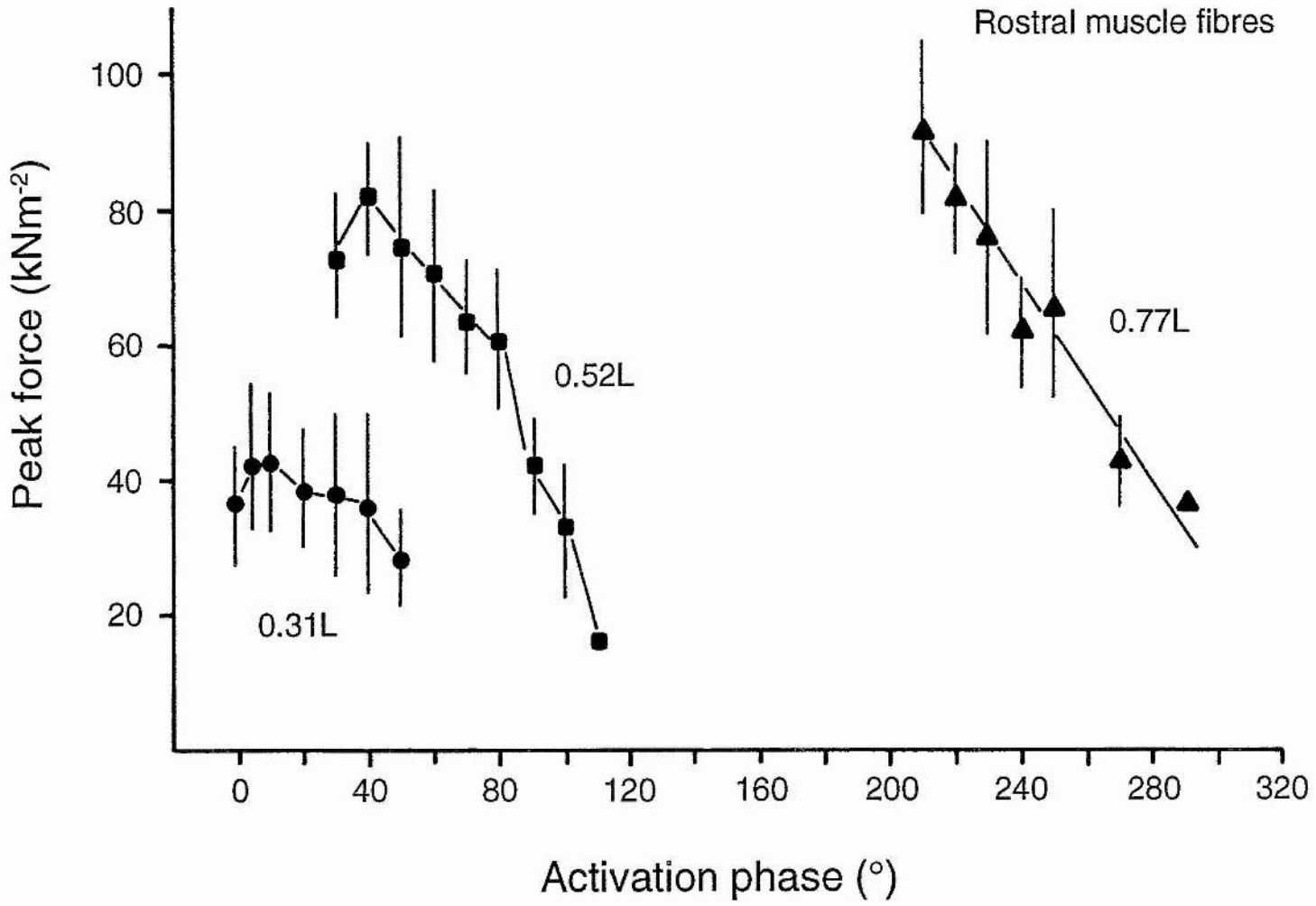

Rostral muscle fibres
Peak force (kNm-2)
100
80
60
40
20
0.31L
0.52L
0.77L
0
40
80
120
160
200
240
280
320
Activation phase (°)

Figure 4.5.

Figure 4.5. Peak force (kN m^{-2}) of caudal fibres performing oscillatory work with *in vivo* strain patterns at all three positions along the body of the fish, against changing activation phase. A duty cycle of 25 - 32% was found to be optimal in most circumstances. $n = 6$ or 7. Values are means ± S.E.

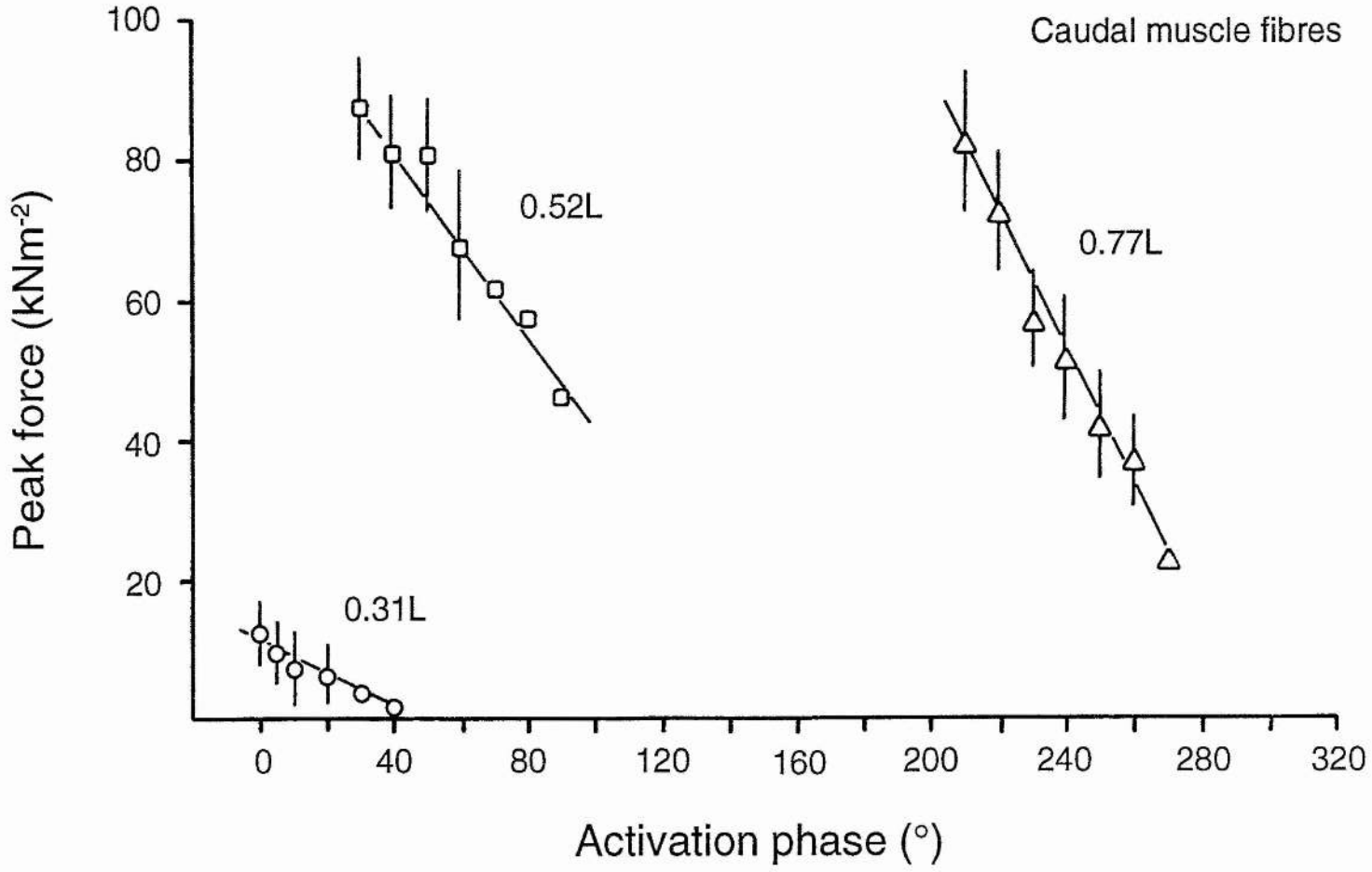

Caudal muscle fibres
Peak force (kNm-2)
100
80
60
40
20
0.52L
0.77L
0.31L
0
40
80
120
160
200
240
280
320
Activation phase (°)

Figure 4.6.

Figure 4.6. Power output (W kg^{-1}) of rostral fibres performing oscillatory work with *in vivo* strain patterns at all three positions along the body of the fish, against changing activation phase. $n = 6$ or 7. Values are means ± S.E.

