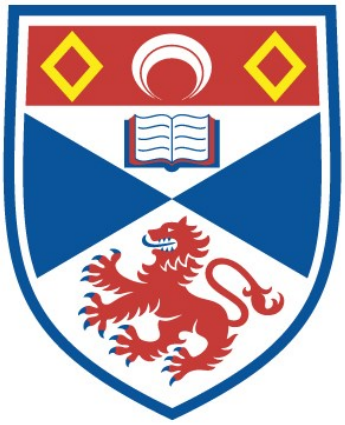
EFFECTS OF ACUTE TEMPERATURE CHANGE AND THERMAL ACCLIMATION ON THE CONTRACTILE PROPERTIES OF TELEOST MUSCLE

Karen S. Langfeld

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1991

EFFECTS OF ACUTE TEMPERATURE CHANGE AND THERMAL ACCLIMATION ON THE CONTRACTILE PROPERTIES OF TELEOST MUSCLE.

BY

KAREN S. LANGFELD

GATTY MARINE LABORATORY,

DEPARTMENT OF BIOLOGY AND PRE-CLINICAL MEDICINE,

UNIVERSITY OF ST. ANDREWS.

A thesis submitted for the degree of

Doctor of Philosophy

September 1990

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

INDEX

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 been previously submitted for a higher degree.

The research was conducted in the Department of Biology and Preclinical Medicine, United College of St. Salvator and St. Leonard, University of St. Andrews, under the direction of Professor Ian A. Johnston.

CERTIFICATE

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

SUMMARY

Chapter 1 - General Introduction

Part I reviews the structure and function of fish muscle, including fibre orientation, the properties of different muscle fibre types and the recruitment of muscle fibres during swimming.

Part II concerns the effects of acute temperature changes on fish muscle and describes a variety of mechanisms underlying temperature adaptation, with particular emphasis on the mechanical performance of fish muscle.

Chapter 2 - Temperature and the mechanical properties of live muscle fibres from the teleost *Myoxocephalus scorpius*.

Small bundles of fast fibres were isolated from the myotomal muscle of the teleost *Myoxocephalus scorpius*. The temperature dependence of isometric contractile properties and the force-velocity (P-V) relation were studied. Fibres were found to deteriorate above 18° C, and the force plateau during tetanic stimulation was not maintained above 15° C. Twitch and tetanic tension (P_o) showed optima at around 8° C. Force-velocity curves were fitted using either Hill's hyperbolic equation or a hyperbolic-linear (hyp-lin) equation (Marsh, R.L. & Bennett, A.F. 1986, *J. exp. Biol.* 126, 63-77). The best fit to the data was provided by the

hyp-1in equation, which gave consistently higher values for unloaded contraction velocity (V_{max}): 4.3, 8.1 and 9.5 muscle lengths s^{-1} at 1° C, 8° C, and 12° C respectively. Both isometric and isotonic data from live fibres was compared with skinned fibres and live fibres from other vertebrates. The P-V relation was found to become progressively more curved at higher temperatures. Muscle power output calculated from the hyp-1in equation was 124 Wkg^{-1} at 1° C and 256 Wkg^{-1} at 8° C. Curves normalised for P_o and V_{max} at each temperature show that the change in curvature is sufficient to increase the relative power output of the muscle by around 15% on decreasing the temperature from 8° C to 1° C.

Chapter 3 - **The myology of the pectoral fin of the common carp *Cyprinus carpio* L. and variation in fibre composition with temperature acclimation.**

Common carp (*Cyprinus carpio* L.) were acclimated to either 8° C or 20° C for 8 weeks (12h light:12h dark). The myology and skeletal structure of the pectoral assemblage of the carp was studied. Cross-sections of the entire assemblage were taken for histochemistry in order to determine the distribution of fibre types within the musculature. The *abductor superficialis* (*AB.S.*) muscle was chosen for more detailed study of the effects of acclimation. The percentage cross-sectional area of slow

fibres was significantly greater in the 8° C-acclimated fish relative to the 20° C-acclimated fish (31.4±0.30% and 26.5±0.22% respectively), the mean number of slow fibres per muscle was also greater for the 8° C fish than the 20° C fish (2392±40 and 2127±29 fibres respectively). Acclimation did not significantly alter the mean fibre diameter of either slow or fast fibres, confirming that the increase in cross-sectional area of slow fibres is caused by the increase in their numbers rather than in an increase in the diameter of existing fibres. Examination of the fibre diameter range data reveals a larger number of smaller slow fibres in the muscle of 8° C-acclimated fish, commensurate with a hypothesis that the slow fibre mass is proliferating during cold-acclimation by producing new slow fibres.

Chapter 4 - **Temperature acclimation in the common carp: force-velocity characteristics and myosin subunit composition of slow muscle fibres.**

Common carp were acclimated to either 8° C or 20° C for 6-12 weeks (12h light:12h dark). Bundles of 20-50 fibres were isolated from the superficial region of the pectoral fin *abductor superficialis* muscle. Histochemical studies showed preparations to contain 93-100% slow muscle fibres.

The maximum tetanic tension (P_o) produced by fibre bundles was similar when measured at the acclimation temperature of each group. However, at 8° C, P_o was significantly higher in 8° C- than in 20° C-acclimated fish

(202±8 and 153±4 kNm^{-2} repectively). For isometric tetani at 8° C, the time to 50% peak force and from peak force to 50% relaxation was 15–20% smaller in preparations from cold- than warm-acclimated carp. Force-velocity (P–V) curves were fitted using either Hill's equation or a hyperbolic-linear equation. The curvature of the P–V relationship was found to be independent of acclimation temperature. Unloaded contraction velocity (V_{max}) was 17% higher at 8° C in fibres from 8° C- than 20° C- acclimated fish (1.18±0.04 and 0.98±0.04 muscle lengths s^{-1}, respectively). Calculated values for maximum power output at 8° C were 26.5 Wkg^{-1} for cold-acclimated and 18.05 Wkg^{-1} for warm-acclimated fish.

Native myosin was purified from isolated fibre bundles using sodium pyrophosphate gel electrophoresis. The mobility of myosin heavy chains on 8% SDS PAGE gels was similar for both acclimation groups. Myosin light chain subunits were separated on 15% SDS PAGE gels. Fibre bundles from warm-acclimated fish contained almost exclusively slow myosin light chains ($LC1_s$ and $LC2_s$). Preparations from cold-acclimated fish contained a significant proportion of fast myosin light chains ($LC1_f$ and $LC2_f$) in addition to $LC1_s$ and $LC2_s$.

It was concluded that cold-acclimation results in modest improvements in the contractile performance of slow muscle fibres at low temperatures. The mechanism may involve the expression of myosin light chain isoforms which are normally associated with faster contracting fibre types.

Chapter 5 - General Discussion

The results detailed in this thesis are discussed in relation to the effects of temperature on fish swimming. Recent developments in technique are decribed and some suggestions for further work using these techniques are outlined.

CHAPTER 1

General Introduction

PART I – The structure and function of fish muscle

The aquatic environment accounts for many of the specialised features of fish muscle. The higher density of water over air means that drag on the body and thus power expenditure to maintain locomotion is greater in water, especially at high speeds. On land, power requirements generally increase linearly with the speed of locomotion, compared to the increase in power proportional to velocity cubed which occurs during swimming (Webb, 1975). However, weight considerations are lessened in water due to the buoyancy of a fish, so fish can carry a large bulk of body musculature in order to achieve fast swimming speeds. The trunk musculature of a fish can constitute between 40 and 60% of the total body mass, the bulk of which is made up of fast contracting white fibres, which can develop power rapidly and are used for burst swimming (Bone, 1978).

The axial musculature of elasmobranchs and teleosts is segmentally arranged into myotomes. Each myotome is made up of fibres inserted at each end via tendons into sheets of connective tissue, the myosepta. The septa form several series of cones, stacked parallel to the longitudinal axis, resulting in a complex "W" shape when the structure is

viewed in longitudinal section (Alexander, 1969). Each cone makes an acute angle to the longitudinal axis in the opposing direction to the acute angle made by the fibre to the longitudinal axis. The arrangement of fibres within the myotome is complex, varying not only between classes, but within the fish, depending on the distance of the myotome along the body, the dorso-ventral position and the depth of the fibres within the muscle (Alexander, 1969). Fibre orientation between successive myotomes describes a succession of helices, the axis lying parallel to the long axis of the fish, and the angles between cones and fibres becoming more acute towards the tail. Within a myotome, the superficial fast fibres generally run parallel to this long axis, whilst the deeper fibres may make angles of up to 40° with it. Alexander (1969) concluded that these arrangements enabled similar degrees of sarcomere shortening during the body flexures associated with swimming, irrespective of the position of each fibre within the myotome. Alexander (1969) calculated the mechanical advantage (the ratio of the fractional change in length of the fibres to the fractional change in length of the muscle block) during body flexion. If the muscle distorts like a uniform solid, the mechanical advantage would actually be a disadvantage, so Alexander (1969) suggested that the medial muscle blocks may slide and shorten more than the overall geometry of the fish. However, recent analysis of the geometric arrangement of selachian muscle fibres by Gardner-Medwin & Curtin (1990)

has shown large mechanical advantages are possible without such sliding if the septa have suitable properties.

Fibre types

In most vertebrates, locomotory muscles contain a mixture of different fibre types. Fish muscle, however, shows anatomical separation of the different types (Bone, 1966). In most fish, the myotomal musculature is made up of two main fibre types, tonic and twitch.

Tonic fibres are found in a few elasmobranchs (Bone & Chubb, 1978; Bone, Johnston, Pulsford & Ryan, 1986) and teleosts (Kilarski & Kozlowska, 1987). Bone & Chubb (1978) detected fibres similar to vertebrate tonic fibres in the dogfish. They form an interrupted single layer on the outer border of the myotomes and are characterised by an intense staining for glucose-phosphate-isomerase. They have a large diameter and low volumes of mitochondria and thus SDH activity and low ATPase activities (Bone *et al.*, 1986). Bone *et al.* (1986) have suggested that these fibres may have a role in maintaining body tone and/or attitude when the dogfish is at rest on the sea floor, *i.e.* a postural rather than a locomotory function.

Twitch fibres are sub-divided into various types. The red, slow fibres usually form a superficial layer just under the skin (beneath the tonic fibres, if present). This layer extends all over the trunk, as in the dogfish *Scyliorhinus canicula* (Bone & Chubb, 1978), or forms a more discrete

wedge around the lateral line, as in the brook trout *Salvelinus fontinalis* (Johnston & Moon, 1980b). There are a few special cases. The skipjack tuna *Katsuwonus pelamis*, for example, has an internalised red muscle mass in addition to a wedge of red muscle around the lateral line. This specialisation is associated with a counter current vascular heat exchanger system which enables the tuna to maintain elevated red muscle temperatures (Sharp & Pirages, 1978). The internalised red muscle can be distinguished from the superficial red by a smaller fibre size and higher SDHase activity (Bone, 1978b). Tuna are good high speed swimmers and utilise ram ventilation, where the myotomal rather than buccal musculature is associated with ventilation. The proportion of slow fibres in the myotomal muscle is related to the activity and lifestyle of the fish, being highest in pelagic fish and lowest in sedentary bottom dwellers, deep sea fish and those fish which swim mainly by using fin movements. Actual percentages vary from 0.5–29% of the muscle mass (values from Greer-Walker & Pull, 1975, a study on 84 species of marine fish).

Beneath the slow fibres lies the white, fast muscle mass, constituting the bulk of the myotomal musculature. In some fish other fibre types have been identified, for example intermediate or pink fibres exist in a number of species, such as the carp *Cyprinus carpio* (Johnston, Davison & Goldspink, 1977), and are situated between the slow and fast muscle.

Fibre types can be distinguished and sub-divided by means of their histochemical, ultrastructural, biochemical and physiological properties. Bone (1978), for example, distinguished five fibre types in the dogfish by means of differences in their innervation, histochemical staining and ultrastructural properties, these being superficial (tonic), outer red, inner red, outer white and inner white muscle fibres.

Red, slow fibres are small diameter fibres, with a low myofibrillar ATPase activity (Johnston, Patterson, Ward & Goldspink, 1974). Ultrastructural studies reveal that volume fraction of myofibrils in slow muscle fibres is 30% lower than in fast fibres (Bone, 1978: Johnston, 1981). Slow fibres stain heavily for the mitochondrial enzyme succinic dehydrogenase (SDH), and ultrastructural studies confirm they have a high volume of mitochondria, the volume ranging between 16 and 38% (Johnston, 1981). Capillary density is higher in slow muscle, in fact slow muscle from the more advanced teleosts shows a good correlation between the density of mitochondria and that of capillaries (Johnston & Bernard, 1982; Egginton & Johnston, 1983). High capillary density is associated with high aerobic capacity.

White, fast fibres have a high myofibrillar ATPase activity (Barany, 1967; Bone, 1978; Johnston, 1981). The volume fraction of myofibrils in fast muscle ranges between 80 and 96% (Johnston, 1981), and the myofibrillar packing is also more regular than in slow muscle. SDH activity and

thus mitochondrial volumes are much lower in fast muscle, between 0.5 and 4% (Johnston, 1981). Capillary density is also lower in fast fibres, but capillarisation does vary with the habit of the species, being more developed (higher unit density) in certain pelagic species (Mosse, 1979). All these features of fast muscle reflect the lower aerobic capacity relative to slow muscle.

Intermediate, or pink fibres have been identified in the myotomes of a number of fish species (Johnston *et al.*, 1974; Patterson, Johnston & Goldspink, 1974). These fibres have also been called fast oxidative glycolytic fibres, and possess values for capillary and mitochondrial volume density, ATPase activity, and aerobic capacity intermediate between those of slow and fast fibres (Johnston *et al.*, 1977; Johnston & Maitland, 1980; Akster, 1985) and are also distinguishable histochemically on the basis of the pH lability of the myosin ATPase reaction (Johnston *et al.*, 1974). Aerobic capacity is intermediate and pink fibres utilise both oxidative and glycolytic energy pathways.

Contractile protein isoforms

Most myofibrillar proteins can exist as isoforms, each with distinguishable functional properties. Isoforms can occur as multiple gene families, for example myosin heavy chains (MHC), which are expressed in a developmental stage and tissue specific mannner (Richter, Young & Moriarty, 1989). Cloning studies performed on carp indicate the

presence of a minimum of 28 different myosin chain genes (Gerlach, Turay, Malik, Lida, Scutt & Goldspink, 1990). Contraction speed and force production of muscle are thought to be determined largely by the type of myosin isoform expressed (Reiser, Moss, Giulian & Greaser, 1985; Lännergren, 1987). Myosin has a common sub-unit structure in fish muscle, it has two heavy chains of 200 kDa and four light chains (LC) of 17-26 kDa (Focant, Jacob & Huriaux, 1981; Rowlerson, Scapolo, Mascarello, Carpene & Vegetti, 1985; Karanski & Kilarski, 1989). The two heavy chains form a coil over their C-terminal halves and then separate to form a globular head containing one alkali light chain and one light chain which can be phosphorylated. The heavy and light chain composition varies between different fibre types. Peptide mapping has shown that slow, intermediate and fast fibre types each contain a distinct isoform of MHC, which are HC_f, HC_i and HC_s respectively (Scapolo & Rowlerson, 1987). Slow muscle fibres have two light chain isoforms, $LC1_s$ and $LC2_s$ (Focant *et al.*, 1976; Rowlerson *et al.*, 1985), whereas fast muscle has three, $LC1_f$, LC2f and $LC3_f$ (Johnston *et al.*, 1977; Yancey & Johnston, 1982). $LC1_f$ and $LC3_f$ are alkali light chains, distinguishable only by the sequence at the NH_2 terminus, and are thought to be produced by an alternate RNA splicing mechanism from a single primary transcript (Nabeshima, Fujii-kuriyama, Muramatsu & Ogata, 1984).

Fast and slow fibres also contain distinct forms of troponin C, I and T (Johnston, unpublished results). Two

different isoforms of troponin I have been distinguished in the fast muscle of the carp (Crockford & Johnston, 1990). The isoforms have similar isoelectric points but slightly different molecular weights. Troponin I isoforms, like the alkali myosin light chains, are thought to arise from an alternate splicing mechanism from a single gene.

Innervation and Electrophysiology

In all the species of fish so far examined, slow fibres display a distributed multiple innervation pattern (Bone & Ono, 1982). At least two axons innervate each fibre, either found passing into the fibre from the ends, or running across the surface of the muscle innervating a number of fibres. Terminations are of the en-grappe type. Slow myotomal fibres of the tench (Barets, 1961) are activated by junction potentials (JPs) and do not generate action potentials (APs), and thus a mechanical twitch, in response to depolarising pulses, neither do red fibres from the *m. adductor operculi* of *Tilapia* (Flitney & Johnston, 1979). Stanfield (1972), however, found that similar fibres in elasmobranchs could be capable of doing so, and twitch responses have been obtained from slow fibres of the cod and cuckoo ray (Johnston, 1982), and slow fibres from the *m. hyohoideus* muscle of the carp (Granzier, Wiersma, Akster & Osse, 1983). Recent work on the teleost *Myoxocephalus scorpius* has shown that slow myotomal muscles are activated by both JPs and overshooting APs (Altringham & Johnston,

1988). Fish slow fibres are clearly a more physiologically heterogenous group than previously expected (Granzier *et al.* 1983).

Fast fibres show a much more variable pattern of innervation. Elasmobranch fast fibres are focally innervated. In dogfish fast muscle, for example, each fibre is innervated by two large diameter axons which fuse to a single end plate (Bone, 1972). Elasmobranch fast fibres show an overshooting AP on depolarisation (Hagiwara & Takahashi, 1967). The fast fibres of primitive teleosts appear to resemble those of elasmobranchs (Bone, 1964; Bone & Ono, 1982). The functional significance of this dual innervation pattern is not yet known.

In the majority of teleosts, fast fibres are multi-terminally (polyneuronally) innervated. The nerves run down the myosepta and then branch out across the myotomal surface. The branching overlaps so each fibre can recieve as many as 23 terminations (Barets, 1961; Bone, 1964; Hudson, 1969; Altringham & Johnston, 1981) which are usually embedded in the sarcolemma (Nishihara, 1967). Each fibre can recieve axons derived from more than one spinal root, for example Hudson (1969), discovered that a single white myotomal muscle fibre from *Myoxocephalus scorpius* can recieve innervation from as many as five axons from each of four spinal nerves, but in almost all cases, no more than one end plate on each fibre is derived from a single axon. Recent work on the same species by Altringham & Johnston

(1989) has shown, however, that the majority of fast fibres are innervated solely by the two immediately adjacent nerves and preterminal branching accounts for many of the endplates found on each fibre. Polyneuronal innervation in the fast muscle of the zebrafish has also been studied (Westerfield, McMurray & Eisen, 1986). The fast muscle motorneurones are divided into two classes, primary and secondary by means of the cell body sizes and positions. Each individual fibre recieves inputs from a single primary motorneurone, and up to three secondary motorneurones. Each of the three primary neurones on the side of each segment of the body musculature innervates a specific subset of fibres in mutually exclusive areas of the segment.

Hudson (1969) found that electrical stimulation of the spinal nerves innervating the fast fibres produced either overshooting APs, resulting in a fast mechanical twitch, or JPs, associated with graded local contraction. In old preparations however, only JPs could be elicited. Altringham & Johnston (1988) found that white fibres always give APs when the specimens and preparations are in good physiological condition, so they concluded that the normal mode of activation is by a propagated overshooting AP.

Studies on fast fibres from fin muscle show widely varying results. AP's rarely had overshoots and have been described as of regular occurrence (Hagiwara & Tagahashi, 1967), rare (Yamamoto, 1972) or not produced (Gilly & Aledjem, 1987).

Polyneuronal innervation has evolved independently on several occasions during adaptive radiation of the teleosts, (Bone & Ono, 1982), so there is obviously strong selection pressure for the pattern. The functional significance remains obscure, however, although the key to understanding probably lies in a deeper understanding of the neural circuitry associated with the polyneuronal pattern (Altringham & Johnston, 1989).

Mechanical properties

The complex arrangement and myoseptal insertions of fibres in the myotomal muscles of fish makes mechanical studies difficult. Early studies of mechanical properties were restricted mainly to fin or jaw muscles (Hidaka & Toida, 1969; Yammamoto, 1972; Flitney & Johnston, 1979).

The twitch and tetanus characteristics of fast myotomal fibre bundles from the cod and the cuckoo ray *Raia naevus* have been compared by Johnston (1980), significant differences are found between the stimulation characteristics of the multiply innervated cod and the focally innervated ray. Typical tetanic fusion frequencies on multiple stimulation are 40–50 Hz for the cod compared to 5–10 Hz for the ray and the stimulation frequency needed to obtain maximal isometric tensions in cod was up to 300 Hz, more than ten times that required in the ray.

A demembranated, or skinned, myotomal fibre preparation was developed by Altringham & Johnston (1982). In skinned fibres, the normal excitation-contraction mechanism is uncoupled and tension generation can be studied in isolation. Isometric tensions derived from skinned fibres were comparable to values found for other vertebrate skinned fibres and much higher than those obtained previously from intact fibres (Flitney & Johnston, 1979). This may be due to damage caused to the sarcolemma of intact fibres during dissection, resulting in a preparation with a high proportion of inactive fibres.

The skinned slow and fast fibres of the cod gave values for maximum isometric tension of 83.4 and 186.5 kNm^{-1} respectively. Slow fibres gave lower isometric tensions, which can be partly attributed to the lower myofibrillar volume, and partly to the tension generated per myofibril being greater in fast muscle (Altringham & Johnston, 1982). Maximum contraction velocity is two to three times higher in fast fibres.

Skinned fibres generate lower tensions than intact fibres in other vertebrates. This has been attributed to the swelling of fibres after membrane removal causing an over-estimate in cross sectional area and thus an under-estimate in tension (Godt & Maugham, 1977), and to differences between the intercellular environment of the intact fibre and the bathing solutions used in experiments. Even though compensation for the first error is possible and skinned fibres are clearly a useful tool for studying muscle

mechanics, a good intact fibre preparation would be more useful, epecially for quantitative studies. Differences in the mechanical characteristics of skinned and fast fibres have been demonstrated in muscle from other vertebrates; for example in frog fibres, the shape of the force-velocity curves obtained using the two preparations differs, indicating that the skinning procedure and following treatment produces significant changes in force-velocity properties (Julian, Rome, Stephenson & Striz, 1986).

Recently, electrically excitable live fibre preparations have been developed for elasmobranch (Curtin & Woledge, 1988) and teleost (Altringham & Johnston, 1988) fibres, and values for maximum isometric tensions are higher than those obtained from skinned fibres and comparable to the values obtained for intact fibres from other vertebrates. Curtin & Woledge (1988) also discovered a difference between the force-velocity properties of skinned and intact dogfish fibres similar to that found in frog fibres (Julian *et al.*, 1986). This difference, combined with the lower forces obtained using skinned fibres, gives much lower values for power output in skinned fibres, so it seems likely that the skinned fibre is not an adequate model of the living fibre since it has lost a large part of its ability to produce mechanical power (Curtin & Woledge, 1988). Skinned fibre preparations are still useful for comparative studies and have many experimental advantages including ease and speed of preparation, and skinned fibres prepared using freeze drying (Steinem, Guth & Rüegg, 1983;

Crockford & Johnston, 1990) can be stored for long periods, enabling the sampling of a large number of specimens in a short time.

Fibre recruitment and implications for locomotion

In elasmobranchs and primitive teleosts electromyographical (EMG) studies during swimming have shown a complete functional division between the slow, and the focally innervated fast, motor systems (Bone, 1966). In the dogfish, only the slow fibres are active during low sustainable swimming speeds. As speed increases, fast fibres are recruited and then rapidly fatigue, in only 1-2 minutes at burst speeds (Bone, 1966). A similar pattern is shown in the Pacific herring *Clupea harengus pallasi* (Bone, Kicenuik & Jones, 1978).

Teleosts with multi-terminally innervated fast muscle do not show such a clear division between the motor systems, recruitment of fast fibres occurring over a broader range of swimming speeds. At low steady speeds only slow fibres are active, but fast fibres become active very early, for example in the coalfish *Pollachius virens*, at a speed of 0.8-0.9 body lengths per second (Johnston & Moon, 1980a). Rome, Funke, Alexander, Lutz, Aldridge, Scott & Freadman, (1988) investigated the continous swimming and startle response of carp. Optimum power is produced during slow swimming by slow fibres, then they start to recruit their fast muscle because the slow muscle alone can no longer

generate enough mechanical power. The fast fibres of carp shorten at optimal speed and thus produce maximum mechanical power during the startle response. The recruitment of pink, intermediate fibres has been studied in the carp (Johnston *et al.*, 1977) and order of recruitment shown to be slow > intermediate > fast. The order of recruitment of fast fibres also depends on the depth of the fibre within the myotome, superficial fibres being recruited before deep ones (Johnston & Moon, 1980a).

EMG records from fast muscle at low speeds resemble those from slow muscle (Johnston *et al.*, 1977; Johnston & Moon, 1980a; Rome, Loughna & Goldspink, 1984). Higher amplitude spike potentials are seen during high speed and burst swimming.

The caudally running wave of lateral bending occuring during continuous swimming is produced by the sequential stimulation of the axial musculature on alternate sides of the body. The bending moments acting on the body proceed as a standing wave, but run faster than the wave of lateral bending (Hess & Videler, 1984). Electromyographic studies have also shown that the velocity of the mechanical wave is greater than that of the electromyographical wave, thus phase differences between force and length cycles will occur along the body. This would imply that the muscle fibres are active at different strain ranges along the body (Grillner & Kashin, 1976; Williams, Grillner, Smojaninov, Wallen, Kashin & Rossignol, 1989).

Van Leeuwen, Lankheet, Akster & Osse (1990) analysed the recruitment and power output of the slow myotomal muscle of the carp using synchronised electromyography and cinematography. The ultrastructure of the fibres along the trunk were also measured using electron microscopy. A model to estimate strain fluctuations and power output along the trunk was developed, taking into account sarcomere properties and the modulation of cross-bridge force due to the force-velocity relationship and tension variation with stimulation and fibre properties. The model confirmed that during continuous swimming the strain range and contraction speed at which fibres were active varied along the trunk. A period of negative power production was followed by a period of positive power production, with the positive phase being more important anteriorly, producing activity during shortening and net positive work over the entire swimming cycle. Posteriorly, net negative work was done, the active fibres mainly being stretched, and near the anus, the amounts of positive and negative work done balanced each other. Most work is thus done by the anterior myotomes, and this results in the stretching of the posterior muscle and collagen fibres.

PART II – The effects of acute temperature change and thermal acclimation on the contractile properties of fish muscle.

In fish, body temperature is defined by, and varies with, the temperature of the environment. In spite of the marked temperature sensitivity of most biological rate processes, fish muscles have evolved to operate in the temperature extremes of the aquatic environment, varying from -2° C in the Antarctic, up to 45° C in geothermal hot springs.

In many terrestrial organisms, behavioural strategies can help to moderate the effects of environmental temperature variation, but aquatic environments offer fewer opportunities for such moderation. Since fish are unable to control body temperature (although a few, like the tuna, can modify it to some extent), the only alternative is to adapt rate processes when temperatures change. This involves changes in the phenotype (the limits are set by the genotype) and this, when observed in the field, is called acclimatisation. A variation in temperature in the natural environment can be associated with other variables such as day length or food availability. In the laboratory, where specific controlled conditions are applied, the adaptation is termed acclimation.

Precht (1958) classified the patterns of adaptation in the following way. Resistance adaptations modify the upper and/or lower lethal limits and so define the temperature

range over which normal function can be maintained. Capacity adaptations modify rate processes to compensate for the effects of temperature over the normal thermal range of the fish. Precht (1958) compared rate processes immediately after transferring an animal to a different temperature with those after a period of acclimation and was able to further divide capacity adaptations into five types; 1 - overcompensation; 2 - perfect compensation; 3 - partial compensation; 4 - no compensation; 5 - inverse or paradoxical compensation.

The mechanical performance of fish muscle is greatly influenced by temperature, and the variation of isometric contractile properties of skinned fibres with temperature is obviously correlated with the environmental temperature range undergone by a species (Altringham & Johnston, 1985, 1986; Johnston, 1990). For example, at 0° C, muscle fibres from Antarctic fish produce higher isometric tensions than those from temperate and tropical species (Johnston & Altringham, 1985), but the fibres from Antarctic fish cease to be viable at the lowest temperature as the temperature is raised. Only minor interspecific differences in myosin ATPase activity are found, but together with the higher isometric tensions of Antarctic species at low temperatures, economy of contraction is highest in Antarctic species (Altringham & Johnston, 1986).

The value of a/P_o, the measure of curvature of the force-velocity relationship, increases with temperature (*ie.*

the relation becomes less curved) for the species studied by Johnston & Altringham (1985), but it also increases in the order tropical < temperate < Antarctic when measured at the normal body temperature of each species. As a/P_o increases, velocity and thus power output increase for a given load, this together with the higher maximum tensions observed providing a mechanism for increasing power output at low temperature in cold-adapted species.

When data from intact muscle preparations of other vertebrates is added to the limited data presently available for intact fish fibres, it is clear that the viability of intact fibres with temperature is also related to the normal body temperature range of the animal (Johnston & Altringham, 1988).

From all the data available, it is clear that evolutionary adaptations of muscle contractile performance are present in fish.

