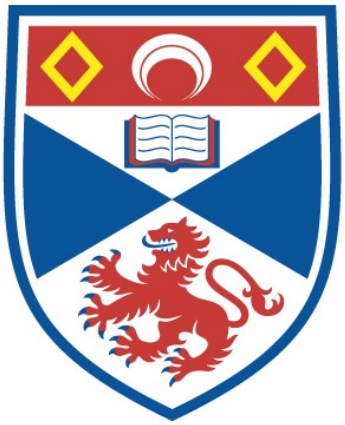
# THE PHENOTYPIC PLASTICITY OF WHOLE ANIMAL AND MUSCLE PERFORMANCE DURING FAST-STARTS IN COTTIDAE

Genevieve Kate Temple

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



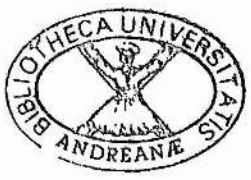
1998

# The Phenotypic Plasticity of Whole Animal and Muscle Performance During Fast-starts in Cottidae

Submitted for the degree of Doctor of Philosophy to the University of St. Andrews by,

Genevieve Kate Temple

School of Environmental and Evolutionary Biology
Gatty Marine Laboratory
University of St. Andrews




January, 1998

## Declaration

I hereby declare that the research represented 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 School of Environmental and Evolutionary Biology, United College of St. Salvator and St. Leonard, University of St. Andrews, under the direct supervision of Professor I. A. Johnston.

Signed

Date 1/1/98

## Certificate

I hereby declare that Genevieve Kate Temple 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 accompanying thesis for the degree of Doctor of Philosophy.

Signed

Date 1/1/98

## CONTENTS

## SUMMARY

### *Chapter 1*

Fast-starts are used by most fish species in order to capture prey and escape predators. An introduction to this mode of fish locomotion and the structure and function of the muscle powering swimming movements, is given. Temperature has the potential to alter fast-start behaviour at various levels of organisation ranging from the whole animal to the molecular and can act over time scales extending from the immediate to the evolutionary. The thermal dependence of fast-start performance is discussed.

### *Chapter 2*

The effects of acclimation and acute temperature on the kinematics of the escape response in two species of marine Cottidae, the short-horn sculpin (*Myoxocephalus scorpius* L.) and the long-spined sea scorpion (*Taurulus bubalis* Euphr.) were examined. Hypotheses were formulated based on relevant studies and the natural history of the fish to test the idea that seasonal temperature acclimation conferred a fitness advantage and to examine whether acclimation responses were constant through development. Fish were acclimated to 5, 15 and 20 °C and filmed using high speed cinématography at 0.8, 5.0, 15.0 and 20.0 °C. Maximum length-specific speed ($\hat{U}_{max}$), acceleration ($\hat{A}_{max}$), angular velocity ($\omega_{max}$) and cumulative turning angle (CTA) were measured as relevant variables of escape performance. At 20.0 °C, in adult short-horn sculpin, $\hat{U}_{max}$ and $\hat{A}_{max}$ were 110 % and 55 % higher, respectively, in 15 °C- than 5 °C-acclimated fish. No evidence was obtained for improved fast-start performance at 0.8 °C or 5.0 °C following cold-acclimation. In the long-spined sea scorpion acclimation to 5 and 15 °C did not improve $\hat{U}_{max}$ or $\hat{A}_{max}$ over fish acutely exposed to these temperatures, although acclimation to 5 °C increased $\omega_{max}$ ($P$=0.005). When tested over the most extreme thermal range found in the field, all variables were improved at a test temperature of 0.8 in 5 °C- compared to 15 °C-acclimated sea scorpion. Acclimation therefore appeared to be beneficial in some instances in both species. How this affects relative fitness is uncertain. The scaling of $\hat{U}_{max}$ with acclimation to 5 and 15 °C, was examined in both species over the test range

5.0-15.0 °C. Temperature acclimation did not affect scaling relationships of $\hat{U}_{max}$ in long-spined sea scorpion ranging in total body length ($L$) from 45 to 160 mm. At a test temperature of 15.0 °C, the scaling of $\hat{U}_{max}$, for sculpin ranging in length from 43 to 270 mm $L$, changed from $aL^{-0.98}$ in 5 °C-acclimated short-horn sculpin, to $aL^{-0.50}$ in 15 °C-acclimated fish ($P$<0.01). In short-horn sculpin therefore, the ability to modify escape performance with temperature acclimation was found to vary during ontogeny, potentially paralleling a change in thermal habitats during growth.

***Chapter 3***

The effect of seasonal thermal acclimation on the *in vivo* strain and power output of the fast muscle fibres during escape responses in the short-horn sculpin was examined. Fish were acclimated to 5 and 15 °C and tested at 5 and 15 °C. Muscle strain and activation were measured during the C-bend and contralateral contraction using implanted sonomicrometry crystals and electromyography (EMG) wires, respectively. Muscle activation times and strain waveforms were abstracted to create cyclic events for use in work loop experiments. At 15 °C, the *in vivo* shortening velocity was 60 and 154 % higher, for the C-bend and contralateral contraction, respectively, in 15 °C- than 5 °C-acclimated fish ($P$<0.05). There was no significant difference in shortening velocity between the acclimation groups at 5 °C. Acclimation temperature significantly affected the instantaneous power output in both stages of the escape response. Maximum instantaneous power output at 15 °C was about 54 % higher in fibres from 15 °C-acclimated than 5 °C-acclimated fish ($P$<0.05). Mean muscle mass-specific power output for the C-bend and contralateral contraction showed a similar trend. At 15 °C, power output was around 150 % higher during both stages of the escape response in 15 °C- than in 5 °C-acclimated fish ($P$<0.001). There was also some compensation in power output during the C-bend following 5 °C-acclimation. At 5 °C, the power output was 204 % higher in 5 °C- than in 15 °C-acclimated fish ($P$<0.05).

### *Chapter 4*

The kinematics, *in vivo* muscle action, power output and energetics of escape and prey capture responses in the short-horn sculpin are discussed. Fast-starts were filmed using high speed video synchronised with sonomicrometry and EMG. The *in vivo* muscle strain and activation recordings were abstracted for use in work loop experiments. Changes in the metabolic substrates following work loops from the two different types of fast-starts were analysed using high performance liquid chromatography (HPLC). All escape responses and 43 % of prey capture responses were C-starts. Mean maximum velocities were not significantly different between the two fast-starts, although the time to maximum velocity and the mean muscle strain cycle duration were longer during prey capture than during escape responses. Muscle shortening velocities were about 3 fibre lengths per second for both responses. Maximum instantaneous and mean power outputs were comparable between the two fast-starts. Similarly, the economy of muscle contraction (the net positive work per unit energy expended) and the efficiency (the ratio of work done to energy used) were not significantly different between the two responses. It was estimated that one successful prey capture of *Crangon crangon* would enable around 250 further fast-start responses.

### *Chapter 5*

The velocity of the wave of curvature ($\hat{U}$) passing down the fish and the power requirements during fast-start escape responses were calculated non-invasively using a mathematical modeling approach devised by Dr. James Wakeling (Gatty Marine Laboratory, University of St. Andrews). This was carried out on both cottid species acclimated to 5 and 15 °C and filmed using high speed cinématography at 0.8, 5.0, 15.0 and 20.0 °C. The power requirements for the contralateral contraction were 20 $W.kg^{-1}$ muscle in 5 °C-acclimated fish escaping at 5 °C and 58 $W.kg^{-1}$ muscle in 15 °C-acclimated fish swimming at 15 °C. Comparative values of power output measured from work loop experiments in Chapter 3 were 33 and 66 $W.kg^{-1}$, respectively. Acclimation temperature had a significant effect on the $\hat{U}$ of the escape response in the short-horn sculpin. At 20.0 °C there was a 130 % increase in $\hat{U}$ following acclimation to 15 °C compared to 5 °C ($\hat{U}$=11.1 and 4.8, respectively). However, there was no trade-off in

this performance parameter at low temperature. There was a clear acclimation effect on $\hat{U}$ in long-spined sea scorpion. Fish swimming at 20.0 °C had greater values of $\hat{U}$ when they were acclimated to 15 °C than 5 °C, whereas fish swimming at 0.8 °C had greater $\hat{U}$ when they were acclimated to 5 °C than 15 °C. Power was significantly higher at 20 °C in the 15 °C- (98 $W.kg^{-1}$) than the 5 °C-acclimated group (42 $W.kg^{-1}$) ($P<0.05$). In addition, the power requirements were significantly higher for 5 °C- than 15 °C-acclimated fish at 0.8 °C (11.26 and 2.7 $W.kg^{-1}$, respectively) ($P<0.05$).

***Chapter 6***

The major results of the studies are discussed and possible avenues for future work in this area are suggested.

*Chapter 1*

# GENERAL INTRODUCTION

"Let us take the case of a wolf, which preys on various animals, securing some ...by fleetness; and let us suppose that the fleetest prey, a deer for instance...increased in numbers. I can under such circumstances see no reason to doubt that the swiftest and slimmest wolves would have the best chance of surviving, and so be preserved or selected..." (Darwin, 1859). In such a context, superior locomotory performance would appear to be of the utmost importance for the success of prey capture, and escape for that matter, and thus for Darwinian or competitive fitness. In the present day, Darwin's "fleetness" would be referred to as an activity capacity, of which burst speed, defined as "the greatest velocity an animal attains over a short distance" (Bennett, 1991), would probably be the most suitable measure. For physiological traits to be susceptible to natural selection they must be heritable, variable within a population and persist through time. Activity capacities have all of these factors; an obvious example of heritability is its application in breeding for burst speed and endurance in racehorses (Gaffney and Cunningham, 1988). Considerable variability and persistence in activity capacities, particularly maximal exertion and endurance, have been found in garter snakes (Jayne and Bennett, 1990*a*, *b*).