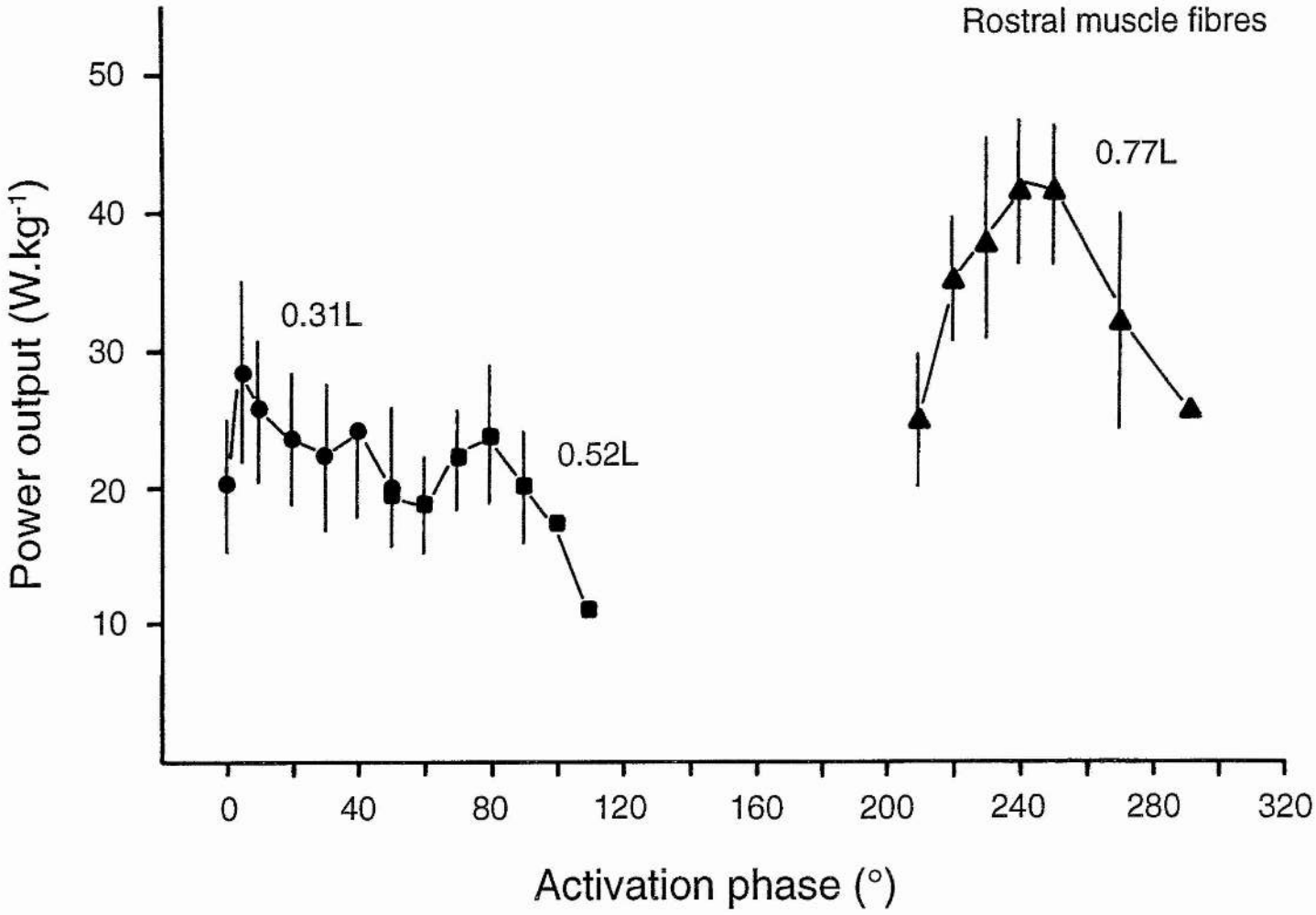
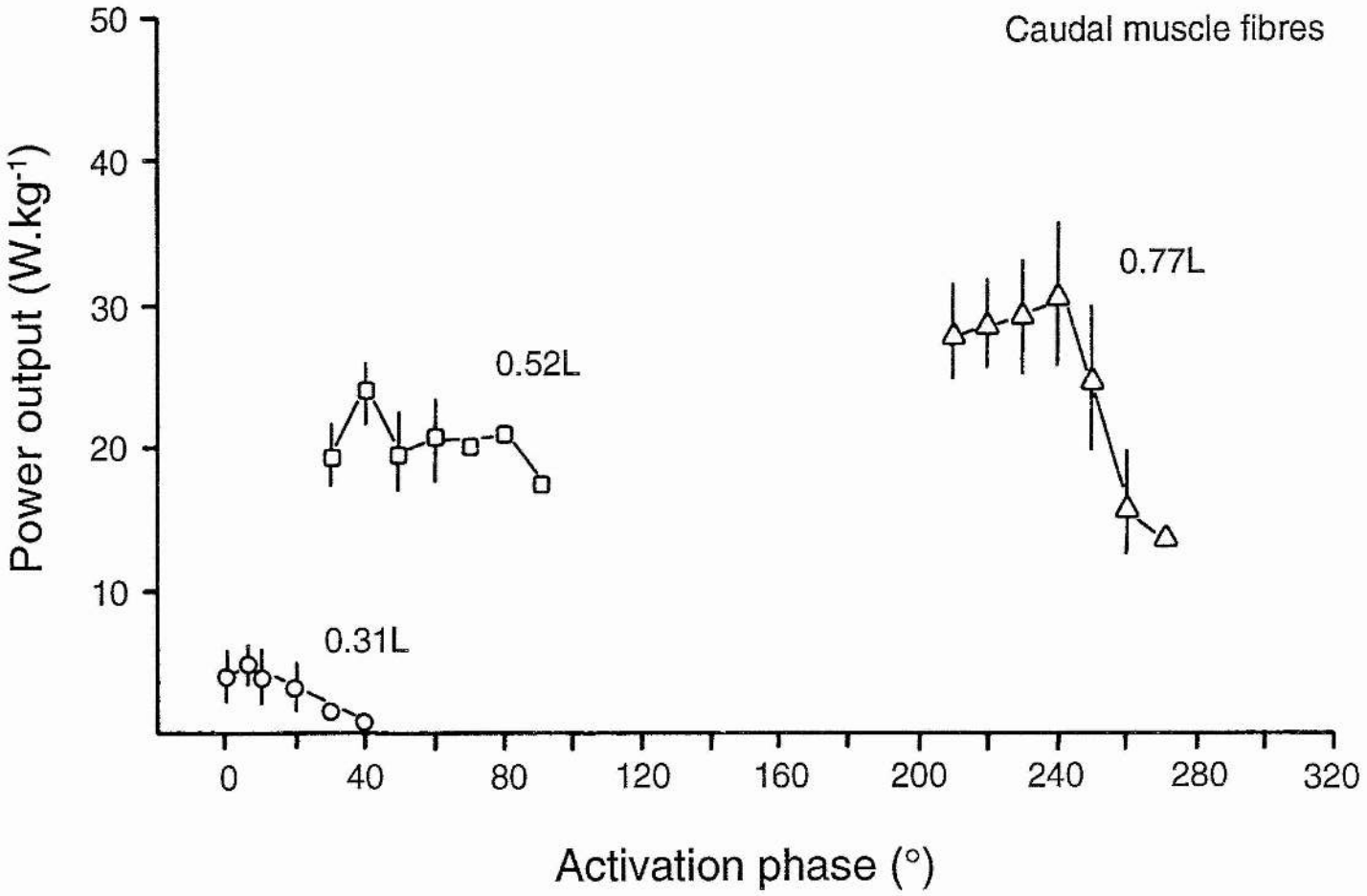

Rostral muscle fibres
0.31L
0.52L
0.77L
Power output (W.kg-1)
50
40
30
20
10
0
40
80
120
160
200
240
280
320
Activation phase (°)

Figure 4.7.

Figure 4.7. Power output (W kg^{-1}) of caudal fibres performing oscillatory work with *in vivo* strain patterns at all three positions along the body of the fish, against changing activation phase. $n = 6$ or 7. Values are means ± S.E.


Caudal muscle fibres
Power output (W.kg-1)
50
40
30
20
10
0.77L
0.52L
0.31L
0
40
80
120
160
200
240
280
320
Activation phase (°)

the left hand corner of the axis, thus producing less work per cycle. Rostral fibres simulating *in vivo* fluctuations at point 0.77L produce optimum work per cycle at higher activation phases of around 70° (d). At these high activation phases the work loop becomes much fuller than when stimulated at earlier phases in the length change cycle (c). Similarly, caudal fibres simulating 0.77L on the body also produce maximum work per cycle at 70° phase (f). However, *in vivo* strain fluctuations of caudal fibres at 0.31L produce work loops with a component of negative work, which would reduce the net positive work obtained per cycle (e).

Discussion

Sinusoidal *versus* calculated waveform

Johnson and Johnston (1991a) also estimated the power output of rostral muscle fibres from summer-caught sculpin at 15°C and found values of 30 W kg^{-1} using sinusoidal length change cycles. This is similar to values obtained when imposing both sinusoidal and *in vivo* strain fluctuations at 0.77L on the fibres in this study (Fig. 4.3).

Fibre properties along the body of the fish

The local environment of the fibre was found to affect muscular performance. The ability of rostral fibres to produce 86% greater power output than caudal fibres at position 1 ($P < 0.001$) suggests an adaptation in the contractile properties of the muscle from the anterior region of the fish. The mechanisms behind this adaptation are unknown, though could be due to differences in peak force generation

of rostral and caudal fibres at position 1 ($P < 0.05$; Figs. 4 & 5). Another contributing factor could be slightly faster half-activation and relaxation rates of the rostral, compared to the caudal fibres. Johnston *et al.*, (1993) found no difference in the contractile properties of rostral compared to caudal fibres of sculpin at 5°C. However, Johnston *et al.*, (1993) used rostral fibres isolated from the dorsal region of the sculpin, whereas in this study fibres were isolated from a ventral region of the abdominal wall. Also, differences in contractile rates could be accentuated by the shorter half-activation and relaxation times found at 15°C, compared to the longer times exhibited at 5°C. Fibres at 0.77L undergo a small pre-stretch prior to activation (Fig. 4.1), resulting in force enhancement by stretch activation (Altringham & Johnston, 1990a). Force-enhancement could therefore improve both caudal and rostral performance at position 3, relative to positions 1 and 2, a prediction first made by Leeuwan *et al.*, (1990) (Figs. 4.6 & 4.7).

Rostral muscle fibres perform equally well as the caudal fibres at positions 0.52L and 0.77L, and better at the 0.31L position down the body. Why then do the sculpin not have the rostral fibres throughout their locomotory musculature? It seems likely that caudal fibres are also specially adapted to the local environment of the tail, in such a way that differences are not exposed in this study. Properties of caudal fibres may confer advantages at slower swimming speeds or allow greater manoeuvrability during swimming.

During typical caudal fin propulsion, the tail is believed to exert maximum thrust on the water to power swimming (Lighthill, 1971). These results support the idea that the caudal and middle regions of fish locomotory musculature, contribute a significant proportion of the total

power output required for swimming. Similar results were found for the red muscle fibres of scup, with anterior fibres producing significantly greater power than posterior fibres, in the anterior position of the fish (Rome, Swank & Corda, 1993). A contributing factor to the greater power output was that anterior fibres had significantly faster activation and relaxation times compared to posterior fibres. Anterior fibres were therefore able to relax completely between strain cycles imitating the front of the scup, whereas, the posterior fibres were only partially relaxed. It should be noted that the strains imposed on the scup fibres were relatively small (Rome *et al.*, 1993), whereas those used in this study were directly comparable to a fish performing a fast-start. Using sinusoidal contractions Altringham *et al.*, (1993) found contrasting results in the saithe. E.M.G. studies showed that the caudal fibres were activated while the muscle was being stretched, thereby maximising muscle stiffness (Wardle & Videler, 1993). Altringham *et al.*, (1993) suggested that the caudal fibres are used primarily to transmit power generated by the rostral muscles to the tail blade.

This experimental technique used in this study enables the realistic calculation of the mechanical power generated by the fibres during swimming. This is important as it may be useful in testing models of fish swimming and muscle functional design.