Phenotypic adaptations of contractile properties with temperature occur in some eurythermal fish, particularly the cyprinids, but are not shown in all fish that experience wide environmental temperature variation, the flounder *Platichthys flesus* (Johnston & Wokoma, 1986) and the striped bass *Morone saxatilis* (Moerland & Sidell, 1986) being examples.

The first demonstrated adaptation in contractile properties was the higher myofibrillar ATPase activity of

muscle fibres from cold- than warm-acclimated fish (Johnston, Davidson & Goldspink, 1975; Sidell, 1980; Penney & Goldspink, 1981; Heap, Watt & Goldspink, 1985). For example, the activity of myofibrillar ATPase from the fast muscle of the goldfish (*Carassius auratus*) was 2.8 times higher in 1° C than 26° C acclimated fish when both groups were assayed at 1° C. The ATPase isolated from the cold acclimated fish was also more susceptible to thermal denaturation than that from the warm-acclimated fish (Johnston *et al.*, 1975). When carp myofibrillar ATPase activity was determined under force generating conditions in skinned fibres (by measuring ADP production using high performance liquid chromatography) at 7° C, ATP turnover per myosin head was 50% lower, and power obtained per ATP hydrolysed was 50% higher in cold- relative to warm-acclimated fish (Altringham & Johnston, 1985).

Johnston, Sidell & Dreidzic (1985) investigated the contractile properties of skinned fibres from common carp acclimated to either 7° C or 23° C and found that contractile properties were dependent on acclimation temperature. P_o and V_{max} for both slow and fast fibres were twice as high in the 7° C acclimated fish as in the 23° C fish, when measured at 7° C. When fibres from 7° C fish were maximally activated at 23° C, they failed to relax completely, the resulting calcium insensitive force component being associated with the development of abnormal cross-bridge formation and very slow contraction velocities (Johnston *et al.*, 1985).

Contractile properties do not vary continuously with acclimation temperature. Crockford & Johnston (1990) found that maximum tensions for carp fibres at 0° C were similar in fish acclimated to temperatures ranging from 2° C to 10° C, whilst fish acclimated to 11° C, 15° C and 23° C showed significant differences in P_o and V_{max}.

These results all indicate a partial capacity adaptation of power output with temperature, but since skinned fibres produce lower maximum tensions than intact fibres, quantitative measurement of the degree of compensation is not possible.

It is probable that the molecular mechanisms responsible for the changes in contractile properties with temperature acclimation involve changes in at least some of the myofibrillar proteins. By measuring protein synthesis rates before and during acclimation, Loughna & Goldspink (1985) found that carp were able to modify their protein metabolism as a result of temperature acclimation. Additional evidence is shown by the lack of change in myofibrillar activity with thermal acclimation in starved carp, where levels of protein synthesis are considerably reduced (Heap, Watt & Goldspink, 1986).

Although myofibrillar ATPase activities are higher in cold-acclimated fish, desensitised actomyosin preparations from goldfish (the tropomyosin-troponin complex was extracted) were found to have similar ATPase activities irrespective of acclimation temperature (Johnston, 1979).

This implies that the acclimatory responses may be associated with changes in one or more of the thin filament proteins. Crockford & Johnston (1990) discovered a second isoform of Troponin I in the fast muscle of warm-acclimated carp, but state that further studies are required to confirm this and determine whether the expression of the second isoform has any physiological significance.

Myosin composition is known to be a major determinant of contractile properties (Reiser *et al.*, 1985; Lännergren, 1987). Temperature acclimation may involve changes in the expression of myosin heavy chains (MHC) (Gerlach *et al.*, 1990), although Crockford & Johnston (1990) found no electrophoretic evidence for different MHCs in carp fast muscle. The latter study did discover evidence for a super-fast light chain isoform ($LC3_{sf}$) in the fast muscle of cold-acclimated carp, together with a difference in the $LC3_f$:$LC1_f$ ratio (the ratio was higher in cold-acclimated carp). This is especially significant in relation to recent evidence that the LC3 content has a role in modulating the contractile properties of rabbit muscle (Greaser, Moss & Reiser, 1988). Studies of single muscle fibres from other vertebrates have shown expression of variable proportions of both heavy and light chain myosin isoforms, giving rise to a large number of theoretical isomyosins (Reiser *et al.*, 1985). Carp possess a large number of different myosin chain genes, so theoretically opportunities exist for a considerable number of isomyosins, and so an explanation for

the great plasticity of the contractile properties of muscle.

The ultrastructure of muscle changes with thermal acclimation. The volume of mitochondria in muscle fibres is higher in cold- compared to warm-acclimated fish (Johnston & Maitland, 1980; Egginton & Sidell, 1989), a similar proliferation of mitochondria occuring with seasonal temperature acclimation in teleosts (Johnston & Dunn, 1987). Surface and volume fractions of muscle capillaries are also higher in cold-acclimated fish (Johnston, 1982), these factors together with increased mitochondrial density resulting in an increased potential for aerobic ATP production. Biochemical studies also show that the maximal activities of some of the enzymes involved in the aerobic energy pathways increased following cold-acclimation. These elevated activities either reflect the higher activities of the enzymes in the mitochondria of cold-acclimated fish or are simply due to the increase in the volume of mitochondria.

When oxygen consumptions of cold- and warm-acclimated fish are compared at an intermediate experimental temperature, cold-acclimated fish use more oxygen, which is likely to result in a higher metabolic rate and an increase in the range of swimming speeds sustainable at low temperatures (Fry & Hochachka, 1970; Rome, Loughna & Goldspink, 1985). Higher concentrations of muscle energy stores are also found in cold-acclimated fish, glycogen concentrations in the muscle of crucian carp acclimated to

2° C being twice those measured in 28° C fish (Johnston & Maitland, 1980). Egginton & Sidell (1986) found that volume densities of lipid droplets in slow muscle fibres from striped bass were 15 times higher in cold- compared to warm-acclimated fish.

Temperature affects fibre recruitment. Electromyography in carp has shown that a reduction in water temperature from 20° C to 10° C decreases the recruitment threshold for fast fibres from 2.6 to 1.4 body lengths/s (Rome *et al.*, 1984). This indicates that the slow fibres alone cannot provide sufficient power to sustain speeds above 1.4 l/s at 10° C. After acclimation to 8° C, however, the recruitment threshold speed for fast fibres increases, so temperature acclimation enables fish to maintain higher sustainable swimming speeds at low temperatures (Rome *et al.*, 1985). By considering the difference between the swimming speeds of the two acclimatory groups, Rome *et al.* (1985) calculated that there was a 2.4-fold increase in the power output of the aerobic muscle. Critical swimming speeds are also affected by thermal acclimation (Heap & Goldspink, 1986). Swimming ability of cold-acclimated fish was greater at 10° C, whereas at 20° C warm-acclimated carp exhibited improved swimming ability. These results provide evidence that acclimation of contractile properties are beneficial over a wide temperature range.

Alterations in the properties of the individual muscle fibres are not the only way of compensating for temperature effects. The proportions of the fibre types in the muscle body can also change following acclimation, the volume of slow fibres, and thus the total aerobic capacity of the muscle, being greater in cold- compared to warm-acclimated fish (Johnston & Lucking, 1978; Jones & Sidell, 1982; Heap, Watt & Goldspink, 1987).

CHAPTER 2

Temperature and the mechanical properties of live muscle fibres from the teleost *Myoxocephalus scorpius*

INTRODUCTION

Studies using skinned (demembranated) fibres have shown that in fish muscle the force-velocity (P-V) relation becomes less curved as temperature decreases (Johnston & Altringham, 1985). This is reflected in the increase in the value of the constant "a" in Hill's (1938) equation. This may provide a mechanism to partially compensate for the decrease in power output as both force and contraction velocity decline (Johnston & Altringham, 1985; Johnston & Wokoma, 1986). However, skinned fibre preparations differ in their P-V relationships from those of live fibres. Data from skinned fibres are generally well described by Hill's (1938) equation, but data from live fibres deviate from a hyperbola at both high and low loads (*e.g.* Edman, 1988; Julian, Rome, Stephenson & Striz, 1986; Marsh & Bennett, 1986; Altringham & Johnston, 1988). Furthermore, skinned fibre preparations develop low tensions relative to live preparations from the same muscle (Altringham & Johnston, 1988). The difference is likely to be due to a combination of different factors, including the replacement of the

sarcoplasm with an artificial medium, sarcomere length inhomogenity during activation (Altringham & Johnston, 1988) and possibly swelling of fibres after skinning causing overestimates of fibre diameter (Godt & Maughan, 1977; Altringham & Johnston, 1982).

Recently, isolated live (electrically excitable), preparations from myotomal muscle have been developed for elasmobranchs (Curtin & Woledge, 1988) and teleosts (Altringham & Johnston, 1988; Rome & Sosnicki, 1990). The tensions generated by these preparations (when normalised to cross-sectional area) are comparable to those of a wide range of whole vertebrate muscles, suggesting their suitability for quantitative modelling of muscle power output.

The aim of the present study was to investigate the effects of temperature on both the isometric contraction properties and the P-V curve of live fibre preparations and compare the results with published data from skinned fibres. The data have been used to calculate the degree of compensation of power output achieved by changes in the curvature of the P-V relation. All experiments were carried out on the fast myotomal muscle of the sculpin *Myoxocephalus scorpius* L., a teleost found in the North Sea at depths of 0-60m and which experiences a seasonal temperature range of around 2-17° C.

MATERIALS AND METHODS

Fish

Specimens of the sculpin *Myoxocephalus scorpius* L. were obtained in the Firth of Forth between November 1987 and May 1988. All fish were held in seawater tanks at 5–7° C for 1–14 days prior to use. Fish were killed by a blow to the head, followed by decapitation. The mean length of the fish used was 21.7±0.8cm, and mean weight was 282±16g (±S.E., N>20).

Isolation of muscle fibre bundles

The preparation consisted of a bundle of 5–20 fibres, isolated from the fast muscle of the abdominal myotomes. A strip of tissue approximately 5mm wide and 3 myotomes in length was removed and pinned out at resting length on a silicone elastomer base (Sylgard 184, Dow Corning) on a cooled plate (5° C). The tissue was bathed in Ringer solution (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 7.4 at 5° C), which was changed frequently. The skin was removed from the tissue and the peritoneum removed from the surface of the central myotome. This myotome was pared down to a small bundle of undamaged fibres. The muscle tissue from the adjacent myotomes was then removed, leaving the preparation anchored by use of the remaining peritoneum. Aluminium foil

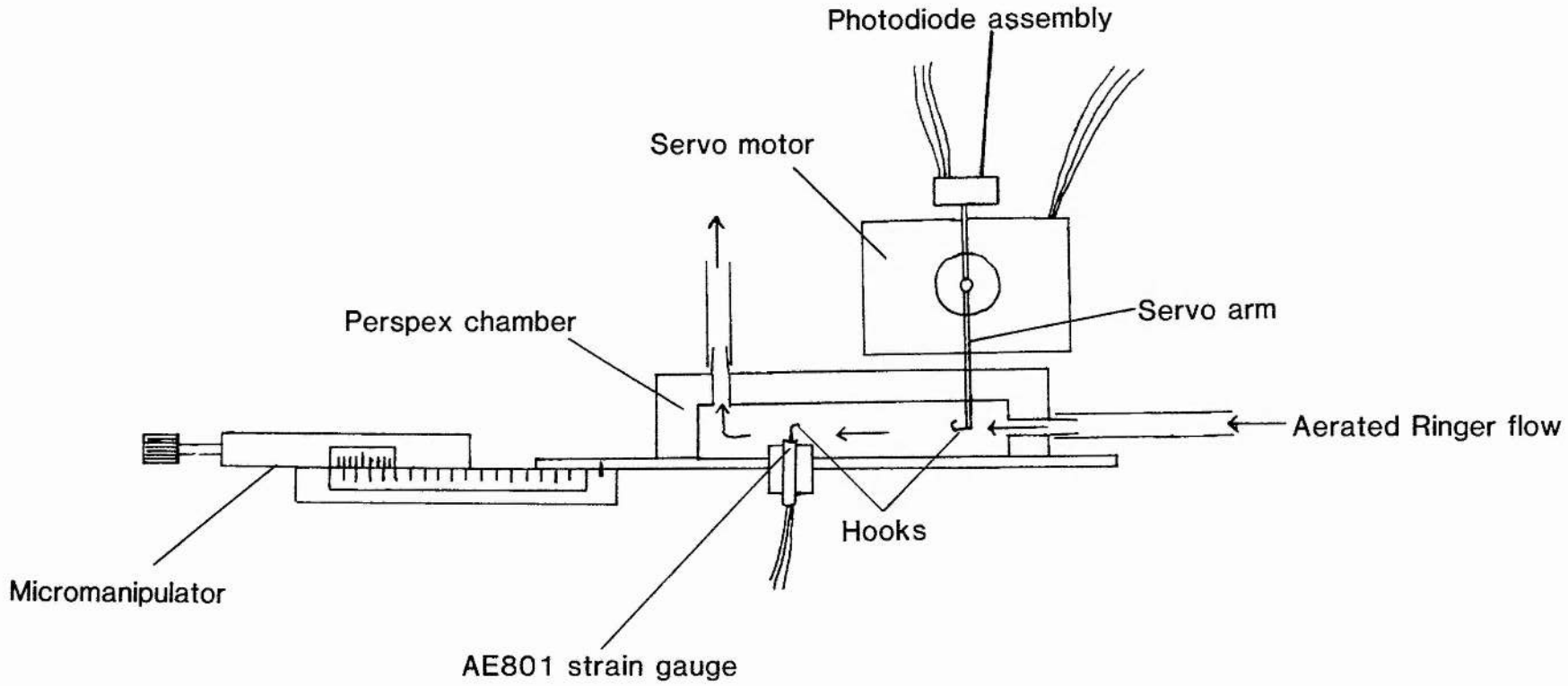
clips were attached to the peritoneum close to the fibre insertions.

Measurement of contractile properties

The preparation was transferred to the experimental chamber and the foil clips placed onto stainless steel hooks, one of which was attached to a strain gauge (AE 801, AME Horten, Norway), the other to a servo motor (MFE model R4-077, Emerson Electronics, Bourne End, Bucks.), controlled by a unit built in the laboratory. This apparatus, shown in Figure 2:1, was used to control and measure the length of the preparation during isovelocity releases. Aerated Ringer circulated constantly through the perspex experimental chamber, and temperature was controlled to ±0.1° C. The preparation was stimulated via two platinum wire electrodes using 1.5ms pulses at 1.2x the voltage generating maximum tension. The sarcomere length of the preparation (measured by laser diffraction) was set to 2.3μm at the beginning of an experiment (this length gave a maximal twitch response). Data were collected and analysed on a Nicolet 3091 digital oscilloscope and stored on a BBC microcomputer.

The effects of temperature on isometric properties were studied by varying the temperature of the Ringer in the chamber and giving a single twitch and tetanus at 10 minute intervals. Over a range of 1-18° C the effects were reversible and so force records from each preparation could be obtained over several cycles of heating and cooling.

FIGURE 2:1. Mechanical apparatus used for experiments. The micromanipulator allows the distance between the hooks to be adjusted. The photodiode assembly permits measurement of the movement of the servo arm.


Photodiode assembly
Servo motor
Servo arm
Perspex chamber
Aerated Ringer flow
Hooks
Micromanipulator
AE801 strain gauge

Maximum twitch and tetanic tensions were measured from each record. Rates of rise and relaxation of tension were measured as the time taken to rise to half maximal tension ($T_{0.5a}$) and to relax to half maximal tension ($T_{0.5r}$). The method is illustrated in Figure 2:2a.

The force-velocity (P-V) relationship was determined by measuring contraction velocity at various loads by means of iso-velocity releases given during the plateau phase of tetanus (Figure 2:2b and detailed by Altringham & Johnston, 1988). P-V curves were obtained at 3 temperatures, but each preparation was studied only at a single temperature.

Fitting of P-V data

P-V data for individual fibres were fitted to Hill's (1938) hyperbolic function:

$$V=b(P_o+a)/(P+a)-b$$

where V is velocity, P is tension, P_o is maximum tension and a and b are constants. The equation was linearised as:

$$V=CZ-b$$

where $C=b(P_o+a)$ and $Z=1/(P+a)$.

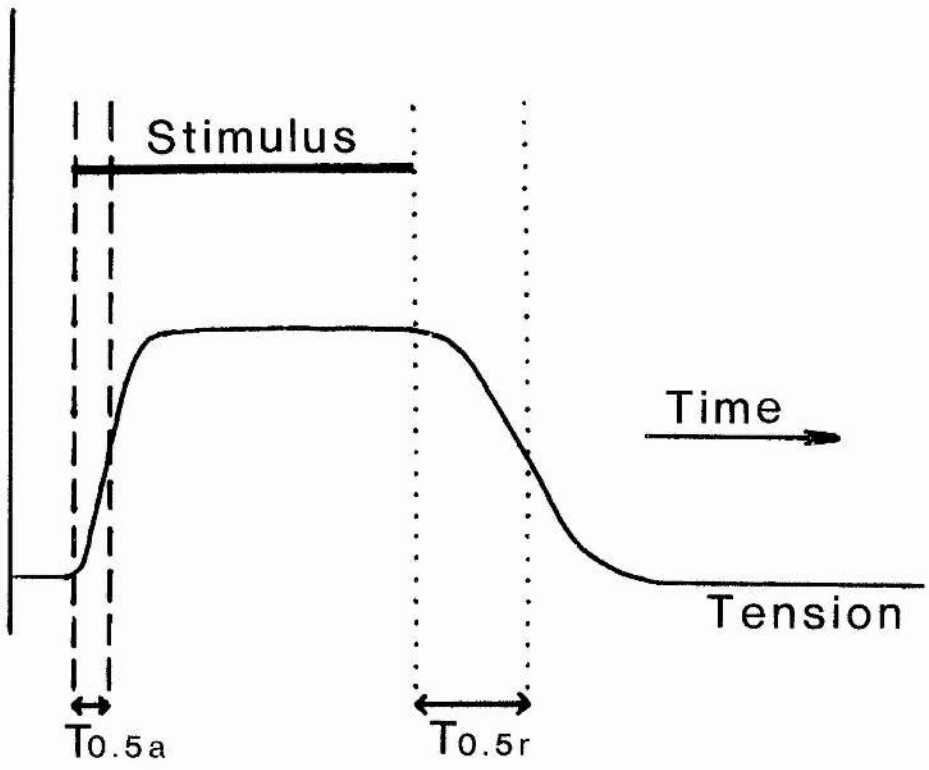
A least squares regression was iteratively fitted to the data by computer, without constraining the curve to go through P_o. Starting with a wide range of values for each constant and stepping through them with progressively narrower ranges and smaller increments, values giving the minimum mean squared differences between observed and predicted data were obtained (Altringham & Johnston, 1988).

FIGURE 2:2.

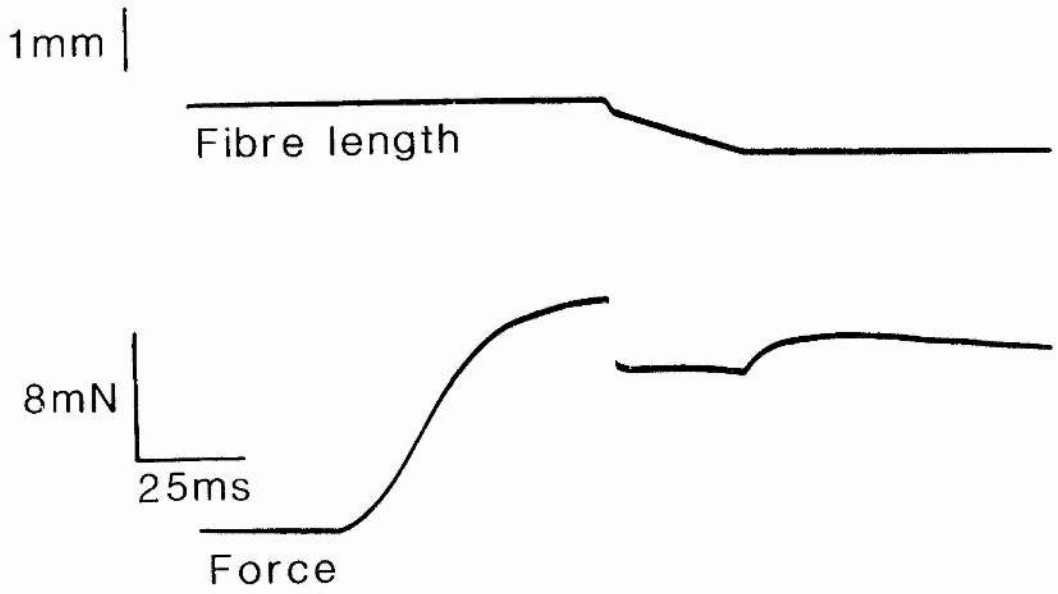
a. Measurement of rate of rise ($T_{0.5a}$) and relaxation ($T_{0.5r}$) of tension. $T_{0.5a}$ is time taken to reach half maximum tension, time measured from start of stimulus. $T_{0.5r}$ is time taken for tension to fall to half maximum tension, measured from end of stimulus.

b. Iso-velocity release of sculpin muscle preparation to illustrate "load clamping" method.

a


Stimulus
Time
Tension
T0.5a
T0.5r

b


1mm
Fibre length
8mN
25ms
Force

In fitting to Hill's equation, data above $0.8P_o$ were omitted, since it has been shown that they consistently deviate from the curve (Edman *et al.*, 1976). The curvature of the P–V relation is inversely related to a/P_o.

The data were also iteratively fitted, in the same way as for Hill's equation, to a hyperbolic–linear (hyp–lin) curve described by Marsh & Bennett (1986):

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

where A is dimensionless and B and C have dimensions of velocity. The entire data set was fitted since data above $0.8\ P_o$ fell on the fitted line. The ratio $\dot{W}_{max}/V_{max}.P_o$, where $\dot{W}_{max}$ is maximum power output, is inversely related to the curvature of the P–V relation, and has been calculated using values derived from both curve fitting procedures.

Statistical analysis

The standard error of the estimate (SEE) for both curve fitting procedures was determined for each fibre from the equation:

$$SEE = \sqrt{RSS/n-2},$$

where RSS = residual sum of squares. The results derived using the two equations were compared with a paired "t" test. Estimates of maximum contraction velocity derived from the two methods were also compared with a paired "t" test. Changes in the curvature of the P–V relation with temperature were tested for with a standard "t" test.

RESULTS

Isometric properties

Representative twitch and tetanus records at various temperatures are shown in Figure 2:3. The force plateau was not maintained during tetanus above 15° C, and preparations deteriorated irreversibly above 18° C. Fused tetani were obtained at 40–50Hz at 1° C, but fusion frequency increased with temperature to 75Hz at 18° C. Both maximum tetanic tension and twitch tension increased with temperature up to a maximum at around 8° C, then decreased as temperature was increased further (Figure 2:4a,b). Although changes in twitch and tetanic tension both showed the same overall pattern, the magnitude of the change varied, giving the variation in twitch–tetanus ratio shown in Figure 2:4c. Evidently, twitch tension is more susceptible to temperature than tetanic tension.

Rates of rise and relaxation of tension were measured as the time taken to rise to half maximal tension ($T_{0.5a}$) and to relax to half maximal tension ($T_{0.5r}$). $T_{0.5a}$ and $T_{0.5r}$ for twitch and $T_{0.5r}$ for tetanus decreased with temperature, Q_{10} (2–12° C) = 1.8–2.0. In contrast, $T_{0.5a}$ for tetanus was relatively independent of temperature (Figure 2:5). Isometric properties are summarised in Table 2:1.

FIGURE 2:3. Isometric force records from a single preparation (12 fibres) at a series of temperatures. Each record shows a single twitch and tetanus.

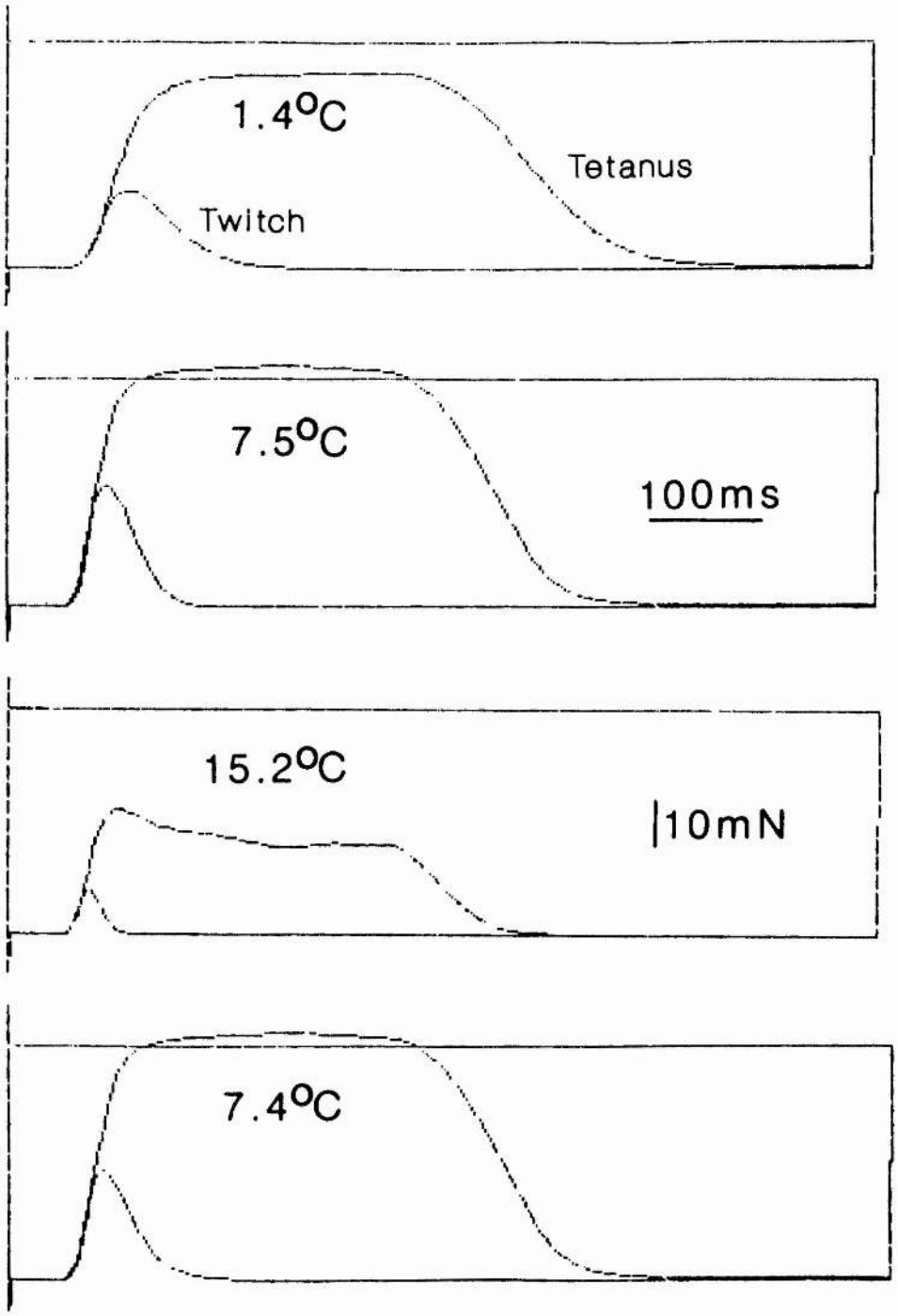

1.4°C
Tetanus
Twitch
7.5°C
100ms
15.2°C
10mN
7.4°C

FIGURE 2:4.

a. Twitch tension plotted against temperature. Different symbols identify different preparations, data normalised to maximum tension.

b. Effects of temperature on tetanic tension.

c. Effects of temperature on twitch-tetanus ratio.

Data±S.E. and grouped in 2° C intervals.

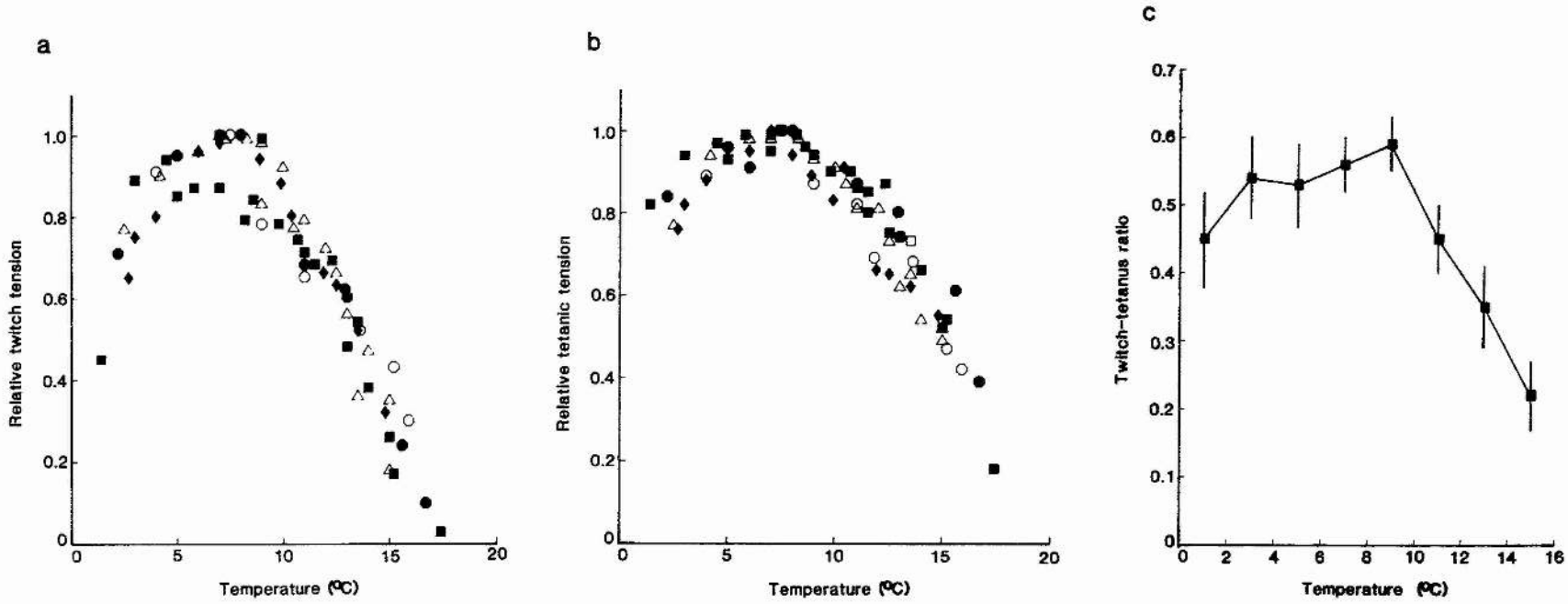

a
Relative twitch tension
Temperature (°C)
b
Relative tetanic tension
Temperature (°C)
c
Twitch–tetanus ratio
Temperature (°C)

FIGURE 2:5. Half rise and relaxation times plotted against temperature. Data±S.E. and grouped in 2° C intervals, a. tetanus, b. twitch.