Many aquatic organisms rely on burst speed in predator-prey encounters. Beamish (1978) classified burst swimming as that which lasts less than 20 seconds. Wardle (1980) recognised the important implications it may have for survival, and correlations between burst swimming and fitness have been both measured and implied. For example, Taylor and McPhail (1985) suggested that differences in burst swimming performance in two populations of Coho salmon (*Oncorhynchus kisutch*) may reflect differences in predation pressure. Law and Blake (1996) believed that high predation pressure was the selective force responsible for high fast-start escape performance of two species of threespine stickleback (*Gasterosteus* spp.) inhabiting different niches. Andraso (1997) suggested that due to having a superior escape response, an unarmoured morph of brook stickleback (*Culaea inconstans*) was as successful as an armoured morph that could better defend itself. Watkins (1996) found burst swimming speed of anuran tadpoles to be an important

determinant of surviving attacks by garter snake predators. Furthermore, Swain (1992) showed that superior burst swimming performance by certain vertebral phenotypes of stickleback (*Gasterosteus aculeatus*) larvae was matched by high frequencies of those phenotypes in the field. Thus, in these cases it seems possible that selection may be acting directly on burst speed.

Environmental variables have the potential to disrupt or alter all biological systems. Temperature is one of the most important and principle factors influencing the physiology and behaviour of ectothermic organisms. This introduction will focus on fish locomotion with particular emphasis on burst swimming, including the mechanisms of movement at the whole animal, cellular, biochemical and molecular levels. This is followed by a discussion of how temperature, over time scales ranging from the immediate to the evolutionary, can affect burst swimming at these different levels of organisation.

### 1.1 Swimming

Fish swim in a diversity of ways and various modes have been categorised according to the period of time that they can be maintained. In addition to "burst," swimming can also be described as "sustained," "prolonged" and "critical" (Brett, 1964; Beamish, 1978). Critical is probably the most commonly measured and refers to the maximum velocity a fish can maintain for a particular period of time (Brett, 1964).

Irrespective of speed, most swimming modes involve undulations of both the fins and body to propel the fish through water; as Videler (1985) wrote, "Oscillatory swimming movements are the basic elements of fish behaviour." Breder (1926) categorised swimming into "anguilliform," "carangiform" and "ostraciiform" swimming modes. Carangiform was further divided into several groups which Lindsey (1978) described as "subcarangiform," "carangiform" and "thunniform." A brief description of all of these swimming styles is given below. A fish may show more than one mode of swimming and there appears to be no evolutionary connection between similar swimming styles exhibited in different fish groups.

The main characteristic of fish swimming is a wave of curvature passing posteriorly down the body of the fish (Gray, 1933). This occurs to a great extent in

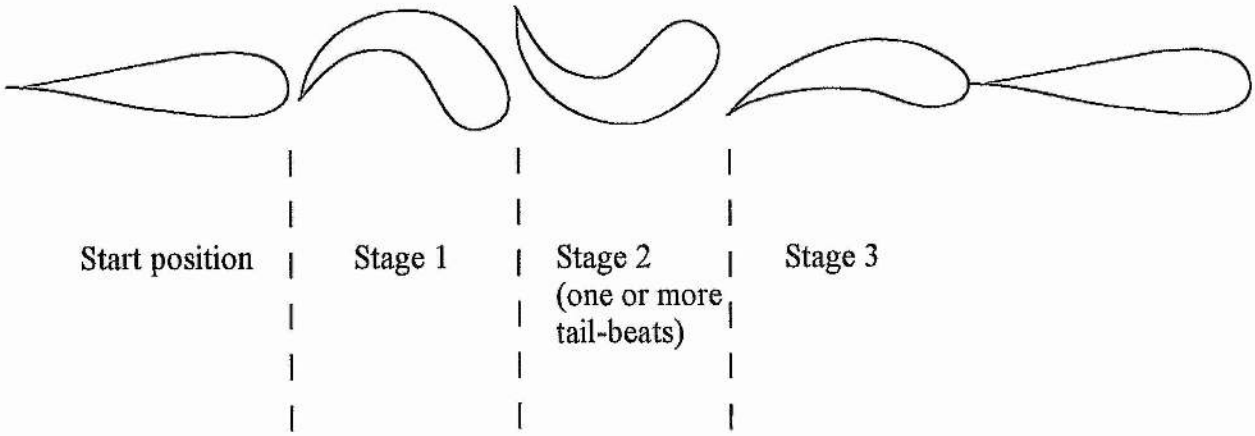
anguilliform swimming, which as the name implies refers to an eel-like mode of swimming. There is large side-to-side wave amplitude along the whole body which becomes even greater towards the tail. Subcarangiform swimming occurs in species such as rainbow trout, in which these side-to-side undulations are small anteriorly and expand only in the posterior portion of the body. Carangiform swimming is the form of locomotion employed by some scombrids, where only the posterior third of the body can flex. A "vortex sheet" forms in the "V" shape of the tail fin when the fish is swimming giving a high aspect-ratio (square of the maximum fin height/fin area) (Lighthill, 1969). Thunniform swimming is used by tuna which can attain very high speeds. High velocities are aided by a streamlined body with the mass concentrated towards the front. Thrust is generated solely by the stiff caudal fin attached *via* a very narrow caudal peduncle which is wider than it is deep due to lateral keels. The tail has a high aspect ratio, and the angle of inclination is altered throughout the tail-beat. Whales and dolphins also have thunniform swimming with the tail in the horizontal plane. Ostraciiform swimming is used by boxfish, where the body can not laterally flex and swimming is achieved by oscillations of a rigid tail. In this case the body is poorly streamlined resulting in low speeds.

### *1.1.1 Burst swimming and fast-starts*

Fast-starts are the typical burst swimming manoeuvres employed by fish during prey capture and escape responses. They involve the same wave of curvature passing down the fish but are further distinguished by rapid accelerations, often from rest (Eaton *et al.* 1977; Webb, 1978*b*; Morley and Batty, 1996; Wakeling and Johnston, 1998). The median fins are usually raised to increase the body depth to maximise thrust (Eaton *et al.* 1977; Webb, 1978*a*). Fast-starts are short-duration, unsteady actions, involving unequal changes in distance moved, direction, acceleration, velocity, and muscle length fluctuations, with successive tail-beats (Johnston *et al.* 1995; Franklin and Johnston, 1997).

The kinematics of fast-starts can be studied using high speed cinématography or video. Marey used cinématography for the first time in 1895, to observe detailed fish locomotion. Today, high speed ciné cameras can record ballistic movements at up to

Figure 1.1. Schematic diagram of a C-start fast-start.


Start position
Stage 1
Stage 2
(one or more
tail-beats)
Stage 3

3000 frames per second. This is ideal for studies on involuntary locomotion, such as forced escape responses. Alternatively, high speed video is ideal for studies in which movement can not be predicted, as is the case with prey capture. Although frame rates and resolutions are lower, the film can be continuously run and easily replayed. Fast-starts can be studied by encouraging a fish to elicit an escape response to a stimulus such as sound (e.g. Blaxter and Batty, 1985), striking the side of the tank (e.g. Johnson and Bennett, 1995), electric shock (e.g. Fuiman, 1986), or a tactile stimulus (e.g. Batty and Blaxter, 1992). Alternatively, fast-starts during prey capture can be examined by introducing prey items into the swim tank (e.g. Beddow *et al.* 1995). This allows for additional measurements on strike distance (initial distance of the snout tip to the prey item).

Fast-starts have been divided into three kinematic stages in fish employing subcarangiform swimming (Weihs, 1973) (Fig. 1.1). Stage 1 is a preparatory stage involving initial contraction of the myotomal muscles on one side of the fish and in some cases maximum acceleration (Webb, 1978*b*). Stage 2 is a propulsive stage initiated by contraction of the contralateral myotomal muscles and involving maximum velocity (Webb, 1978*b*). This stage may last for one or more tail-beats. Stage 3 is a steady swimming or gliding stage. These starts have furthermore been categorised into C- or S-starts, depending on the shape of the fish at the end of stage 1 (see Domenici and Blake, 1997, for review). C-starts have predominantly been found during escape responses and S-starts during prey capture (Hoogland *et al.* 1956; Webb, 1978*a*; Frith and Blake, 1991).

Eaton *et al.* (1981) found that the latency between a stimulus and neuromuscular activity of an escape response may be less than 10 ms. This fast reaction has been attributed to a pair of brainstem neurons, known as the Mauthner cells (Eaton *et al.* 1977). The Mauthner cells reside in the reticulospinal system that coordinates complex sequences of movements (Peterson, 1984). During an escape response one Mauthner cell is activated and fires one action potential. Among the motor neurons that are excited by the Mauthner axon are the cranial motor neurons which cause the mouth to close and the spinal motor neurons which activate the white and red fibres of the ipsilateral trunk musculature. In addition, the fins are activated for propulsive, stabilising or defensive purposes (see Ritzman and Eaton, 1997, for review). The Mauthner neurons have also

been found to fire in goldfish during the last phase of prey capture when the fish performs a C-shaped flexion (Canfield and Rose, 1993).