CHAPTER 6

General discussion

The major adaptations found in this study were the ability of sculpin fast muscles to generate substantially greater force at 15°C following thermal acclimation to 15°C (Chapter 2). Fibres from cold acclimated fish produce 3.6-times less force than warm acclimated sculpin at 15°C, which severely limits the potential muscle power output at that temperature (Chapter 3). Although V_{max} increased significantly at 15°C following warm acclimation, only modest increases were found in the rates of force development and decline (Chapter 2). Studies on other fish species and different phylogenetic groups suggest that the effects of temperature on muscle contractile rates have become relatively constrained over an evolutionary time scale (see Guderley & Blier, 1988; Johnson & Johnston, 1991b; Rome, 1990). In contrast to this study, cold acclimation does have significant effects on rates of muscle force development and decline in some cyprinid species (Fleming *et al*, 1990). These changes appear to be the exception rather than the rule.

The properties of the isolated muscle fibres and whole animal burst swimming performance of sculpin were similarly affected by temperature and thermal acclimation. Johnstrude and Webb (1985) measured power output (42.4 W kg^{-1}) and force (11.8 kN m^{-2}) of rainbow trout fast-starts *in situ*. These values were comparable to those obtained from isolated muscles simulating locomotion (Altringham & Johnston, 1990a & b; Johnson & Johnston, 1991a; Chapter 5). Therefore, it seems probable that the properties of the musculature do set the upper limits for locomotion and that losses of power, via mechanical linkages and the skin, are minimal. Isolated fast fibre bundles would appear to provide a good model on which to study other aspects of burst speed swimming such as: energetics, scaling,

neuromuscular function and possibly the effects of some biological pollutants.

Comparative physiologists often attempt to correlate observed structural and/ or functional differences in biological processes, to changes in the environmental conditions (Pogson, 1988). Adaptative changes can occur in response to selective alterations in genes coding for processes that affect fitness. Also, adaptation can also refer to the adjustments that arise from the phenotypic plasticity of the genome (Wells, 1990). Some physiological processes may appear to be non-adaptive as they lack correlation with changing environmental conditions; however, this may be a reflection of the large plasticity in the functional capacity of the system studied (Wells, 1990). Adaptationists must therefore be careful in assigning whole animals the functional properties of their cells. As pointed out by Prosser (1986), the chemical component of a cell does not reveal its function and neither is an organism the sum of those cellular processes. Living organisms are composed of complex and integrated physiological processes. Therefore, adaptations in isolated physiological processes with changing environmental conditions should be not be assumed to occur at whole animal level.

The greater the complexity and integration of a biological system the more restricted its physiological range (Prosser, 1986). Decreased functional capacity with increasing organisation is well illustrated by the effects of temperature on Antarctic fish. The peripheral nerves of Antarctic fish fail to propagate action potentials at 31°C (Macdonald, 1981). In conjunction with skeletal muscle, the temperature at which neuromuscular function degenerates falls to 12 - 16°C (Macdonald &

Montgomery, 1982). However, whole animal locomotion and function will only occur to a maximum of 4°C (Somero & DeVries, 1967).

Limits to burst speed swimming

During a fast-start all the fast fibres are assumed to be activated (Rome, 1990; Wardle & Videler, 1980). The fast muscle relies on limited phosphocreatine or glycogen stores found within the cell to fuel contraction. Therefore fast muscle can only contract maximally while sufficient fuel stores are available, which partly accounts for the short duration of the burst speed response. Due to the short duration and high speed of the fast-starts the thermal sensitivity of the response is difficult to determine. Relative temperature independence was observed in the burst speed response of herring (*Clupea harengus*), flounder (*Pseudopleuronectes americanus*) and the sockeye salmon (Beamish, 1966; Brett, 1967). However, burst speed swimming capabilities were found to increase with temperature in rainbow trout, striped mullet (*Mugil cephalus*), spot (*Leiostomus xanthurus*) and pinfish (*Lagodon rhomboides*) (Rulifson, 1977; Webb, 1978b). Sculpin are the first species shown to exhibit an increase in burst speed swimming capability following temperature acclimation. The effects of temperature on the fast-starts of other fish species should therefore be considered in relation to their thermal history.

Wardle (1975) measured the twitch contraction times of blocks of fast muscle at different temperatures. Like this and many other studies, contraction times decreased with increasing temperature (see Rome, 1990; Bennett, 1990; Langfeld *et al*, 1989, Johnson & Johnston, 1991b; Chapter 2). The maximum twitch contraction times were used to predict the upper limit of maximum tail-beat frequencies of fish

(Wardle, 1975). At low temperatures, the duration of contraction increases and therefore so would the duration of the propulsive stroke (Webb, 1978a). This would cause a reduction in the maximum velocity and acceleration rate of burst speed swimming with decreasing temperature (Rulifson, 1977; Webb, 1978a). Slight decreases in tail-beat frequency, velocity and acceleration rates were observed in fast-starts of 5°C-acclimated sculpin with decreasing temperature (Chapter 4). However, due to the decreased power output of fibres from 5°C-acclimated sculpin at 10°C and 15°C the actual distance travelled was greatest at 5°C (Chapter 4).

Walters and Fiersteine (1964) reported yellowfin tuna (*Thunnus albacares*) and Wahoo (*Acanthocybium solanderi*) moving at twice the speed predicted by the twitch contraction times calculated by Wardle (1975). Similar observations have been made for other fish species (Wardle & Videler, 1980). Wardle & Videler (1980) suggested that the fish could increase the swimming speed by halving the wavelength of the contraction wave down the body. This would effectively double the distance moved per stride (Wardle & Videler, 1980). A difference was also found in the duration of *in vivo* twitch times (Johnstrude & Webb, 1985). It was suggested that in order for the fish to complete one tail-beat, *in vivo* contraction times were of a longer duration than that of a single isometric twitch (Johnstrude & Webb, 1985; Webb, 1980). Simply multiplying contraction times does not now appear adequate to accurately predict maximum swimming speeds of fish. This is due to possible differences in the *in vivo* action of the muscle fibres during swimming (eg. different contraction times, power output and internal temperature).

Energetics

Woledge (1989) suggested that during evolution, high power outputs may have been achieved at the expense of reduced contraction efficiency. Johnson (1990) examined the economy (positive work/ unit energy expended) and efficiency (work done: free energy of driving the reaction) of fast muscle from summer acclimatised sculpin. Summer and 15°C-acclimated sculpin have developed high power outputs at 15°C (Johnson, 1990; Chapter 3). Correspondingly the energetic efficiency was found to decrease between 5 and 15°C (Johnson, 1990). It appears that the increase in maximum swimming performance (Chapter 4) could be achieved at the expense of muscle efficiency. Efficiency appears to be retained to some extent by maintaining the V/ Vmax following temperature acclimation (see Chapter 3). Increased speeds of unloaded contraction velocity enables an increase in the V at which the muscle shortens *in vivo*. Therefore, muscle velocity increases but the V/ Vmax is kept relatively constant and maximum power output is produced at high efficiencies.

Adaptations in the nervous system ?

Preliminary experiments on sculpin revealed that reduced Ca^{2+} concentration in external media reduced the twitch and tetanic tension of live fast fibres, at 4°C (Johnson, 1990). No effect was observed on the time dependent contractile properties with changing Ca^{2+} concentration. Tension recovered when calcium was re-applied to the Ringer. Although external calcium is thought to have a modulating role it is not essential for contraction in most vertebrate muscle (see Dulhunty & Gage, 1988). The reason [Ca^{2+}] affects isometric tension is possibly associated with polyneuronal innervation of the fast and slow fibres of

sculpin. The addition of a neuromuscular blocker α-bungarotoxin (BGT) was tested on muscle fibres from both focally and polyneuronally innervated teleosts (Johnson, 1990). BGT made fast and slow muscle fibres from polyneuronally innervated species almost inexcitable. However, BGT had virtually no effect on the neuro-transmission of fibres from focally innervated species. Johnson (1990) concluded that end plate transmission and therefore calcium were essential for the activation of these live fibre preparations. It is therefore possible that the reduced fast-start capabilities of cold-acclimated sculpin at 10°C and 15°C, is due to failure of the motor neurones to recruit muscle fibres (Johnson, 1990; Rome 1990). Heat block of neuromuscular transmission has already been found in Antarctic fish species (Macdonald, 1981). The nervous system seems to have played a major role in the evolutionary adaptation of fish to low temperatures. Live fibre bundles of carp red muscle also failed to be activated by electrical stimulation alone (Rome & Sosnicki, 1990). Acetylcholine release from the nervous tissue in the preparation is required for maximum tension generation (Rome & Sosnicki, 1990). Activation was enhanced in the carp red fibres by the addition of caffeine (a twitch potentiator thought to increase Ca^{2+} release from the SR) and eserine (which increases depolarisation by inhibiting acetylcholinerase). The addition of caffeine and eserine to fast fibre preparations isolated from cold-acclimated sculpin failed to enhance force production at 10 and 15°C (Chapter 2). High-potassium however, did elicit contractures with 53% greater tension than tetanic P_o. The increased force following maximal activation indicates that crossbridge function is not impaired in fast fibres from the cold-adapted fish at 15°C. Fatigued muscle also shows a similar decline in tension combined with a marked slowing of the relaxation rate. The mechanisms involved

during fatigue are complex and unclear, with changes in both crossbridge function and excitation-activation coupling being indicated. Metabolite levels that change during prolonged activity are associated with declining tension, with both H^+ dependant and H^+ independent processes being involved (Cady, Jones, Lynn & Newham, 1989; Edwards, Hill, Jones, 1975; Sahlin, Edström, Sjöholm & Hultman, 1981). However, such changes in metabolite levels are generally associated with slowing of relaxation which is not observed in the fast muscles of the cold-adapted sculpin at 15°C. Fibres from cold-acclimated sculpin may have decreased T-tubule membrane excitability, possible due to changes in the intracellular metabolites, which is by-passed following the addition of potassium. Alternatively, potassium could cause additional Ca^{2+} release from stores associated with the T-tubule system. Though still unclear, it seems likely that the major cause of force decline is failure of some step in the excitation-coupling process.