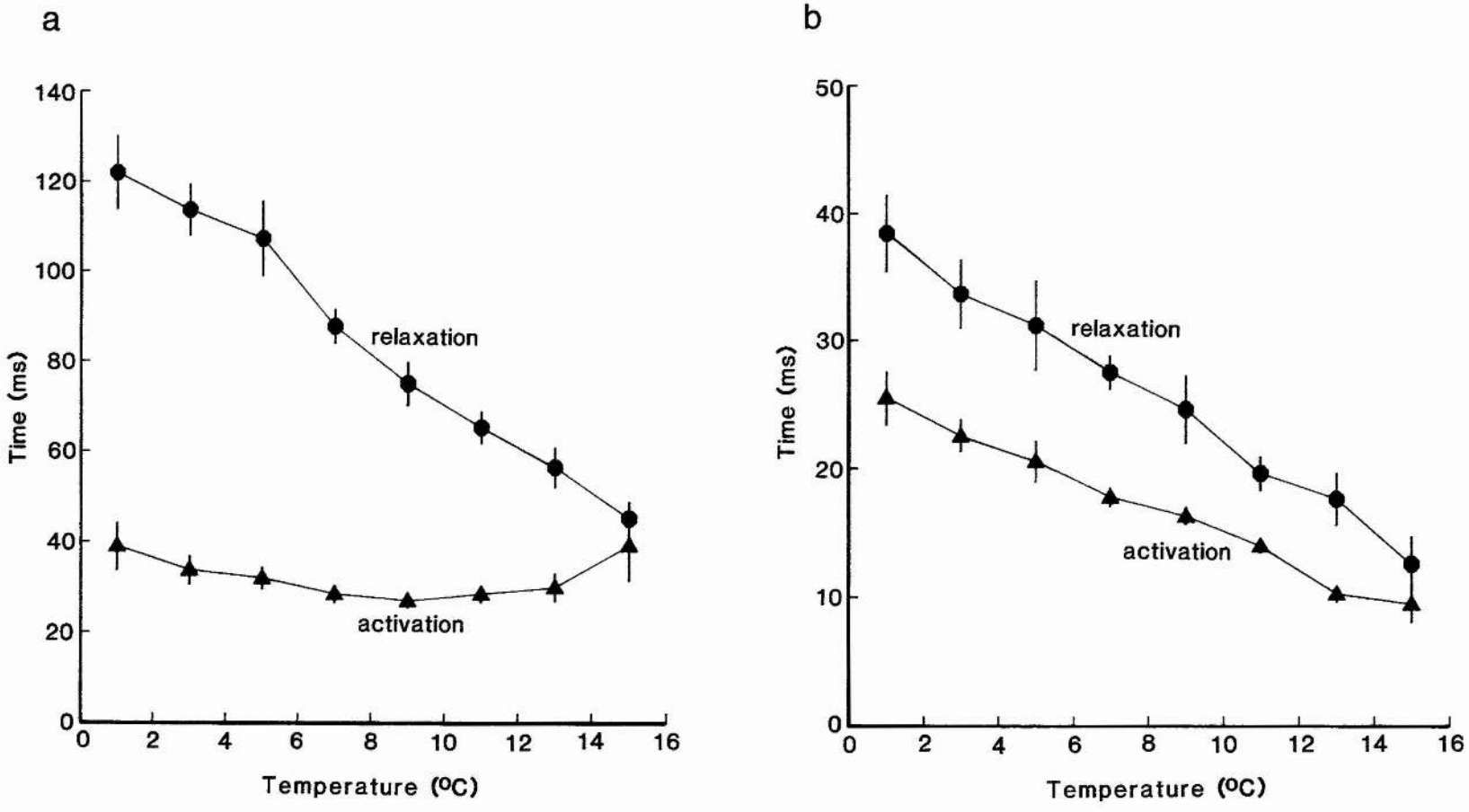

a
140
120
100
80
60
40
20
0
Time (ms)
relaxation
activation
0
2
4
6
8
10
12
14
16
Temperature (°C)
b
50
40
30
20
10
0
Time (ms)
relaxation
activation
0
2
4
6
8
10
12
14
16
Temperature (°C)

TABLE 2:1. Contractile properties of fast myotomal muscle fibres.

	2° C	8° C	12° C	16° C
Maximum isometric tension (kNm^{-2})	242	315	255	172
Twitch-tetanus ratio	0.55±0.07	0.66±0.04	0.55±0.05	0.32±0.05
Tetanic fusion	50Hz	60Hz	65Hz	75Hz
Half twitch rise time (ms)	25.5±2.1	17.7±0.7	13.9±0.47	9.4±1.4
Half twitch relaxation time (ms)	38.4±3.0	27.4±1.3	19.5±1.3	12.5±2.1
Half tetanus rise time (ms)	39.0±5.1	28.3±1.8	28.2±1.8	38.7±7.7
Half tetanus relaxation time (ms)	121.9±8.1	87.1±3.7	64.6±3.4	44.6±3.6

Values represent mean±S.E., N=5. Maximum isometric tensions are extrapolated from tension derived at 3° C in a previous study (Altringham & Johnston, 1988).

Force-velocity relation

The P-V relationship was studied at 1° C, 8° C (optimum) and 12° C. The results from both curve-fitting procedures, for each temperature, are summarised in Table 2:2, and representative P-V curves shown in Figures 2:6 (Hill's) and 2:7 (hyp-lin). A better fit to the data was achieved using the hyp-lin equation ($P<0.01$, Table 2:2).

V_{max} was calculated using both Hill's and the hyp-lin equation, values being 4.08, 7.10 and 8.10 Ls^{-1} (Hill's) and 4.27, 8.14 and 9.46 Ls^{-1} (hyp-lin) at 1, 8 and 12° C respectively. V_{max} values calculated using the hyp-lin equation were significantly higher at all temperatures (Table 2:2), and exhibited less scatter than values from Hill's equation. Q_{10} values for V_{max} were 1.82 for Hill's and 2.01 for the hyp-lin equation.

The P-V relation becomes significantly more curved with increasing temperature (see Table 2:2). a/P_o is inversely related to the degree of curvature and decreased from 0.32 at 1° C, to 0.27 at 8° C and 0.24 at 12° C. Similarly the value for $\dot{W}_{max}/V_{max}.P_o$ derived from the hyp-lin plot decreased with increasing temperature, from 0.136 at 1° C, to 0.126 at 8° C and 0.119 at 12° C. $\dot{W}_{max}/V_{max}.P_o$ values from Hill's equation also decreased between 1° C and 12° C (Table 2:2).

TABLE 2:2. Data represent mean±S.E., N=6. V_{max} is extrapolated maximum contraction velocity, $\dot{W}_{max}$ is maximum power output, a and b are constants from the Hill equation, A, B and C constants from the hyp-lin equation. Ls^{-1} is muscle lengths per second. *=P<0.05, **=P<0.01, ^{ns}=not significant. In the first two sections of the table, values at 8° C and 12° C have been compared to those at 1° C. In the third section, the standard error of the mean (SEE) derived from the two fitting procedures are compared at each temperature. The hyp-lin equation yields significantly higher V_{max} values than Hill's equation at all temperatures (1° C, P<0.05; 8° C and 12° C, P<0.01).

TABLE 2:2. Summary of force-velocity data at three temperatures.

	1° C	8° C	12° C
HILL'S EQUATION			
V_{max} (Ls^{-1})	4.08±0.05	7.10±0.2	8.10±0.20
$\dot{W}_{max}$ (Wkg^{-1})	123	256	223
Load for max. power output	0.31P_o	0.30P_o ns	0.28P_o *
a/P_o	0.27±0.02	0.22±0.02 ns	0.17±0.02**
b (Ls^{-1})	0.98±0.08	1.35±0.12	1.17±0.10
$\dot{W}_{max}/V_{max}.P_o$	0.125±0.002	0.115±0.003*	0.109±0.004**
r^2	0.98	0.98	0.96
HYP-LIN EQUATION			
V_{max} (Ls^{-1})	4.27±0.08	8.14±0.22	9.46±0.16
$\dot{W}_{max}$ (Wkg^{-1})	140	313	292
Load for max. power output	0.44P_o	0.47P_o**	0.50P_o**
A	0.10±0.02	0.06±0.01	0.04±0.01
B (Ls^{-1})	0.26±0.05	0.27±0.02	0.21±0.05
C (Ls^{-1})	1.60±0.14	3.22±0.13	3.90±0.22
$\dot{W}_{max}/V_{max}.P_o$	0.136±0.002	0.126±0.002**	0.119±0.002**
r^2	0.99	0.99	0.99
SEE			
Hill's	0.027±0.004	0.041±0.005	0.055±0.007
Hyp-lin	0.010±0.001**	0.012±0.002**	0.019±0.007**

FIGURE 2:6. Force-velocity data from single, representative preparations fitted to Hill's equation. Data above $0.8P_o$ were omitted and the curve was not constrained to pass through P_o. a/P_o is 0.27 at 1° C, 0.22 at 8° C and 0.17 at 12° C.

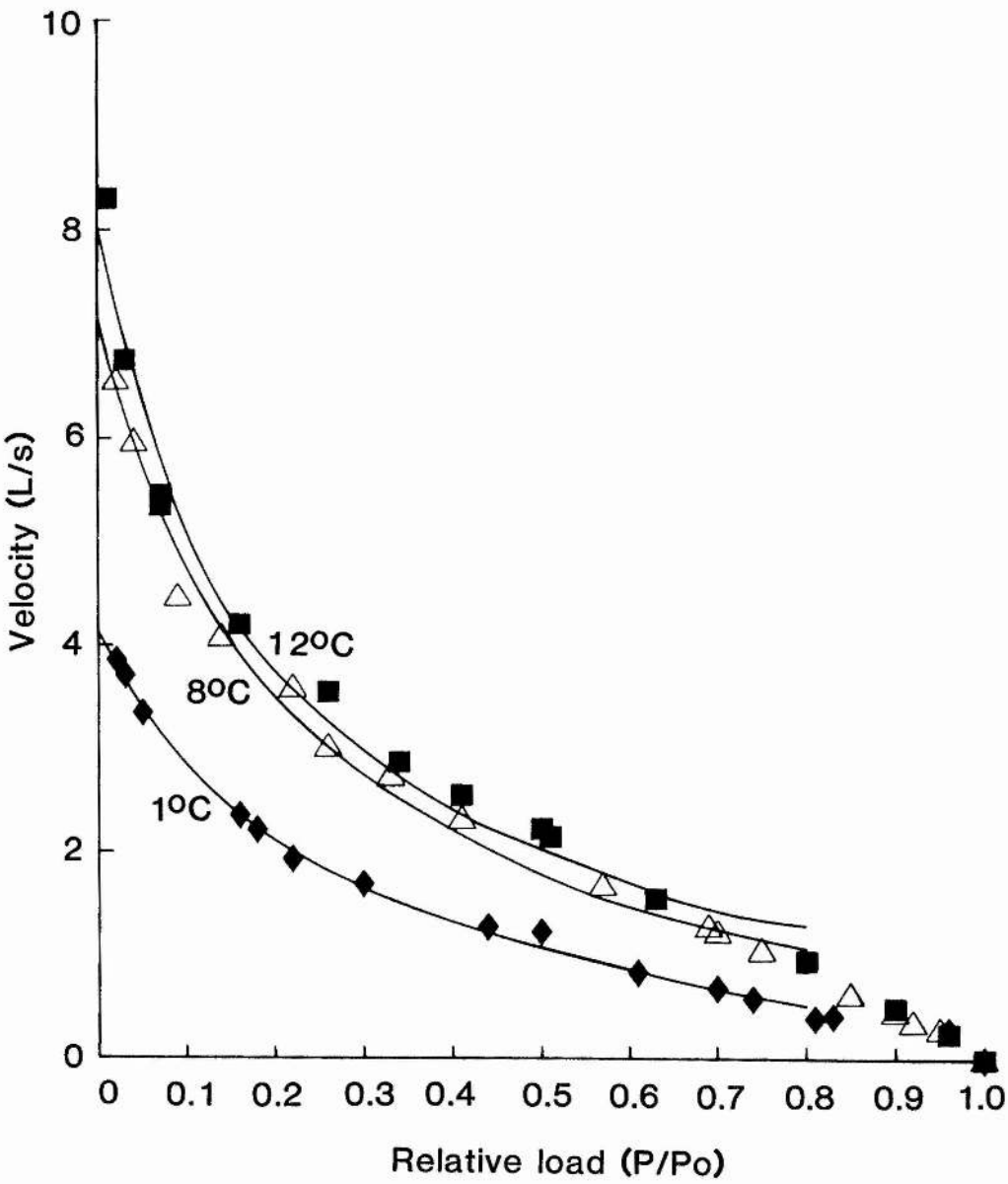
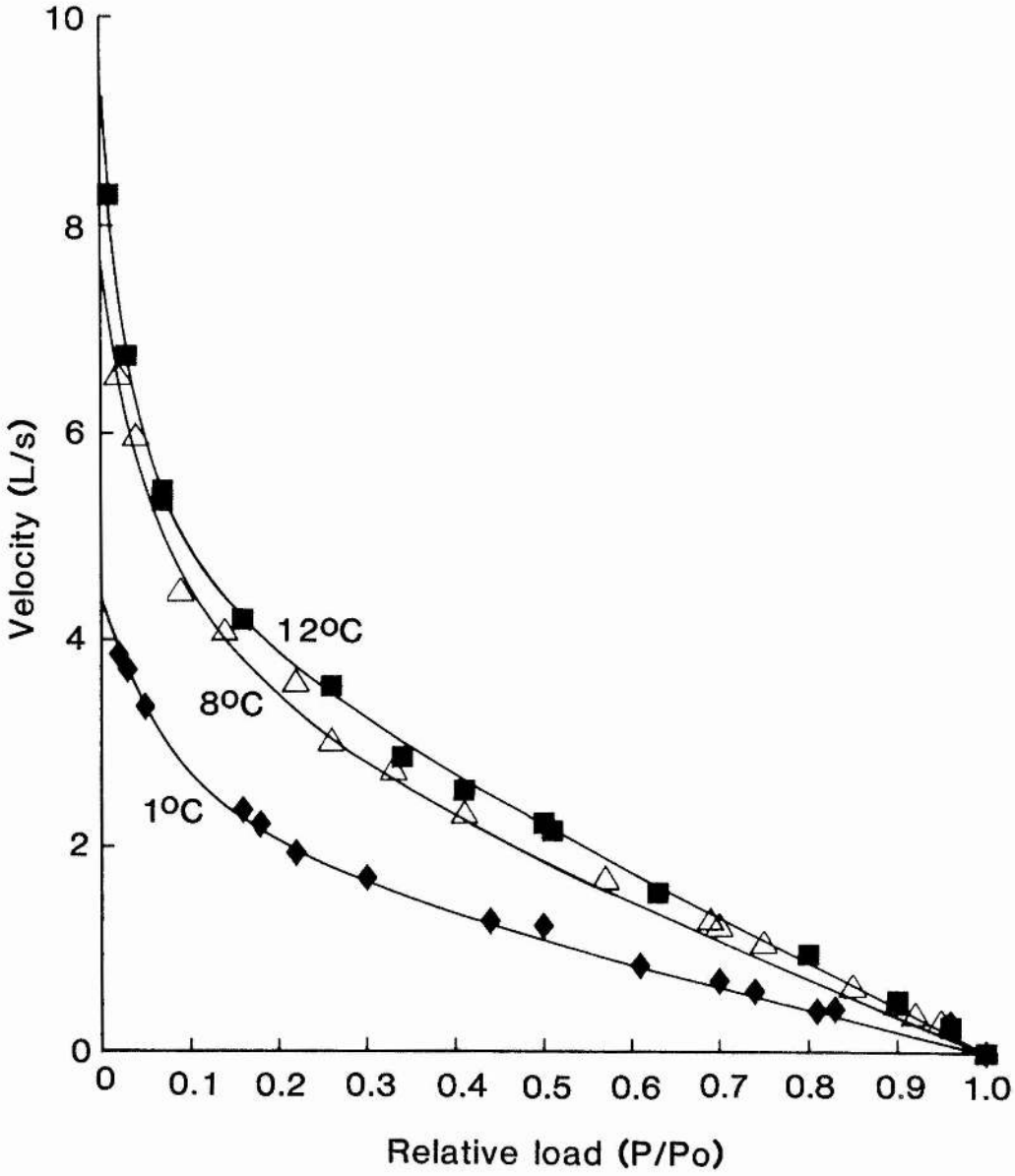

10
8
6
4
2
0
Velocity (L/s)
12°C
8°C
1°C
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Relative load (P/Po)

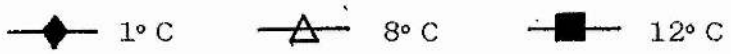
FIGURE 2:7. Data from preparations used for Figure 3 fitted to the hyp-lin equation. The entire data sets were fitted. $\dot{W}_{max}.V_{max}/P_o$ is 0.136 at 1° C, 0.126 at 8° C and 0.119 at 12° C.


10
8
6
4
2
0
Velocity (L/s)
12°C
8°C
1°C
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)

Power output

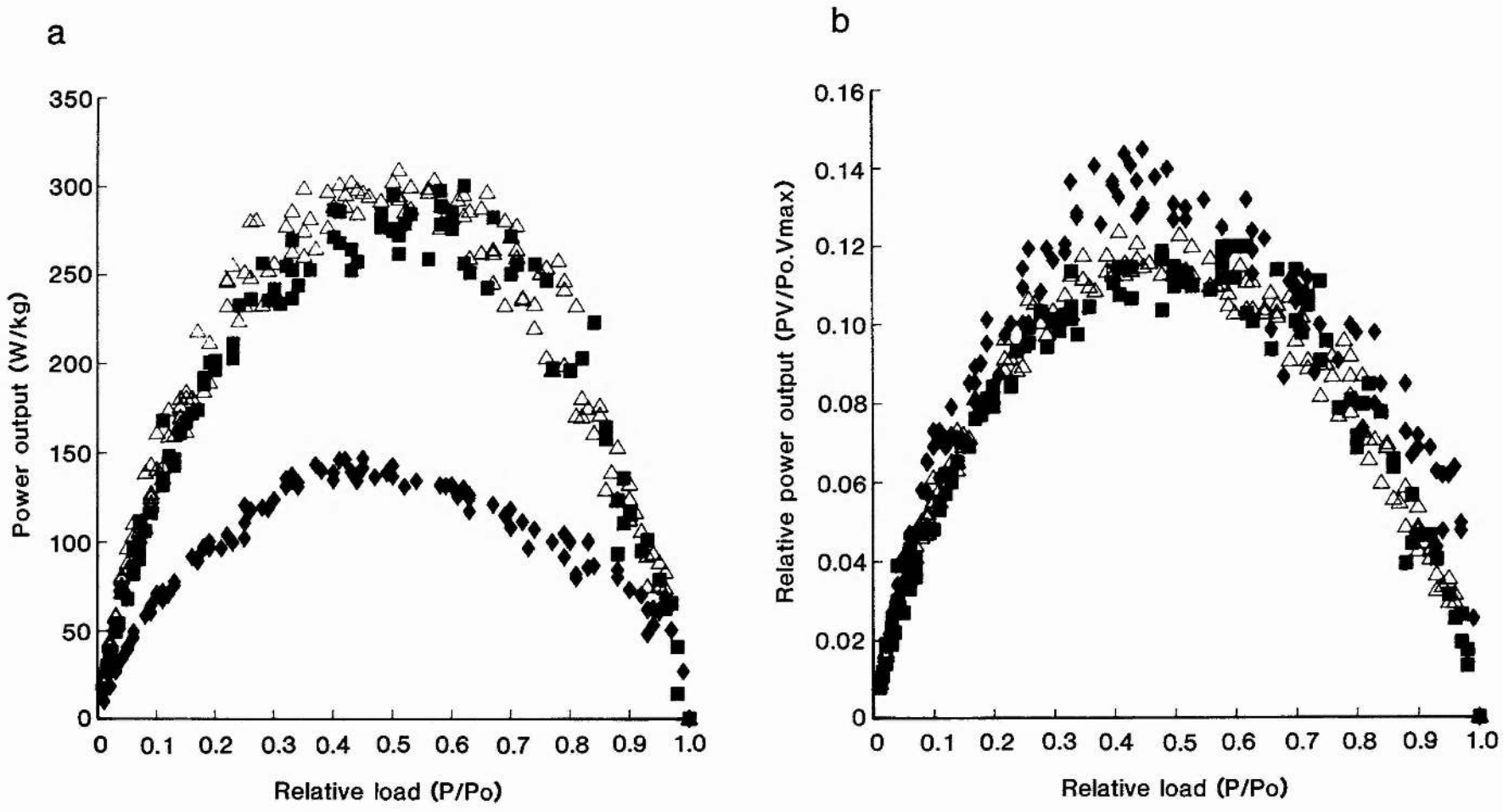
P-V data were used to plot power output (PxV) against load for all temperatures for all fibres. In Figure 2:8 absolute power output (a) has been plotted alongside curves normalised for temperature induced changes in both force and velocity (b). Power output was calculated using the hyp-lin equation, and a mean value for absolute tension (normalised to cross sectional area) derived in a previous study on this species (Altringham and Johnston, 1988). In this way, differences between preparations were normalised so that data from all fibres could be plotted on the same graph. Figure 2:8b shows the change in power output due only to changes in the curvature of the P-V relation. The curvature change between 8° C and 1° C increased the relative power output at 1° C by around 15%. The load at which maximum power was obtained, calculated using the hyp-lin equation, increased with increasing temperature, from $0.44P_o$ at 1° C to $0.50P_o$ at 12° C ($P<0.01$, Table 2). This trend is readily apparent in the raw data, shown in Figure 2:8a. In contrast, the load for maximum power output calculated using Hill's equation decreases slightly, but significantly, with increasing temperature.

FIGURE 2:8



a. Power output calculated using parameters derived from fitting with the hyp-lin equation plotted against relative load at 3 temperatures.

b. Power output normalised for temperature induced changes in force and velocity plotted against relative load, $(P.V/P_o.V_{max})$ v (P/P_o).


a
Power output (W/kg)
350
300
250
200
150
100
50
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
Relative power output (PV/Po.Vmax)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
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)

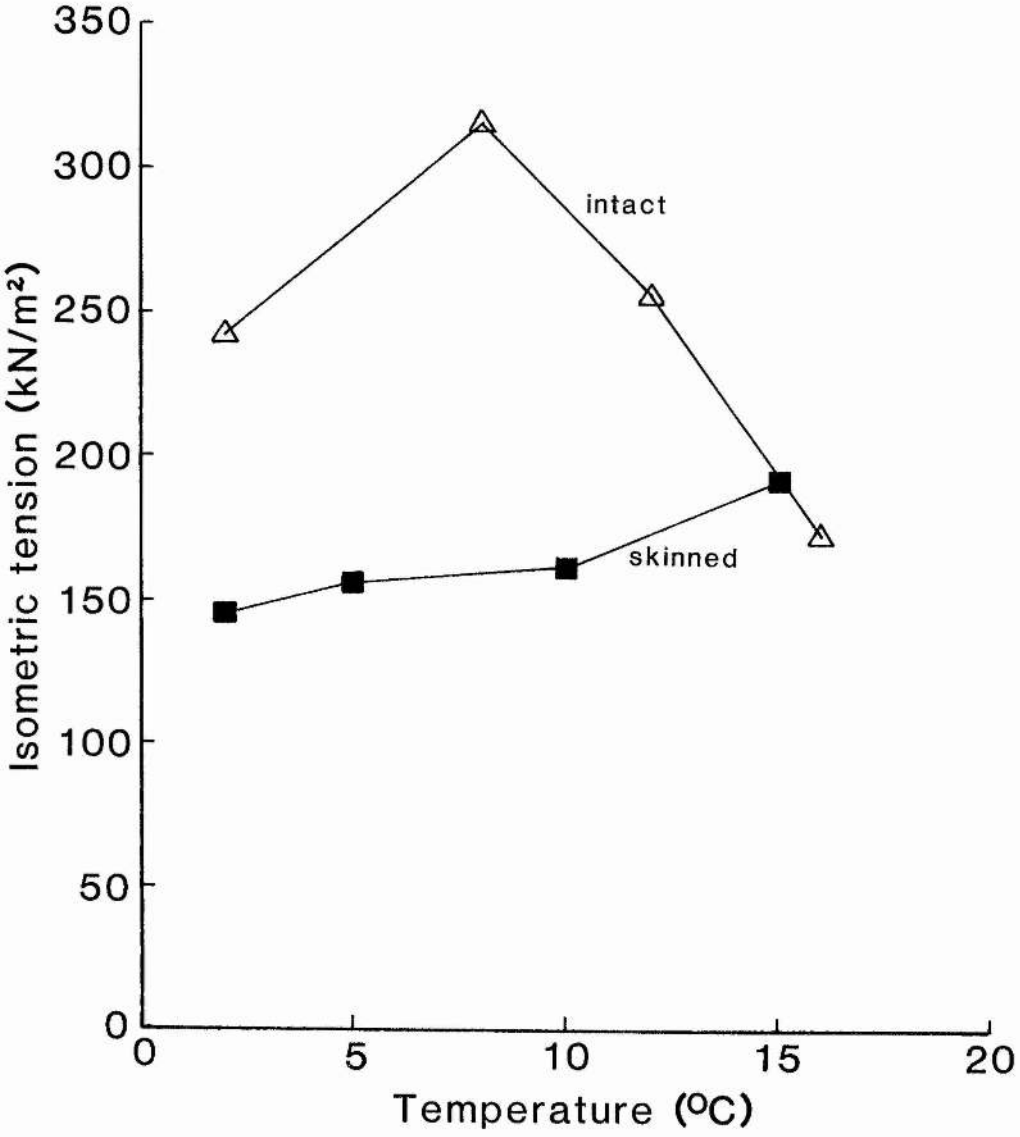
DISCUSSION

Isometric properties.

In sculpin live fibre preparations both twitch and tetanic tensions show a clear optimum at around 8-10° C, the midpoint of the normal environmental temperature range (ET). This is in marked contrast to results from skinned fibre preparations from fish where maximum tension increases with increasing temperature, or is largely temperature independent, over the ET of each species (Johnston & Sidell, 1984; Johnston & Altringham, 1985; 1988; Altringham & Johnston, 1986). Figure 2:9 compares data from this study with skinned fibre data from the same species obtained by Johnston & Sidell (1984). The experiments on skinned fibres indicate that the force-generating mechanism itself is relatively independent of temperature. The decline in tension observed at high temperatures in intact preparations suggests a marked temperature dependence, or failure, of excitation-contraction coupling.

The temperature range over which isolated live fibres remain viable is correlated with body temperature. Fibres from the Antarctic icefish (ET -1.9 to 2° C), rapidly deteriorate above 10° C (Johnston, 1987). In contrast, fibres from the desert iguana (preferred body temperature (PBT) 40° C) give stable contractions at 22-44° C, but tension declines with time below 15° C (Marsh & Bennett, 1986). Phylogenetic dissimilarities complicate the interpretation

FIGURE 2:9. Changes in maximum isometric tension over the environmental temperature range of *Myoxocephalus scorpius* for fast skinned and intact muscle fibres from this species. Skinned fibre data from Johnston & Sidell, (1984), intact fibre data from this work.


350
300
250
200
150
100
50
0
Isometric tension (kN/m²)
intact
skinned
0
5
10
15
20
Temperature (°C)

of interspecific adaptations in contractile properties. To overcome these problems John-Alder & Bennett (1987) investigated muscle contractile properties in 3 genera of Australian skinks. The result indicated that even relatively small differences in average body temperature are sufficient to produce resistance type adaptations in muscle contractility. Translational capacity adaptations are also evident in maximum tetanic tension (Precht, 1958). They can be seen both within Classes, *e.g.* in reptiles (Bennett, 1985; John-Alder & Bennett, 1987) and between Classes. As an example of the latter, maximum tetanic tension for fast muscle fibres occurs at 5° C in the icefish (ET -1.9 to 2° C) (Johnston, 1987), 20° C in the tiger salamander (ET 5 to 25° C) (Else & Bennett, 1987) and at 40° C in the desert iguana (PBT 40° C) (Marsh & Bennett, 1986). With an optimum tension at 8° C (ET 2 to 17° C), the sculpin fits appropriately into this sequence (Figure 2:10).