Larvae also exhibit fast-starts, and in fact the escape response C-start, or "startle response" is one of the earliest behaviours to develop in larval zebra danios (*Danio* [*Brachiodanio*] *rerio*) (Eaton and DiDomenico, 1986). Herring (*Clupea harengus*) larvae which hatch at about 6-8 mm total body length (*L*) (Batty, 1984), exhibit startle responses in reaction to tactile stimuli and predators when 10-12 mm *L*. Directional responses in reaction to acoustic stimuli, however, occur later in development (>22 mm *L*) relying on complete development of the head lateral line coupled to a gas filled otic bulla (Blaxter and Batty, 1985). This early behavioural development is not surprising considering that marine fish larvae experience mortality rates of up to 30 % a day, of which predation is thought to be the major cause (Miller *et al.* 1988; Batty and Blaxter, 1992).

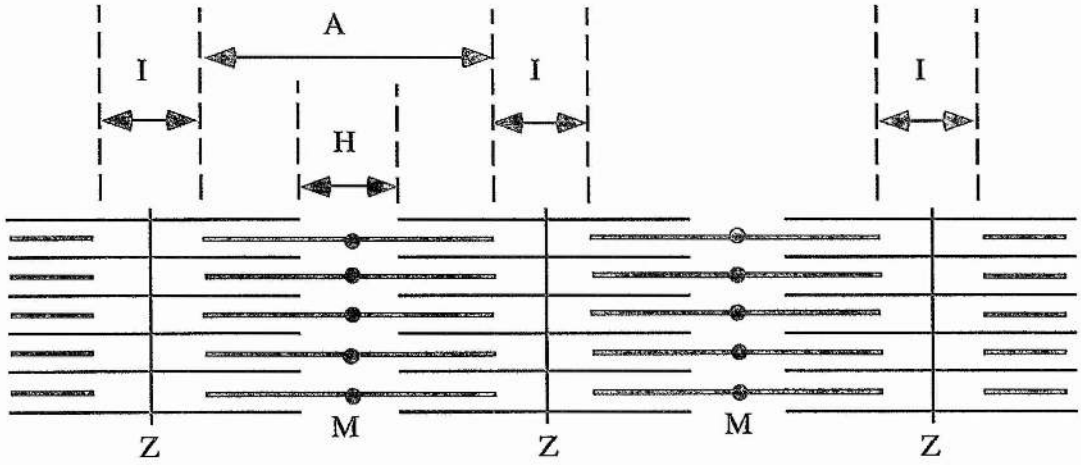
## 1.2 Muscle

All vertebrate locomotion relies on muscle and in fish the locomotory musculature makes up 40-60 % of the total body mass (Bone, 1978). Muscle is needed to generate power that will enable both routine and burst swimming.

### *1.2.1 Structure of muscle*

Fish myotomal muscle is striated muscle, which is made up of a large number of parallel fibres with a diameter of 0.1-0.01 mm. These contain myofibrils which are composed of thick and thin filaments interdigitating with one another (Huxley, 1953) creating a striated or banded appearance (Huxley and Hanson, 1954; Huxley and Niedergerke, 1954) (Fig. 1.2). There are two main bands; a dark A band and a lighter I band. A refers to anisotropic and I to isotropic which reflects their light scattering properties. The thick filaments are coincident with the A band which has a simple lattice structure in teleosts, but a superlattice structure in all other major phylogenies (Squire, 1981; Luther *et al.* 1996). The thin filaments are attached to the Z-bands, constitute the I bands and extend through into the A bands. The unit of length between two Z-bands is a sarcomere. The H zone is the less dense region of the A band between the ends of two sets of thin filaments and is bisected by the M-band.

Figure 1.2. Schematic representation of striated muscle. Letters represent the various regions as described in the text. After Huxley (1953).


A
I
I
I
H
Z
M
Z
M
Z

The Z-bands provide a link between antiparallel thin filaments of opposite polarity in adjacent sarcomeres and can have one of two structures: small-square or basket-weave. They can furthermore be wide or narrow, the latter often being referred to as the "simple" Z-line (Luther, 1991). Z-bands are composed mainly of α-actinin but also titin (Vigoreaux, 1994). Titin is a giant protein (about 3 mDa) which extends from the Z-band to the M-band, probably defines the length of the thick filaments and provides stability by positioning the A-band in the middle of the sarcomere (Labeit and Kolmerer, 1995; Trinick, 1996). Stability is furthermore provided by the M-band which maintains thick filament orientation *via* M-bridge connections (Luther *et al.* 1995). Stability of the myofibrils is also thought to be due to the large nebulin molecule (Horowits *et al.* 1986).

The two types of filament are made of different proteins. Thick filaments are composed of myosin (Hanson and Huxley, 1953) which is a complex protein with a molecular weight of around 500,000 Da. It consists of two globular heads known as the subfragment 1 (S1) region and a rodlike S2 section. The heads can interact with actin and have ATPase activity, thus hydrolysing ATP into ADP and inorganic phosphate (Engelhardt and Ljubimowa, 1939; Gergely, 1994). The myosin molecule consists of the protein chains, myosin heavy chain and myosin light chain (Gazith *et al.* 1970; Weeds and Lowey, 1971; Focant and Huriax, 1976). In the rod region the heavy chains are arranged in a coiled-coil α-helix arrangement (Lowey *et al.* 1969). The heads contain only short sections of this structure. Myosin light chain 1 (LC1), an alkali light chain, and myosin light chain 2 (LC2; naming has been in order of decreasing molecular weight), a regulatory light chain, also occur here, although different muscle types can have different light chains present. LC2 is thought to be an important determinant of the speed of muscle contraction (Lowey *et al.* 1993).

Thin filaments are made of actin (Hanson and Huxley, 1953), tropomyosin (Bailey, 1948) and the troponin complex (Ebashi and Kodama, 1966*a, b*). Actin forms the backbone of the thin filaments and is a double helix of "beads". When isolated, it has two forms, G-actin, a fairly globular molecule and F-actin, a fibrous polymer of G-actin. Tropomyosin is long and thin, and molecules are attached to each other end-to-end, forming a very thin threadlike structure that lies in the grooves between the actin helix. Troponin is attached to each tropomyosin molecule, and is a complex of three proteins:

troponin-C (TnC), troponin-I (TnI), and troponin-T (TnT) (Greaser and Gergely, 1973). The TnC molecule has four calcium binding sites in addition to binding to TnI and TnT.

### *1.2.2 Muscle contraction*

The contraction process involves interaction between the myosin heads of the thick filaments and the actin of the thin filaments. In relaxed muscle, tropomyosin blocks the myosin-binding site (the "off" state) on the actin thus preventing this interaction. Contraction is initiated when a nerve impulse arrives at a motor end-plate. A transmitter substance, usually acetylcholine, is released and causes the muscle fibre membrane or sarcolemma to depolarise (Kuffler, 1946). This action potential is propagated along the fibre and into the T-system, which is a complex system of tubular invaginations of the sarcolemma responsible for rapid communication to each myofibril. T-tubules usually lie at the level of the Z-band adjacent to the terminal cisternae of the sarcoplasmic reticulum (SR); an arrangement known as a triad. The SR is a system of flattened vesicles which surround each myofibril. In the relaxed state calcium is localised within the SR but during contraction the propagated action potential triggers the release of calcium ions (Heilbrunn and Wiercinski, 1947). The concentration of $Ca^{2+}$ in the sarcoplasm of the muscle cell rises from about 0.1 μM to 10 μM (Bagshaw, 1993). $Ca^{2+}$ bind to TnC, releasing the inhibitory effect of TnI on ATPase activity. This has a knock-on effect on the structure of TnT which is bound strongly to tropomyosin. The latter thus moves away from the myosin-binding site (the "on" state) and the structure of the actin changes (Cohen, 1975; Lehrer, 1994; Al-Khayat *et al.* 1995; Geeves and Conibear, 1995). Relaxation is achieved by the SR actively re-accumulating $Ca^{2+}$ sometimes with the aid of calcium binding parvalbumins (Baron *et al.* 1975; Gillis *et al.* 1982).

In the 1950s the "Sliding filament model" of muscle contraction emerged, in which it was confirmed that neither thick nor thin filaments changed in length during this process (Huxley, 1953; Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). Instead, contraction was shown to involve sliding of the thin filaments between the thick filaments, due to cyclic attachment and detachment of the myosin heads, or cross-bridges, between the two filaments. The myosin heads attach to the actin of the thin filaments at a certain angle. During contraction, they undergo a conformational change causing the

bridges to move, pulling the thin filaments past the thick. To shorten significantly each cross-bridge must attach, detach and then re-attach at a point further along the thin filament. The force developed by the muscle is related to the amount of overlap of the filaments (Gordon *et al.* 1966*a*, *b*). However, the actual mechanism has, until recently, remained rather vague. Several models of cross-bridge action have been proposed, including rotation of the S1 head about its point of attachment to the actin filament (Huxley, 1969), a shortening of the S2 rod (Harrington, 1979) or a change in the shape of the S1 head (Toyoshima *et al.* 1987). Evidence has now accumulated in support of the lever-arm concept, in which the bulk (catalytic domain) of the myosin head binds to actin with a constant orientation and only the distal part of the myosin head, the neck domain, moves (Rayment *et al.* 1993*a*, *b*; Jontes *et al.* 1995; Whittaker *et al.* 1995). Using *in vitro* motility assays, in which actin filaments are moved across a sheet of S1 molecules irrigated with ATP, it has been found that the sliding velocity is linearly related to the length of the neck (Uyeda *et al.* 1996). A similar result was obtained when the neck was replaced with artificial lever arms (Anson *et al.* 1996).