Further studies

Primarily it would be necessary to determine the exact role of the nervous system in response to acute temperature changes and acclimation. Studies on skinned fibre preparations where all extraneous tissues are removed (i.e. nerves and membranes) leaving the contractile apparatus intact need to be carried out. Then the effects of temperature on the force generating mechanism of the muscle itself could be tested. Also, a more detailed pharmacological study on the live fibres may elucidate the exact cause of tension decline in the cold adapted sculpin, at 15°C. Having established an acclimatory response in the muscle fibres, possible changes in the myosin composition could be examined using gel and capillary electrophoresis.

By closely re-enacting the fibre length changes that occur during specific swimming activities accurate measurements of muscle power output can now be obtained. Extending this study, further combinations of video analysis and work loop experiments could be used to determine the power output of fast muscle fibres at different temperatures. The effects of temperature acclimation on the muscle power output of the fibres could then be assessed for different fibre positions along the body. Ultimately, a whole host of species with different swimming strategies could be filmed and the fibre action according to position along the body of the fish could be determined. This technique will enable ideas and theories on the functional design of fish locomotory musculature to be tested. The effects of temperature, salinity, drugs and pollutants on muscle power output could then be assessed.

References

Abbott, B.C. (1951). The heat production associated with the maintenance of a prolonged contraction and the extra heat produced during large shortening. *J. Physiol., Lond.* **112**, 438-445.

Alexander, R. McN. (1967). *Functional design in fishes.* London: Hutchinson University Library.

Alexander, R. McN. (1969). The orientation of muscle fibres in the myomeres of fishes. *J. Mar. Biol. Assoc. U.K.* **49**, 263-290.

Alexander, R. McN. (1983). The history of fish biomechanics. In: *Fish biomechanics*. pp 1-35 (eds P.W. Webb & D. Weihs). New York: Praeger Press.

Alexandrov, V. Ya. (1977). Cells, molecules and temperature. *Ecological studies* **21**. Berlin, Heidelberg, New york: Springer Verlag.

Altringham, J.D. and Johnston, I.A. (1981). Quantative histochemical studies of the peripheral innervation of cod (*Gadus morhua*) fast myotomal muscle fibres. *J. comp. Physiol.* **143**, 123-127.

Altringham, J.D. and Johnston, I.A. (1985). Changes in tension generation and ATPase activity in skinned muscle fibres of the carp following temperature acclimation. *Pflügers Arch.* **403**, 449-451.

Altringham, J.D. and Johnston, I.A. (1986). Evolutionary adaptation to temperature in fish muscle crossbridge mechanisms: tension and ATP turnover. *J. comp. Physiol* B. **156**, 819-821.

Altringham, J.D. and Johnston, I.A. (1988a). The mechanical properties of polyneuronally innervated myotomal muscle fibres isolated from a teleost fish (*Myoxocephalus scorpius*). *Pflügers Arch.*. **412**, 524- 529.

Altringham, J.D. and Johnston, I.A. (1988b). Activation of multiply innervated, fast and slow myotomal muscle fibres of the teleost *Myoxocephalus scorpius*. *J. exp. Biol.* **140,** 313-324.

Altringham, J.D. and Johnston, I.A. (1989). The innervation pattern of fast myotomal muscle in the teleost *Myoxocephalus*

scorpius: a reappraisal. *Fish Physiol. Biochem.* **6**, 309-314.

Altringham, J.D. and Johnston, I.A. (1990a). Modelling muscle power output in a swimming fish. *J. exp. Biol.* **148**, 395-402.

Altringham, J.D. and Johnston, I.A. (1990b). Scaling effects on muscle function: power output of isolated fish muscle fibres performing oscillatory work. *J. exp. Biol.* **151**, 453-467.

Altringham, J.D., Wardle, C.S. & Smith, C.I. (1993). Myotomal muscle function at different points in the body of a swimming fish. *J Exp. Biol.* **182**, 191-206.

Anderson M.E. & Johnston, I.A. (1992). Scaling of power output in fast muscle fibres of the Atlantic cod during cyclical contractions. *J. Exp. Biol.* **170**, 143-154.

Anderson, R.L., Minton, K.W., Li, G.C. and Hahn, G.M. (1981). Temperature induced homeoviscous adaptations of Chinese hamster ovary cells. *Biochem. Biophys. Acta.* **641**, 334-348.

Archer, S.D. and Johnston, I.A. (1989). Kinematics of labriform and subcarangiform swimming in the Antarctic fish *Notothenia neglecta*. *J. exp. Biol.* **143**, 195-210.

Aristotle. Fourth century B.C. b) *Movement of animals*. c) *Progression of animals* (English trans. E.S Forster. 1937. London: Heinemann).

Bainbridge, R. (1958). The speed of swimming of fish as related to size and to the frequency and amplitude of the tail beat. *J. Exp. Biol.* **35**, 109-133.

Bainbridge, R. (1961). Problems of fish locomotion. In: *Vertebrate locomotion, Symp. Zool. Soc. London.* **5**, 13-32.

Baldwin, J. and Hochachka, P.W. (1970). Functional significance of isozymes in thermal acclimatization: acetylcholinesterase from trout brain. *Biochem. J.* **116**, 883-887.

Barets, A. (1961). Contributions a l'etude des systemes moteur lent et rapide du muscle lateral des teleoststeens. *Arch. Anat. Morph. exp.* **50** (suppl), 91-187.

Baross, J. & Deming, J. (1984). Methanogenic bacteria from hot vents and high pressure. *Nature, Lond.* **303**, 423-426.

Bass, E.L. (1971). Temperature acclimation in the nervous system of the brown bullhead (*Ictalurus nebulosus*). *Comp. Biochem. Physiol.* A. **40**, 833-849.

Beamish, F.W.H. (1966). Swimming endurance of Northwest Atlantic fishes. *J. fish. Res. Bd. Can.* **23**, 341-7.

Bennett, A.F. (1985). Temperature and muscle. *J. exp. Biol.* **115**, 333-344.

Bennett, A.F. (1990). Thermal dependance of locomotor capacity. *Am. J. Physiol.* **259**, R253-R258.

Blake, R.W. (1983). *Fish locomotion.* Cambridge University Press. London.

Blaxter, J.H.S. (1969). Swimming speeds of fish. *FAO Fish. Report.* **62**, 69-100.

Block, B. & Carey, F. (1985). Warm eye and brain temperatures in sharks. *J. Comp. Physiol.* B. **156**, 229-236.

Bone, Q. (1964). Patterns of muscular innervation in the lower chordates. *Int. Rev. Neurobiol.* **6**, 99-147.

Bone, Q. (1966). On the function of the two types of myotomal muscle fibres in elasmobranch fish. *J. mar. biol. Ass. U.K.* **46**, 657-665.

Bone, Q. (1972). The dogfish neuromuscular junction: Dual innervation of vertebrate striated muscle fibres? *J. Cell. Sci.* **10**, 657-665.

Bone, Q. (1978). Locomotor muscle. In *Fish Physiology.* Vol. **7**, (eds W.S. Hoar and D.J.Randall) pp361-424. New York: Academic Press.

Bone, Q. and Ono, R.D. (1982). Systematic implications of innervation patterns in teleost myotomes. *Breviora.* **470**, 1-23.

Borelli, I.A. (1680). *De motu animalium pars I.* Rome: Bernabo.

Breder, C.M. (1926). The locomotion of fishes. *Zoologica* (N.Y.) **4**, 159- 297.

Bressler, B.H. (1981) Isometric contractile properties and instantanious stiffness of amphibian skeletal muscle in the temperature range of 0 to 20°C. *Can. J. Physiol. Pharmacol.* **59**: 548-554.

Brett, J.R. (1963). The energy required for swimming by young sockeye salmon with a comparison of the drag force on a dead fish. *Trans. R. Soc. Can.* **1**, 441-57.

Brett, J.R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd. Can.* **21**, 1183-1226.

Brett, J.R. (1967). Swimming performance of sockeye salmon in relation to fatigue time and temperature. *J. Fish. Res. Bd. Can.* **24**, 1731-41.

Brill, R.W. & Dizon, A.E. (1979). Red and white fiber activity in swimming skipjack (*Katsuwanus pelamis*). *J. Fish Biol.* **15**, 679- 685.

Cady, E.B, Jones, D.A., Lynn, J. & Newham, D.J. (1989). Changes in force and intracellular metabolites during fatigue of human skeletal muscle. *J. Physiol.* **418**, 311-325.

Cayley, G. (1810). On aerial navigation, part III. *Nicholson's J. Nat. Phil. Chem. NS.* **25**, 161-169.

Connell, J.J. (1958). Studies on the proteins of fish skeletal muscle 5. Molecular weight and shape of cod fibrillar proteins. *Biochem. J.* **70**, 81-91

Connell, J.J. (1961). The relative stabilities of skeletal muscle myosins of some animals. *Biochem. J.* **80**, 503-538.

Constantin, L.L. (1971). Biphasic potassium contractures in frog muscle fibres. *J. Gen. Physiol.* **58**, 117-130.

Cossins, A.R. (1983). The adaptations of membrane structure and function to changes in temperature. In *Cellular Acclimatization to Environmental Change* (ed. A.R. Cossins and P. Sheterline), pp3-32. Cambridge: Cambridge University Press.

Cossins, A.R. and Bowler, K. (1987). *Temperature biology of animals.* London, New York: Chapman and Hall.

Crockford, T.C. and Johnston, I.A. (1990). Temperature acclimation and the expression of contractile protein isoforms in the skeletal muscles of common carp (*Cyprinus carpio* L.). *J. comp. Physiol.* B. **160**, 23- 30.

Curtin, N.C. and Woledge, R.C. (1988). Energetic cost of power output by isolated fibre bundles from dogfish white muscle. *J. Physiol., Lond.* **407**, 74P.

Davies, M. & Johnston, I.A. (1992). Muscle fibres in rostral and caudal myotomes of the Atlantic cod have different contractile properties. *J. Physiol.* **459**, 8p.

Davison, W., Goldspink, G. & Johnston, I.A. (1976). Division of labour between fish myotomal muscles during swimming. *J. Physiol. Lond.* **263**, 185-186.

Dean, B. (1895). "Fishes, Living and Fossil". Columbia Univ. Press, New York.

DeVries, A.L. and Lin, Y. (1977). The role of glycopeptide antifreezes in the survival of Antarctic fishes. In: *Adaptation within Antarctic Ecosystems* (eds. G.A. Llano), pp. 439-458. Washington, DC. Smithsonian Institution.

Donovan, E. (1808). *The natural History of British Fishes.* Vol. **1**. London. Republished 1978. New York. Arno Press.