Maximum contraction velocity.

V_{max} values for live preparations from the sculpin were higher than those calculated for skinned fibres by Johnston & Sidell (1984) and compare well with data from other vertebrate fast muscles (Table 2:3). Q_{10} values of around 2 are similar to those for other live fibre preparations from *Rana* (Edman, Mulieri & Scubon-Mulieri, 1976) and *Xenopus* (Lännergren, Lindblom & Johansson, 1982). V_{max} was found to be more temperature dependent at the lower end of the

FIGURE 2:10. The relationship between normalised maximum isometric tension and experimental temperature. The bars indicate the normal environmental temperature range experienced by these aquatic animals, sculpin —●— (data from this work), icefish *Chaenocephalus auratus* —◊— (Johnston, unpublished), tiger salamander *Ambystoma tigrinium nebulosum* —△— (Else & Bennett, 1987). The arrow indicates the preferred body temperature (PBT) of the desert iguana *Dipsosaurus dorsalis* —■— (Marsh & Bennett, 1985).

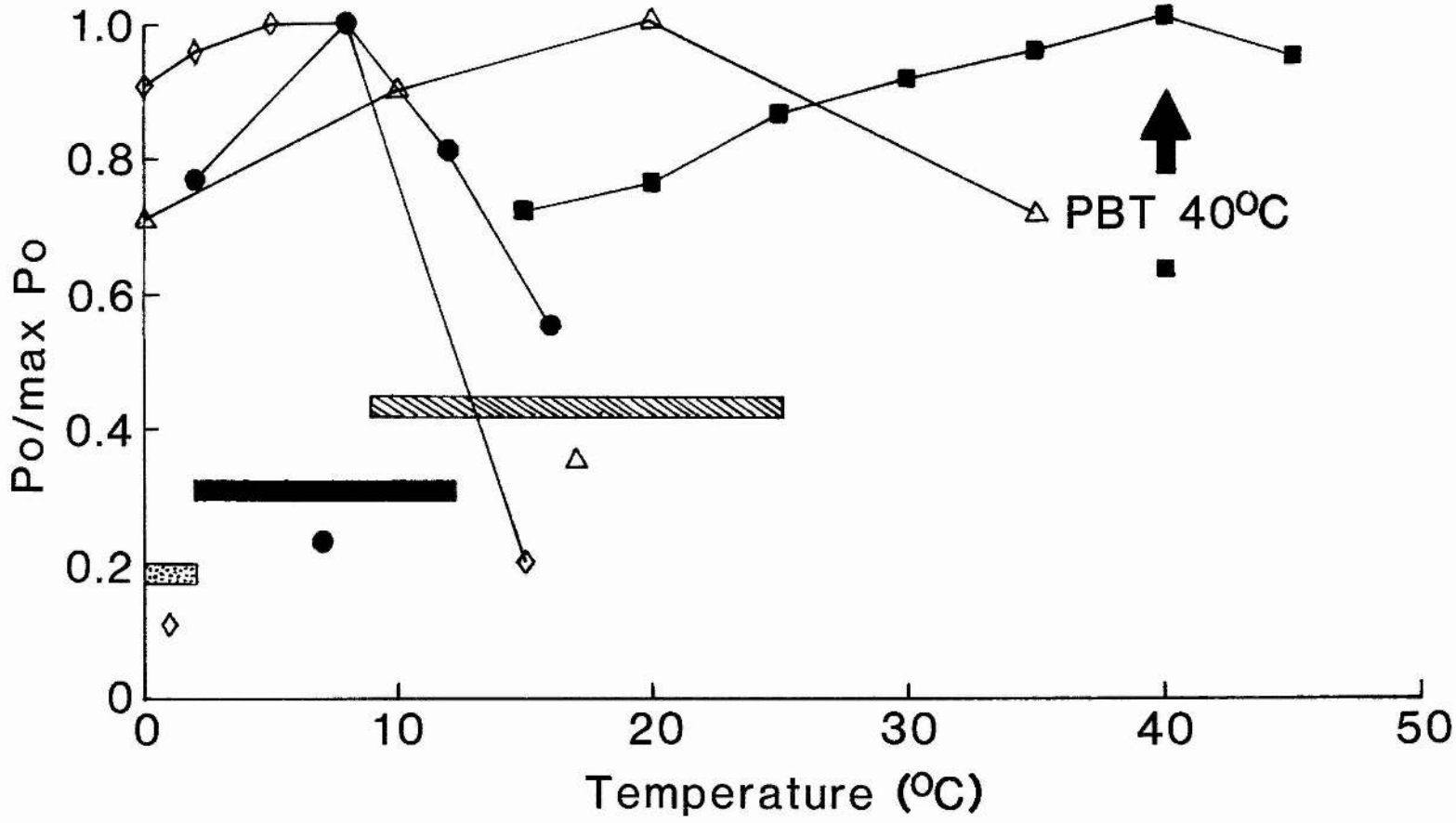

1.0
0.8
0.6
0.4
0.2
0
Po/max Po
0
10
20
30
40
50
Temperature (°C)
PBT 40°C

TABLE 2:3. Summary of Vmax and a/Po determinations of fast muscle fibres taken from the literature.

	V_{max} (Ls^{-1})	a/P_o	Experimental details
Fish			
Dogfish[1]	4.8	0.274	white myotomal intact
Tilapia mossambica[2]	2.57	–	18° C intact opercular muscle bundles
Sculpin[3]	0.55	0.064	2° C skinned myotomal
Amphibia			
Rana temporaria[4]	1.29	0.26	0° C, whole sartorius
Rana temporaria[5]	4.05	0.216	4° C, intact fibres of anteror tibialis
Xenopus laevis[6]	6.34	0.38	22° C intact single fibres of iliofibularis
Ambystoma tigrinium nebulosum[7]	3.01	0.352	20° C whole iliotibialis pars anterior
Reptiles			
Desert Iguana[8]	18.7	–	40° C intact bundle of iliofibularis
Mammals			
Rat[9]	43µm sarcomere^{-1}	0.25	35° C whole fast twitch

References: 1. Curtin & Woledge, 1988. 2. Flitney & Johnston, 1979. 3. Sidell & Johnston, 1984. 4. Hill, 1938. 5. Julian *et. al.*, 1986. 6. Lännergren, 1978. 7. Else & Bennett, 1987. 8. Marsh & Bennett, 1985. 9. Close, 1964.

studied temperature range, as reported in amphibia (Lännergren *et al.*, 1982) and reptiles (*e.g.* John-Alder & Bennett, 1987).

P-V relation.

The hyp-1in equation provides a better description of the P-V data for intact fibres than Hill's equation, in agreement with previous results on intact myotomal fibres (Altringham & Johnston, 1988) and on reptilian muscles (Marsh & Bennett, 1986). Data derived from skinned fibres, however, could be adequately fitted to Hill's equation, as long as data above $0.8P_o$ was omitted and the curve was constrained to pass through P_o. This better fit to Hill's equation of data obtained from skinned fibres than that from intact fibres is also found in frog muscle (Julian *et al.*, 1986).

The extrapolated value of V_{max} from the hyp-1in equation was significantly higher than that from Hill's equation. The fitted line for Hill's equation consistently fell below the data at very low loads causing an underestimation of V_{max}. Deviation of the data from the fitted line at high loads, first noted by Edman *et al.* (1976), was also evident. This has also been seen in a previous study of sculpin fast muscle (Altringham & Johnston, 1988) and in other vertebrate muscle, *e.g. Xenopus laevis* (Lännergren *et al.*, 1982). Edman (1988) has recently

studied this part of the P–V curve in detail in the frog *Rana temporaria*. He demonstrated that the P–V relation undergoes a clear inflection around $0.8P_o$. The data above and below this load were fitted to two separate hyperbolic curves. Edman (1988) suggests that cross bridge kinetics change at this point, and proposes that either the rate of cross-bridge attachment decreases, as a consequence of the high density of attached bridges, or a high proportion of bridges enter a low force state. P–V data from sculpin live fibres also show an inflection above $0.8P_o$, but undergo a reversal of curvature at high loads (Altringham & Johnston, 1988).

The P–V relation of live fibres became more curved with increasing temperature, a characteristic also observed in fish skinned fibres (Johnston & Altringham, 1985; Johnston & Wokoma, 1986) and in carp intact red muscle fibres (Rome & Sosnicki, 1990). In contrast, curvature has been shown to be independent of temperature in all other ectotherms studied, *e.g.* in amphibia (Lännergren, 1978; Edman, 1988) and reptiles (Marsh & Bennett, 1985; 1986; Else & Bennett, 1987; John-Alder & Bennett, 1987; Mutungi & Johnston, 1987), with the exception of *Xenopus* type 1 fibres (Lännergren *et. al.*, 1982). Values of a/P_o obtained for live sculpin fibres are much higher than those obtained for skinned fibres from the same species, Johnston & Sidell (1984) obtained values of around 0.06, but the values obtained in this study lie within the range found in other vertebrate muscles (Table 2:3).

Muscle power output

A less curved force-velocity relation (ie. higher a/P_o or $\dot{W}_{max}/V_{max}.P_o$) yields a higher velocity, and thus a greater power output, for a given load. When calculated from Hill's equation maximum power is produced at lower force as curvature increases (Woledge, Curtin & Homsher, 1985). In contrast, the power curves obtained using the hyp-lin equation show maximum power to be produced at lower forces as curvature decreases (Table 2:2, Figure 2:8).

The change in curvature of the P-V relation in intact fish fibres may be a mechanism for offsetting the decrease in power output induced by decreasing temperature. The observed change in curvature between 8 and 1° C increased the relative maximum power output by about 15% (Figure 2:8b). A similar effect has since been noted in carp red fibres by Rome & Sosnicki (1990), the change in curvature between 20° C and 10° C increasing power output by 16%. When compared at their normal environmental temperatures, muscle fibres from fish adapted to cold environments have a less curved P-V relation than those from warm-water species (Johnston & Altringham, 1985). Thus, although V_{max} shows no systematic capacity adaptation between fish from different thermal environments (Johnston & Altringham, 1985), changes in the curvature of the P-V relation may contribute to capacity adaptations in muscle power output and efficiency. However, this mechanism must be of secondary importance to the complete capacity adaptation of maximum isometric tension

observed in fibres of fish from different thermal environments (Johnston & Altringham, 1985; 1988; Altringham & Johnston, 1986).

CHAPTER 3

The myology of the pectoral fin of the common carp *Cyprinus carpio* L. and the variation in fibre composition with temperature acclimation.

INTRODUCTION

Most fish initially respond to a change in temperature by a corresponding change in metabolic rate. During acclimation, compensation of metabolic rate changes occur, sometimes accompanied by compensation of locomotory activity. Species of fish that acclimate tend to show an enhanced capacity for aerobic energy supply following cold-acclimation (Hazel & Prosser, 1974), ensuring a relative constancy of energy supply for locomotion. Locomotory performance is also highly dependent on the contraction rates of the different muscle fibre types, so a study of acclimation responses must include measurements of the responses both between and within fibre types.

The maximal activities of some enzymes involved in aerobic energy pathways increase following cold-acclimation (Hazel, 1972; Sidell, 1980; Johnston *et al.*, 1985; Johnston & Wokoma, 1986), probably as a result of the higher volume density of mitochondria in cold-acclimated muscle (Johnston & Maitland, 1980; Egginton & Sidell, 1989). An increase in myofibrillar ATPase enzyme activity has also been seen

following cold-acclimation in several cyprinids (Johnston *et al.*, 1975; Altringham & Johnston, 1985; Heap *et al.*, 1985). Histochemical studies have shown that the numbers and size of oxidative fibre types increase during cold-acclimation (Johnston & Lucking, 1978, Jones & Sidell, 1982; Heap *et al.*, 1987). Measurement of dissection weights (Smit, Van den Berg & Klijne-Den Hartog, 1974) and proportions of biochemical constituents (Sidell, 1980) confirm that thermal acclimation can be accompanied by a change in the relative proportion of fibre types in addition to biochemical adaptations within each fibre type.

The common carp swims using distinct propulsive styles at different swimming speeds. Sustained low speed swimming, up to 1 bodylength/second (1/s), is achieved using the pectoral fins, with an occasional undulatory kick of the tail. Between 1 1/s and 2.5 1/s, the myotomal muscle is recruited, the fish being propelled by undulatory movements of the body and tail, with the pectoral fins acting to steer the fish. Above 2.5 1/s, the pectoral fins are tucked in to reduce drag (Johnston, unpublished data). So, in order to have good manoeuvrability, the muscles of the pectoral fin must maintain their functional efficiency over the full temperature range experienced by the fish. The structure of the entire pectoral assemblage of the carp was determined in this study. The structure of the *abductor superficialis* muscle of the pectoral assemblage was chosen for more detailed analysis because the muscle could be dissected out discretely, and the fibres within the muscle ran in a fairly

uniform direction, enabling accurate analysis of the cross-sectional area occupied by each fibre type.

MATERIALS & METHODS

Fish

Common carp (*Cyprinus carpio* L.) were obtained from Humberside Fisheries, Skerne Farm, Driffield, Hull. These were maintained in tanks with partly recirculated fresh water at 8° C and 20° C for 8 weeks (12h light: 12 dark). Fish were fed to satiation once daily with commercial fish pellets (Ewos Aquaculture Ltd.).

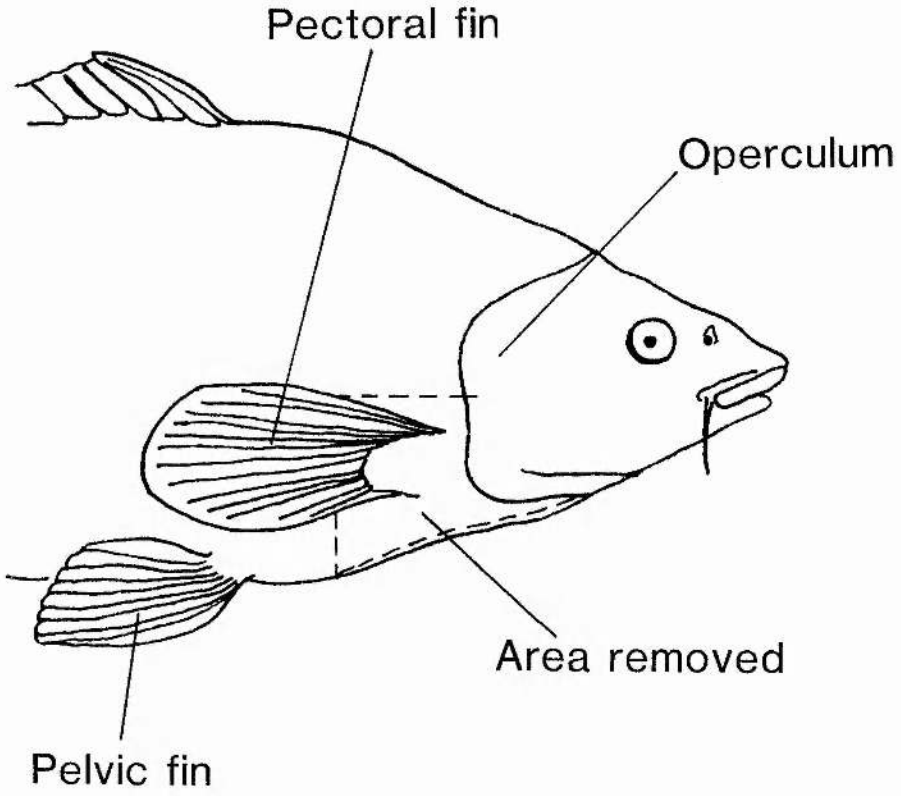
Fish were killed with a blow to the head, followed by decapitation. The mean length of the fish used was 17.1±0.5cm and the mean mass was 149±17g (±S.E., N=24).

Dissection of pectoral assemblage

The pectoral muscle assemblage was removed from the fish, the cleithrum being severed at the level of the lateral line (Figure 3:1). The assemblage and its associated skeleton was then pinned out on a silicone elastomer base (Sylgard 184, Dow Corning, Midland, MI, USA) and immersed in Carp Ringer's solution (in $mmol\,l^{-1}$: NaCl 119.0; sodium pyruvate 10.0; KCl 2.7; $NaHCO_3$ 2.5; $CaCl_2$ 1.8; $MgCl_2$ 1.0; pH 7.4 at 5° C).

The skin was removed from the assemblage and the muscles carefully dissected out to discern the structure of

FIGURE 3:1. The common carp *Cyprinus carpio* L. The dotted lines indicate where the pectoral assemblage was separated from the fish.


Pectoral fin
Operculum
Area removed
Pelvic fin

the assemblage. Drawings of the pectoral muscles in situ and of the pectoral skeleton were made. Where resolution was difficult, the tissue was immersed in a 0.5% solution of Toluidine Blue (Sigma Chemicals, Poole) which helped to highlight details. A total of six fish were dissected.

Histochemistry

Histochemical studies of the assemblage were carried out on three specimens at each of the two acclimation temperatures. The assemblage was prepared for histochemistry by removing the pectoral fin blade just above the entry of the fin rays into the muscle mass. The body wall musculature was removed from around the assemblage, apart from a small piece adjacent to the *abductor superficialis* which was left on to support that side of the section. The tissue remaining was then mounted fin ray end upwards on a chilled brass cryostat chuck in Tissue-Tek OCT embedding compound (Miles Scientific). The block was immediately frozen by immersion in isopentane (2-methyl butane, BDH Chemicals Ltd., Poole) cooled by liquid nitrogen to near its melting point (-160° C). The block was then placed into a Bright cryostat set at -20° C for one hour before sectioning.

Series of six sections 15 microns thick were taken at the level of the foramen and mounted on coverslips. The blocks were sectioned from differing orientations to eliminate distortion due to cutting and obtain an accurate

cross-sectional picture. The blocks were large, so damage to part of the sections nearly always occurred during cutting. To check the accuracy, smaller blocks consisting of either the abductor or adductor sides of the assemblage were also prepared and sectioned.

In six specimens from each temperature group the *abductor superficialis* (*AB.S.*) muscle was isolated from the assemblage. This was then mounted fin ray end up, frozen and sectioned in the same way.

The sections obtained were stained for myofibrillar ATPase, succinic dehydrogenase and glycogen in order to discern structure and the composition of fibre types in the muscle.

All incubation and staining procedures were carried out at room temperature (approximately 20° C).

Myofibrillar ATPase: Sections were stained for myofibrillar ATPase using the modification of the method of Guth & Samaha (1969; 1970) described by Johnston *et al.* (1974). Results were found to be improved, however, by soaking the sections in Carp Ringer's solution for 5 minutes before initiating the staining procedure. Staining was carried out with and without alkaline pre-incubation in order to distinguish intermediate fibres, which have a higher pH stability, but unfortunately pH values high enough to inactivate both fast and slow fibres also caused extensive cellular damage, preventing accurate estimates of intermediate fibre distribution.

The stained sections were washed thoroughly in distilled water, dried in air and mounted under glycerol-gelatin.

Succinic dehydrogenase (SDH): Sections were stained for SDH as described by Johnston *et al.* (1974). Optimum time in the incubation medium in the dark, at room temperature was 30 minutes. The sections were washed and mounted as above.

Glycogen: The PAS reaction (Bancroft & Stevens 1972) was used to stain sections for glycogen. The sections were incubated in 1% periodic acid for 45 minutes, rinsed in distilled water and transferred to Schiff's reagent for 20 minutes. The sections were then thoroughly washed in three changes of 0.5% sodium metabisulphite, rinsed in distilled water and mounted.

The serial sections of the entire pectoral assemblage were used to build up a detailed cross-sectional picture of the muscle structure and the organisation of fibre types within them.

The total area of the *AB.S.* and the diameters of slow and fast fibres from each acclimation group were measured from the isolated *AB.S.* muscle sections. This was done by making diagrams of the sections using a drawing arm in conjunction with a binocular light microscope, and analysing the results using a digitising pad - the Imagan system (Kompira, Strathclyde, Scotland). The percentage area of slow fibres was calculated and the total number of slow

fibres in each section counted. Photographs of the sections were taken with a Zeiss photomicroscope using Kodak Technical Pan film and printed on Ilford multigrade paper.

Statistical analysis

Fibre diameters and areas were compared using a standard "t"-test.

RESULTS

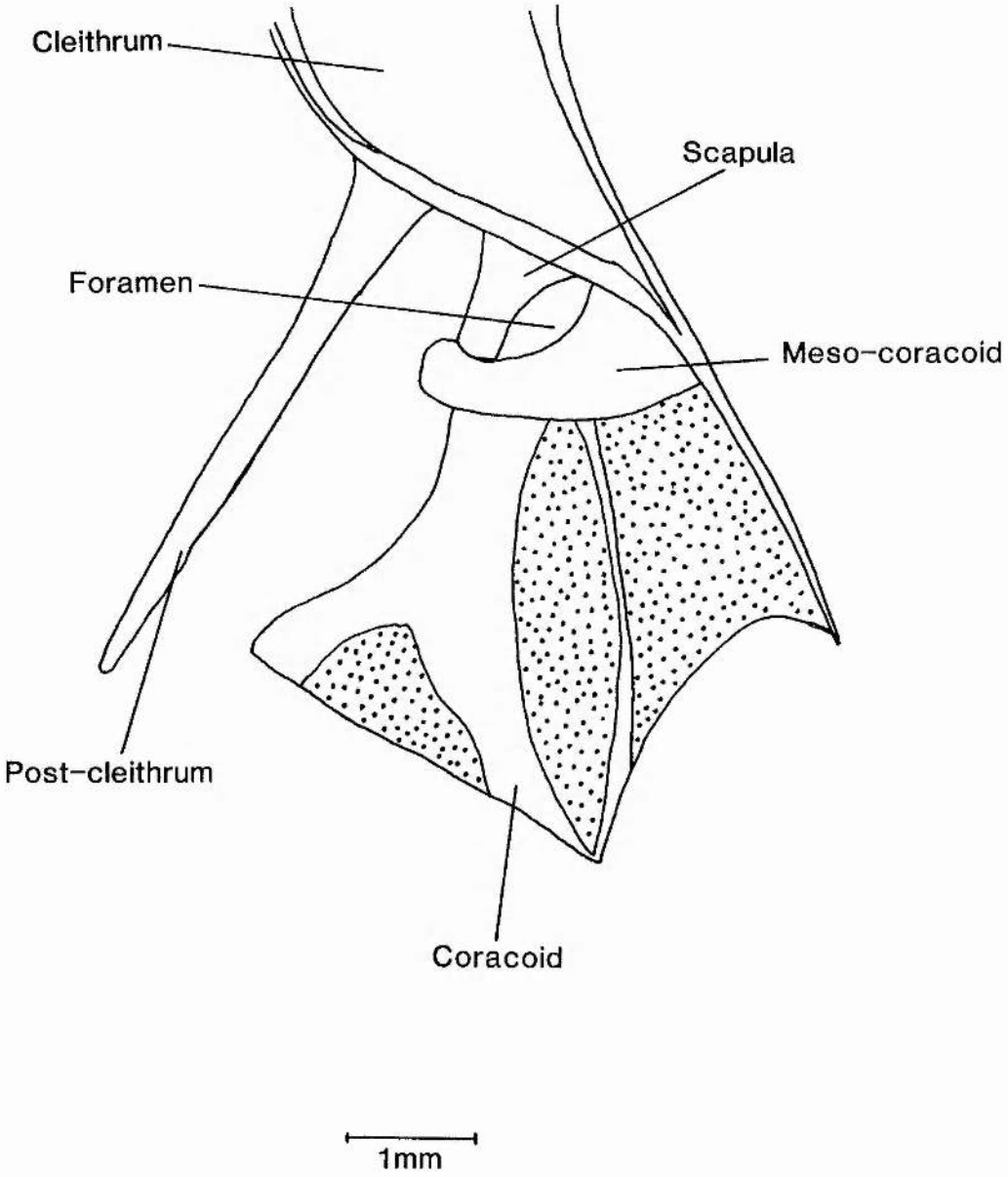
Pectoral skeleton

The pectoral skeleton is shown in Figure 3:2, and nomenclature is based on that of Starks (1930).

The cleithrum is curved and extends from the cranial region to the ventral surface of the fish. The post-cleithrum originated from the medial surface of the cleithrum and was embedded for most of its length in the musculature of the body wall.

The scapula originates at the cleithrum and extends ventrally to attach to the mesocoracoid, which arches to meet the cleithrum. The scapula and mesocoracoid enclose a foramen. The mesocoracoid is supported by the coracoid. A fine membranous cartilage fills the areas between the processes of the coracoid (represented by stippled areas in Figure 3:2).

FIGURE 3:2. Lateral view of the pectoral skeleton of the common carp following the removal of the pectoral musculature. The stippled areas represent a membranous cartilage.


Cleithrum
Scapula
Foramen
Meso-coracoid
Post-cleithrum
Coracoid
1mm

Pectoral muscles

The myology of the pectoral assemblage is shown in Figures 3:3 and 3:4. Nomenclature is based on that of Winterbottom (1974).

Abductor muscles:

Following removal of skin, the structure of the following muscle was clearly visible:

Abductor superficialis (*AB.S.*). This muscle originates along the lateral border of the cleithrum and the anterior of the coracoid. The insertions are tendinous at the entire bases of fin rays 2–17, but only tendinous on the ventral portion of fin ray 1. The muscle fibres run parallel to each other and at right angles to the cleithrum for the ventral three-quarters of the muscle, but the dorsal part of the muscle appears pinnate (Figure 3:3a). When this muscle contracts, the fin appears to be drawn forward & slightly downward, and the rays close.

On removing the *AB.S.*, the structure of the other two abductor muscles could be seen (Figure 3:3b,c):

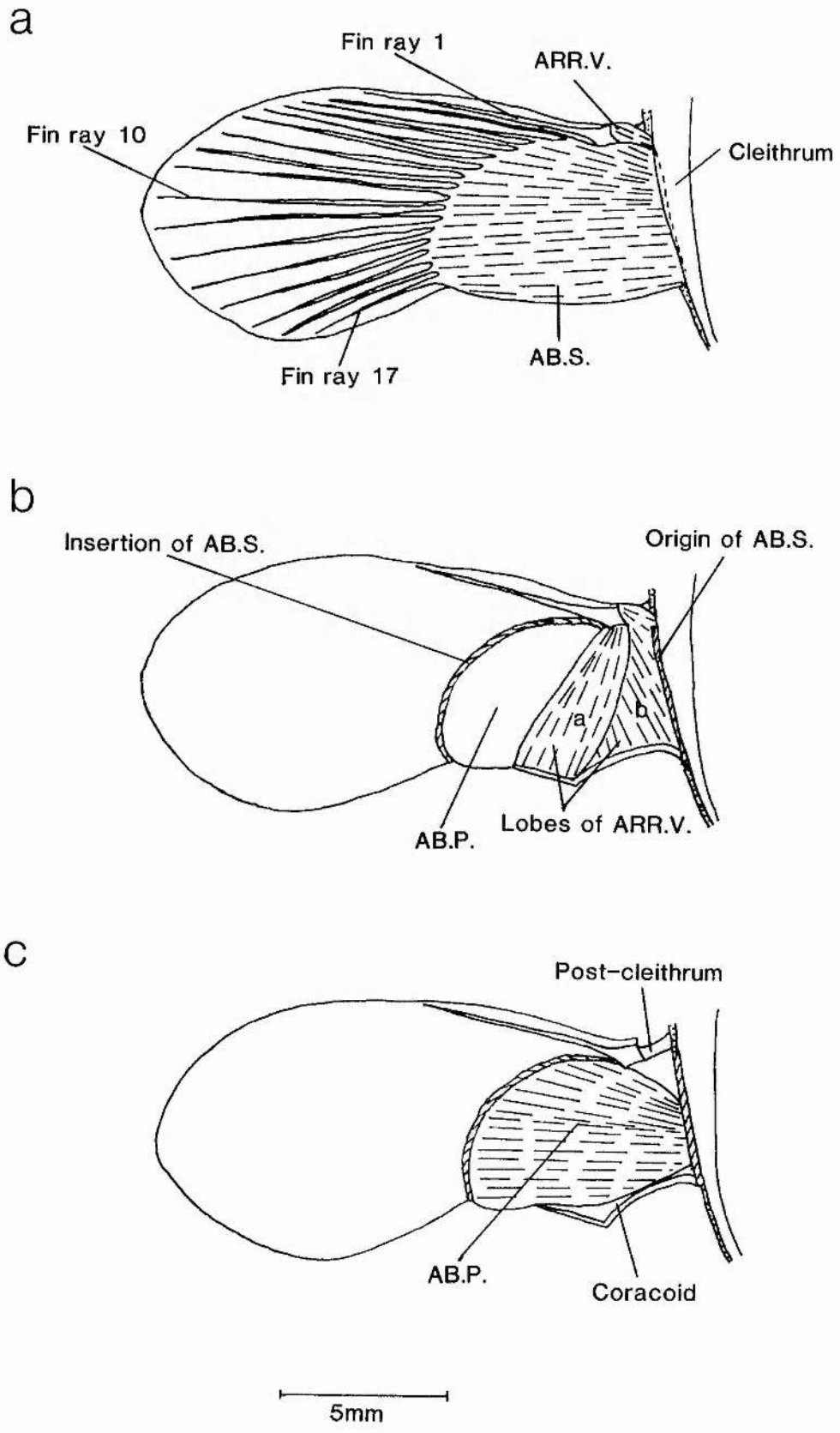
Arrector ventralis (*ARR.V.*). This is a two lobed muscle with an extensive origin on the cleithrum and a large area of the clavicle. The dorsal lobe (a) inserts onto the dorsal side of fin ray 1, and the ventral lobe (b) onto the ventral side. The fibres in each lobe taper from the extensive origin to the single insertion (Figure 3:3b).