Each cycle of attachments requires expenditure of energy. The ATP molecule binds temporarily to the myosin head forming an active complex that will attach to the actin strand. The myosin head will not detach from the actin strand again until combined with a new ATP molecule. ATP can be formed by the complete oxidation of carbohydrates, fats, proteins or amino acids, resulting in high molar yields of ATP. The simplest metabolic pathway of generating ATP, however, is phosphogen mobilisation, e.g. creatine phosphate (PCr) in vertebrates or arginine phosphate in invertebrates (Hochachka, 1994). Creatine is accumulated in the muscles and forms PCr by the action of creatine phosphokinase (CPK) (Walker, 1979). The phosphate group of the PCr is transferred to ADP to form ATP, a process which requires no oxygen and is catalyzed by cytosolic isozymes of CPK (Hochachka, 1994). This is ideal for burst swimming in which oxygen is in low supply in the muscles. Thus the highest CPK activities occur in animals capable of burst speeds. CPK occurs in various locations: as a soluble fraction, bound to myosin ATPase, to the sarcolemma (Blum *et al.* 1991) and to the SR (Rossi *et al.* 1990). The end products of utilising PCr are creatine and Pi; the former is mainly reconverted to the phosphogen form, the latter is a highly reactive metabolite which puts

a limit on the amount of phosphogen that can be stored. When phosphogen supplies are depleted, anaerobic glycolysis of muscle glycogen is able to replenish ATP, which enables about 10 times the amount of work sustainable by PCr. Anaerobic glycolysis results in lactate and proton end products (Hochachka, 1983) and as in all anaerobic ATP-synthesizing pathways, recovery involves oxidative metabolism (Curtin *et al.* 1997).

### *1.2.3 Muscle fibre types in fish*

The wide range of power requirements needed for different swimming modes may be met by both the recruitment of different numbers and types of fibres, and by changing the conditions under which they operate. Fish swim at a range of speeds; low speed requires economy whereas short bursts of high speed demand high power from the muscles. Therefore at least two types of muscle fibre have evolved, although the existence of this simply dichotomy rarely occurs and is only seen in primitive teleosts and some elasmobranchs (Bone, 1978).

The two main fibre types, slow and fast, were characterised in teleosts in 1875 by Arloing and Lavocat. Slow muscle, also known as red and slow-oxidative muscle, has a high myoglobin content which is responsible for the red colouration. Slow muscle fibres are small (about 40 μm), well vascularised, contain many mitochondria in association with lipids and glycogen granules and have a high oxidative enzyme activity (Luther *et al.* 1995). These characteristics enable histochemical identification by staining with the enzyme succinic dehydrogenase (SDH), a mitochondrial marker for oxidative metabolism (Bone, 1978). The slow fibres are multiply innervated (Barets, 1961; Bone, 1964, 1966, 1970; Best and Bone, 1973). Fast, fast-glycolytic or white muscle fibres have a low myoglobin content and can be more than 300 μm in diameter. They are poorly vascularised, have fewer and smaller mitochondria with less tightly packed cristae, lack lipid droplets and use glycogen to rapidly produce ATP *via* anaerobic glycolysis (Bone, 1978; Luther *et al.* 1995). White fibres can be focally or multiply innervated (Barets, 1961; Bone, 1964, 1970; Hudson, 1969), depending on the degree of phylogenetic complexity; focally innervated white fibres tend to be found in primitive teleosts, multiply innervated fibres in advanced species (Bone, 1970).

Multiply innervated white fibres are small with regularly packed myofibrils, an extensive SR and well developed glycolytic capacity (Johnston and Moon, 1981). These fibres may have a higher aerobic capacity than the fast fibres found in primitive fish, and hence may also have higher mitochondrial volume densities (Johnston, 1983; Johnston and Moon, 1981). They tend to have a large number of closely spaced nerve terminals along the length of the fibre (Johnston, 1983). However multiply innervated muscle fibres have been found to behave similarly to focally innervated fibres; stimulation of the multi-terminally innervated fast fibres of the short-horn sculpin (*Myoxocephalus scorpius*) resulted in action potentials yielding fast twitches (Altringham and Johnston, 1988, 1989).

Some fish have a third type of muscle fibre usually referred to as intermediate, fast-oxidative-glycolytic or, due to an intermediate amount of myoglobin, pink. Johnston *et al.* (1977) reported on "fast red" muscle fibres in carp which had a high glycolytic enzyme activity, and an aerobic capacity and myofibrillar ATPase enzyme activity intermediate between that of slow and fast muscle. They also showed these fibres to be active at intermediate speeds, with an orderly recruitment of slow - fast red - fast white with increasing speed (Johnston *et al.* 1977). Coughlin *et al.* (1996) found the pink muscle in the scup (*Stenotomus chrysops*) relaxed and contracted faster and had higher power production than that of the red muscle. The pink muscle was positioned anteriorly where muscle strain during swimming is low and therefore it was reasoned that the pink muscle supplemented the low power production of the red muscle in these regions. In the hagfish, the intermediate fibres are thought to be a common fibre during muscle development (Bone, 1978). Some species possess quite a myriad of fibre types; for example, histochemistry has revealed 7 fibre types in cod myotomal muscle (Korneliussen *et al.* 1978) and Bone (1978) reported on 5 muscle fibre types in the dogfish (*Scyliorhinus canicula*).

Many studies have revealed differences in myofibrillar protein isoform (proteins with similar structures and functions, but different amino acid sequences) expression during development. In larval mullet (*Dicentrarchus labrax*) there was a sequential expression of myosin isoforms in both red and white fibres during ontogeny (Scapolo *et al.* 1988). The larval muscle fibres of the Atlantic herring (*Clupea harengus*) were

distinct from either fast or slow fibres of the adult, since they contained characteristic isoforms of myosin heavy chain, TnI, TnT, and myosin LC2 (Crockford and Johnston, 1993; Johnston and Horne, 1994; Johnston *et al.* 1997). Complex changes in isoform expression have also been found in plaice (*Pleuronectes platessa*) (Brooks and Johnston, 1993), eel (*Anguilla anguilla*) (Chanoine *et al.* 1992) and Arctic charr (*Salvelinus alpinus*) (Martinez and Christiansen, 1994).

### *1.2.4 Recruitment patterns of muscle fibres*

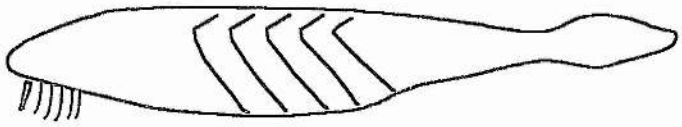
Fish are convenient models on which to carry out studies of muscle since the functionally different fibre types occur in discrete tissue masses, and moreover, of all the vertebrates, fish show the greatest differences between slow and fast fibres (Morgan and Proske, 1984). The main mass of myotomal fast muscle often constitutes 90 % of the musculature (Greer-Walker and Pull, 1975). Slow fibres usually exist in a thin superficial layer over the fast muscle, with a thicker triangle of muscle at the horizontal septum. Some active species have an additional band of slow muscle near the spine. Continuous swimmers, such as anchovy and mackerel, tend to have the greatest amounts of slow muscle (Johnston, 1983; Luther *et al.* 1995).

Electromyographic (EMG) recordings (the implantation of electrodes to measure electrical muscle activity, e.g. Jayne and Lauder, 1993, 1994; Johnston *et al.* 1993), of muscle activity during swimming, have revealed the low power demand of low-speed swimming to be met by the small volume of slow muscle fibres (Johnston *et al.* 1977; Bone *et al.* 1978). However, activity occurs within the deeper zone of fast fibres from the bulk of the myotomal muscle mass, at higher speeds (Bone, 1966; Johnston *et al.* 1977; Rome *et al.* 1984; Jayne and Lauder, 1993, 1994). In primitive fish species, fast fibres are recruited solely during burst swimming (Bone, 1964; Johnston, 1983). However, in higher teleosts where fast fibres are multiply innervated, evidence has accumulated to suggest that they may be recruited at low speeds as well as during burst swimming (Johnston, 1983).

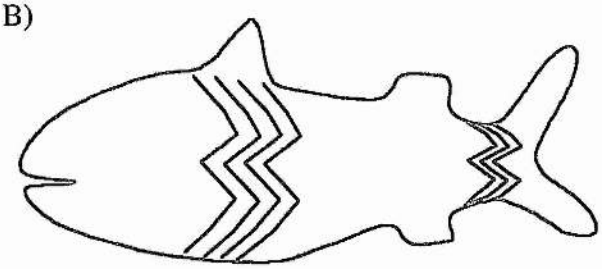
Duthie (1982) found elevated levels of lactic acid in the fast muscle of flounder (*Platichthys flesus*) during moderate swimming speeds indicating anaerobic metabolism of the fast fibres. Johnston *et al.* (1977) found the threshold speed for recruitment of fast

Figure 1.3. Diagram of the arrangement of fish myotomes A) in a lamprey and B) in an advanced teleost.