Dorn, P., Johnson, L. & Darby, C. (1979). The swimming performance of nine species of common california inshore fishes. *Trans. Am. Fish. Soc.* **108**, 366-372.

Dulhunty, A.F. and Gage, P.W. (1988). Effects of extracellular calcium concentration and dihydropyridines on contraction in mammalian skeletal muscle. *J. Physiol., Lond.* **399**, 63-80.

Eaton, R.C., Bombardieri, R.A. & Meyer, D.L. (1977). The Mauthner-initiated startle response in teleost fish. *J. Exp. Biol.* **66**, 65-85.

Edman, K.A.P. (1979). The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *J. Physiol. Lond.* **291**, 143-159.

Edman, K.A.P. and Flitney, F.W. (1982). Laser diffraction studies of sarcomere dynamics during 'isometric' relaxation in isolated muscle fibres of the frog. *J. Physiol. Lond.* **329**: 1-20.

Edwards, R.H., Hill, D.K. & Jones, D.A. (1975). Metabolic changes associated with the slowing of relaxation in fatigued mouse muscle. *J. Physiol.* **251**, 287-301.

Eggington, S.D. and Siddell, B.D. (1989). Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am. J. Physiol. Lond.* **373**, 78p.

Ehrenbaum, E. (1932). *Myoxocephalus scorpius*. In L. Joubin (ed.) *Faune ichtyol. Atlant. N.*,55-57. Copenhagen.

Else, P.L. and Bennett, A.F. (1987). The thermal dependence of locomotor performance and muscle contractile function in the salamander *Ambystoma tigrinum nebulosum. J. exp. Biol.* **128**, 219- 233.

Farmer, G.J. & Beamish, F.W.H. (1969). Oxygen consumption of *Tilapia nilotica* in relation to swimming speed and salinity. *J. Fish Res. Bd. Can.* **26**, 2807-2821.

Fleming, J.R., Crockford, T., Altringham, J.D. and Johnston, I.A. (1990). The effects of temperature acclimation on muscle relaxation in the carp, a mechanical, biochemical and ultrastructural study. *J. exp. Zool.* **255**, 286-295.

Fletcher, G.L., Addison, R.F., Slaughter, D. and Hew, C.L. (1982). Antifreeze proteins in the Arctic shorthorn sculpin (*Myoxocephalus scorpius*). *Arctic*, **35 (2)**, 302-306.

Flitney, F.W. & Johnston, I.A. (1979). Mechanical properties of isolated fish red and white muscle fibres. *J. Physiol.* **295**, 49-50.

Focant, B., Huriaux, F. & Johnston, I.A. (1976). Subunit composition of fish myofibrils: the light chains of myosin. *Int. J. Biochem.* **7**: 129- 133.

Focant, B., Jacob, M.F. & Huriaux, F. (1981). Electrophoretic comparison of the proteins of some perch (*Perca fluviatilis* L.) head muscles. *J. Mus. Res. Cell. Motil.* **2**, 295-305.

Freadman, M.A. (1979). Role partitioning of swimming musculature of striped bass, *Morone saxatalis* and bluefish *Pomatus saltatrix. J. Fish Biol.* **15**, 417-424.

Frith, H.R. & Blake, R.W. (1991). Mechanics of the startle response in the northern pike, *Esox lucius. Can. J. Zool.* **69**, 2831-2839.

Fry, F.E.J. and Hart, J.S. (1948). Cruising speed of goldfish in relation to water temperature. *J. Fish. Res. Bd. Can.* **7**, 169-175.

Fryer, M.W & Neering, I.R. (1989). Actions of caffeine on fast- and slow-twitch muscles of the rat. *J. Physiol.* **416**, 435-454.

Gerlach, G., Turay, L., Mailik, K., Lida, J., Scutt, A. and Goldspink, G. (1990). The mechanisms of seasonal temperature acclimation in carp: a combined physiological and molecular biology approach. *Am. J. Physiol.* **259**, R237-R244.

Gero, D.R. (1952). The hydrodynamic aspects of fish propulsion. *Am. Mus. Notiv.* **1601**, 1-32.

Goldman, Y.E. McCray, J.A. and Ranatunga, K.W. (1987). Transient tension changes initiated by laser temperature jumps in rabbit psoas muscle fibres. *J. Physiol. Lond.* **392**: 71-95.

Graham, J.B. (1983). Heat transfer. In: *Fish Biomechanics* (eds. Webb, P.W. & Weihs, D.). Praeger Scientific.

Granzier, H.L.M., Wiersma, J., Akster, H.A. and Osse, J.W.M. (1983). Contractile properties of a white- and a red fibre type of the *M. hyohyoideus* of the carp (*Cyprinus carpio* L.). *J. comp. Physiol.* **149**, 441-449.

Gray, J. (1933a). Studies in animal locomotion. I. The movement of fish with special reference to the eel. *J. Exp. Biol.* **10**, 88-104.

Gray, J. (1933b). Studies in animal locomotion. II. The relationship between waves of musculature contraction and the propulsive mechanism of the eel. *J. Exp. Biol.* **10**, 386-390.

Gray, J. (1933c). Studies in animal locomotion. III. The propulsive mechanism of the whiting (*Gadus merlangus*). *J. Exp. Biol.* **10**, 391- 400.

Gray, J. (1936). Studies in animal locomotion. VI. The propulsive powers of the dolphin. *J. Exp. Biol.* **13**, 192-199.

Gray, J. (1968). "Animal Locomotion". Weidenfeld & Nicolson, London.

Greaser, M.L., Moss, R.L. & Reiser, P.J. (1988). Variations in contractile properties of rabbit single muscle fibres in relation to troponin T isoforms and myosin light chain. *J. Physiol.* **406**, 85-98.

Greer-Walker, M. and Pull, G.A. (1975). A survey of red and white muscle in marine fish. *J. Fish. Biol.* **7**, 295-300.

Grillner, S. & Kashin, S. (1976). On the generation and performance of swimming. In: *Neural control of locomotion,* (ed. Herman, R.M., Grillner, S. Stein, P.S.G. & Stuart, D.G.) pp. 181-201. New York. Plenum Press.

Guderley, H. and Blier, P. (1988) Thermal acclimation in fish: conservative and labile properties of swimming muscle. *Can. J. Zool.* **66**, 1105-1115.

Guderley, H. and Foley, L. (1990). Anatomic and metabolic responses to thermal acclimation in the ninespine stickleback, *Pungitius pungitius. Fish. Physiol. Biochem.* **8**, 465-473.

Günther, A. (1888). Report on the fishes obtained by Mr J. Murray in deep waters on the north-west coast of Scotland, between April 1887. *Proc. R. Soc. Edinb.* **15**, 205-220.

Hagiwara, S. & Takahashi, K. (1967). Resting and spike potentials of skeletal muscle fibres in salt water elasmobranch and teleost fish. *J. Physiol. Lond.* **190**, 499-518.

Harper, A.A., Shelton, J.R. and Watt, P.W. (1989). The temperature dependence of the time course of growth and decay of miniature end-plate currents in carp extraocular muscle following thermal acclimation. *J. Exp. Biol.* **147**, 237-248.

Harper, D.G. & Blake, R.W. (1990). Fast-start performance of rainbow trout *Salmo gairdneri* and northern pike *Esox lucius. J. exp. Biol.* **150**, 321-342.

Harper, D.G. & Blake, R.W. (1991). Prey capture and fast-start performance of northern pike *Esox lucius. J. Exp. Biol.* **155**, 175-192.

Haschemeyer, A.E.V. (1969). Rates of polypeptide chain assembly in liver *in vivo*: relation to the mechanism of temperature acclimation in *Opsanus tau. Proc. natn. Acad. Sci.* USA. **62**, 128-135.

Hazel, J.R. (1972). The effect of acclimation temperature upon succinic dehydrogenase activity from the epaxial muscle of the common carp (*Carassius auratus* L.) II. Lipid reactivation of the soluble enzyme. *Comp. Biochem. Physiol.* B. **43**, 863-882.

Hazel, J.R. and Prosser, C.L (1974). Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.* **54**, 620- 677.

Heap, S.P., Watt, P.W. and Goldspink, G. (1986). Myofibrillar ATPase activity in the carp *Cyprinus carpio*: Interactions between starvation and environmental temperature. *J. Exp. Biol.* **123**, 373-382.

Hess, A. (1970). Vertebrate slow muscle fibres. *Physiol. Rev.* **50**, 40-62.

Hess, F. and Videler, J.J. (1984). Fast continuous swimming of saithe (*Pollachius virens*): a dynamic analysis of bending moments and muscle power. *J. exp. Biol.* **109,** 229-251.

Hertel, H. (1966). *Structure, form and movement.* Reinhold Publishing Corp. New York. 251p.

Hew, C.L., Slaughter, D., Fletcher, G.L. and Joshi, S.B. (1981). Antifreeze glycoproteins in the plasma of Newfoundland Atlantic cod (*Gadus morhua*). *Can. J. Zool.* **59**. 2186-2192.

Hill, A.V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B.* **126**, 136-195.

Hill, A.V. (1950). The dimensions of animals and their muscular dynamics. *Sci. Prog.* **38**, 209-30.

Hill, A.V. (1951). The influence of temperature on the tension developed in an isometric twitch. *Proc. R. Soc. B.* **138**, 349-354.

Hill, A.V. (1964). The efficiency of mechanical power development during muscular shortening and its relation to load. *Proc. R. Soc. B.* **159**, 319-324.

Hillaby, B.A. & Randall, D.J. (1979). Acute ammonia toxicity and ammonia excretion in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Can.* **36**, 621-629.

Hochachka, P.W. (1965). Isozymes in metabolic adaptation of a poikilotherm: subunit relationships in lactate dehydrogenases of isolated hepatocytes from the European eel *Anguilla anguilla* L. *J. Therm. Biol.* **6**, 201-208.

Hou, T.-T. and Rall, J.A. (1987). Rate of relaxation in single skeletal muscle fibres of the frog as a function of tetanus duration and temperature (Abstract). *Biophys. J.* **51**: 475a.

Houssay, S.F. (1912). *Forme, prissance et stabilite des poissons*. Paris: Hermann.

Hudson, R.C.L. (1969). Polyneuronal innnervation of the fast muscles of the marine teleost *Cottus scorpius* L. *J. exp. Biol.* **59**, 47-67.

Hughes, G.M. & Shelton, G. (1962). Respiratory mechanisms and their nervous control in fish. *Adv. Comp. Physiol. Biochem.* **1**, 275-364.