FIGURE 3:3. The abductor muscles of the carp.

a. Lateral view of the abductor muscles following removal of the skin.

b. Lateral view of the two lobes of the *Arrector ventralis* (ARR.V.) and part of the *Abductor profundis* (AB.P.) following removal of the *Abductor superficialis* (AB.S.).

c. Lateral view of the entire AB.P. following removal of the ARR.V.


a
Fin ray 1
ARR.V.
Fin ray 10
Cleithrum
AB.S.
Fin ray 17
b
Insertion of AB.S.
Origin of AB.S.
a
b
AB.P.
Lobes of ARR.V.
c
Post-cleithrum
AB.P.
Coracoid
5mm

The muscle appears to rotate the fin and cause fin ray spreading by contracting downwards on the base of fin ray 1.

Abductor profundis (*AB.P.*). This muscle originates along the ventral portion of the cleithrum and onto the adjacent coracoid. There are tendinous insertions onto fin rays 2–17, but insertion only to the ventral edge of fin ray 1 (Figure 3:3c). Contraction of this muscle appears to draw the fin downwards and away from the body.

Adductor muscles:

After removing the skin and bending back the pectoral fin, all three adductor muscles were visible (Figure 3:4):

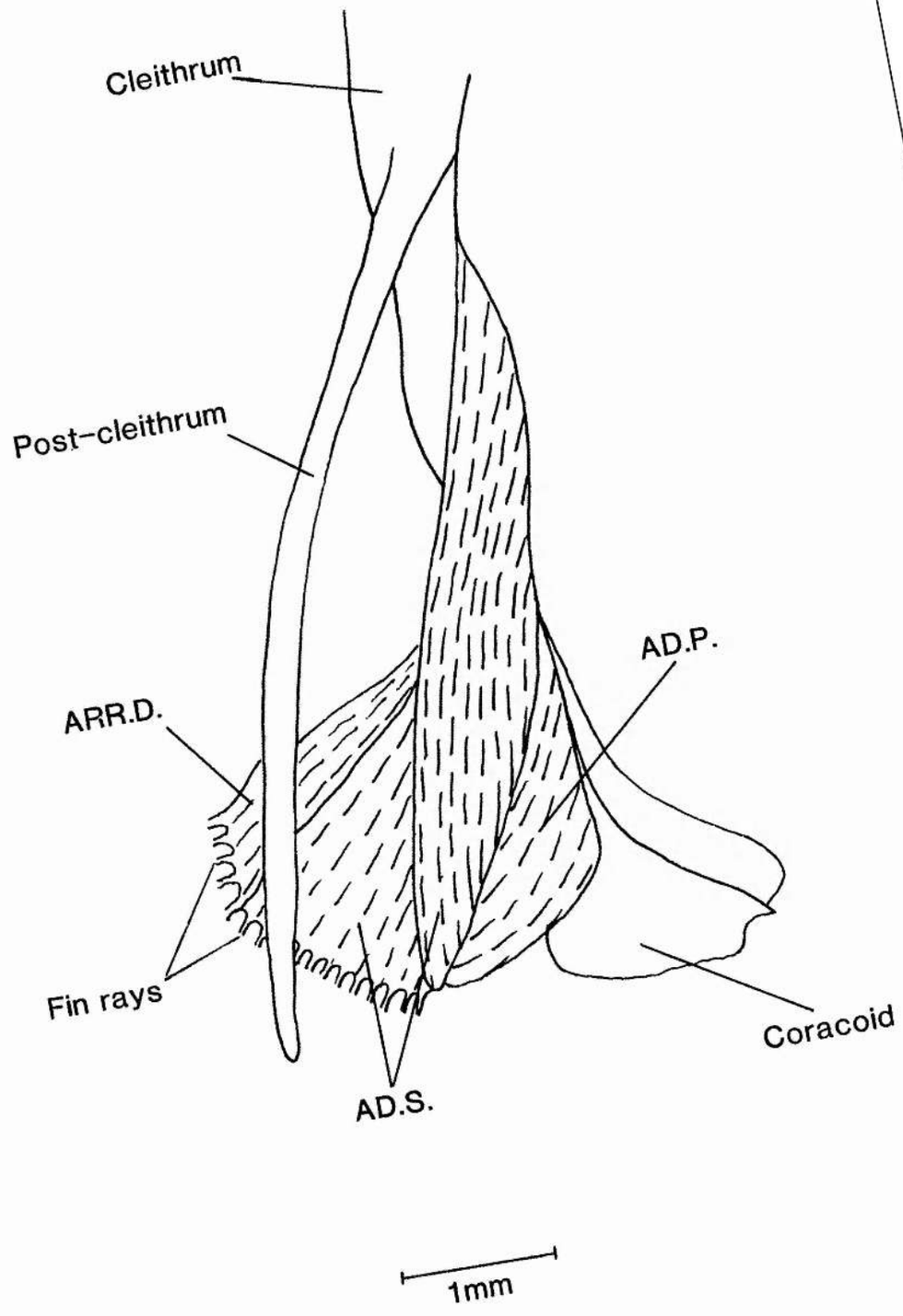
Adductor superficialis (*AD.S.*). This muscle has an extensive origin along the medial surface of the cleithrum and inserts by means of tendons to fin rays 5–17. The muscle appears to 'cross over' itself, with the more dorsomedial fibres serving the more ventral fin rays, while the more ventrolateral fibres serve the more dorsal rays. This means that the two extreme fibre directions occur at right angles to each other, with a complete gradation of direction between them.

Adductor profundis (*AD.P.*). This muscle originates from the medial surface of the scapula and the ventral portion of the cleithrum and inserts by means of tendons to fin rays 5–17. Contraction of this muscle appears to draw the fin towards the body.

Arrector dorsalis (*ARR.D.*). This muscle originates from the medial surface of the cleithrum and the adjoining

FIGURE 3:4. The adductor muscles of the carp.

a. Medial view of the adductor muscles following removal of the skin, AD.S. *Adductor superficialis*, AD.P. *Adductor profundis*, ARR.D. *Arrector dorsalis*.


Cleithrum
Post-cleithrum
AD.P.
ARR.D.
Fin rays
Coracoid
AD.S.
1mm

area of the coracoid and the insertions are tendinous upon the bases of fin rays 1-4.

Fibre types

A cross sectional picture of the pectoral assemblage showing the fibre composition of the various muscles is shown in Figure 3:5. Due to the failure of the alkaline pre-incubation in the ATPase assay to reliably distinguish intermediate fibres, any present are included within the fast muscle blocks. The bulk of the muscle seems to be composed of fast fibres, the slow fibres, if present, appearing in a layer along the surface of the muscles.

Abductor superficialis study

In both acclimatory groups, it was found that the *AB.S.* was composed mainly of fast fibres, with a layer of slow fibres along the outside edge, adjacent to the skin. There appeared to be considerable overlap of the fibre types. Figure 3:6 shows serial sections of a part of the *AB.S.* stained for SDH, Glycogen and ATPase.

The mean cross-sectional area of the *AB.S.* was calculated for each acclimatory group and no significant difference was found between groups. The mean diameters of each fibre type for both acclimatory groups are shown in Figure 3:7. Acclimation did not significantly alter the mean diameter of either fast or slow fibres.

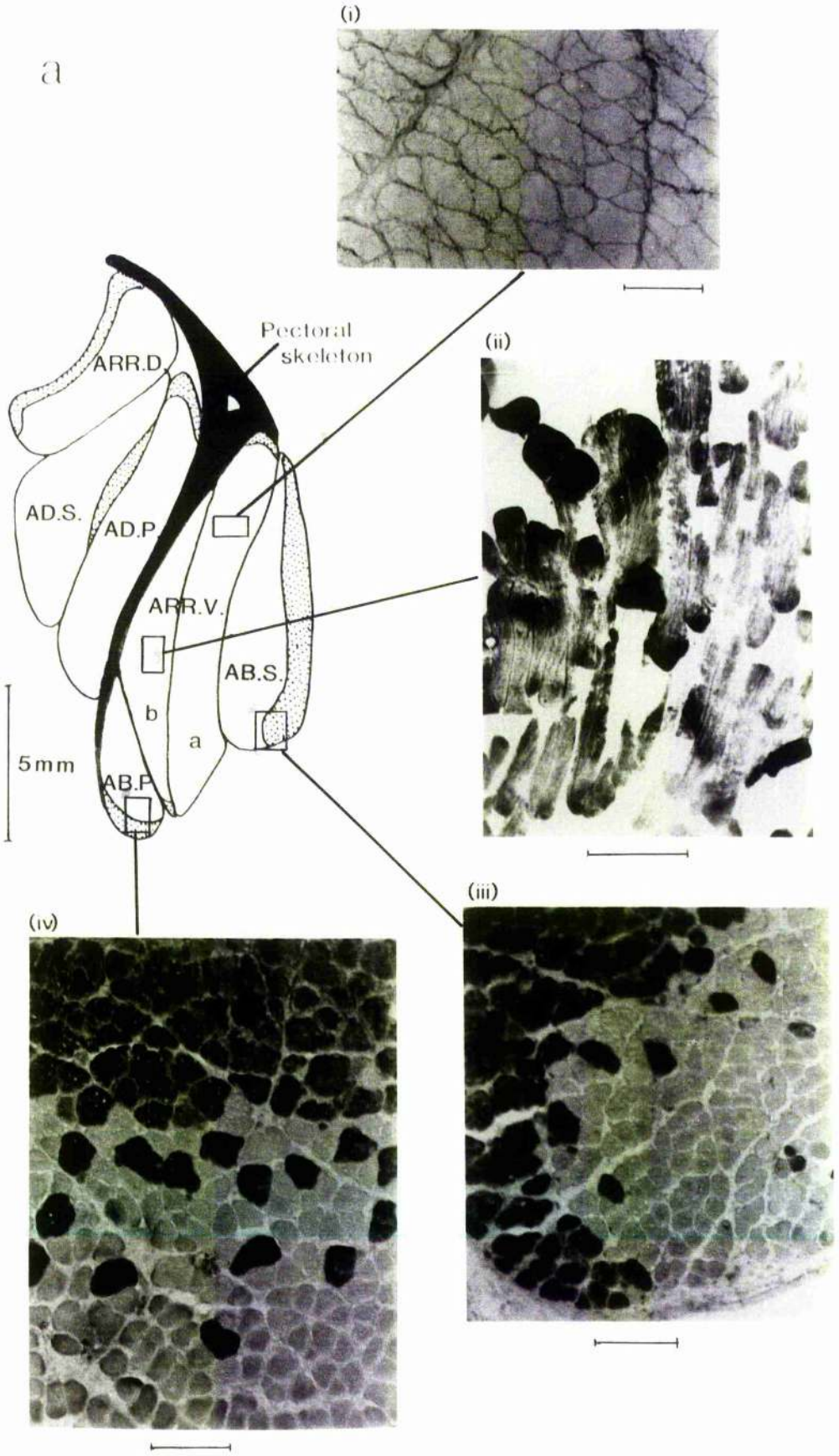
FIGURE 3:5. Cross sectional representation of a transverse section through the pectoral muscle assemblage at the level of the foramen.

AB.S. *Abductor superficialis;* AB.P. *Abductor profundis;* ARR.V. *Arrector ventralis;* AD.S. *Adductor superficialis;* AD.P. *Adductor profundis;* ARR.D. *Arrector dorsalis.*

a. Selected areas of the abductor muscles and ARR.V. muscle are shown stained for i. glycogen, ii.-iv. ATPase. Note the difference in fibre orientation of the two lobes of the ARR.V.

b. Selected areas of the adductor muscles and the ARR.D. shown stained for i. ATPase, ii. SDH, iii. ATPase.

All scale bars for stained sections represent 100µm.


a
(i)
Pectoral
skeleton
ARR.D.
AD.S.
AD.P.
ARR.V.
AB.S.
b
a
5mm
AB.P.
(ii)
(iii)
(iv)

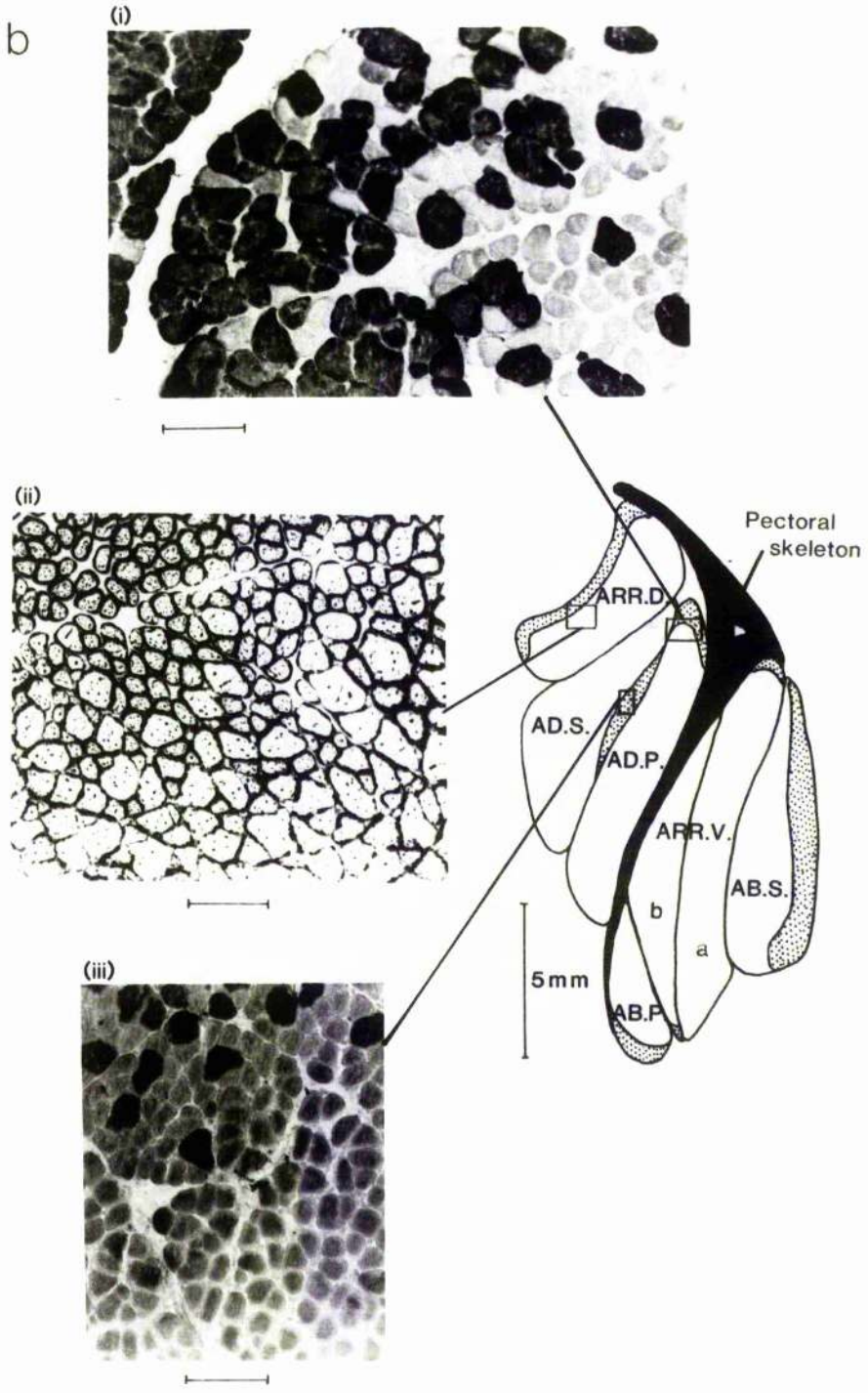
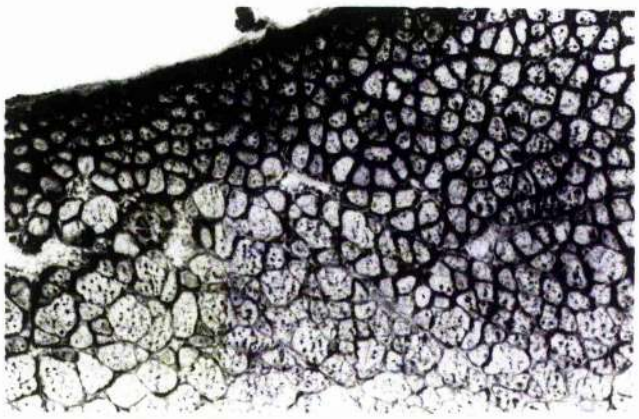
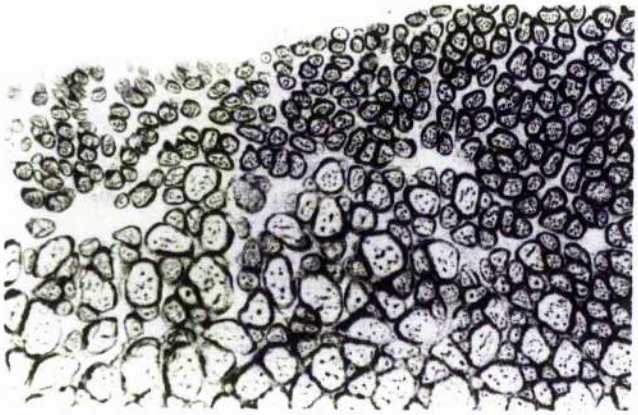

b
(i)
(ii)
(iii)
Pectoral
skeleton
ARR.D.
AD.S.
AD.P.
ARR.V.
AB.S.
b
a
5mm
AB.P.

FIGURE 3:6. *Abductor superficialis* muscle, serial sections stained for i. Glycogen; ii. SDH; iii. ATPase. Scale bars represent 100µm.

a



b



c

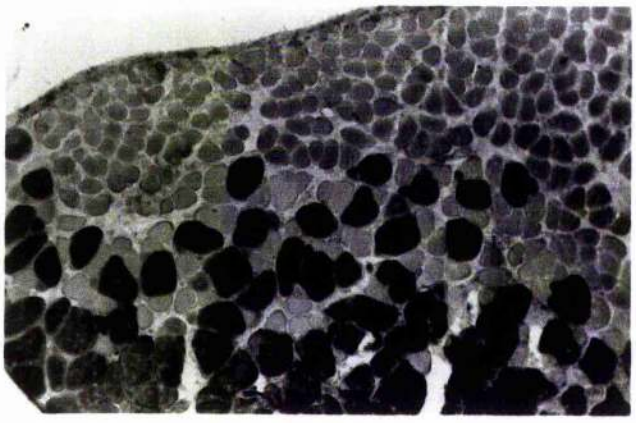


FIGURE 3:7. Histogram showing the mean diameters of slow and fast fibres from the *abductor superficialis* muscle of the common carp for both acclimatory groups. Values are mean±S.E., N=6.

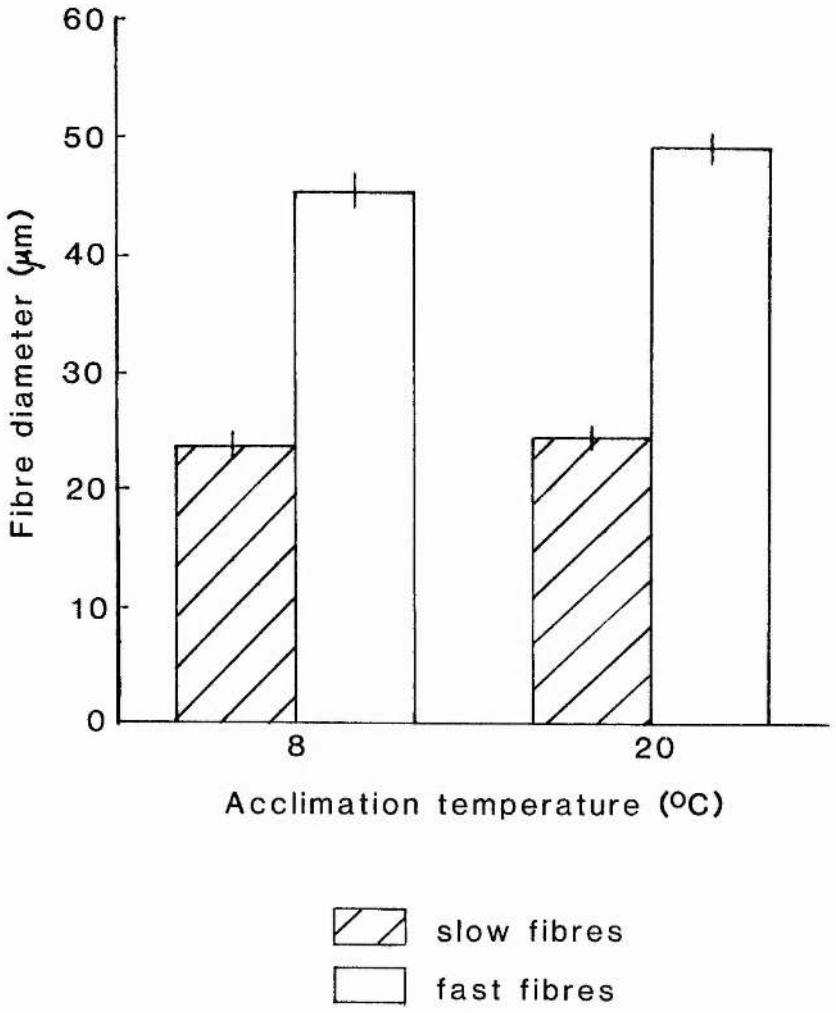

60
50
40
30
20
10
0
Fibre diameter (μm)
8
20
Acclimation temperature (°C)
slow fibres
fast fibres

Figure 3:8 shows frequency histograms of fibre diameters for each fibre type and temperature. Although mean diameter is not significantly different, examination of the range data in Figure 3:8 shows that there is a greater percentage of very small slow fibres in the 8° C acclimation group.

The mean diameters and histochemical staining properties of slow and fast fibres from each acclimatory group are given in Table 3:1.

The percentage cross-sectional area of slow fibres and the mean number of slow fibres per muscle was significantly higher in the 8° C-acclimated fish, 31.4±0.30% and 2392±40 fibres respectively, compared to 26.5±0.22% and 2127±29 fibres for the 20° C-acclimated fish (Mean±S.E., N=6, $P<0.01$). The results are displayed in Table 3:2 and Figure 3:9.

DISCUSSION

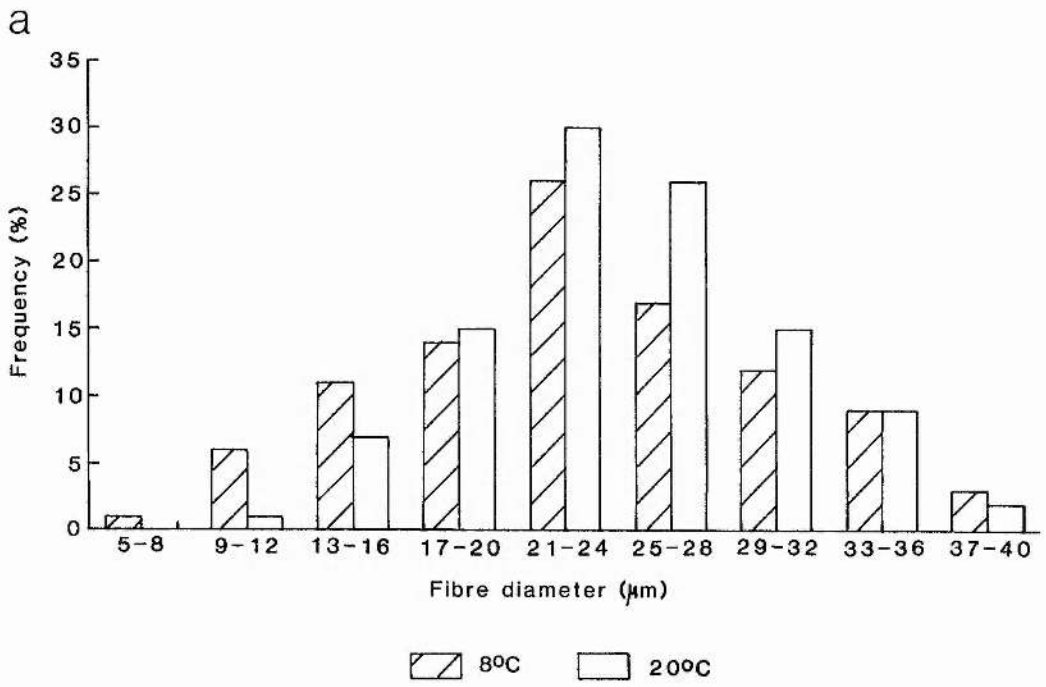
Pectoral skeleton and myology

Starks (1930) studied the general structure of the pectoral skeleton or primary shoulder girdle in many species of bony fish. The generalised description of the cyprinid shoulder given by Starks broadly resembles the description drawn from this study, although he makes no mention of the post-cleithrum, perhaps because the structure is embedded in the myotomal musculature and is thus not directly part of the pectoral structure.

FIGURE 3:8. Frequency histograms showing the range of fibre diameters in the *abductor superficialis* muscle of common carp.

a. Slow fibres from both acclimatory groups.

b. Fast fibres from both acclimatory groups.


a
35
30
25
20
15
10
5
0
Frequency (%)
5-8
9-12
13-16
17-20
21-24
25-28
29-32
33-36
37-40
Fibre diameter (μm)
8°C
20°C

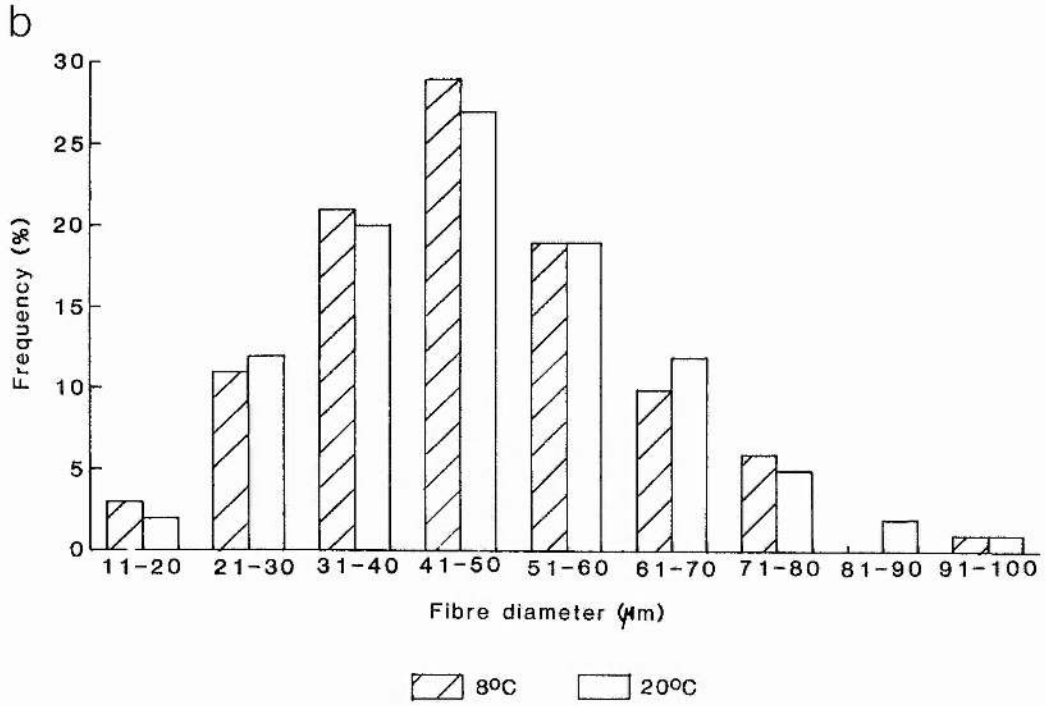

b
30
25
20
15
10
5
0
Frequency (%)
11-20
21-30
31-40
41-50
51-60
61-70
71-80
81-90
91-100
Fibre diameter (μm)
8°C
20°C

TABLE 3:1. Diameters and histochemical staining properties of the fibres contained in the *Abductor superficialis* muscle.