A)



B)



fibres during sustained swimming in carp (*Cyprinus carpio*) to be 2.1 body lengths per second ($s^{-1}$), a speed that can be maintained without fatigue. In addition, Jayne and Lauder (1993) studied the escape behaviour of the bluegill sunfish (*Lepomis macrochirus*) performed from a standstill and during steady swimming. EMGs were essentially the same irrespective of initial level of activity and red and white muscle was simultaneously recruited. In 1994, the same workers studied rapid burst and glide swimming in this species and found white muscle activity increased with speed as would be expected, but the activity of the red muscle fibres decreased. This differed from the general pattern of muscle recruitment, in which the fast fibres are recruited in addition to the slow fibres.

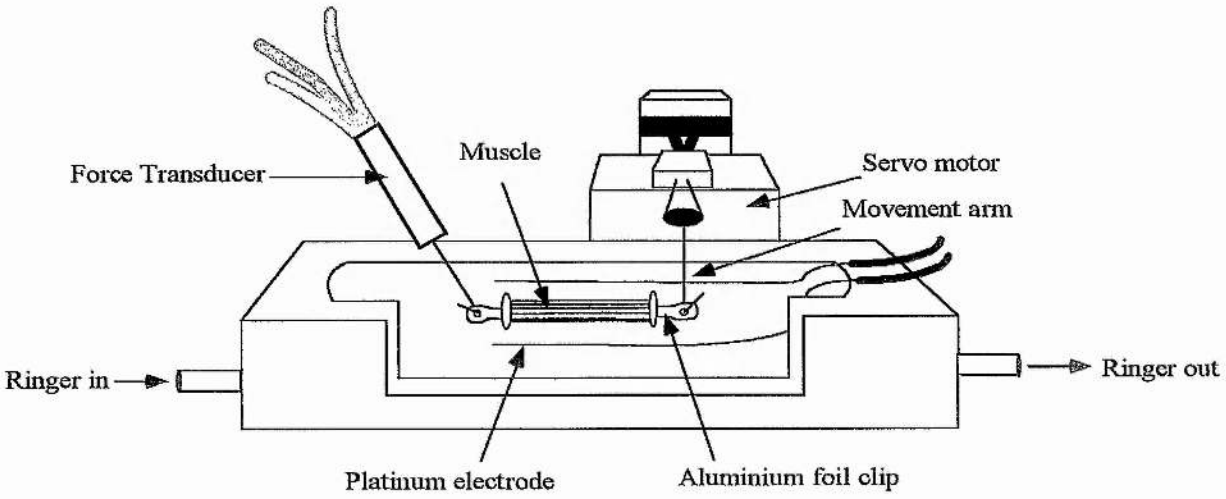
### *1.2.5 Anatomical arrangement of muscle fibres in fish*

The body musculature of fish is composed of lateral bands of muscle divided transversely into successive segments called myotomes. These correspond in number with the vertebrae, but alternate with them. Myotomes are arranges in a simple "V" or a more complex "W" following a phylogenetic increase in complexity (Bone, 1978) (Fig. 1.3). Ontogeny often recapitulates phylogeny and this is reflected in the development of higher fish groups where somites first form a primitive "V" before folding into the adult and more advanced "W". The middle of the "W" lies on the horizontal septum which separates the dorsal epaxial and ventral hypaxial musculature. Additional flexures can cause as many as 5 zigzag arms within a myotome.

The orientation of muscle fibres within the myotomes is also not constant. The red superficial fibres run parallel to the long axis of the fish and all contract at the same rate. Fast fibres, on the other hand, do not run parallel to the long axis; if this were the case, the more medial fibres would shorten less during bending, and their rate of contraction would not be the same. This potential problem is overcome by having fast fibres arranged at angles of up to 35 ° from the horizontal plane, so that all can contract to the same fraction of their initial length (Alexander, 1969).

Most fibres attach to sheets of connective tissue separating adjacent myotomes, called myosepta. Successive fibres which lie end-to-end on opposite sides of the myosepta are called muscle trajectories. White fibre trajectories may take on one of two

Figure 1.4. Diagram of the apparatus used for *in vitro* experiments on bundles of muscle fibres.


Force Transducer
Muscle
Servo motor
Movement arm
Ringer in
Ringer out
Platinum electrode
Aluminium foil clip

forms, the chondrichthyean or teleostean (Alexander, 1969). The two patterns differ in the angle of the muscle fibres relative to the myosepta. The former is found in the elasmobranchs, primitive teleosts, *Anguilla* and *Salmo*, and also in higher teleosts where it is restricted to the last few myotomes. In the teleostean pattern, trajectories form segments of helices with axes roughly parallel to the body. Helices are arranged in coaxial bundles with 4 on each side. This arrangement of fibres results in faster bending for the same rate of contraction of the muscle fibres than in the chondrichthyean pattern, and it has been reported that teleosts swim faster than sharks of the same size (Bainbridge, 1958; Bone, 1978).

## 1.3 Mechanical properties of muscle

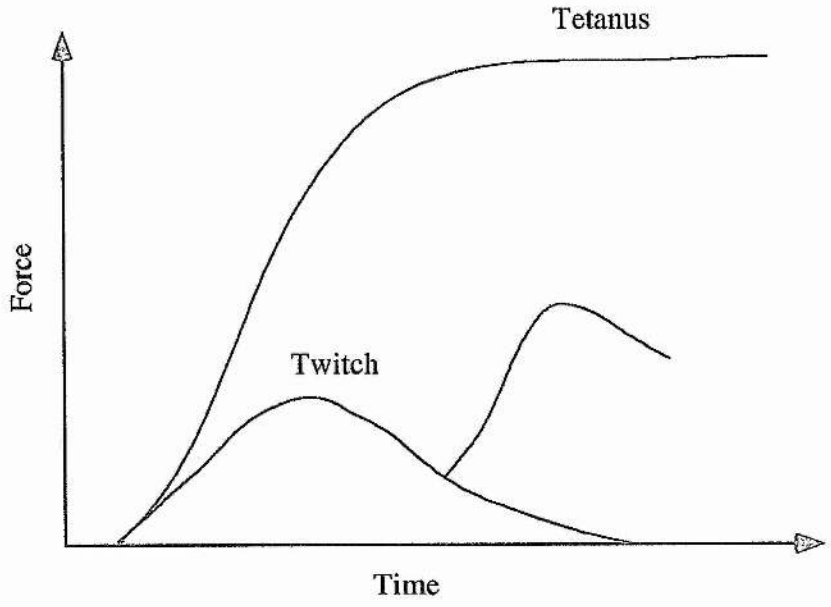
"The most important functional capacity of a muscle is its ability to shorten against a load and thus to do work" (Josephson, 1985).

*In vitro* studies of muscle contraction allow measurements of length, tension, contraction rates, work and power. These studies involve attaching one end of a muscle preparation, or whole muscle, to a tension recording transducer and the other end often to a movement arm. The muscle is stimulated by electrodes (Fig. 1.4). In isometric contractions, the muscle is not allowed to shorten and the tension it produces is measured. A single stimulus of sufficient amplitude produces a rapid increase in force, known as a twitch, which then decays (Fig. 1.5). If a second stimulus is applied before the tension from the first twitch has fallen to zero, mechanical summation occurs. This produces tension in the second twitch which is higher than that of the first. Repetitive stimulation at a sufficient frequency produces a tetanus (Fig. 1.5).

### *1.3.1 The force-velocity relationship*

During isotonic contractions the shortening of the muscle is measured whilst the tension in the muscle is maintained constant. The contraction of the muscle during constant velocity length changes is known as isovelocity shortening. Using different loads it has been found that the velocity at which a muscle shortens is inversely related to the force on the muscle. This is the essence of the force-velocity (*P-V*) relationship (Fig. 1.6), a curve originally described by the Hill equation (Hill, 1938):

Figure 1.5. The change in force produced by a muscle during a twitch, mechanical summation and a tetanus.


Tetanus
Force
Twitch
Time

$$(F + a)(V + b) = (P_0 + a)b,$$

where $F$ is force, $V$ is velocity, $P_0$ is the maximum isometric tension of the muscle (the intercept of the force-velocity curve with the force axis), and $a$ and $b$ are constants. The ratio of $a/P_0$ is used to express the curvature of the force-velocity curve. Low values indicate a strongly curved plot. Marsh and Bennett (1986) have also described the relationship using a hyperbolic-linear equation. These authors proposed the use of the power ratio to indicate the radius of curvature of the *P-V* relationship:

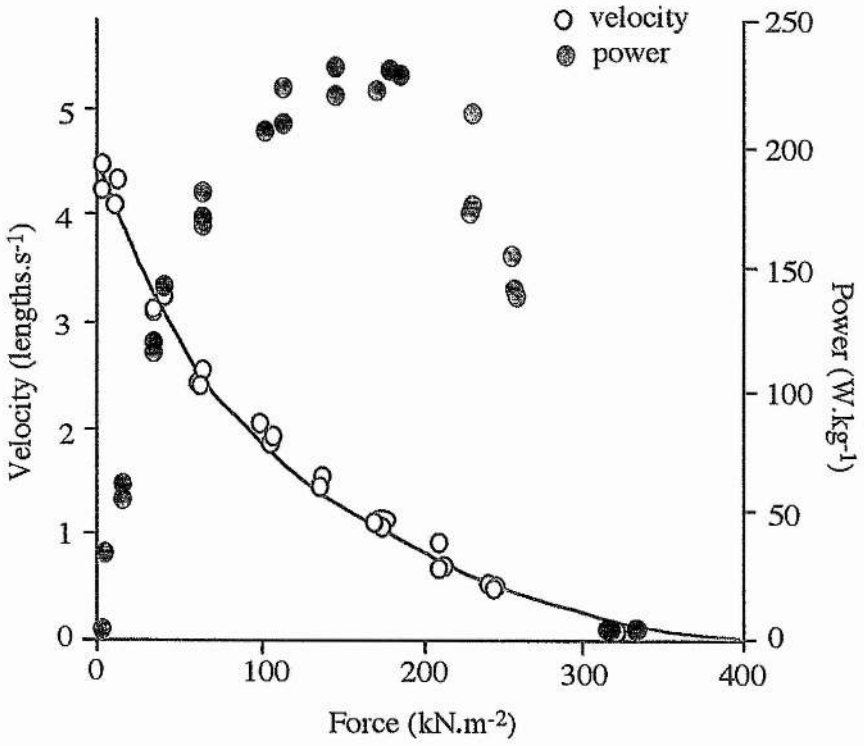
$$\dot{W}_{max} / V_{max}. P_0$$

where $\dot{W}_{max}$ is the maximum power determined from the *P-V* relationship and $V_{max}$ is the maximum shortening velocity of the muscle extrapolated from the *P-V* curve at zero force. Low values for the power ratio indicate a highly curved plot.