Hunter, C.F. and Rodwell, V.W. (1980). Regulation of vertebrate liver HMG-CoA reductase via reversible modulation of its catalytic activity. *J. Lipid. Res.* **21**, 399-405.

Hunter, J.R. & Zweifel, J.R. (1971). Swimming speed, tail beat frequency, tail beat amplitude and size in jack mackerel *Trachurus symmetricus*, and other fishes. *Fishery Bulletin.* **69**, nº. 2.

Huriaux, F. & Focant, B. (1977). Isolation and characterisation of the three light chains from carp white muscle myosin. *Archs. Int. Physiol. Biochem.* **85**: 917-929.

Huxley, A.F. (1957). Muscle structure and the theories of contraction. *Prog. Biophys. Biophys. Chem.* **7**: 255-318.

Hwang, G.C., Watabe, S. and Hashimoto, K. (1990). Changes in carp myosin ATPase induced by temperature acclimation. *J. comp. Physiol.* **160**, 233-239.

Hwang, G.C., Ochiai, Y. Watabe, S. and Hashimoto, K. (1991). Changes in carp myosin subfragment-1 induced by temperature acclimation. *J. comp. Physiol.* **161**, 141-146.

Inmann, V.T., Saunders, J.B. de C.M., & Abbott, L.C. (1944). Observations on the function of the shoulder joint. *J. Bone Joint Surg.* **26**, 1-30.

Jayne, B.C., Bennett, A.F. & Lauder, G.V. (1990). Muscle recruitment during terrestrial locomotion: How speed and temperature affect fibre type use in a lizard. *J. Exp. Biol.* **152**, 101-128.

Jenkins, J.T. (1936). *The fishes of the British Isles, both freshwater and salt*. 2nd ed. London. Warne & Co

John-Alder, H.B. and Bennett, A.F. (1987). Thermal adaptations in lizard muscle function. *J. comp. Physiol.* B. **157**, 241-252.

Johnson, T.P. (1990). The influence of temperature on the mechanics and energetics of contraction in fish muscle. Ph.D. Thesis. University of St. Andrews, Scotland.

Johnson, T.P. and Johnston, I.A. (1991a). Power output of fish muscle fibres performing oscillatory work: Effects of acute and seasonal temperature change. *J. exp. Biol.* **157**, 409-423.

Johnson, T.P. and Johnston, I.A. (1991b). Temperature adaptation and the contractile properties of live muscle fibres from teleost fish. *J. Comp. Physiol.* B. **161**, 27-36.

Johnston, I.A. (1979). Calcium regulatory proteins and temperature acclimation of actomyosin ATPase from a eurythermal teleost (*Carassius auratus* L.) *J. Comp. Physiol.* **129**, 163-167.

Johnston, I.A. (1981). Structure and function of fish muscles. *Symp. zool. Soc., Lond.* **48**, 71-113.

Johnston, I.A. (1982). Capillarisation, oxygen diffusion distances and mitochondrial content of carp muscle following acclimation to summer and winter temperatures. *Cell. Tiss. Res.* **222**, 325-337.

Johnston, I.A. (1985). The effects of temperature on force velocity relationship of skinned fibres isolated from icefish (*Chaenocephalus aceratus*) skeletal muscle. *J. Physiol., Lond.* **361**, 40P.

Johnston, I.A. (1987). Respiratory characteristics of muscle fibres in a fish (*Chaenocephalus aceratus*) that lacks haem pigments. *J. exp. Biol.* **133**, 415-428.

Johnston, I.A. and Altringham, J.D. (1985). Evolutionary adaptation of muscle power output to environmental temperature: force velocity characteristics of skinned fibres isolated from Antarctic, temperate and tropical marine fish. *Pflügers. Arch.* **405**, 136-140.

Johnston, I.A. and Brill, R. (1984). Thermal dependance of contractile properties of single skinned muscle fibres isolated from

Antarctic and various Pacific marine fishes. *J. comp. Physiol.* B. **155**, 63-70.

Johnston, I.A., Clarke, A. and Ward, P. (1991). Temperature and metabolic rate in antarctic fish: constraints on adaptation. *Marine Biol.* **109**, 191-195.

Johnston, I.A., Davison, W. and Goldspink, G. (1975). Adaptations in Mg^{2+} -activated myofibrillar ATPase activity induced by temperature acclimation. *FEBS Lett.* **50**, 293-295.

Johnston, I.A., Davison, W. and Goldspink, G. (1977). Energy metabolism of carp swimming muscles. *J. comp. Physiol.* **114**, 203-216.

Johnston, I.A. and Dunn, J.F. (1987). Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. In *Temperature and Animal Cells* (ed. K. Bowler and B.J. Fuller), pp. 67-93. Cambridge: *Symposia of the Society for Experimental Biology number XXXXI.*

Johnston, I.A., Fleming,, J.D. and Crockford, T.C. (1990). Thermal acclimation and muscle contractile properties in cyprinid fish. *Am. J. Physiol.* **259,** R231-R236.

Johnston, I.A., Franklin, C.E. & Johnson, T.P. (1993). Recruitment patterns and contractile properties of fast muscle fibres isolated from rostral and caudal myotomes of the short-horned sculpin. *J. Exp. Biol.* (In press).

Johnston, I.A. and Maitland, B. (1980). Temperature acclimation in crucian carp (*Carassius carassius* L.); morphometric analysis of muscle fibre ultrastructure. *J. Fish. Biol.* **17**, 113-125.

Johnston, I.A. and Moon, T.W. (1980). Exercise training in the skeletal muscle of brook trout (*Salvelinus fontinalis*). *J. exp. Biol.* **87**, 177- 194.

Johnston, I.A., Patterson, S., Ward, P. and Goldspink, G. (1974). The histochemical demonstration of myofibrillar adenosine triphosphatase activity in fish muscle. *Can. J. Zool.* **52**, 871-877.

Johnston, I.A. and Sidell, B.D. (1984). Differences in the temperature dependance of muscle contractile properties and myofibrillar ATPase activity in a cold-temperate fish. *J. exp. Biol.* **111,** 179-189.

Johnston, I.A., Sidell, B.D. and Driedzic, W.R. (1985). Force-velocity characteristics and metabolism of carp muscle fibres following temperature acclimation. *J. exp. Biol.* **119**, 239-249.

Johnston, I.A. and Walesby, N.J. (1977). Molecular mechanisms of temperature adaptation in fish myofibrillar adenosine triphosphatases. *J. comp. Physiol.* **119**, 195-206.

Johnston, I.A. and Wokoma, A. (1986). Effects of temperature and thermal acclimation on contractile properties and metabolism of skeletal muscle in the flounder (*Platichthys flesus* L.). *J. exp. Biol.* **120**, 119-130.

Johnstrude, C.L. and Webb, P.W. (1985) Mechanical properties of the myotomal musculo-skeletal system of rainbow trout, *Salmo gaidneri. J. exp. Biol.* **119**, 71-83.

Josephson, R.K. (1981). Temperature and the mechanical performance of insect muscle. In: *Insect Thermoregulation,* (ed. B. Heinrich), pp. 19-44. New York: John Wiley & Sons.

Josephson, R.K. (1985). Mechanical power output from striated muscle during cyclical contraction. *J. exp. Biol.* **114**, 493-512.

Julian, F.J., Rome, L.C., Stephenson, D.G. & Striz, S. (1986). The maximum speed of shortening in live and skinned frog muscle fibres. *J. Physiol.* **370**, 181-199.

King, P.A., Fives,J.M. and Dunne, J. (1983). Littoral and benthic investigations on the west coast of Ireland--XVIII. The biology of the short-spined sea scorpion *Myoxocephalus scorpius* (L.) in the Galway Bay area. *Proc. R. Ir. Acad.* **83B**, 325-334.

Kleckner N. W. & Sidell, B. (1985). Comparison of maximal activities of enzymes from tissues of thermally acclimated and naturally acclimatised chain pickerel (*Esox Niger*). *Physiol. Zool.* **58**(1): 18- 28.

Kolok, A.S. (1991). Photoperiod alters the critical swimming speed of juvenile Largemouth Bass, *Micropterus salmoides*, acclimated to cold water. *Copeia*, **4**, 1085-1090.

Konishi, J. & Hickman, Jr., C.P. (1964). Temperature acclimation in the central nervous system of rainbow trout *Salmo gairdneri. Comp. Biochem. Physiol.* **13**, 433-442.

Kuhn, H.J., Guth, K., Drexler, B., Berberich, W., and Ruegg, J.C. (1979). Investigation of the temperature dependenceof the crossbridge parameters for attachment as deduced from mechano-chemical studies in glycerinated single fibres from the dorsal longitudinal muscle of *Lethocerus maximus*. *Biophys. Struct. Mech.* **6**: 1-29.

Langfeld, K.S., Altringham, J.D. and Johnston, I.A. (1989). Temperature and the force-velocity relationship of live muscle fibres from the teleost *Myoxocephalus scorpius*. *J. exp. Biol.* **144**, 437-448.

Langfeld, K.S., Crockford, T.C. and Johnston, I.A. (1991). Temperature acclimation in the common carp: force-velocity characteristics and myosin subunit composition of slow muscle fibres. *J. exp. Biol.* **155**, 291-304.

Lannergren, J. (1978). The force-velocity relation of isolated twitch and slow muscle fibres of *Xenopus laevis*. *J. Physiol. London*. **283**, 501-21.

Lannergren, J. (1987). Contractile properties and myosin isoenzymes of various kinds of *Xenopus* twitch muscle fibres. *J. Mus. Res. Cell. Mot.* **8**, 260-273.

Lannergren, J., Lindblom, P. & Johansson, B. (1982). Contractile properties of of two varieties of twitch muscle fibres in *Xenopus laevis*. *Acta Physiol. Scand.* **114**, 523-535.

Lannergren, J. & Westerblad, H. (1989). Maximum tension and force-velocity properties of fatigued, single *Xenopus* muscle fibres studied by caffeine and high K^+. *J. Physiol.* **409**, 473-490.

Leeuwen, J.L., Lankheet, M.J.M., Akster, H.J. and Osse, J.W.M. (1990). Function of red axial muscles of carp (*Cyprinus carpio* L.): recruitment and normalised power output during swimming in different modes. *J. Zool. Lond.* **220**, 123-145.

Lighthill, M.J. (1960). Note of the swimming of slender fish. *J. Fluid Mech.* **9**, 305-17.

Lighthill, M.J. (1971). Large amplitude elongated- body theory of fish locomotion. *Proc. R. Soc.* **B179**, 125-138.