	Acclimation temperature			
	8° C		20° C	
Fibre type	Red	White	Red	White
Fibre diameter (μm, mean±S.E.)	23.6±0.8	45.4±1.4	24.4±0.6	49.0±1.2
Diameter range (μm)	8.2–39.7	15.1–79.8	12.1–39.9	16.5–94.8
Staining intensities				
a. Glycogen	+++	+	+++	+
b. SDH	+++	+	+++	+
c. ATPase	+	+++	+	+++

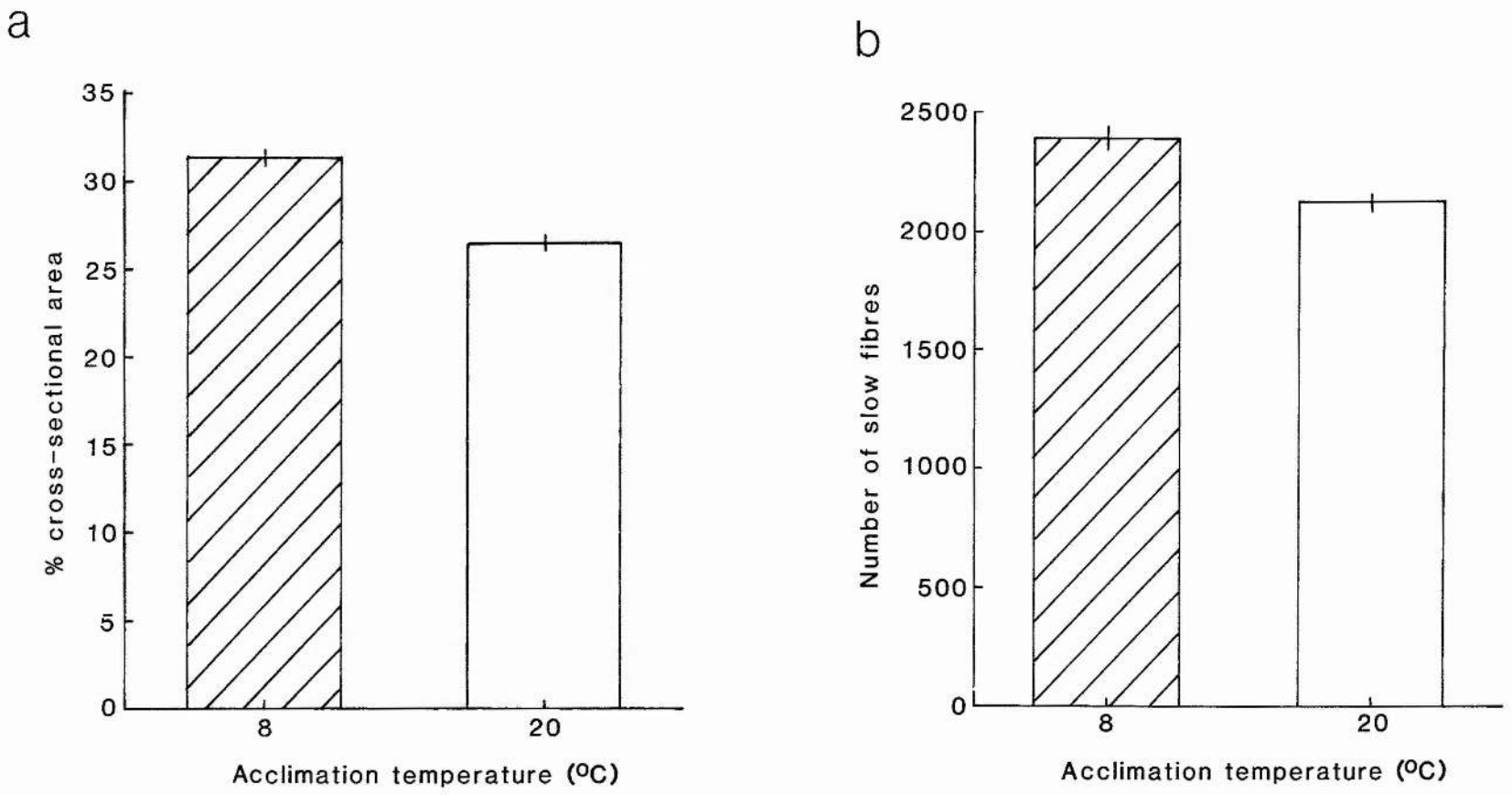
+ lighly stained +++ heavily stained. Differences between white and red fibre diameters highly statistically significant ($P<0.01$), differences between acclimation groups for both fibre types not significant ($P>0.05$).

TABLE 3:2. Slow fibre percentage area and total slow fibre number in the *Abductor superficialis* muscle.

	Acclimation temperature	
	8° C	20° C
Percentage area of slow fibres	31.4±0.30	26.5±0.22
Total slow fibre number	2392±40	2127±29

All values are given as mean±S.E., N=6. Values at 8° C have been compared with those at 20° C, the differences between the acclimation groups being highly significant ($P<0.01$) in both cases.

FIGURE 3:9. Fibre composition of the *AB.S.* following temperature acclimation. Values are mean±S.E., N=6.
a. Percentage cross-sectional area of slow fibres.
b. Total number of slow fibres.


a
% cross-sectional area
35
30
25
20
15
10
5
0
8
20
Acclimation temperature (°C)
b
Number of slow fibres
2500
2000
1500
1000
500
0
8
20
Acclimation temperature (°C)

In the common carp, six muscles articulate the movement of the pectoral fin blade, the general arrangement conforming to the general teleost plan described by Shann (1920). The two abductor and two adductor muscles articulate the fin in an anterio-posterior direction, whilst the two arrector muscles rotate the fin about a dorso-ventral axis.

Ping, Pao & Yang (1958) looked at the skeletal musculature of the carp. Although their terminology differs, the muscular structure is similar, excepting their omission of the *arrector dorsalis* muscle or equivalent. Winterbottom (1974) states that the *arrector dorsalis* structure is often mis-interpreted. The structure in this study also differs from that described by Winterbottom, tendons from the *ARR.D.* serving fin rays 1-4, not just 1, and the possibility of error has been checked as he describes. Perhaps the difference is due to the size of the fish concerned, Winterbottom studied carp of around 8cm, while specimens double that size were used in this study. Fish size can affect the pectoral myology. For example, the *abductor profundis* muscle of smaller carp originates wholly on the coracoid rather than on both the cleithrum and the coracoid, and is thus much shorter (S.K.J. Cochrane, pers comm).

Fibre Types

Unlike the myotomal muscle of common carp (Johnston *et al.*, 1974; Johnston & Lucking, 1978), intermediate and fast fibres in the pectoral muscle could not be reliably differentiated on the basis of the alkaline stabilities of their myosin ATPases. It is expected that a layer of intermediate fibres does exist beween the fast and slow fibres, since such a layer exists in the myotomal musculature of this species and in the pectoral musculature of the goldfish, a closely related species (Heap *et al.*, 1987).

The bulk of the pectoral muscle appeared to be composed of fast (or fast and intermediate) muscle, the slow muscle, if present, appearing along the surface of the individual muscles. The volume of slow fibres in the pectoral musculature would be expected to reflect the locomotory style of the species. In the Antarctic teleost *Notothenia neglecta*, for example, the bulk of the pectoral musculature is made up of slow fibres, with thin overlayers of fast fibres (Harrison, Nicol & Johnston, 1987). The notothenoid performs sustained, low speed swimming using a labriform mode of locomotion (Twelves, 1972; Montgomery & McDonald, 1984, Archer & Johnston, 1988) and the pectoral fins are large in relation to the body. The very low proportion and lower aerobic capacity of slow muscle in the trunk of *N. neglecta* also confirms the importance of the labriform mode for sustained swimming (Johnston & Camm,

1987; Archer & Johnston, 1989). Carp do use their pectoral fins at low speeds, but their use is less important than in the notothenoid, and also the notothenoid is a more sedentary benthic species than the carp. The contribution of the pectoral muscles to sustained, aerobically powered swimming would therefore be expected to be greater in the notothenoid than the carp and thus the volume of slow fibres higher in the notothenoid, as is the case. Also, carp species have impressive anaerobic capacities (Johnston, 1975), so carp may not require as high a volume of aerobic fibre types in their musculature as notothenoids in order to maintain a similar locomotory performance.

Effects of Temperature Acclimation

In the *AB.S.* muscle of the carp, the fast fibres have a greater diameter and range of diameters than do the slow fibres (although there is some degree of overlap). This is the same pattern found in the myotomal musculature (Nag, 1972; Johnston, Ward & Goldspink, 1975).

Cold-acclimation increases the total cross-sectional area of slow fibres in the *AB.S* muscle of carp. Measurement of total slow fibre number and measurement of fibre diameters confirm that the increase in area is caused by an increase in the number of slow fibres rather than an increase in the size of those already present. This is in general agreement with data from the goldfish (Johnston & Lucking, 1978), although a slight increase in fibre diameter

is found in the goldfish following cold-acclimation. Although there appears to be no significant effect on mean diameter of either fibre type following acclimation in the carp, qualitative examination of the range diameter reveals a larger number of very small slow fibres in the 8° C acclimation group. If the slow fibre mass is proliferating during temperature acclimation, an increase in the number of immature, smaller diameter fibres would be expected. This measure is qualitative, but if firm quantitative data could be produced, it would confirm the hypothesis that new slow muscle fibres are produced, rather than fast muscle fibres being converted to slow muscle fibres.

Although the pattern of recruitment of different fibre types is similar over a wide temperature range in the carp, a fall in temperature causes a compression of the recruitment order (Rome *et al.*, 1984). To maintain locomotory performance at low temperatures, fish recruit more muscle fibres, which means they start to recruit faster fibre types at lower swimming speeds. This in turn means that although the fish are able to generate the mechanical power to maintain performance, the speeds at which they can sustain locomotion are reduced, because the fast, anaerobic muscle fatigues rapidly. Proliferation of aerobic muscle during cold-acclimation should permit the fish to reach a higher swimming speed before initial anaerobic fibre recruitment, and electromyographical studies of fibre recruitment following acclimation in carp (Rome *et al.*,

1985) and the striped bass *Morone saxatilis* (Sisson & Sidell, 1986) confirm this. The proliferation of aerobic fibre types, together with evidence that mechanical power per fibre of slow, aerobic muscle can be increased following cold-acclimation (Altringham & Johnston, 1985; Heap *et al.*, 1985), enables compensation for a fall in temperature. In carp, cold-acclimation results in an increase of the aerobic capacity of the pectoral muscle and provides an enhanced capacity for sustained swimming at low temperatures.

CHAPTER 4

Temperature acclimation in the common carp: force-velocity characteristics and myosin subunit composition of slow muscle fibres

INTRODUCTION

The maximum cruising speed of some freshwater fish is increased at low temperatures and decreased at high temperatures after several weeks cold acclimation (Fry & Hart, 1948; Heap & Goldspink, 1986). The mechanisms underlying this plasticity in swimming performance are complex and include: changes in the relative proportions of different muscle fibre types (Johnston & Lucking, 1978; Jones & Sidell, 1982), altered patterns of muscle fibre recruitment (Rome *et al.*, 1985; Sisson & Sidell, 1987), and adaptations in the properties of membranes and nerves (Harper, Shelton & Watt, 1989). In cyprinids, muscle contractile properties also vary with acclimation temperature (see Johnston *et al.*, 1990). As an example, in the goldfish (*Carassius auratus* L.), the ATPase activity of fast muscle myofibrils is around 3-times higher at 1° C in fish acclimated to 1° C- than 26° C (Johnston *et al.*, 1975). Similar increases in ATPase activity with cold-acclimation occur in common carp (*Cyprinus carpio* L.) and roach (*Rutilus rutilus*) (Heap *et al.*, 1986), but may not be widespread

among teleosts (Walesby & Johnston, 1981; Jones & Sidell, 1982; Sidell & Johnston, 1985).

Johnston *et al.* (1985) used skinned fibres to investigate the force-velocity characteristics of fast and slow myotomal muscles in common carp acclimated to either 7° C or 23° C. Both the maximum tension (P_o) and contraction velocity (V_{max}) at 7° C were about 1.5-2.0 times higher in fibres from the cold- than warm-acclimated fish. There is evidence that these adaptations in contractile properties involve changes in the expression of both myosin heavy chains (Gerlach *et al.*, 1990) and myosin light chains (Crockford & Johnston, 1990; Johnston *et al.*, 1990).

Recent studies have shown differences in the properties of skinned and live fish muscle fibres. Skinned fibres have lower tensions and shortening speeds than live fibres, and the force-velocity relationship of skinned and live fibre preparations differs, making skinned fibres unsuitable for accurate quantitative calculations of muscle power output (Altringham & Johnston, 1988; Curtin & Woledge, 1988). Unfortunately, all attempts to obtain a suitable live fibre preparation from carp myotomal muscle have proved unsuccessful (see also Rome *et al.*, 1988). However, we have found that stable live fibre preparations can be isolated from the superficial region of the pectoral fin *abductor superficialis* muscle of the common carp *Cyprinus carpio* L. Histochemical studies have shown that these preparations contain contain predominantly slow twitch muscle fibres. In the present study, the preparation has been used to

calculate muscle power output from the force-velocity relationship in carp acclimated to either 8° C or 20° C. The effect of temperature acclimation on the subunit composition of slow muscle myosin has also been investigated.

MATERIALS & METHODS

Fish

Common carp (*Cyprinus carpio* L.) were obtained from Humberside Fisheries, Driffield, England. The total length and body mass of the fish studied was 31.0±2.5cm and 735±10g (mean±SD; N=24). Fish were held in tanks of partially re-circulated filtered freshwater at either 8° C or 20° C for 6-12 weeks (12h light:12 dark). Trout pellets were fed daily to satiation.

Isolation of muscle fibre bundles

Carp were killed by a blow to the head followed by decapitation and pithing. The intact pectoral fin and associated skeleton was removed from the fish, the cleithrum being severed at the level of the lateral line. The assembly was pinned out on a silicone elastomer base (Sylgard 184, Dow Corning), with the *abductor superficialis* muscle (*AB.S.*) face down. The preparation was covered with Ringer solution (mmol l^{-1}): NaCl, 119.0; Na pyruvate, 10;

KCl, 2.7; $MgCl_2$, 1; $CaCl_2$, 1.8; $NaHCO_3$, 2.5; pH 7.4 at 5° C). The other muscles of the pectoral assembly were removed and the bone reduced as far as possible without damaging the attachments of the *AB.S.* The preparation was repinned via the skin, and the layer of fast fibres removed from the ventral surface. Finally, the skin was removed from the surface of the muscle, and the slow fibre region was pared down to a bundle of 20-50 fibres (Figure 4:2). Aluminium foil clips were attached as close to the bone as possible (200-300μm), and the bone further reduced. Dissection was performed on a cooled plate (8° C) and the Ringer was changed frequently.

Measurement of contractile properties

The apparatus consisted of a perspex chamber through which aerated Ringer was circulated at constant temperature (±0.1° C). Force was measured using a silicon beam strain gauge (AE 801, AME, Horten, Norway). Muscle length was measured and controlled via a servo motor (MFE model R4-077, Emerson Electronics, Bourne End, Bucks.) and control unit built in house (Altringham & Johnston, 1988). Sarcomere length was measured by laser diffraction and set to 2.3μm (this length gave a maximal twitch response). Fibres were stimulated via two platinum wire electrodes lying on either side, using 1.5ms pulses at 1.2x threshold voltage. Data was collected and analysed on a Nicolet 3091 digital oscilloscope, and stored on a BBC microcomputer.

The isometric properties of fibre bundles from both acclimation groups were measured at 8° C and 20° C. The effects of temperature were reversible and so several cycles of heating and cooling were performed on each preparation. Fibre bundles from 8° C fish deteriorated if maintained at an experimental temperature of 20° C for long periods. Contraction velocity was measured at various loads using isovelocity releases. During the plateau phase of tetanus (2s, 25Hz) the preparations were given an initial 2ms release, of varying magnitudes, to lower the tension. A second, slower release was adjusted to hold the tension constant after the step (Altringham & Johnston, 1988 and Figure 4:1).

Fitting of P–V data

Force–velocity (P–V) curves were constructed by plotting velocity against the relative tension over the first 10ms interval after release.

P–V data for individual fibres were fitted to Hill's (1938) hyperbolic function:

$$V=b(P_o+a)/(P+a)-b$$

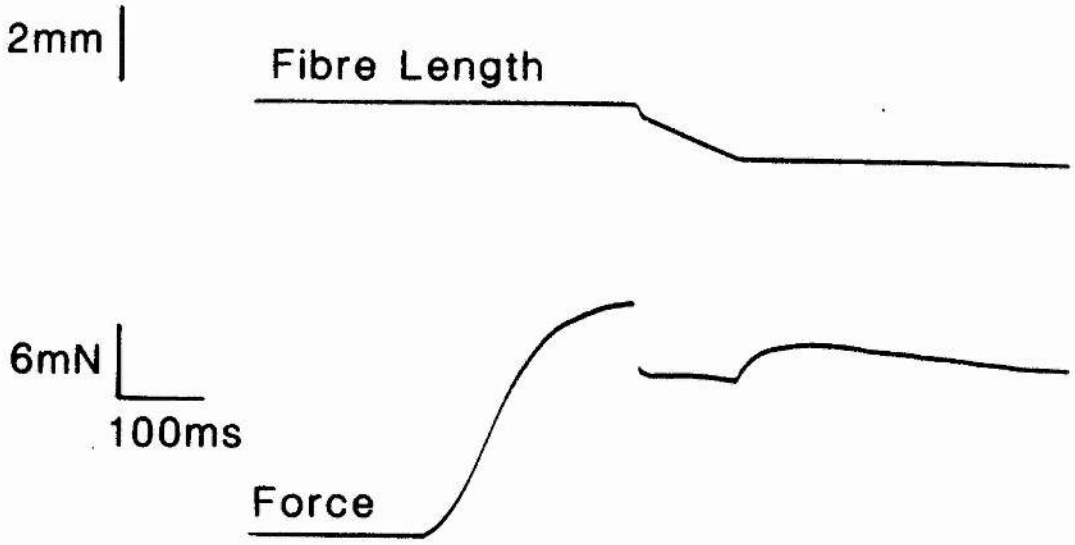
where V is velocity, P is tension, P_o is maximum tension and a and b are constants. The equation was linearised as:

$$V=CZ-b$$

where $C=b(P_o+a)$ and $Z=1/(P+a)$.

A least squares regression was iteratively fitted to the data by computer, without constraining the curve to go

FIGURE 4:1. A typical isovelocity release of a carp slow fibre preparation to illustrate the stability of force records during shortening.


2mm
Fibre Length
6mN
100ms
Force

through P_o. Starting with a wide range of values for each constant and stepping through them with progressively narrower ranges and smaller increments, values giving the minimum mean squared differences between observed and predicted data were obtained (Altringham & Johnston, 1988). In fitting to Hill's equation, data above $0.8P_o$ were omitted, since it has been shown that they consistently deviate from the curve (Edman *et al.*, 1976). The curvature of the P-V relation is inversely related to a/P_o.

P-V data for individual fibres were also iteratively fitted to a hyperbolic-linear (hyp-lin) curve described by Marsh & Bennett (1986):

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

where B and C have dimensions of velocity and A is dimensionless. The entire data set was fitted since data above $0.8P_o$ fell on the fitted line. The ratio $\dot{W}_{max}/V_{max}.P_o$, where $\dot{W}_{max}$ is maximum power output, is inversely related to the curvature of the P-V relation, and has been calculated using values derived from both curve fitting procedures.

Statistical analysis

The standard error of the estimate (SEE) for both curve fitting procedures were determined for each fibre from the equation:

$$SEE=\sqrt{RSS/n-2}$$

where RSS=residual sum of squares.

Data for the two acclimation groups was compared using an unpaired "t" test.

Histochemistry

At the end of each experiment, the preparation was frozen at its resting length using isopentane cooled to its melting point in liquid nitrogen (-159° C). Frozen fibre bundles were inserted into a block of liver tissue mounted on a cryostat chuck and re-frozen. Frozen sections (10μm) were cut and stained for myofibrillar ATPase and succinic dehydrogenase activity (Johnston *et al.*, 1975). The cross-sectional area of muscle fibres was determined using a digitizing pad interfaced to a microcomputer.

Electrophoresis

Sample preparation

The myosins from 7 warm-acclimated and 6 cold-acclimated carp were studied electrophoretically. Muscle fibre bundles (5-18mg) were placed in a test tube containing 25vols 40 mmol l^{-1} NaCl, 3 mmol l^{-1} phosphate buffer pH 7.0 at 2° C and crushed with a glass rod. The buffer was removed and replaced with 25vol of a solution containing (mmol l^{-1}): $Na_4P_2O_7$, 50; EGTA, 2.5; β-mercaptoethanol, 1; 50% glycerol, pH 8.8 at 2° C. The sample was homogenised using a hand held pestle, and left to extract for 60min on ice. The myosin

extract was centrifuged at 40,000g for 60min at 1° C, and stored at −20° C.

Polyacrylamide gel electrophoresis

Sodium pyrophosphate gels contained: 25 mmol l^{-1} $Na_4P_2O_7$ (pH 8.8 at 1° C), 38g/l acrylamide, 2g/l BIS, 10% glycerol, 0.1% TEMED, 0.05% ammonium persulphate. The electrode buffer which contained 25 mmol l^{-1} $Na_4P_2O_7$ (pH 8.8 at 1° C) and 10% glycerol was recirculated at 700ml/min. Electrophoresis was carried out at 1–2° C. Samples (20µl for analytical and 60µl for preparative gels) were loaded and run on to the gel for 30min at 35V. The voltage was then increased to 100V and run for 18h. The myosin bands were cut out from preparative gels rapidly stained with coomassie blue, and incubated for 30min at 30° C in an equal volume of 125 mmol l^{-1} Tris–HCl pH 6.75, 4% SDS, 5% β–mercaptoethanol, 20% glycerol, 0.004% bromophenol blue. The subunit compositions of myosins was examined using 8% and 15% SDS PAGE gels (Laemmli, 1970).

Staining

Gels were stained for 2h at 30° C in 0.1% coomassie blue, 50% methanol, 7% acetic acid, and destained in 50% methanol, 7% acetic acid. A rapid method was used for preparative gels, the gel was placed in the same staining solution as above until the bands became visible, about

5min, then washed for 10min in five changes of ultrapure water (Milli-Q). The bands were then ready for cutting out for SDS PAGE. SDS PAGE gels of the myosin light chains were silver stained using the SIGMA silver stain kit. Protein bands were characterised using myosin light chain markers from carp myotomal muscles (Crockford & Johnston, 1990; Johnston *et al.*, 1990) and proteins of known molecular mass (14-200 kDa) (SIGMA). Gels were scanned at 550nm using a Shimadsu CS 9000 densitometer.

RESULTS

Histochemistry

The arrangement of pectoral fin muscles in the carp is shown in Figure 4:2. Isolated fibre bundles were fibre-typed using serial frozen sections stained for myofibrillar ATPase and succinic dehydrogenase activity (Figure 4:2). The preparations were mostly composed of slow fibres which have a high SDHase activity and a low myofibrillar ATPase activity which was readily inactivated at pH 10.4 (Johnston *et al.*, 1974). Some preparations also contained a small percentage of fibres which stained intensely for myofibrillar ATPase activity following alkaine preincubation (Figure 4:2f). These correspond to fast oxidative or "intermediate" muscle fibres (Johnston *et al.*, 1974). The percentages of fast oxidative fibres in the preparations

FIGURE 4:2.

a. Lateral view of the pectoral fin assemblage of *Cyprinus carpio* following removal of the skin to show *Abductor superficialis* (Ab.s.) muscle, from which preparations were dissected. The vertical line indicates the level of the transverse section.

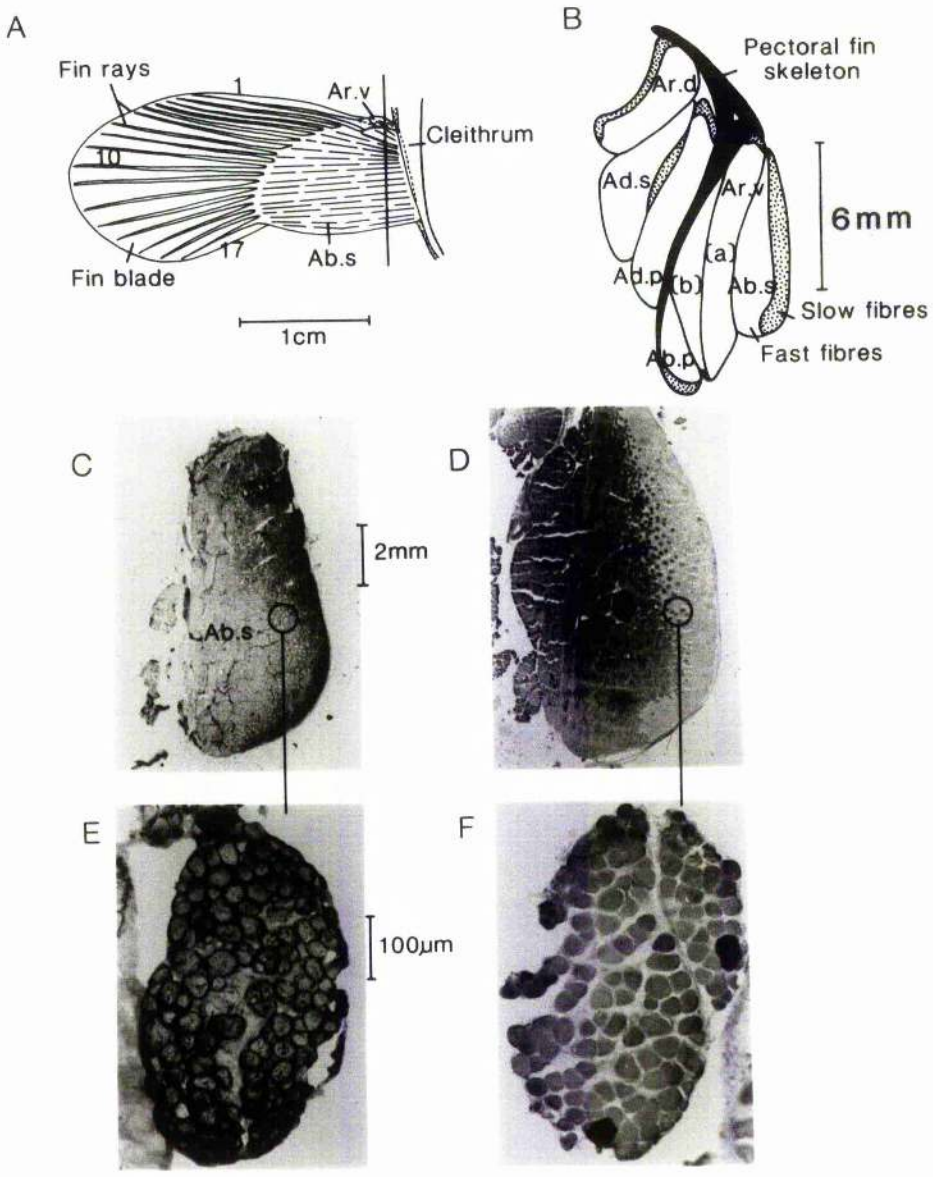
b. Representation of a transverse section through the pectoral assemblage to show the muscles, Ab.s. *Abductor superficalis*, Ab.p. *Abductor profundis*, Ar.v. *Arrector ventralis* (2 lobes, a & b), Ad.s. *Adductor superficialis*, Ad.p. *Adductor profundis*, Ar.d. *Arrector dorsalis*.

c. Transverse section of the *Abductor superficalis* muscle stained for succinic dehydrogenase activity, showing the location of fibres used for a preparation.

d. Transverse section of the same muscle stained for ATPase.

e. Transverse section of a typical preparation stained for succinic dehydrogenase activity.

f. Transverse section of the same preparation stained for ATPase.


A
Fin rays
1
Ar.v
Cleithrum
10
17
Ab.s
Fin blade
1cm
B
Pectoral fin skeleton
Ar.d
Ad.s
Ar.v
6mm
(a)
Ad.p
(b)
Ab.s
Slow fibres
Ab.p
Fast fibres
C
2mm
Ab.s
D
E
100µm
F

were 3.3±3.1 for from 8° C-acclimated and 2.8±2.7 for 20° C-acclimated fish (mean±S.D.).

Isometric properties

Isometric contractile properties showed a range of thermal sensitivities and varied with acclimation temperature (Table 4:1). R_{10} values for maximum tetanic tension (a number analogous to Q_{10} for non-rate variables, Bennett, 1984) were 1.10 and 1.34 for fish acclimated to 8° C and 20° C respectively. At 8° C maximum tetanic tension was 32% higher in fibres from 8° C- than 20° C-acclimated fish ($P<0.01$). Values for tetanic tension measured at the acclimation temperature of each group were similar (Table 4:1). Isometric twitch and tetanic contactions of representative preparations are shown in Figure 4:3.

The times to 50% peak force ($T_{0.5a}$) and from peak force to 50% relaxation ($T_{0.5r}$) were measured (Table 4:1). For isometric twitches $T_{0.5r}$ was much more temperature dependent than $T_{0.5a}$ (Figure 4:3). Q_{10} values were 2.53 and 2.13 for $T_{0.5r}$ and 1.21 and 1.28 for $T_{0.5a}$ in 8° C- and 20° C-acclimated fish respectively. The corresponding Q_{10} values for tetanic contractions were more similar: the Q_{10}'s were 2.14 and 2.17 for $t_{0.5r}$ and 1.69 and 1.86 for $t_{0.5a}$ in 8° C- and 20° C-acclimated fish respectively. For both twitches and tetani $T_{0.5a}$ was 15% and $T_{0.5r}$ was 20% higher at 8° C for fibres from 8° C- than 20° C-acclimated fish (Table 4:1, $P<0.01$). Measured at the respective acclimation

TABLE 4:1. Contractile properties of slow pectoral muscle fibres.