The maximum shortening velocity of a muscle preparation can also be determined using the slack test and in this case is often referred to as $V_0$ (Edman, 1979). Slack tests involve quickly releasing an isometrically contracting muscle a distance great enough to bring the force to zero. The muscle is then allowed to shorten at the new length taking up the slack and the slack time or time to force redevelopment is measured. This is plotted against various release distances, the slope of the line giving the shortening velocity under zero load ($V_0$). Values of $V_0$ are often found to be greater than those of $V_{max}$. This is thought to occur because $V_0$ measures just the fastest fibres, whereas $V_{max}$ is a measure of a population of fibres (see Josephson, 1993, for review).

The mechanical power output of a muscle can be calculated from the *P-V* relationship, since power is the product of force × velocity. It is maximum at about 0.3 $V_{max}$ or 0.3 $P_0$ (Fig. 1.6) (see Josephson, 1993, for review). The maximum power measured from the *P-V* relationship overestimates the average sustainable power available from a muscle during swimming. It can also underestimate the maximum power output during locomotion, due to the effects of active pre-stretch, where the muscle is active during lengthening. Therefore the *P-V* relationship has little relevance for locomotion. During repetitive contraction, a muscle shortens doing work and also lengthens having work done upon it. This may cause the net work done over a whole cycle to be less than the work done during the shortening phase. The shortening velocity

Figure 1.6. The force-velocity relationship. After Josephson (1993).


velocity
power
Velocity (lengths.s-1)
Power (W.kg-1)
Force (kN.m-2)
0
1
2
3
4
5
0
50
100
150
200
250
0
100
200
300
400

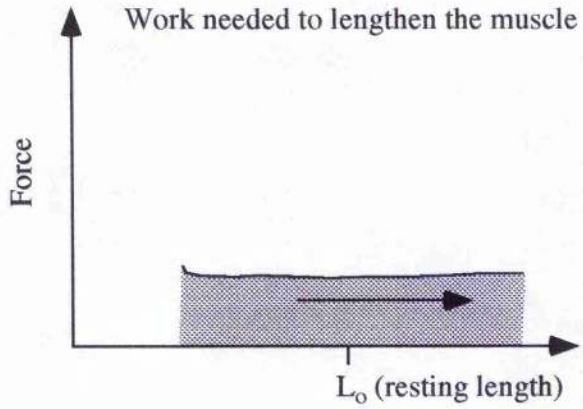
of a muscle is also rarely constant or optimum for power output. For repetitively active muscle the length change is usually sinusoidal, which means that maximum power is 10-20 % less than would be expected from a muscle shortening at its optimum velocity (Josephson, 1989). Furthermore, muscle activation and relaxation are not instantaneous and overlap into each half of the cycle reducing the net work and power over the full cycle.

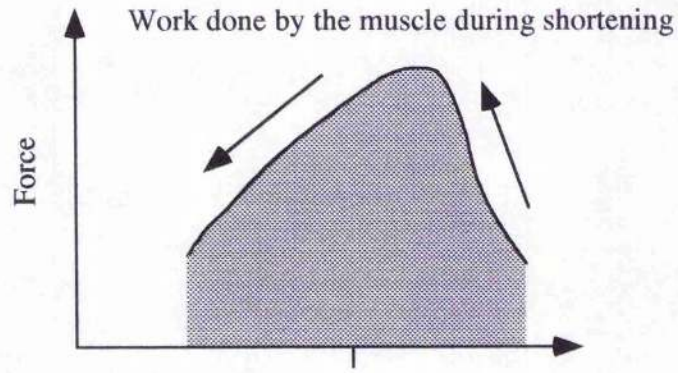
### *1.3.2 Work loop experiments*

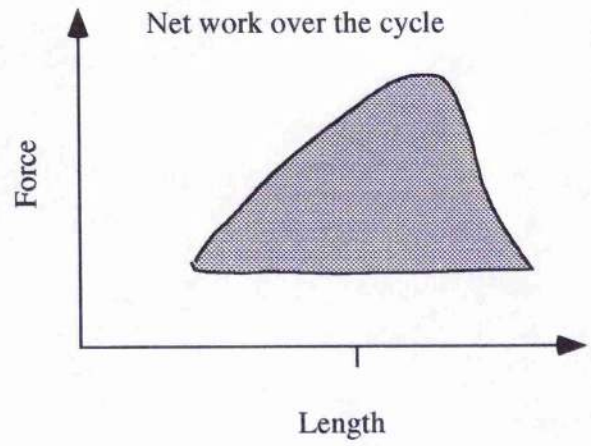
In order to measure the sustainable power output of a muscle, the force of the muscle is measured whilst it is stimulated during length changes imposed by a movement arm. In order to mimic cyclic muscle contraction as, for example, during steady swimming, the prescribed length changes are often sinusoidal (Hess and Videler, 1984). A plot of force versus length produces what is known as a work loop (Fig. 1.7). Machin and Pringle (1959) developed the work loop technique for asynchronous insect muscle, but Josephson (1985) later adapted it for synchronous muscle. If a loop is traversed in an anticlockwise direction net positive work is done over the cycle. The area below the loop, from minimum to maximum length, is equal to the work needed to stretch the muscle. The area below the upper part of the loop is the work done by the muscle during shortening and the difference between the two is the net work done over the cycle. This, multiplied by the cycle frequency, is the mean mechanical power output of the muscle over the cycle. Stevens (1993) has extended the work loop idea to the power loop, where force is plotted against velocity. This is useful for comparisons with force-velocity curves.

A number of parameters affect the power output obtained from work loops. These include cycle frequency/duration, muscle length change (strain), strain trajectory (e.g. sinusoidal, linear), stimulation onset (phase) and duration, and temperature. The stimulus phase that is optimum for work output is that at which muscle activation and active force production are high throughout the shortening phase and low during the lengthening phase of the cycle. This is generally such that muscle activation begins shortly before the end of the lengthening phase. During fish locomotion, electrical activity occupies a constant proportion of the tail-beat cycle and therefore the number of stimuli delivered to

Figure 1.7. The work loop - the area of the loop is the net work done per cycle and is the difference between the work required to lengthen the muscle and the work done by the muscle during shortening. After Josephson (1993).


Work needed to lengthen the muscle
Force
$L_o$ (resting length)


Work done by the muscle during shortening
Force


Net work over the cycle
Force
Length

a muscle during a cycle *in vitro*, will decrease with increasing cycle frequency (Grillner and Kashin (1976).

Altringham and Johnston (1990*a*) varied a number of parameters in order to obtain the maximum power output against tail-beat frequency in the slow and fast fibres of the short-horn sculpin. They found, for example, power output to be maximal at ±5 % of resting fibre length, over a narrow phase shift (the delay between peak force and maximum length) range, during which the muscle was stretched slightly just prior to shortening. Slow fibres were found to develop maximum power output at around 2 Hz, which is a tail-beat frequency that can be sustained for long periods, whereas fast fibres produced around five times more power at a frequency of 5-7 Hz but these were more quickly limited by fatigue due to a reliance on anaerobic metabolism. These authors (1990*b*) also examined the cycle frequency for maximum work and power output of fast muscle fibres from cod (*Gadus morhua*) of different sizes. With increasing size, the cycle frequency decreased, consequently affecting the strain rate which decreased from 2.2 muscle lengths per second ($s^{-1}$) in a 10 cm fish to about 0.7 $s^{-1}$ in a 100 cm fish. Luiker and Stevens (1992) investigated the effect of varying stimulus parameters on the force and work produced in the adductor muscle of the sunfish (*Lepomis gibbosus*) and reported that duty cycle (the fraction of the cycle during which the muscle is activated) was the most important stimulus parameter affecting work. They found values of work comparable to those in the white muscle of the short-horn sculpin. Josephson (1993) reported that maximum power available from fast muscle was about 150 $W.kg^{-1}$, and from aerobic muscle 100 $W.kg^{-1}$.