Lin, H. & Randall, D.J. (1990). The effect of varying water pH on the acidification of expired water in rainbow trout. *J. Exp. Biol.* **149,** 149-160.

Love, R. (1980). *Chemical biology of fishes.* London: Academic, Vol.II.

Lowey, S., Guillermina, S. & Trybus, M. (1993). Skeletal muscle myosin light chains are essential for physiological speeds of shortening. *Nature Lond.***365**, 454-456.

Macdonald, J.A. (1981). Temperature compensation in the peripheral nervous system: antarctic vs temperate species. *J. Comp. Physiol.* **142**, 411-18.

Macdonald, J.A. and Montgomery, J.C. (1982). Thermal limits of neuromuscular function in an Antarctic fish. *J. Comp. Physiol. A. Sens. Neural Behav. Physiol.*. **147**, 237-250.

Machin, K.E. and Pringle, J.W.S. (1959). The physiology of insect myofibrillar muscle. II. Mechanical properties of beetle flight muscle. *Proc. R. Soc.* B. **151**, 204-225.

Marey, E.J. (1895). "Movement". Heinemann, London.

Marsh, R.L. and Bennett, A.F. (1985). Thermal dependance of isotonic contractile properties of skeletal muscle and sprint performance of the lizard *Dipsosaurus dorsalis. J. comp. Physiol.* B. **155**, 541-551.

Marsh, R.L. and Bennett, A.F. (1986). Thermal dependance of sprint performance of the lizard *Sceloporus occidentalis. J. exp. Biol.* **126**, 79-87.

Matheson, D.F. and Roots, B.I. (1988a). Effect of acclimation and fixation temperatures on the number of lamellae and periodicity of myelin in fibres of the optic nerve of goldfish. *Exp. Brain. Res.* **72**, 63-70.

Matheson, D.F. and Roots, B.I. (1988b). Effect of acclimation temperature on the axon and fiber diameter spectra and thickness of myelin of fibers of the optic nerve of goldfish. *Exp. Neurol.* **101**, 29-40.

McArdle, H.J. and Johnston, I.A. (1980). Evolutionary temperature adaptation in fish muscle sarcoplasmic reticulum. *J. comp. Physiol.* B. **135**, 157-164.

Moerland, T.S. and Sidell, B.D. (1986a). Biochemical responses to temperature in the contractile protein complex of striped bass, *Morone saxatilis*. *J. exp. Zool.* **238**, 287-295.

Moerland, T.S. and Sidell, B.D. (1986b). Contractile responses to temperature in the locomotory musculature of striped bass, *Morone saxatilis*. *J. exp. Zool.* **240**, 25-33.

Montgomery, J.C. and Macdonald, J.A. (1984). Performance of motor systems in Antarctic fishes. *J. comp. Physiol.* A. **154**, 241-248.

Montgomery, J.C., McVean, A. and McCarthy, D. (1983). Effects of lowered temperature on spontaneous eye movements in a teleost fish. *J. comp. Physiol.* **160**, 289-293.

Moon, T.W. and Johnston, I.A. (1980). Starvation and the activities of glycolytic and gluconeogenic enzymes in skeletal muscles and liver of the plaice *Pleuronectes platessa*. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **136**, 31-38.

Nelson, J.A. (1989). Critical swimming speeds of yellow perch *Perca flavescens*: comparison of populations from a naturally acidic lake and neutral water. *J. Exp. Biol.* **145**, 239-254.

Nursall, J.R. (1956). The lateral musculature and the swimming of fish. *Proc. Zool. Soc. London.* **126**, 127-143.

Ono, R.D. (1983). Dual motor innervation in the axial musculature of fishes. *J. Fish. Biol.* **22**, 365-408.

Osse, J.W.M. (1969). Functional morphology of the head of the perch (*Perca fluviatilis* L.): An electromyographical study. *Neth. J. Zool.* **19**, 289-392.

Penny, R.K. and Goldspink, G. (1980). Temperature adaptation of sarcoplasmic reticulum of fish muscle. *J. Thermal. Biol.* **5**, 63-68.

Pogson, G.H. (1988). Constraints on the genetic process of biochemical adaptation. *Can. J. Zool.* **66**, 1139-1145.

Precht, H. (1958). Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In *Physiological Adaptation* (ed. C.L. Prosser), pp50-78. Washington DC: Amer. Assn. Adv. Science.

Prosser, C.L. (1973). *Comparative Animal Physiology*. Philadelphia: Saunders.

Prosser, C.L. (1986). *Adaptational biology: Molecules to organisms.* Wiley-Interscience Pub. N.Y.

Putnam, R.W. and Bennett, A.F. (1981). Thermal dependance of behavioural performance of anuran amphibians. *Anim. Behav.* **29**, 502-509.

Putnam, R.W. and Bennett, A.F. (1982). Thermal dependance of isometric contractile properties of lizard muscle. *J. comp. Physiol.* **147**, 11- 20.

Rand, D.M. & Lauder, G.V. (1981). Prey capture in the chain pickerel *Esox niger*: Correlations between feeding and locomotory behaviour. *Can. J. Zool.* **59**, 1072-1078.

Randall, D. & Brauner, C. (1991). Effects of environmental factors on exercise in fish. *J. Exp. Biol.* **160**, 113-126.

Reeves, R.B. (1977). The interaction of body temperature and acid-base balance in ectothermic vertebrates. *Ann. Rev. Physiol.* **39**, 559-586.

Reiser, J., Moss, L., Guilian, G. and Greaser, M.L. (1985). Shortening velocity in single fibres from adult rabbit Soleus muscles is correlated with myosin heavy chain composition. *J. Biochem. Chem.* **260**, 9077-9080.

Renaud, J.M. and Stevens, E.D. (1981a). Effect of acclimation temperature and pH on contraction of frog sartorius muscle. *Am. J. Physiol.* **240** (*Reg. Integ. comp. Physiol.* **9**), R301-R309.

Renaud, J.M. and Stevens, E.D. (1981b). The interactive effects of temperature and pH on the isometric contraction of toad sartorius muscle. *J. comp. Physiol.* **145**, 67-71.

Renaud, J.M. and Stevens, E.D. (1984). The extent of short-term and long-term compensation to temperature shown by frog sartorius muscle. *J. Exp. Biol.* **108**, 57-75.

Rome, L.C. (1982). The energetic cost of running with different muscle temperatures in the Savannah Monitor Lizards. *J. Exp. Biol.* **97**, 411-426.

Rome, L.C. (1983). The effect of long-term exposure to different temperatures on the mechanical performance of frog muscle. *Physiol. Zool.* **56**, 33-40.

Rome, L.C. (1986). The influence of temperature on muscle and locomotory performance. In: *Living in the cold: Physiological and biochemical adaptations* (ed. Heller, H.C., Musacchia, H.J & Wang, L.C.H.) New York: Elsevier. pp 485-495.

Rome, L.C. (1990). Influence of temperature on muscle recruitment and muscle function *in vivo*. *Am. J. Physiol.* **259**, R210-R222.

Rome, L.C., Choi, I., Lutz, G. & Sosnicki, A. (1992). The influence of temperature on muscle function in the fast swimming scup. (I). Shortening velocity and muscle recruitment during swimming. (II) The mechanics of red muscle. *J. Exp. Biol.* **163**, 259- 295.

Rome, L.C., Funke, R.P., Alexander, R.McN (1990). The influence of temperature on muscle velocity and sustained performance in swimming carp. *J. Exp. Biol.* **154**, 163- 178.

Rome, L.C., Funke, R.P., Alexander, R.McN., Lutz, G., Aldridge, H., Scott, F. and Freadman, M. (1988). Why animals have different muscle fibre types. *Nature., Lond.* **335**, 824-827.

Rome, L.C., Loughna, P.T. and Goldspink, G. (1984). Muscle fibre activity in the carp as a function of swimming speed and temperature. *Am. J. Physiol.* **247**, R272-R279.

Rome, L.C., Loughna, P.T. and Goldspink, G. (1985). Temperature acclimation: improved sustained swimming performance in carp at low temperature. *Science*. **228**, 194-196.

Rome, L.C. & Sosnicki, A.A. (1990). The influence of temperature on mechanics of red muscle in carp. *J. Physiol., Lond.* **427**, 151-169.

Rome, L.C. & Sosnicki, A.A. (1991). Myofilament overlap in swimming carp. II. Sarcomere length changes during swimming. *Am. J. Physiol.* **260**, C289-C296.

Rome, L.C., Swank, D. & Corda, D. (1993). How fish power swimming. *Science*. **261**. 340-343.

Rowlerson, A., Scapolo, P.A., Mascarello, F., Carpene, E. & Vegetti, A. (1985). Comparative study of myosins present in the lateral muscle of some fish: species variation in myosin isoforms and distribution in red, pink and white muscle. *J. Mus. Res. Cell. Motil.* **6**, 601-640.

Rulifson, R.A. (1977). Temperature and water velocity effects on the swimming performance of young of the year striped mullet, spot and pinfish. *J. Fish. Res. Bd. Can.* **34**, 2316-2322.

Sahlin, K., Edström, L., Sjöholm, H. & Hultman, E. (1981). Effects of lactic acid accumulation and ATP decrease on muscle tension and relaxation. *Am. J. Physol.* **240**, C121-C126.

Scapolo, P.A. & Rowlerson, A. (1987). Pink lateral muscle in the carp (*Cyprinus carpio* L.): histochemical properties and myosin composition. *Experentia.* **43**, 384-386.

Schvenke, M. and Wodtke, E. (1983). Cold-induced increase in Δ9- and Δ6- desaturase activities in endoplasmic reticulum. *Biochim. Biophys. Acta.* **734,** 70-75.

Selivonchick, D.P. and Roots, B.I. (1976). Variation in myelin lipid composition induced by change in environmental temperature of goldfish (*Carassius auratus* L.). *J. Thermal. Biol.* **1**, 131-135.

Shaklee, J., Christiansen, J.A. & Sidell, B.D. (1977). Molecular aspects of temperature acclimation in fish. Contributions of changes in enzyme activities and isozyme patterns to metabolic reorganisation in the green sunfish. *J. Exp. Zool.* **201**, 1-20.

Sidell, B.D. (1977). Turnover of cytochrome c in skeletal muscle of green sunfish (*Lepomas cyanellus* R.) during thermal acclimation. *J. exp. Zool.* **199**, 233-250.