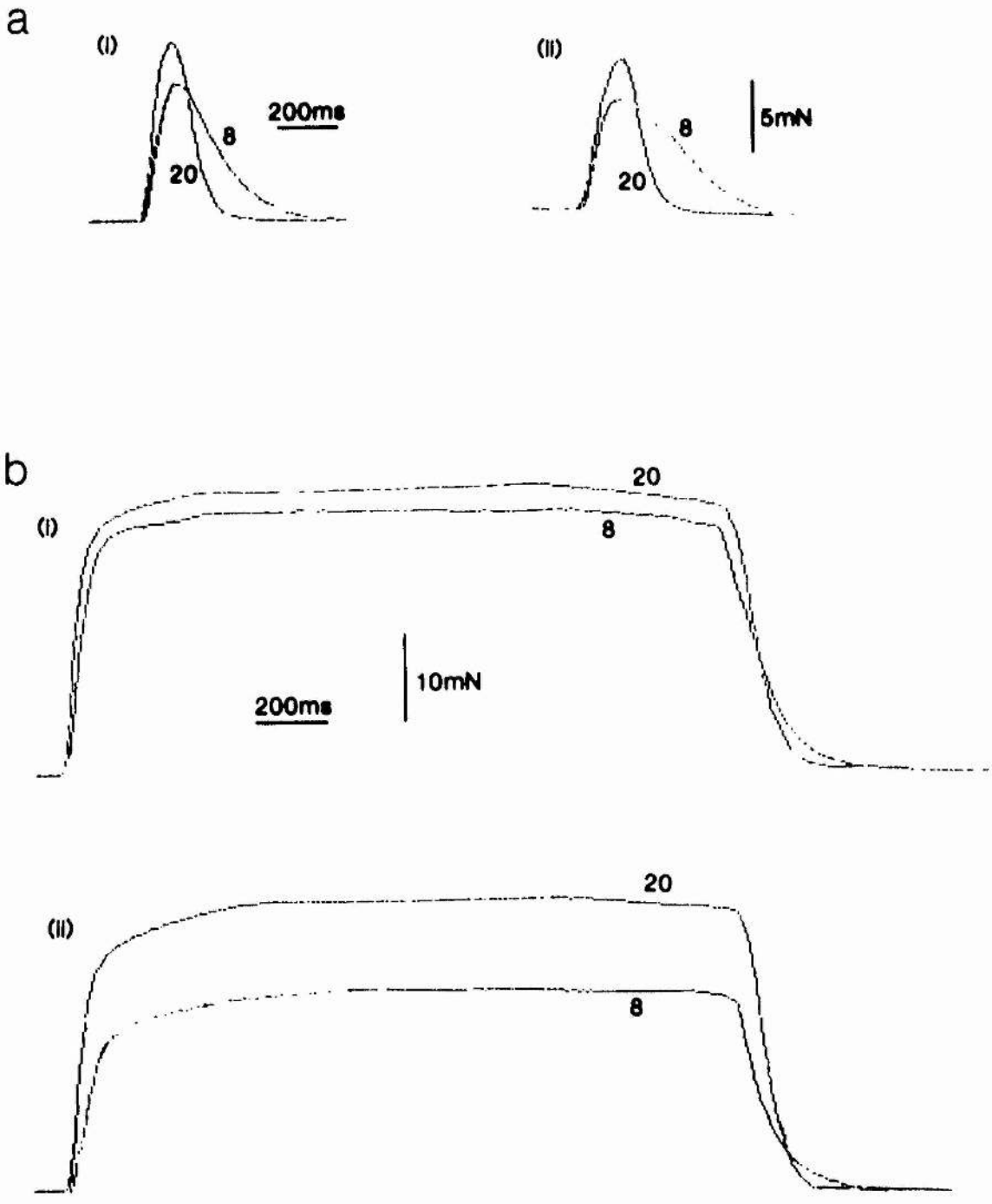
	8° C fish		20° C fish	
	at 8° C	at 20° C	at 8° C	at 20° C
Maximum isometric tension (kNm^{-2})	202±8	226±19*	153±4**	218±8^{ns}
Half twitch rise time (ms)	46.2±1.6	36.6±2.9**	53.1±1.8*	39.6±0.7**
Half twitch relaxation time (ms)	141.3±3.1	46.2±1.8**	169.2±2.9**	68.4±2.8**
Half tetanus rise time (ms)	125.4±4.3	50.2±2.7**	143.9±3.6*	76.4±4.6**
Half tetanus relaxation time (ms)	156.6±4.1	61.9±4.4**	189.3±8.5*	89.8±3.6**

Values represent mean±S.E., N=6. *=P<0.05, **=P<0.01, ^{ns}=not significant.

FIGURE 4:3.

a. Isometric twitch contractions of preparations from (i) 8° C-acclimated and (ii) 20° C-acclimated carp at 8° C and 20° C.

b. Tetanic contractions of preparations from (i) 8° C-acclimated and (ii) 20° C-acclimated carp at 8° C and 20° C.


a
(I)
200ms
8
20
(II)
8
5mN
20
b
(I)
20
8
10mN
200ms
(II)
20
8

temperatures $T_{0.5a}$ was 1.6 times and $T_{0.5r}$ was 1.7 times faster in 20° C than in 8° C-acclimated fish. Thus the rates of tension development and relaxation exhibited capacity adaptations in the region of 13-17% following cold acclimation.

Force-velocity relationship

Since preparations from 8° C fish deteriorated rapidly if maintained at 20° C, and the aim of the experiments were to investigate any capacity adaptations to low temperature following cold-acclimation, the P-V relationship of fibres from both acclimation groups were compared at 8° C. The hyp-1in equation gave a better fit to the data than the Hill equation, as found in previous studies (Altringham & Johnston, 1988; Langfeld *et al.*, 1989), for both acclimation groups. Representative P-V curves from cold- and warm-acclimated carp, calculated using the hyp-1in equation, are shown in Figure 4:4, and all the data is summarised in Table 4:2. The unloaded contraction velocity (V_{max}) of fibres was 17% higher in 8° C than 20° C-acclimated fish ($P<0.05$). $\dot{W}_{max}/V_{max}.P_o$, which provides a measure of the curvature of the force-velocity relationship (Marsh & Bennett, 1986), was not affected by acclimation temperature. The calculated maximum mechanical power output was 47% higher at 8° C in 8° C- than 20° C-acclimated fish, largely due to greater force production in the cold-acclimated fish (Table 4:2, Figure 4:5).

FIGURE 4:4. Force-velocity data from representative preparations fitted to the hyp-1in equation. $\dot{W}_{max}.V_{max}/P_o$ is 0.111 for 8° C-acclimated fish and 0.120 for 20° C-acclimated fish.

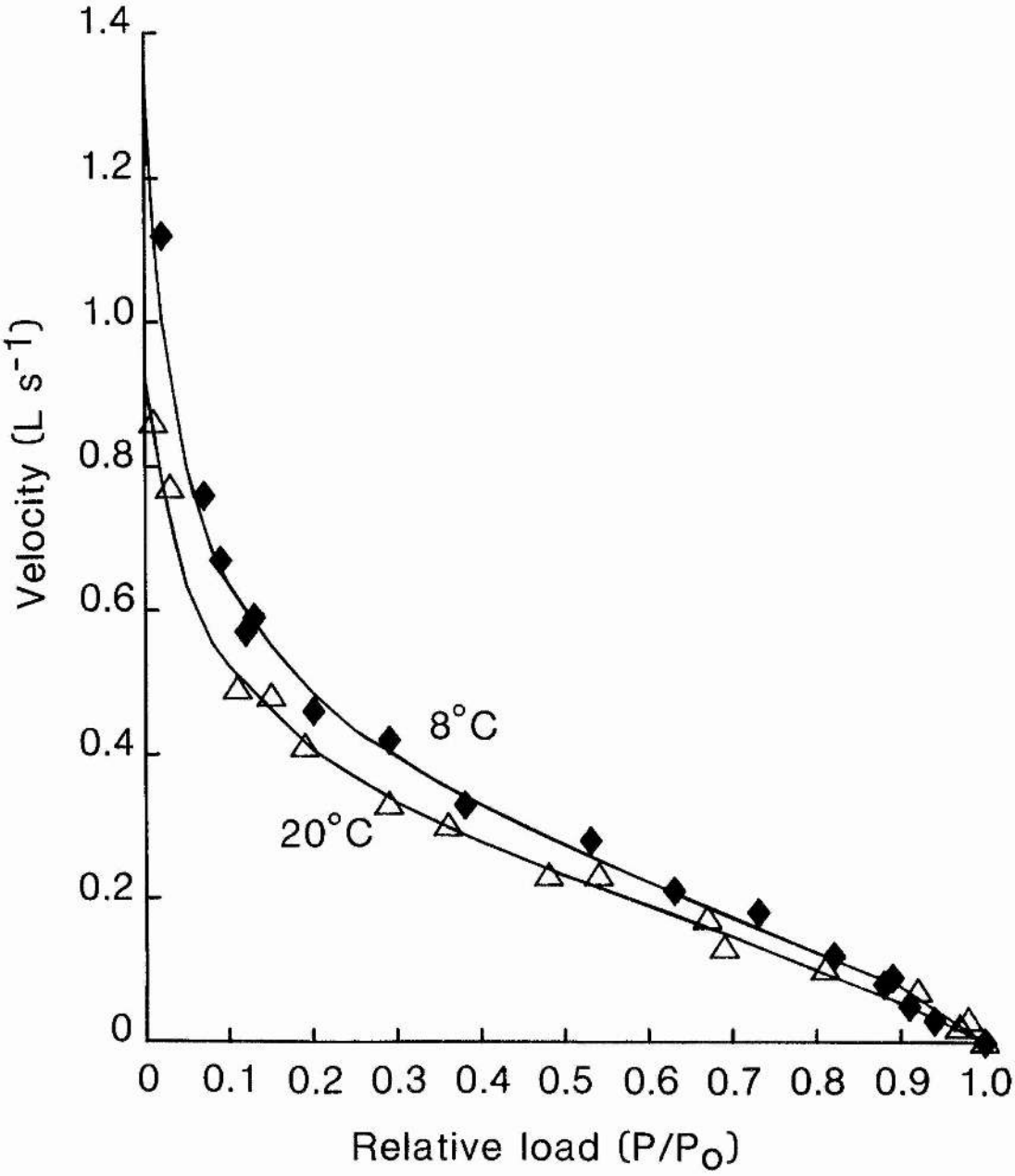

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Velocity (L s-1)
8°C
20°C
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Relative load (P/Po)

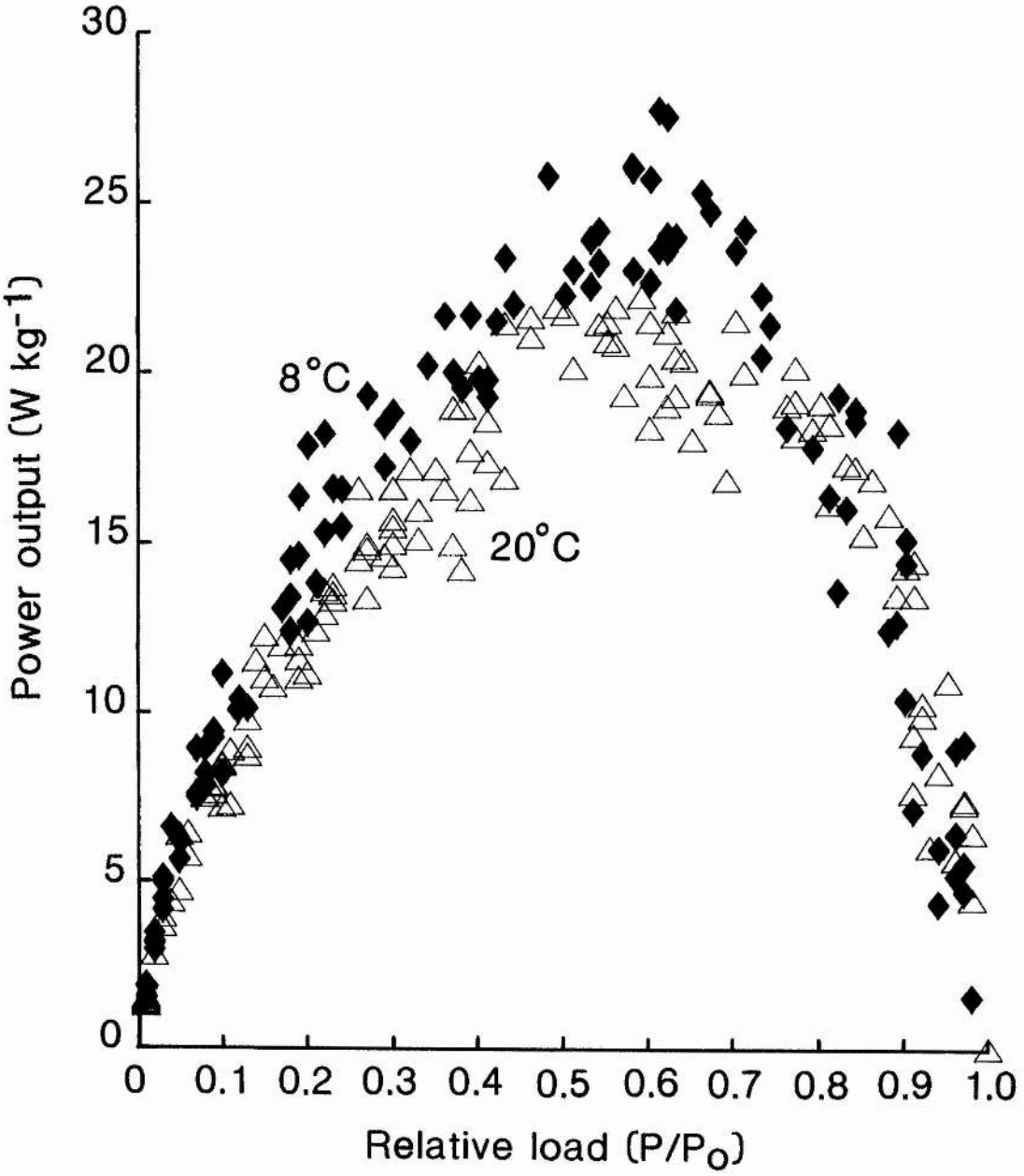
TABLE 4:2. Data represent mean±S.E., N=6. V_{max} is extrapolated maximum contraction velocity, $\dot{W}_{max}$ is maximum power output, A, B and C are constants from the hyp-lin equation, a and b are constants from Hill's equation.

Ls^{-1}=muscle lengths per second. *=P<0.05, **=P<0.01, ^{ns}=not significant. In the third section of the table, the standard error of the mean (SEE) derived from the two curve-fitting procedures are compared for each acclimation group.

TABLE 4:2. Summary of force-velocity data.

	8° C	20° C
HYP-LIN EQUATION		
V_{max} (Ls^{-1})	1.18±0.04	0.98±0.04*
$\dot{W}_{max}$ (Wkg^{-1})	26.46	18.04**
Load for max. power output	0.48P_o	0.46P_o*
A	0.040±0.006	0.046±0.004
B (Ls^{-1})	0.032±0.005	0.031±0.003
C (Ls^{-1})	0.32±0.03	0.32±0.02
$\dot{W}_{max}/V_{max}.P_o$	0.111±0.007	0.120±0.007ns
r^2	0.98	0.98
HILL'S EQUATION		
V_{max} (Ls^{-1})	0.95±0.05	0.82±0.03*
$\dot{W}_{max}$ (Wkg^{-1})	18.38	13.16**
Load for max power output	0.23P_o	0.24P_o ns
a/P_o	0.092±0.009	0.105±0.007ns
b (Ls^{-1})	0.086±0.006	0.086±0.004
$\dot{W}_{max}/V_{max}.P_o$	0.093±0.007	0.105±0.005ns
r^2	0.97	0.97
SEE		
Hyp-lin	0.004±0.001	0.004±0.001
Hill's	0.009±0.002**	0.009±0.001**

FIGURE 4:5. Power output calculated using parameters derived from fitting with the hyp-lin equation plotted against relative load for both acclimatory groups.


30
25
20
15
10
5
0
Power output (W kg-1)
8°C
20°C
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)

Electrophoresis

On sodium pyrophosphate gels, myosins from the slow region of the *AB.S.* co-migrated with slow myotomal muscle myosin. No differences were observed in the relative mobilities of native myosins (pyrophosphate gels) or myosin heavy chains (8% SDS PAGE gels) between cold- and warm-acclimated fish.

Electrophoretically purified native myosins were examined on 15% SDS PAGE gels. Fibre preparation from 20° C-acclimated carp contained almost exclusively slow muscle light chain isoforms ($LC1_s$ and $LC2_s$) (Figures 4:6 & 4:7). There were also traces of fast muscle myosin isoforms (LC1f and LC2f). Preparations of *AB.S.* from 8° C -acclimated carp contained a much higher proportion of the two fast muscle myosin isoforms (Figures 4:6 & 4:7). $LC3_f$ was either not present or it co-migrated with $LC2_s$ (both peptides have a similar apparent molecular mass in carp (Crockford & Johnston, 1990).

DISCUSSION

Cold-acclimation resulted in modest increases in the rates of force development and relaxation in slow muscle fibres at low temperatures (Figure 4:3, Table 4:1). Considerably larger changes in twitch duration occur in fast myotomal fibres. In a myotomal nerve-muscle preparation

FIGURE 4:6. SDS 15% polyacrylamide gel to show the myosin light chains from cold- and warm-acclimated carp purified on sodium pyrophosphate gels. The gel was silver stained. Lanes A and B were from 20° C-acclimated carp, lanes C and D were from 8° C-acclimated carp. $LC1_s$, myosin light chain 1 slow; $LC1_f$, myosin light chain 1 fast; $LC2_s$, myosin light chain 2 slow, $LC2_f$, myosin light chain 2 fast.

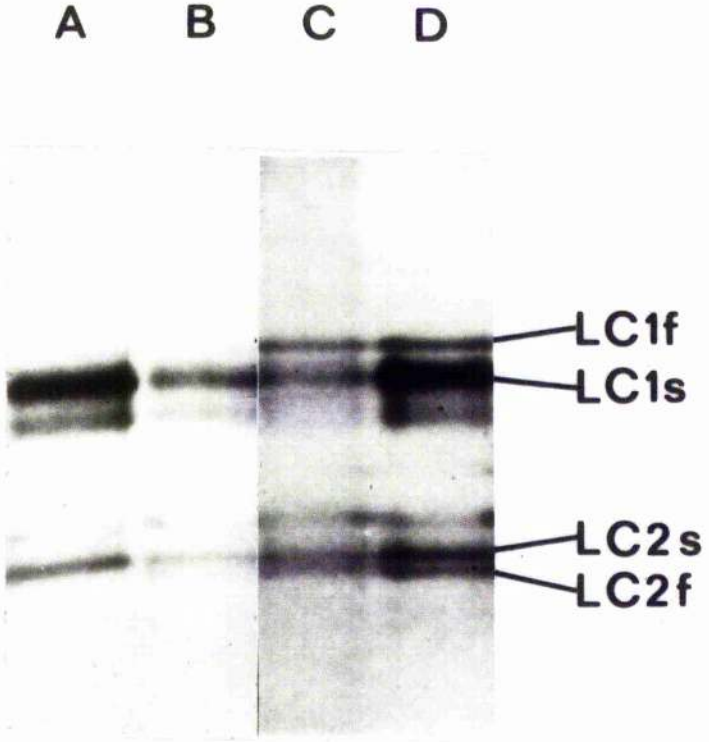
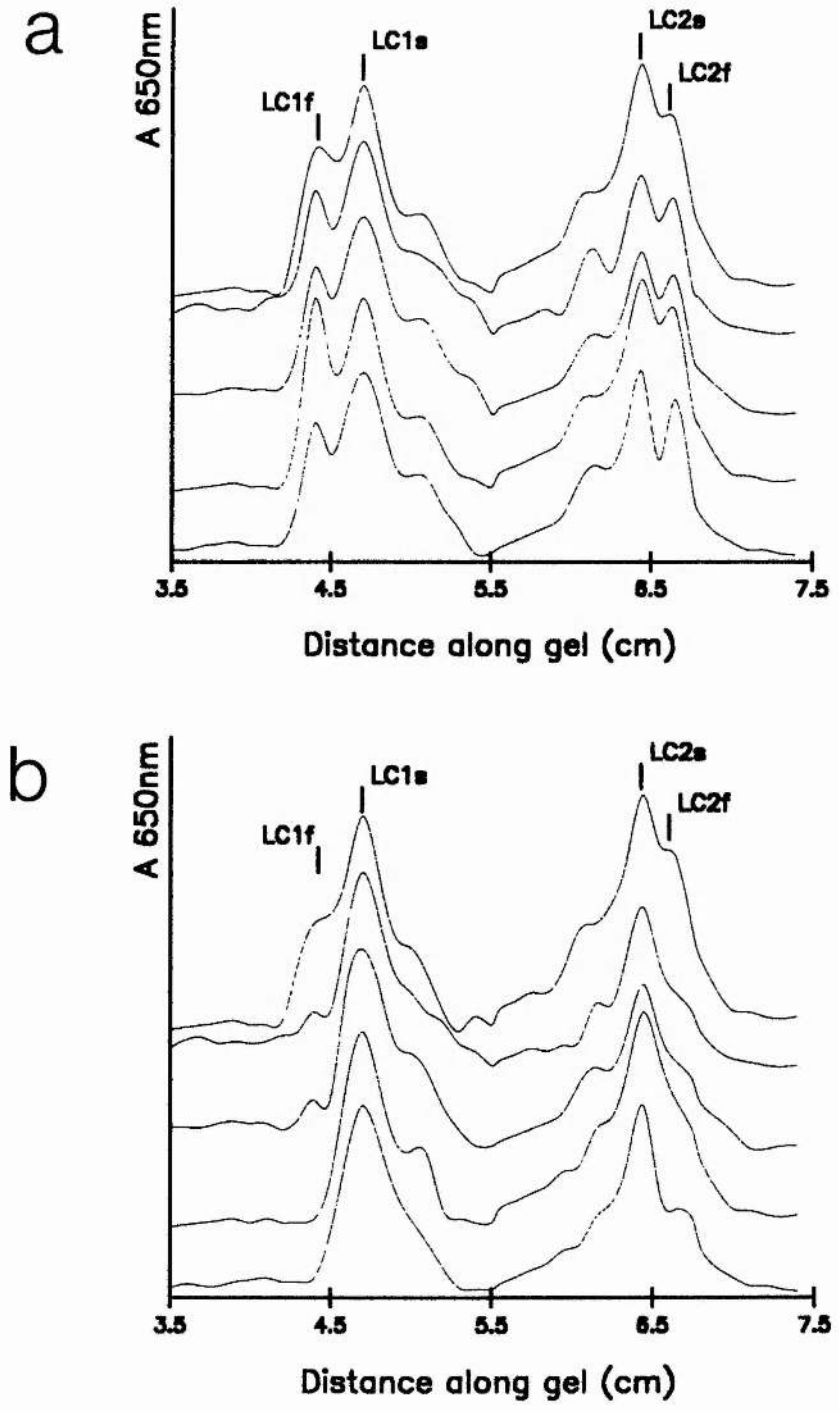

A
B
C
D
LC1f
LC1s
LC2s
LC2f

FIGURE 4:7. Densiometric scans of myosin light chains from several individual fish acclimated to either a. 8° C or b. 20° C.


a
A 650nm
LC1f
LC1s
LC2s
LC2f
3.5
4.5
5.5
6.5
7.5
Distance along gel (cm)
b
A 650nm
LC1f
LC1s
LC2s
LC2f
3.5
4.5
5.5
6.5
7.5
Distance along gel (cm)

from carp, half times for twitch activation and relaxation were 2-times shorter at 8° C in cold- than warm-acclimated fish (Fleming *et al.*, 1990). Unlike goldfish (Penney & Goldspink, 1980), the surface and volume density of sarcoplasmic reticulum (SR) in the myotomal muscles of the common carp were not altered by temperature acclimation, neither were the pCa-tension relationship or parvalbumin levels (Fleming *et al.*, 1990). Faster relaxation of tension is thought to be associated with an increase in SR Ca^{2+}-ATPase activity (Fleming *et al.*, 1990; Johnston *et al.*, 1990).

Rome & Sosnicki (1990) studied the influence of temperature on the slow myotomal muscle of carp. Unlike the preparations obtained from the pectoral muscle used in this study, the fibre bundles gave low activation levels when stimulated electrically. Even when activation levels were boosted by the addition of caffeine and eserine, maximum isometric tensions were considerably lower than those obtained in this study, only 116 kNm^{-2} as compared to 218 kNm^{-2}. Pectoral slow muscle preparations are clearly capable of action potentials. V_{max} values obtained by Rome & Sosnicki (1990), however, were more than double those obtained in this study, resulting in larger calculated power outputs for myotomal carp fibres. The disparity in V_{max} values, and thus power output, may be explained by an interaction of experimental factors, including the differing activation procedures, differences in the methods of measuring muscle length and determining the P-V data, or

simply by the explanation that fish slow muscles are a more physiologically heterogenous group than might be expected (Granzier *et al.*, 1983).

This is the first quantitative study on the effects of temperature acclimation on force production and power output by live muscle fibres in fish. The values obtained for maximum tension generation (P_o) by live fibres in the present study are higher than for skinned slow fibre preparations from the same species (Johnston *et al.*, 1985). Acclimation to 8° C resulted in a perfect capacity adaptation of force production in slow fibres (Table 4:1), *ie.* acclimation completely offsets the decline in tension which results from an acute decrease in temperature. Other studies have shown that P_o does not vary continuously with acclimation temperature, but reaches upper & lower limits (Penney & Goldspink, 1981). For example, P_o for fast myotomal fibres at 0° C is similar in carp acclimated to 2–11° C, but declines progressively at higher acclimation temperatures (Crockford & Johnston, 1990). In the crucian carp, *Carassius carassius*, the volume density of myofibrils in fast and slow muscle fibres is slightly higher in warm- than cold-acclimated fish (Johnston & Maitland, 1980). Thus changes in force production with temperature acclimation are not due to changes in myofibril packing, but reflect differences in the behaviour of myosin cross-bridges. In a X-ray diffraction study using skinned skeletal muscle fibres from the mouse Matsubara *et al.* (1985) found that the mass transferred radially from the thick to thin filaments was

approximately proportional to the relative tension. Differences in the absolute force produced at a given temperature, and in the temperature dependence of P_o; can be explained by differences in the number of bound cross bridges and the relative rates of cross bridge attachment and detachment respectively. Stephenson & Williams (1981) found that in rat skinned fibres rigor force was 15–20 times lower at 5° C than 20° C, irrespective of whether rigor was induced independently at each temperature, or induced at 5° C and the temperature raised. This provides indirect evidence that the number of attached cross bridges increases as the temperature is raised. However, studies with frog live fibres have shown that stiffness is independent of temperature between 0–20° C, whereas tension increased by 50% (Bressler, 1981). Observations using probe and X-ray methods have provided evidence for two classes of attached cross bridges (see Huxley & Kress, 1985). One possibility is that the number of strongly attached force generating cross bridges increases significantly as the temperature is raised wheras the number of weakly attached cross bridges is more constant.

Experiments with both skinned (Johnston & Altringham, 1985) and live (Langfeld *et al.*, 1989) fibres have shown that the force–velocity relationship of fish muscle becomes progressively less curved at low temperatures. Langfeld *et al.* (1989) investigated the P–V relationship of live fast myotomal muscle fibres from the sculpin *Myoxocephalus scorpius*. They found that on normalising curves for P_o and

V_{max} at each temperature, the change in curvature was sufficient to increase relative contraction speed and hence power output of the muscle by around 15% on decreasing the temperature from 8° C to 1° C. Although the curvature of carp fibres at any given temperature is unchanged by acclimation the above mechanism will contribute to temperature compensation of mechanical power output (and see Rome & Sosnicki, 1990). In addition, cold-acclimation results in a decrease in twitch duration and an increase in V_{max} of around 15-20% at 8° C (Figure 4:3; Tables 4:1 & 4:2). This represents a relatively modest capacity adaptation, since both parameters have Q_{10}'s of around 1.6-2.0. Fleming *et al.* (1990) studied a nerve-muscle preparation from the fast myotomal muscle of common carp. They found that the half times for twitch activation and relaxation were around 2-times shorter at 8° C in 8° C-acclimated than 20° C-acclimated fish. Similarly, V_{max} at 7° C, skinned fast fibres is 2-times higher in 7° C- than 23° C-acclimated carp (Johnston *et al.*, 1985). Thus capacity adaptations in contractile performance with cold acclimation are more marked for faster contracting muscle fibre types.

Fish myosin is composed of two heavy chains of 200 kDa and four light chains ranging in mass between 17 and 26 kDa (Focant *et al.*, 1981; Rowlerson *et al.*, 1985). Force production and shortening speed in vertebrate skeletal muscle is thought to be largely determined by heavy chain composition (Reiser *et al.*, 1985; Lännergren, 1987). The

myosin heavy chains are a multi-gene family of proteins that are expressed in a developmental stage- and tissue-specific manner (Richter *et al.*, 1989). Cloning studies indicate that common carp possess a minimum of 28 different myosin chain genes (Gerlach *et al.*, 1990). Crockford & Johnston (1990) found that peptide maps produced from proteolytic digests of purified myosin heavy chains from 8° C- and 20° C-acclimated carp were identical. However, Gerlach *et al.* (1990) using a DNA probe for myosin heavy chains isolated from warm-acclimated carp found increased hybridization to RNA from warm- than cold- acclimated carp. This suggests that temperature acclimation results in changes in myosin heavy chain gene expression. Crockford & Johnston (1990) have obtained electrophoretic evidence for an isoform of myosin light chain 3 which is unique to cold-acclimated carp. Single fast muscle fibres from cold-acclimated carp contained an extra band on isoelectric focusing gels with a pI intermediate between $LC2_f$ and $LC3_f$, and apparent molecular weight of 20 kDa (the same as $LC3_f$). Densiometric scans showed that the ratio $LC2_f$:($LC1_f$ +$LC3_f$ +extra band) was around 1 for both acclimation groups, supporting evidence for the band being an alkali light chain. The ratio of LC1:LC3 was also significantly lower in 8° C than 20° C-acclimated carp (Crockford & Johnston, 1990). These findings are of interest in relation to the recent report that LC3 content has a role in modulating V_{max} of rabbit muscle fibres (Greaser *et al.*, 1988). The relatively large changes in fast muscle fibre contractile properties probably result

from changes in the expression of both heavy and light chains.