### *1.3.3 Muscle efficiency*

Muscle efficiency has been defined as the ratio of mechanical work output and the free energy available (Wilkie, 1960), the ratio of work output and the total energy output (heat plus work), or the net work output for each ATP molecule (Curtin and Woledge, 1993). Maximum efficiency has been found to be higher in muscle undergoing sinusoidal length fluctuations compared to isovelocity shortening (Curtin and Woledge, 1993). An important factor in this is that sinusoidal experiments require fewer stimuli resulting in less energy being used by the $Ca^{2+}$ pumps in the SR. Using heat production methods,

Curtin and Woledge (1994, 1996) have found that maximum efficiency depends on the amplitude of the imposed sinusoidal strain waveform. These authors (1996) also found that altering the duty cycle of a strain wave, can have different effects on power output and efficiency. In white muscle of the dogfish (*Scyliorhinus canicula*), power increased with increasing duty cycle, but efficiency decreased, as a result of heat production during stretch. Thus fast fibres designed for high power have lower efficiencies.

Efficiency and power output are both functions of $V/V_{max}$ where $V$ is the velocity of muscle shortening *in vivo* (Rome *et al.* 1988). Rome *et al.* (1988) compared the velocity of shortening of fast and slow muscle with their $V_{max}$ in order to account for the need for different fibre types in fish. Slow fibres have a low $V_{max}$ whereas fast fibres have a high $V_{max}$; without fast fibres burst swimming would be impossible simply because the slow fibres cannot shorten fast enough. They demonstrated that both fibre types operate within a narrow range of $V/V_{max}$; during slow swimming slow fibres shorten at a velocity that gives peak mechanical power and efficiency, whilst during burst swimming fast fibres shorten at their optimum velocity.

### *1.3.4 Powering fast-starts*

Muscle activity is not constant and will differ along the length of the fish. In addition, different swimming modes appear to produce different recruitment patterns. Using EMGs in the axial muscles of the bluegill sunfish during escape responses, Jayne and Lauder (1993) found that fast and slow muscle was simultaneously recruited, there was no longitudinal lag in muscle activation, but there was a posterior increase in the duration of the electrical activity. During rapid burst and glide swimming, however, there was a lag time between the anterior and posterior positions of approximately 7 ms (Jayne and Lauder, 1994). Johnston *et al.* (1993) found a delay of 10 ms between the activation of the anterior and posterior fast muscle fibres during startle responses and prey capture in the short-horn sculpin.

van Leeuwen *et al.* (1990) suggested that muscle fibres may not always work optimally *in vivo* depending on their position along the trunk. This would be due in part to changes in the onset and duration of electrical activity down the fish. For example, in kick-and-glide and steady swimming, negative work or decreased work is done by the

posterior muscles of some fish, e.g. carp (van Leeuwen *et al.* 1990) and saithe (Altringham *et al.* 1993). Although muscle fibres in these positions produce force over most of the cycle they are active during lengthening and only produce positive work towards the end of the cycle (Altringham *et al.* 1993). Therefore, Altringham *et al.* (1993) suggested that the caudal muscle fibres could have an altered function of transmitting force towards the caudal fin.

Johnston *et al.* (1995) examined power output along the trunk during predation fast-starts in the short-horn sculpin. They calculated strains from a knowledge of the complex arrangement of the muscle fibres and the curvature of the backbone. Therefore, instead of conducting work-loop experiments using sinusoidal length changes on isolated muscle fibres, they used strain fluctuations which were far more unsteady and complex. The altered timing of electrical activity down the fish and the large change in the pattern and amplitude of the muscle strains resulted in appreciable negative power generation by the caudal fibres. Therefore in this mode of swimming, a similar conclusion was reached to that for kick-and-glide and steady swimming (van Leeuwen *et al.* 1990; Altringham *et al.* 1993); the caudal fibres were involved in transmitting power from the middle myotomes to the tail.

### 1.4 Temperature and the thermal dependence of fast-start performance

At certain latitudes the aquatic environment can be very thermostable. Sea water temperatures fluctuate by less than 1 °C in the Southern Ocean around Antarctica (Kock, 1992) and remain fairly stable at about 25 °C in some tropical seas (McConnaughey, 1978). However, in the temperate zone, surface water temperatures can fluctuate by 20 °C both diurnally (Ward and FitzGerald, 1983; Reebs *et al.* 1984) and seasonally (Cossins and Bowler, 1987). Physiological rate processes of poikilotherms are greatly influenced by the thermal environment and thus many species exhibit phenotypic plasticity (Prosser, 1973; Schmidt-Nielsen, 1990; Clarke, 1991). Phenotypic plasticity refers to "the general effect of the environment on phenotypic expression" (Scheiner, 1993) whereby an organism's phenotype includes not only its physiology, but also its morphology and behaviour (Huey and Berrigan, 1996). Reaction norms are the range of phenotypes produced in different environments by the same genotype (Scheiner, 1993).

Table 1.1. $Q_{10}$ values for maximum speed during fast-starts as measured in a number of species.

| Species | Acute temperature range (acclimation temperature) (°C) | Type of fast-start | Stimulus | $Q_{10}$ | Authors |
|---|---|---|---|---|---|
| *Danio [Brachiodanio] rerio* (Larvae) | 21-30 (variable) | ER | electric shock | 1.4 | Fuiman, 1986 |
| *Clupea harengus* (Larvae) | 5-17 (variable) | ER | live predators | 1.5 | Batty *et al.*, 1991; 1993; |
| | 5-15 (10) | | | 1.88 | Batty and Blaxter, 1992 |
| *Pleuronectes platessa* (Larvae) | 5-15 (8) | ER | tactile stimuli | 1.9 | Batty and Blaxter, 1992 |
| *Scophthalmus maximus* | 13-23 (18) | ER | tactile stimuli | 1.77 | Gibson and Johnston, 1995 |
| *Carasius auratus* | 10-35 (35) | ER | striking side of tank/dropping weight in tank | 2.0 | Johnson and Bennet, 1995 |
| *Fundulus heteroclitus* | 10-35 (35) | ER | striking side of tank/dropping weight in tank | 1.2 | Johnson and Bennet, 1995 |
| *Myoxocephalus scorpius* | 5-15 (5) | PC | live prey | 1.22 | Beddow *et al.*, 1995 |

Swimming performance can be plastic and in some fish species changes in performance due to temperature can be ameliorated by acclimation responses. Acclimation involves compensatory changes over a period of time, ranging from instantaneous to evolutionary (Hazel and Prosser, 1974).

### *1.4.1 Effect of acute temperature variation on fast-starts*

Burst swimming has been reported to have a relatively low $Q_{10}$ (1.3-2.0) in cold temperate fish (Bennett, 1978). From the contraction times of fast muscle strips and tail-beat frequencies, Wardle (1980) calculated speeds to increase with temperature, with a $Q_{10}$ of 2.0. Direct measurements during fast-starts, have revealed similar values (Table 1.1).

Several studies, however, report little effect of acute temperature change on maximum speeds (Fuiman, 1991; Beddow *et al.* 1995; Morley and Batty, 1996). Results may differ depending on previous thermal acclimation regime and temperature range over which fish were tested. Morley and Batty (1996) found herring (*Clupea harengus*) larvae altered the kinematics of the S-strike during prey capture by adjusting the body coil (coiled/straight or C/S ratio), and thus maintained a constant velocity at different acute temperatures. This contrasted with the C-start escape response in larvae of this species, where maximum speed increased with increasing acute temperature.

Heat shock is a form of acute temperature change that has been reported to be an ecological problem near power plants (Coutant, 1970; Edsall and Yocom, 1972). This type of thermal stress has been found to alter the vulnerability of fish prey to larger fish predators. Prey vulnerability was found to be higher in heat-shocked juvenile lake whitefish (*Coregonus clupeaformis*), compared to controls, attacked by yellow perch (*Perca flavescens*) (Yocom and Edsall, 1974). Vulnerability has also been found to increase as heat-shock temperature increased in goldfish attacked by rainbow trout (*Oncorhynchus mykiss*) (Webb and Zhang, 1994).

### *1.4.2 Thermal acclimation*

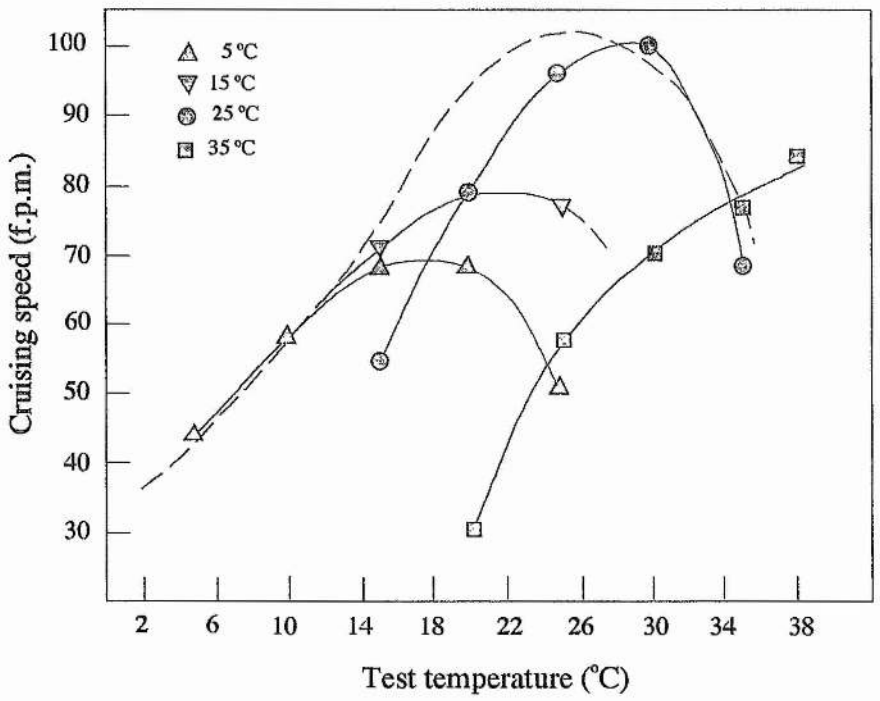
"Acclimation" refers to control of an environmental variable in the laboratory and contrasts with "acclimatisation" which is indicative of natural conditions. An

"acclimation effect" usually refers to some phenotypic response after a period of several days or weeks. It is a physiological example of phenotypic plasticity (Huey and Berrigan, 1996). Precht (1958) classified acclimation responses according to the difference in physiological rate between the previous and current thermal regime. If the rate was the same, then compensation was perfect; if the rate changed initially on introduction to the new temperature, but did not change thereafter, there was no compensation. Compensation could also be partial, excess or inverse.