Sidell, B.D. (1980). Responses of goldfish (*Carrasius auratus* L.) muscle to acclimation temperature: alterations in biochemistry and proportions of different fibre types. *Physiol. Zool.* **53**, 98-107.

Sisson, J.E. and Sidell, B.D. (1987). Effect of thermal acclimation on muscle fiber recruitment of swimming striped bass (*Morone saxatilis*). *Physiol. Zool.* **60,** 310-320.

Somero, G.N. (1981). pH-temperature interactions on proteins: principle of optimal pH and buffer system design. *Marine Biol. Letters.* **2**, 163-178.

Somero, G.N. & DeVries, A.L. (1967). Temperature tolerance of some Antarctic Fishes. *Science.* **156**, 257-8.

Sosnicki, A.A., Loesser, K.E. & Rome, L.C. (1991). Myofilament overlap in swimming carp. I. Myofilament lengths of red and white muscle. *Am. J. Physiol.* **260**, C283-C288.

Smith, M.W. and Ellory, J.C. (1971). Temperature-induced changes in sodium transport and Na^+/ K^+-adenosine triphosphatase activity in the intestine of goldfish *Carassius auratus* L. *Comp. Biochem. Physiol.* A. **39**, 209-218.

Stanfield, P.R. (1972). Electrical properties of white and red muscle fibres of the elasmobranch fish *Scyliorhinus canicula. J. Physiol. Lond.* **222**, 161-186.

Stein, R.B., Gordon, T. and Shriver, J. (1982) Temperature dependence of mammalian muscle contractions and ATPase activities. *Biophys. J.* **40**: 97-107.

Stephenson,D.G. and Williams, D.A. (1981). Calcium-activated force responses in fast- and slow-twitch skinned muscle fibres of the rat at different temperatures. *J. Physiol. (Lond).* **317**, 281-302.

Stevens, E.D. (1979). The effect of temperature on tail beat frequency of fish swimming at constant velocity. *Can. J. Zool.* **57**, 1628-1635.

Taylor, C.R. & Weibel, E.R. (1981). Design of the mammalian respiratory system. 1. Problem and strategy. *Respir. Physiol.* **44**, 1- 10.

Teichert, T. and Wodtke, E. (1987). The influence of acclimation temperature on carp liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Biochim. Biophys. Acta.* **920**, 161-170.

Tyler, S. and Sidell, B.D. (1984). Changes in mitochondrial distribution and diffusion distances in muscle of goldfish (*Carassius auratus*) upon acclimation to warm and cold temperatures. *J. exp. Biol.* **232**, 1-10.

Ushio, H. and Watabe, S. (1993). Effects of temperature acclimation on Ca^{2+}- ATPase of the carp sarcoplasmic reticulum. *J. exp. Zool.* **265**, 9-17.

Videler, J.J. & Wardle, C.S. (1978). New kinematic data from high speed cine film recordings of swimming cod (*Gadus morhua*). *Neth. J. Zool.* **28**, 465-84.

Walesby, N.J. and Johnston, I.A. (1980a). Fibre types in the locomotory muscles of an antarctic teleost *Notothenia rossi. Cell. Tiss. Res.* **208**, 143-164.

Walters, V. & Fierstine, H.L. (1964). Measurements of swimming speeds of yellowfin tuna and wahoo. *Nature, Lond.* **202,** 208-209.

Ward, G. and Fitzgerald, G.J. (1983). Macrobenthic abundance and distribution in tidal pools of a Quebec salt marsh. *Can. J. Zool.***61**, 1358-1361.

Wardle, C.S. (1975). The limit of fish swimming. *Nature., Lond.* **255**, 725-727.

Wardle, C.S. (1985). Swimming activity in fish. In: *Physiological adaptations of marine animals.* (Ed M. S., Laverack). *Symp. Soc. Exp. Biol.* XXXIX, 521-540.

Wardle, C.S. & Videler, J.J. (1980). How do fish break the speed limit? *Nature.* **244** (3). 445-7.

Wardle, C.S. & Videler, J.J. (1993). The timing of emg in the lateral myotomes of mackerel and saithe at different swimming speeds. *J. Fish. Biol.* **42**, 347-359.

Watabe, S. & Hashimoto, (1980). Myosins from white and dark muscles of mackerel. *J. Biochem.* **87**, 1491-1499.

Watabe, S., Hwang, G-C., Nakaya, M., and Okamoto, Y. (1992). Fast skeletal myosin isoforms in thermally acclimated carp. *J. Biochem.* **111**, 113-122.

Watabe, S., Ochai, Y. & Hashimoto, K. (1982). Identification of 5,5'-Dithio-bis-2-nitrobenzoic Acid (DTNB) and Alkali light chains of piscine myosin. *Bull. Jpn. Soc. Sci. Fish.* **48**, 827-832.

Watt, P.W., Marshall,P.A., Heap, S.P., Loughna, P.T. & Goldspink, G. (1988). Protein synthesis in tissue of fed and starved carp, acclimated to different temperatures. *Fish Physiol & Biochem.* **4**, no 4, 165-173.

Webb, P.W. (1970). Some aspects of the energetics of swimming fish with special reference to the rainbow trout. Ph.D. Thesis. University of Bristol.

Webb, P.W. (1971). The swimming energetics of trout. I. Thrust and power output at cruising speeds. II. Oxygen consumption and swimming efficiency. *J. Exp. Biol.* **55**, 489-540.

Webb, P.W. (1975a). Acceleration performance of rainbow trout *Salmo gaidneri* and the green sunfish *Lepomis cyanellus*. *J. exp. Biol.* **63,** 451-465.

Webb, P.W. (1975b). Hydrodynamics and energetics of fish propulsion. *Bull. Fish. Res. Bd. Can.* **190**, 1-158.

Webb, P.W. (1976). The effect of size on fast-start performance of rainbow trout (*Salmo gaidneri*) and a consideration of piscivorous predator-prey interactions. *J. exp. Biol.* **65,** 157-177.

Webb, P.W. (1977). Effects of median-fin amputation on fast-start performance of rainbow trout (*Salmo gaidneri*). *J. exp. Biol.* **68,** 123-135.

Webb, P.W. (1978a). Effects of temperature on fast-start performance of rainbow trout, *Salmo gaidneri. J. Fish Res. Bd Can.* **35**, 1717-1422.

Webb, P.W. (1978b). Fast-start performance and body form in seven species of teleost fish. *J. exp. Biol.* **74,** 211-226.

Webb, P.W. (1980). Temperature effects of acceleration of rainbow trout, *Salmo gairdneri. J. Fish. Res. Bd. Can.* **35**, 1417-1422.

Webb, P.W. (1984a). Body and fin form and strike tactics of four teleost predators attacking fathead minnow (*Pimephales promelas*) prey. *Can. J. Fish. Aquat. Sci.* **41**, 157-165.

Webb, P.W. (1984b). Body form, locomotion and foraging in aquatic vertebrates. *Amer. Zool.* **24**, 107-120.

Webb, P.W. (1986). Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth Bass (*Micropterus salmoides*). *Can. J. Fish. Aquat. Sci.* **43**, 763-771.

Webb, P.W., Kostecki, P.T. and Stevens, E.D. (1984). The effect of size and swimming speed on locomotion kinematics of rainbow trout. *J. exp. Biol.* **109**, 77-95.

Webb, P.W. & Skadsen, J.M. (1980). Strike tactics of esox. *Can. J. Zool.* **58**, 1462-1469.

Weihs, D. (1972). A hydrodynamical analysis of fish turning manoeuvres. *Proc. R. Soc. Lond.* B. **182,** 59-72.

Weihs, D. (1973). The mechanism of rapid starting of slender fish. *Biorheology.* **10**, 343-350.

Weihs, D. (1974). Energetics advantages of burst swimming fish. *J. Theor. Biol.* **48**, 215-229.

Weihs, D. (1980). Hydrodynamics of suction feeding of fish in motion. *J. Fish. Biol.* **16**, 425-433.

Weis-Fogh, T & Alexander, R.McN. (1977). The sustained power output from striated muscle. In: *Scale effects in animal locomotion.* pp 511- 25 (ed. T.J. Pedley). London: Academic Press.

Wells, R.M.G. (1990). Haemoglobin Physiology in Vertebrate Animals: A Cautionary Approach to Adaptationist Thinking. In: *Advances in Comparative and Environmental Physiology,* Vol. **6**. (ed. R.G. Boutlier), pp143-161. Berlin: Springer Verlag.

Westerfield, M., McMurray, J.V. & Eisen, J.S. (1986). Identified motorneurones and their innervation of axial muscles of the zebrafish. *J. Neurosci.* **6**, 2267-2277.

Whalen, R.G., Sell, S.M., Butler-Browne, G.S., Schwarty, K., Bouveret, P. and Pinset-Harstrom, I. (1981). Three myosin heavy-chain isozymes appear sequentially in rat muscle development. *Nature Lond.* **292**, 805-809.

Wheeler, A. (1969). *The fishes of the British Isles and North-West Europe.* London. Macmillan.

Williams, T.L., Grillner, Smoljaninov, V.V., Wallen, P., Kashin, S. and Rossignol, S. (1989). Locomotion in lamprey and trout: the relative timing of activation and movement. *J. exp. Biol.* **143**, 559-566.

Wilson, F.R. (1973). Quantitative changes on enzymes of the goldfish (*Carassius auratus* L.) in response to temperature acclimation. An immunological approach. PhD Thesis: University of Illinois.

Wodtke, E. (1981). Temperature adaptation of biological membranes. Compensation of the molar activity of cytochrome c oxidase in the mitochondrial energy-transducing membrane during thermal acclimation of the carp

Cyprinus carpio L. *Biochim. Biophys. Acta.* **640**, 710-720.

Woledge, R.C. (1989). Energy transformation in living muscle. In: *Energy transformations in cells and organisms* (ed. W. Wieser and E. Gnaiger), pp 36-45. *Proceedings of the 10th conference of the European Society for Comparative Physiology and Biochemistry. Stuttgart*: Georg Thieme Verlag.

Yancey, P.H. & Johnston, I.A. (1982). Effect of electrical stimulation and exercise on the phosphorylation state of myosin light chains from fish skeletal muscle. *Pflügers. Arch.* **393**, 334-339.

Ye, X., Randall, D.J. & He, X. (1991). The effect of acid water on oxygen consumption circulating catecholamines and blood ionic and acid- base status in rainbow trout (*Salmo gairdneri*). *Fish Physiol. Biochem.* **9**, 23-30.

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