Fast and slow myosins can be expressed in the same fibre type (Reiser *et al.*, 1985; Greaser *et al.*, 1988). *AB.S.* fibre preparations from 8° C fish had a higher content of myosin light chain isoforms normally associated with faster fibre types (Figures 4:6 and 4:7). Since the percentage of fast fibres in preparations did not vary with acclimation temperature, it would appear that $LC1_f$ and $LC2_f$ are co-expressed with $LC1_s$ and $LC2_s$ in the slow muscle of 8° C acclimated fish. This may contribute to the higher force production and speeds of contraction observed following cold-acclimation.

CHAPTER 5

General Discussion

Swimming is a highly complex process, with many hydrodynamic and biomechanical constraints operating on the body of a swimming fish. The hydrodynamic constraints involve the effects of the physical properties of the water surrounding the fish and will be affected by the size, body shape and speed of the fish as it moves through the water. Other constraints include the fuelling of metabolic activity, and the function of the various components of the locomotory structure of a fish, such as the skeleton and muscle fibre type, arrangement and quantity. Clearly, many factors need to be taken into consideration when observing the effects of temperature on fish swimming.

Determining the effects of temperature change on the contractile performance of isolated muscle fibres will provide information about the effect of temperature on one component of fish swimming, although the constraints for isolated muscles and those *in vivo* will be different. The maximal forces developed by muscle fibres and their rates of force activation and relaxation, contraction speeds and power output are all altered as the environmental and thus body temperature varies. In species that undergo wide seasonal temperature variation, we might expect to find physiological adaptations that minimise thermal effects, so

that relatively constant biological activity can be maintained.

During this work, the effects of temperature on the mechanical properties of the muscle of two teleosts have been studied using a relatively new preparation, an isolated live fibre bundle, developed by Altringham & Johnston (1988). Results obtained using this preparation have enabled a detailed study of the force-velocity relationship and the calculation of muscle power output during steady shortening. Decreasing temperature causes a decrease in muscle power output, but a change in the curvature of the force-velocity relationship offsets the decrease to some degree. In the fast muscle of *Myoxocephalus scorpius*, the observed change in curvature between 8° C and 1° C increases relative maximum power output by 15% (Chapter 2 and Langfeld *et al.*, 1989). A similar effect is found in slow muscle from the carp *Cyprinus carpio* (Chapter 4).

Following cold-acclimation, the swimming performance of some fish is increased at low temperatures and decreased at high temperatures (Fry & Hart, 1948, Heap & Goldspink, 1986). A number of contributory factors to this plasticity in swimming performance with cold-acclimation have been highlighted by the present study. One method of compensating for the metabolic effects of low temperature would be to increase the volume of slow fibres and thus the total aerobic capacity of the muscle. An increase in the relative proportion of aerobic fibres in the *abductor*

superficalis muscle of carp was seen following cold-acclimation (Chapter 3). Considering this result in conjunction with the evidence produced by Rome *et al.* (1984) for a compression of the fibre recruitment order at low temperatures, it is clear that the presence of a greater number of aerobic fibres will enable the fish to reach a higher swimming speed before the rapidly fatiguing anaerobic fibres need to be recruited, and thus cold-acclimation permits compensation for the effects of low temperature on swimming performance. Electromyographical data produced by Rome *et al.* (1985), for carp indeed confirms that recruitment of anaerobic fibres takes place at higher swimming speeds following cold-acclimation.

An alternative method of compensation to the above would be to modify the properties of individual muscle fibres. This work has found evidence for temperature compensation in some of the contractile properties of the slow, aerobic musculature following cold-acclimation. The rates of force-development and relaxation at low temperatures were higher in cold-acclimated fish. The increase in maximum contraction velocities and maximum tensions observed at low temperatures in cold-acclimated fish result in greater power output of slow fibres at low temperature following cold-acclimation (Chapter 4 & Langfeld *et al.*, 1990). Comparison of twitch contraction parameters from this study with those obtained by Fleming *et al.*, (1990), however, reveal that capacity adaptations in contractile performance following cold-acclimation appear to

be greater in faster contracting muscle fibre types.

The molecular mechanisms associated with the change in contractile properties following acclimation probably involve changes in several of the myofibrillar proteins. Myosin composition is known to be a major determinant of contraction velocity (Reiser *et al.*, 1985; Lännergren, 1987). This study has observed that cold-acclimated fish have a higher content of myosin light chain isoforms normally associated with faster contracting fibre types than do fibres from warm-acclimated fish, $LC1_f$ and $LC2_f$ appearing to be co-expressed with $LC1_s$ and $LC2_s$ in cold-acclimated fish (Chapter 4 & Langfeld *et al.*, 1990). In fast muscle from carp, a super-fast isoform of myosin light chain ($LC3_{s\,f}$) is found in cold-acclimated fish (Crockford & Johnston, 1990), a significant result in the light of recent evidence that LC3 content has a role in modulating the contractile performance of rabbit muscle fibres (Greaser *et al.*, 1988). Although Crockford & Johnston (1990) found no electrophoretic evidence for differences in myosin heavy chains in cold-acclimated carp fast muscle, a study using DNA probes by Gerlach *et al.*, (1990) found increased hybridization to RNA from warm- than cold-acclimated carp, suggesting that changes in myosin heavy gene expression are likely following temperature acclimation. The larger changes in contractile performance of fast muscle relative to slow muscle following acclimation are thus probably due to the greater changes in the expression of myosin chains in fast muscle.

During this work, muscle power output has been calculated from force-velocity curves determined from shortening under constant load. Whilst these measurements give an indication of the potential power output of a given muscle type, caution must be exercised when relating the data obtained to locomotion. During fish swimming, muscle fibres undergo cyclical length changes and so the dynamic effects of muscle lengthening and shortening, the fraction of time that the muscle fibre is active during each cycle and the effects of previous contractions on the crossbridges must also be taken into account when the power output directly relevant to swimming is calculated (Johnston & Altringham, 1988; Altringham & Johnston, 1990).

Josephson (1985), using synchronous insect flight muscle, was the first to measure muscle power output using an approach more applicable to conditions *in vivo*. The wing muscle was subjected to sinusoidal length changes at the normal wing-stroke frequency, and single or multiple stimuli applied at selected phases. Work loops were generated by plotting force against length, allowing the power output of the muscle to be calculated as work per cycle multiplied by frequency.

Altringham & Johnston (1990) have recently applied this method to isolated fish muscle fibres, power output being measured under conditions simulating muscle activity in a fish swimming at different speeds. Power output was maximised for each cycle frequency by adjusting the number

and timing of stimuli. Power output was also dependent on the amplitude of the length changes. Power output was maximal at cycle or tailbeat frequencies of 2 Hz for slow fibres and 5-7 Hz for fast fibres. Since tailbeat frequency is a major determinant of swimming speed (Webb *et al*, 1984), these results can be related to the motion of the fish. At low swimming speeds, power demands are met by the small diameter slow fibres (Johnston *et al.*, 1977; Bone *et al.*, 1985; Rome *et al.*, 1984). The tailbeat frequency of 2Hz, where slow fibres develop maximum power output, can be maintained for long periods. The maximum power output of slow fibres is lower than that of fast fibres, this low value for power output and the aerobic metabolism of slow fibres making them ideal for driving slow, sustained swimming. At higher swimming speeds, more power is required and fast fibres are recruited (Johnston *et al.*, 1977; Bone *et al.*, 1978, Rome *et al.*, 1984), the fast fibres produce five times as much power as slow fibres do at their optimal frequency of 5-7 Hz (Altringham & Johnston, 1990). However the anaerobic metabolism of fast fibres means that they fatigue quickly, so even though they produce more power than slow fibres at the slow fibre optimal frequency, fast fibres would be metabolically uneconomic for slow, sustained swimming.

Values for maximal power output obtained using this technique are necessarily much lower, but more applicable to actual fish swimming than those obtained using the methods described in this study. For example, power output in fast

fibres from *Myoxocephalus scorpius* determined using the cyclical contraction method was 35 Wkg^{-1} (Altringham & Johnston, 1990), compared to a value of 313 Wkg^{-1} obtained in this study (Chapter 2).

Recently, Van Leeuwen *et al.* (1990) examined the reçruitment patterns of slow muscle fibres and swimming movements of the carp using synchronised electromyography and cinematography. When combined with measurements of sarcomere ultrastructure, the data enabled modelling of the strain fluctuations and normalised power along the trunk of the fish for the observed combinations of sarcomere structure and contraction regime. The model takes into account the modulation of force resulting from sarcomere length changes, modulation of cross-bridge force resulting from the force-velocity relationship and changes in rates of tension activation and relaxation resulting from fibre properties and stimulation. One possible problem with the model however, concerns the factor attributable to the deformation of the trunk. Van Leeuwen *et al.* (1990) state that their particular deformation was based upon observations of radiographs of free-swimming fish, but that a major step forwards would be the construction of a dynamic model to calculate the body deformation during swimming from the structural and cybernetic features of the body, in combination with the physical properties of the surrounding water.

The data on power output obtained during this work illustrates the effects of acute temperature change and temperature acclimation on the contractile properties of fish muscle and thus potential for swimming. The technique used by Altringham & Johnston (1990) provides data more directly relevant to fish swimming. Parameters determined from data from observed fibre recruitment patterns and calculated strain ranges (Van Leeuwen *et al.*, 1990) could be inserted. Other factors also need to be taken into account for a more realistic picture of fish swimming to be constructed. The muscle fibres in successive segments operate in different strain ranges and at different contraction speeds (Van Leeuwen *et al.*, 1990) and the depth and orientation of the fibres within a single segment also determines fibre properties. Alexander (1969), for example, predicts that at a given curvature of the body during swimming, the deep muscle fibres would only need to shorten at 25% of the speed of the superficial fibres.

To obtain an accurate picture of how temperature affects fish swimming, a method of bridging the gap between experiments on isolated muscle fibres and whole animals needs to be found. A possible way forward would be to determine strain ranges in the swimming fish using cinematography, and use the data to apply more realistic length change patterns to isolated muscle fibre preparations. A model could then be constructed that would more closely resemble conditions *in vivo*.

REFERENCES

Akster, H.A. (1985). Morphometry of muscle fibre types in the carp (*Cyprinus carpio* L.). Relationships between structural and contractile characteristics. *Cell Tiss. Res.* **241**, 193–201.

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

Altringham, J.D. & Johnston, I.A. (1981). Quantitative histochemical studies of the peripheral innervation of cod (*Gadus morhua*) fast myotomal muscle fibres. *J. Comp. Physiol.* **143**, 123–127.

Altringham, J.D. & Johnston, I.A. (1982). The pCa tension and force–velocity characteristics of skinned fibres isolated from fish fast and slow muscles. *J. Physiol.* **333**, 421–449.

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

Altringham, J.D. & Johnston, I.A. (1988). The mechanical properties of polyneuronally innervated, myotomal muscle fibres isolated from a teleost fish

(*Myoxocephalus scorpius*). *Pflügers Arch.* 412, 524–529.

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

Altringham, J.D. & Johnston, I.A. (1990). The innervation pattern of fast myotomal muscle in the teleost *Myoxocephalus scorpius*: a reappraisal. *Fish Physiol. Biochem.* 6, 309–314.

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

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

Bancroft, J.D. & Stevens, A. (1972). *Theory and practice of histological techniques.* Churchill, Edinburgh.

Bàràny, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J. gen. Physiol.* 51, 197–216.

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

Bennett, A.F. (1984). Thermal dependence of muscle function. *Am. J. Physiol.* 247, R217–R229.

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

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 fibres in elasmobranch fish. *J. Mar. Biol. Assoc. U.K.* 46, 321-349.

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. *Fish Physiol.* Vol VII, pp 361-424, Academic Press.

Bone, Q. & Chubb, A.D. (1978). The histochemical demonstration of myofibrillar ATPase in elasmobranch muscle. *Histochem. J.* 10, 489-494.

Bone, Q., Johnston, I.A., Pulsford, A. & Ryan, K.P. (1986). Contractile properties and ultrastructure of three types of muscle fibre in the dogfish myotome. *J. Muscle Res. Cell Motil.* 7, 47-56.

Bone, Q., Kicenuik, J. & Jones, D.R. (1978). On the role of the different fibre types in fish myotomes at intermediate swimming speeds. *Fish Bull.* 76, 691-699.

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

Brenner, B. (1988). Kinetics of the crossbridge cycle derived from measurements of force, rate of force

development and isometric ATPase. *J. Mus. Res. Cell Motil.* 7, 75–76.

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

Brett, J.R. (1972). The metabolic demand for oxygen in fish, particularly salmonids and a comparison with other vertebrates. *Resp. Physiol.* **14**, 151–170.

Close, R.I. (1964). Dynamic properties of fast and slow skeletal muscles of the rat during development. *J. Physiol.* **188**, 261–271.

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

Curtin, N.A. & Woledge, R.C. (1988). Power output and force–velocity relationship of live fibres from white myotomal muscle of the dogfish *Scyliorhinus canicula*. *J. exp. Biol.* **140**, 187–197.

Edman, K.A.P. (1988). Double-hyperbolic force-velocity relation in frog muscle fibres. *J. Physiol.* **404**, 301–321.

Edman, K.A.P., Mulieri, L.A. & Scubon-Mulieri, B. (1976). Non-hyperbolic force–velocity relationship in single muscle fibres. *Acta Physiol. Scand.* **98**, 143–156.

Egginton, S.D. & Johnston, I.A. (1983). An estimate of capillary anisotrophy and determination of surface and

volume densities of capillaries in skeletal muscles of the conger eel (*Conger conger* L.). *J. exp. Physiol.* 68, 603–617.

Egginton, S. & Sidell, B.D. (1986). Changes in mitochondrial spacing in fish skeletal muscle induced by environmental temperature. *J. Physiol.* 373, 78P.

Egginton, S. & Sidell, B.D. (1989). Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am. J. Physiol.* 256, R1–R9.

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

Fleming, J.R., Altringham, J.D. & Johnston, I.A. (1989). The effects of temperature acclimation on muscle relaxation in the carp, a mechanical, biochemical & ultrastructural study. *J. exp. Zool.* In press.

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

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.

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

Fry, F.E.J. & Hochachka, P.W. (1970). In *Comparative Physiology of Thermoregulation* (ed. G.C. Whittow) pp 97–134. New York & London: Academic Press.

Gardner-Medwin, A.R. & Curtin, N.A. (1990). Implications of the septal structure in fish muscle. *J. Physiol.* **426**, 36P.

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

Gilly, W.F. & Aladjem, E. (1987). Physiological properties of three muscle fibre types controlling dorsal fin movements in a flatfish (*Citharichthys sordidus*). *J. Mus. Res. Cell Motil.* 8, 407–417.

Godt, R.E. & Maughan, D.W. (1977). Swelling of single muscle fibres of the frog. Experimental observation. *Biophys. J.* 19, 103–116.

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

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

Greaser, M.L., Moss, R.L., & Reiser, P.J. (1988). Variation in contractile properties of rabbit single muscle

fibres in relation to troponin T isoforms and myosin light chains. *J. Physiol.* 406, 85–98.

Greer-Walker, M. (1970). Growth and development of the skeletal muscle fibres of the cod (*Gadus morhua* L.). *J. Cons. int. Explor. Mer.* 33, 228–244.

Greer-Walker, M. & 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 fish. In *neural control of locomotion* (eds. R.M. Herman, S. Grillner, P.S.G. Stein & D.G. Stuart) pp 181–201. New York: Plenum Press.

Guth, L. & Samaha, F.J. (1969). Qualitative differences between actomyosin ATPase of fast and slow mammalian muscle. *Exp. Neurol.* 25, 138–152.

Guth, L. & Samaha, F.J. (1970). Proceedure for the histochemical demonstration of actomyosin ATPase. *Exp. Neurol.* 28, 365–367.

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. & Watt, P.W. (1989). The temperature dependence of the time course of growth and decay of minature end-plate currents in carp extra-ocular muscle following temperature acclimation. *J. exp. Biol.* 147, 237–248.

Harrison, P., Nicol, J.M. & Johnston, I.A. (1987). Gross morphology, fibre composition and mechanical properties of pectoral fin muscles in the Antarctic teleost, *Notothenia neglecta* Nybelin. In *Proceedings of the Fifth Congress on European Icthyology*, Stockholm 1985 (ed. S.O. Kullander & B. Fernholm), pp 459–465. Stockholm: Swedish Museum of Natural History.

Hazel, J.R. (1972). The effect of temperature acclimation upon SDH activity from the epaxial muscle of the common goldfish (*Carassius auratus* L.). *Comp. Biochem. Physiol.* 43B, 837–882.

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

Heap, S.P. & Goldspink, G. (1986). Alterations to the swimming performance of carp, *Cyprinus carpio*, as a result of temperature acclimation. *J. Fish Biol.* 29, 747–753.

Heap, S.P., Watt, P.W. & Goldspink, G. (1985). Consequences of thermal change on the myofibrillar ATPase of 5 freshwater teleosts. *J. Fish. Biol.* 26, 733–738.

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

Heap, S.P., Watt, P.W. & Goldspink, G. (1987). Contractile properties of goldfish fin muscles following

temperature acclimation. *J. comp. Physiol.* B. 157, 219–225.

Hess, F. & 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.

Hidaka, T. & Toida, N. (1969). Biophysical and mechanical properties of red and white muscle fibres in fish. *J. Physiol. Lond.* 201, 49–59.

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

Hudson, R.C.L. (1969). Polyneuronal innervation of the fast muscles of the marine teleost *Cottus scorpius* L. *J. exp. Biol.* 50, 47–67.

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

Johnston, I.A. (1975). Anaerobic metabolism in the carp (*Carassius carassius* L.). *Comp. Biochem. Physiol.* 51B, 235–241.

Johnston, I.A. (1980). Contractile properties of fish fast muscle fibres. *Mar. Biol. Lett.* 1, 323–328.

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 muscles

following acclimation to summer and winter temperatures. *Cell Tissue Res.* **222**, 325–337.

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. (1990). Cold adaptation in marine organisms *Phil. Trans. R. Soc. Lond.* B **326**, 655–667.

Johnston, I.A. & 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. & Altringham, J.D. (1988). Muscle energetics and environment in ectotherms. In: *Energy Transformation in Cells and Animals.* (ed. W. Wieser). Stuttgart: Georg Thieme Verlag.

Johnston, I.A. & Altringham, J.D. (1988). Muscle function in locomotion. *Nature* **355**, 767–768.

Johnston, I.A. & Bernard, L.M. (1982). Ultrastructure and metabolism of skeletal muscle fibres in the tench: effects of long term acclimation to hypoxia. *Cell Tissue Res.* **227**, 179–199.

Johnston, I.A. & Camm, J.-P. (1987). Muscle structure and differentiation in pelagic and demersal stages of the Antarctic teleost *Notothenia neglecta*. *Mar. Biol.* **94**, 183–190.

Johnston, I.A., Davidson, W. & Goldspink, G. (1975). Adaptations in Mg^{2+}-activated myofibrillar ATPase

activity induced by temperature acclimation. *FEBS Letts.* 50, 293–295.

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

Johnston, I.A. & Dunn, J.F. (1987). Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. In *Temperature and Animal Cells* (ed. K. Bowler). SEB Symposia XXXXI, 67–93.

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

Johnston, I.A. & Lucking M. (1978). Temperature induced variation in the distribution of different types of muscle fibre in the goldfish (*Carassius auratus*). *J. comp. Physiol.* 124, 111–116.

Johnston, I.A. & Maitland, B. (1980). Temperature acclimation in crucian carp (*Carassius carassius* L.): Morphometric analyses of muscle fibre ultrastructure. *J. Fish. Biol.* 17, 113–125.

Johnston, I.A. & Moon, T.W. (1980a). Endurance exercise training in the fast and slow muscle of a teleost (*Pollachius virens*). *J. comp. Physiol. B* **135**, 147–156.

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

Johnston, I.A., Patterson, S., Ward, P. & Goldspink, G. (1974). The histochemical demonstration of

myofibrillar ATPase activity in fish muscle. *Can. J. Zool.* **52**, 871–877.

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

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

Johnston, I.A., Ward, P.S. & Goldspink, G. (1975). Studies on the swimming musculature of the rainbow trout. I. Fibre types. *J. Fish. Biol.* **7**, 451–458.

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

Jones, P.L. & Sidell, B.D. (1982). Metabolic responses of striped bass *Morone saxatilis* to temperature acclimation: Alterations in metabolic carbon sources and distribution of fibre types in locomotory muscle. *J. exp. Zool.* **219**, 163–172.

Josephson, R.K. (1985). Mechanical power output from striated muscle during cyclic 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 living and skinned frog muscle fibres. *J. Physiol.* **370**, 181–199.

Karanski, J. & Kilarski, W. (1989). Polymorphism of myosin isoenzymes and myosin heavy chains in histochemically typed skeletal muscles of the roach (*Rutilus rutilus* L., Cyprinidae, fish). *Comp. Biochem. Physiol.* **92B**, 727–731.

Kilarski, W. & Kozlowska, M. (1987). Comparison of ultrastructural and morphometric analysis of tonic, white and red muscle fibres in the myotome of teleost fish (*Noemacheilus barbatulus* L.). *Z. mikrosk. anat. Forsch. Leipzig* **101**, 636–648.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Langfeld, K.S., Altringham, J.D. & 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. & Johnston, I.A. (1990). Temperature acclimation in the common carp: force–velocity characteristics and myosin subunit composition of slow muscle fibres. *J. exp. Biol.* In press.

Lännergren, J. (1978). The force–velocity relation of isolated twitch and slow muscle fibres of *Xenopus laevis*. *Acta Physiol. Scand.* **114**, 523–535.

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

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

Leinwand, L.A., Saez, L., McNally, E. & Nadal-Ginard, B. (1983). Isolation and characterisation of human myosin heavy chain genes. *Proc. Nat. Acad. Sci. USA* 80, 3716.

Loughna, P.T. & Goldspink, G. (1985). Muscle protein synthesis rates during temperature acclimation in a eurythermal (*Cyprinus carpio*) and a stenothermal (*Salmo gairdneri*) species of teleost. *J. exp. Biol.* **118**, 267–276.

Magnuson, J.J. (1973). Comparative study of adaptations for continuous swimming and hydrostatic equilibrium of scromboid and xiphoid fishes. *U.S. Fish. & Wildlife Serv. Fish. Bull.* **71**, 337–356.

Marsh, R.L. & Bennett, A.F. (1985). Thermal dependence 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. & Bennett, A.F. (1986). Thermal dependence of contractile properties of skeletal muscle from the lizard *Sceloporus occidentalis* with comments on methods for fitting and comparing force–velocity curves. *J. exp. Biol.* **126**, 63–77.

Moerland, T.S. & Sidell, B.D. (1986). Contractile responses to temperature in the locomotory musculature

of striped bass *Morone saxatilis*. *J. exp. Zool.* 240, 25–33.

Montgomery, J.C. & McDonald, J.A. (1984). Performance of motor systems in antarctic fishes. *J. comp. Physiol.* 154 A, 241–248.

Mosse, P.R.L. (1978). The distribution of capillaries in the somatic musculature of two vertebrate types with particular reference to teleost fish. *Cell Tissue Res.* 187, 281–303.

Mutungi, G. & Johnston, I.A. (1987). The effects of temperature and pH on the contractile properties of skinned muscle fibres from the terrapin, *Pseudemys scripta elegans*. *J. exp. Biol.* 128, 87–105.

Nabeshima, Y., Fujii-kuriyama, Y., Muramatsu, M. & Ogata, K. (1984). Alternative transcription and two modes of splicing result in two myosin light chains from one gene. *Nature* 308, 333–338.

Nag, A.C. (1972). Ultrastructure and adenosine triphosphatase activity of red and white muscle fibres of the caudal region of a fish, *Salmo gairdneri*. *J. Cell. Biol.* 55, 42–57.

Nishihara, H. (1967). Studies on the fine structure of red and white fin muscles of the fish (*Carassius auratus*). *Archvm. histol. jap.* 28, 425–447.

Patterson, S., Johnston, I.A. & Goldspink, G. (1974). A histochemical study of the lateral muscles of five teleost species. *J. Fish. Biol.* 7, 159–169.

Penney, R.K. & Goldspink, G. (1980). Temperature adaptation of sarcoplasmic reticulum of goldfish. *J. Therm. Biol.* 5, 63–68.

Penney, R.K. & Goldspink, G. (1981a). Compensation limits of fish myofibrillar ATPase enzyme to environmental temperature. *J. Therm. Biol.* 5, 269–272.

Penney, R.K. & Goldspink, G. (1981b). Temperature adaptation by the myotomal muscle of fish. *J. Therm. Biol.* 6, 297–306.

Ping, C., Pao, S. & Yang, H.Y. (1958). The skeletal musculature of the carp (*Cyprinus carpio*). *Acta. Zool. Sinica.* 10, 289–315.

Precht, H. (1958). Concepts of the temperature adaptation in unchanging reaction systems of cold-blooded animals. In: *Physiological Adaptation* (ed. C.L. Prosser), pp. 50–78. Amer. Assoc. Adv. Science, Washington.

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

Reiser, P.J., Moss, R.L., Giulan, G.G. & Greaser, M.L. (1985). Shortening velocity in single fibres from adult rabbit soleus muscle is correlated with myosin heavy chain composition. *J. Biol. Chem.* 260, 9077–9080.

Rome, L.C., Funke, R.P., Alexander, R. McN., Lutz, G., Aldridge, H.D.J.N., Scott, F. & Freadman, M. (1988). Why animals have different fibre types. *Nature* 355, 824–827.

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

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

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

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 isofroms and distribution in red, pink and white muscle. *J. Mus. Res. Cell Motil.* **6**, 601–640.

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

Shann, E.W. (1920). The comparative myology of the shoulder girdle and pectoral fin of fishes. *Trans. R. Soc. Edin.* **52**, 531–570.

Sharp, G.D. & Pirages, S. (1978). The distribution of red and white swimming muscles, their biochemistry and the biochemical phylogeny of selected scombrid fishes. In *The physiological ecology of tunas* (ed. G.D. Sharp & A.E. Dizon), pp 41–78. New York & London: Academic Press.

Smit, H., Van den Berg, R.J. & Klijne-Den Hartog, I. (1974). Some experiments on thermal acclimation in the goldfish (*Carassius auratus* L.). *Neth. J. Zool.* 24, 32-49.

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

Sidell, B.D. & Johnston, I.A. (1985). Thermal sensitivity of contractile function in chain pickerel, *Esox niger*. *Can. J. Zool.* 63, 811-816.

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

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

Starks, E.C. (1930). The primary shoulder girdle of the bony fishes. *Stanford Univ. Publ. Ser. Biol. Sci.* 6, 147-239.

Steinem, G.J.M., Guth, K. & Rüegg, J.C. (1983). Force and force transients in skeletal muscle fibres of the frog skinned by freeze-drying. *Pflügers Arch.* 397, 272-276.

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

Twelves, E.L. (1972). Blood volume of two Antarctic fishes. *Br. Antarct. Surv. Bull.* **31**, 85–92.

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

Walesby, N.J. & Johnston, I.A. (1981). Temperature acclimation of $Mg^{2+}Ca^{2+}$ myofibrillar ATPase from a cold-selective teleost, *Salvelinus fontinalis*: a compromise solution. *Experentia* **37**, 716–718.

Webb, P.W. (1975). Hydrodynamics and energetics of fish propulsion. *Bull. Fish Res. Board Can.* **190**, 1–159.

Webb, P.W. (1984). Form and function in fish swimming. *Scient. Am.* **251**, 72–82.

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

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.

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

Winterbottom, R. (1974). A descriptive synonymy of the striated muscles of the teleostei. *Proc. Acad. Nat. Sci. Philadelphia* 125, 225-317.

Woledge, R.C., Curtin, N.A. & Homsher, E. (1985). *Energetic aspects of muscle contraction.* Academic Press, London.

Yamamoto, T. (1972). Electrical and mechanical properties of the red and white muscles in the silver carp. *J. exp. Biol.* 57, 551-567.

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.

ACKNOWLEDGEMENTS

I should like to thank the many people who helped to make this thesis possible, first and foremost, my supervisor Professor Ian Johnston. The help and advice given by Dr. John Altringham was invaluable, and without Dr Tony Crockford, the electrophoretic data in Chapter 4 would not have been possible.

Many thanks are due to Dr Julian Eastwood for writing the curve-fitting programmes, to Pete, Roland and Ian for the unfailing technical support and to the members of the photography unit, whom I badgered and bothered on many an occasion.

I should also like to mention Marie, Jackie, Jeni and Cristal, who gave moral support over many lunches, and thank all the members of the Gatty Marine Lab for making it such a pleasant place to work.

Last, but not least, I should like to thank my mother, and of course Mike and the "family", Jess, Jupi, and also Dash and Abby whom are now, sadly, gone.

This work was funded by an NERC studentship.