A classic experiment on the effect of acclimation temperature on swimming performance was carried out by Fry and Hart (1948) on goldfish (*Carassius auratus*). Following acclimation to temperatures between 5 and 35 °C, a rotating annular chamber was used to examine cruising speed over a two minute period. When measured at the acclimation temperature speed remained fairly stable between 20 and 30 °C, indicating perfect compensation. Below 20 °C, however, compensation was partial, such that acclimation to the lower temperatures did not increase speed up to that of the higher acclimation temperatures. Acclimation also extended the thermal range for locomotory activity (Fig. 1.8).

There have been only a few studies, however, on the effect of acclimation temperature on fast-start performance. Acclimation temperature was found to have an effect on the maximum speed of escape responses in rainbow trout (*Salmo gairdneri*) (Webb, 1978*a*), similar to that of critical swimming in goldfish (Fry and Hart, 1948). Maximum velocity ($U_{max}$) and maximum acceleration in rainbow trout acclimated to temperatures from 5-25 °C, increased with acclimation temperature between 5 and 15 °C, with perfect temperature compensation observed between 15 and 25 °C. The distance travelled was also found to increase, and the response latency and duration of the propulsive strokes decrease, with increasing acclimation temperature. Gibson and Johnston (1995) also found near perfect compensation of maximum speed in the eurythermal turbot (*Scophthalmus maximus*) over a limited temperature range. During escape responses, settled stages of turbot had significantly reduced $U_{max}$ following an acute reduction in temperature from 18-13 °C. However, after a period of three weeks at 13 °C, $U_{max}$ rose to a similar value attained by fish acclimated to 18 °C.

Figure 1.8. The effect of acclimation temperature on the cruising speed (measured in feet per minute) of goldfish (*Carassius auratus*) swimming at various temperatures. Dashed line shows speed when fish were acclimated to test temperatures. Adapted from Fry and Hart (1948).


5 °C
15 °C
25 °C
35 °C
Cruising speed (f.p.m.)
100
90
80
70
60
50
40
30
2
6
10
14
18
22
26
30
34
38
Test temperature (°C)

Yocom and Edsall (1974) found more juvenile whitefish were caught by yellow perch when fish were acclimated to 15 and 18 °C, than when acclimated to 10 °C. It was thought that this was due to the effect of acclimation temperature on the predators rather than the prey, such that the perch, being a warmer water species, would have a lower activity at 10 °C. This implies that perch show limited compensation of prey capture performance following cold acclimation, although acclimation was only carried out for three weeks prior to experiments.

Johnson and Bennett (1995) investigated the improvements afforded by cold acclimation over acute cold exposure, during escape behaviour in goldfish and killifish (*Fundulus heteroclitus*). At 10 °C, $U_{max}$, the distance moved and the maximum angular velocity increased by 35 %, 52 % and 42 %, respectively, in goldfish acclimated to 10 °C compared to those acclimated to 35 °C. Acclimation to 10 °C, however, was not found to have such a significant effect on these variables in the killifish. This was attributed to its more eurythermal, estuarine habitat.

Warm acclimation in the short-horn sculpin (*Myoxocephalus scorpius*) similarly improved maximum speed compared to that attained during acute exposure. At 15 °C, warm acclimation to 15 °C increased $U_{max}$ during prey capture of shrimps (*Crangon crangon*) by 33 %, and the percentage of successful attacks by 74 %, compared to cold acclimation of 5 °C (Beddow *et al.* 1995).

### *1.4.3 Temperature variation during early development*

Fish larvae have a large surface area to mass ratio and therefore the viscosity of the medium plays a key role in determining swimming performance. A larva will move during propulsion, but stop as soon as it ceases to propel itself (Lighthill, 1969). The ratio of inertial forces to viscous forces is described by the Reynolds number (*Re*).

$$Re = UL\rho\mu^{-1},$$

where $U$ and $L$ are the velocity and length of the larva, respectively, and $\rho$ and $\mu$ are the density and viscosity of the medium, respectively. Previously, it was thought that viscous forces would only influence swimming performance at very low *Re* of less than 20. Therefore, larvae would operate under inertial forces during the initial burst of a fast-

start, when *Re* would be greater than 200, and then re-enter the viscous regime upon deceleration (Webb and Weihs, 1986; Batty and Blaxter, 1992). However, Fuiman and Batty (1997) have recently found the viscous hydrodynamic regime to extend to an *Re* of approximately 300, but could be important up to 450. Therefore, viscous forces probably influence performance in larvae throughout a fast-start.

Temperature affects the $\rho$ and $\mu$ components of the *Re* and viscosity alterations, particularly, can be dramatic. As temperature decreases from 25 °C to 0 °C, the viscosity of sea water increases two-fold (Kennett, 1982). Although sea water is at its most dense at 4 °C, density changes with temperature are generally small. Fuiman and Batty (1997) were able to separate the physiological and physical effects of temperature change by manipulating both the viscosity and temperature of the medium. They found both variables contributed to reduced speed during routine swimming in large herring larvae (18 mm *L*). For example, speed was significantly reduced when the kinematic viscosity increased from $1.4\times10^{-6}$ to $1.6\times10^{-6}$ $m^2.s^{-1}$ at 13 °C, but at the same viscosity, swimming speed was greater at 13 °C than 9 °C or 6 °C. In small larvae (<10 mm *L*) the physiological effects of temperature were negligible, and swimming speeds declined with increasing viscosity from $1.3\times10^{-6}$ to $2.0\times10^{-6}$ $m^2.s^{-1}$. As the *Re* increases during ontogeny, locomotor performance, and in particular burst swimming, becomes largely governed by inertial forces. In large fish, a large amount of energy is required to accelerate the fish from rest, and stopping is not instantaneous. Viscosity effects of temperature change were found to be entirely absent in escape responses of 6 cm long goldfish, and mostly so, in 2 cm long guppies (*Poecilia reticulata*) (Johnson *et al.* 1993).

Few studies have examined the effect of rearing, or acclimation, temperature on fast-starts in larval fish. In herring larvae, Batty *et al.* (1993) found that $U_{max}$ during the escape response was not influenced by rearing temperature between 5 and 15 °C, reinforcing previous reports that burst speeds in ectotherms in general, show little thermal compensation (Bennett, 1990).

### *1.4.4 Thermal adaptation over evolutionary time periods*

Due to the stenothermal nature of certain habitats, many species of fish are able to operate within only narrow thermal tolerance limits and tend to have a reduced ability to

acclimate. These fish often occur at the more extreme ends of the thermal gradient, such as the poles and tropical waters or desert springs. The question is, do species from different thermal environments exhibit compensation of fast-start performance? A limited number of studies have set out to examine this.

Wakeling and Johnston (1998) found little temperature compensation over evolutionary time scales in various measures of fast-start performance in adult fish. When body morphology and escape performance was measured in Antarctic, North sea, Mediterranean and Indo-West Pacific species, they found an increase in habitat temperature correlated with an increase in the maximum velocity and maximum acceleration of escape responses. They also found that an increase in the median habitat temperature correlated with an increase in the velocity of the wave of curvature travelling posteriorly along the fish, and a decrease in body curvature. In larvae and juvenile Antarctic fish, there similarly appears to be little compensation in fast-start performance as measured by maximum velocity and tail-beat frequency. In 8 mm larvae, mean escape speeds of the Antarctic fish, *Harpagifer antarcticus* at 0 °C, were found to be about half that of herring and plaice at 9-11 °C, and about 40 % of that of northern anchovy (*Engraulis mordax*) and dolphin fish (*Coryphaena hippurus*) at 17-25 °C (Johnston *et al.* 1991). Similarly, burst swimming speeds and tail-beat frequencies of juveniles of the Antarctic teleost *Notothenia coriiceps* were lower than those of temperate fish (Archer and Johnston, 1989). Franklin and Johnston (1997) found maximum velocities and accelerations, during escape responses in adults of this species, to be at the lower end of those for temperate fish.

### *1.4.5 Mechanisms of thermal acclimation*

Wakeling and Johnston (1998) showed that hydrodynamic power during fast-starts was limited by the muscle power output. Muscle contraction relies on the splitting of ATP, which occurs at the ATPase activity site on the myosin heavy chains (MHC), and is determined by both the MHC and myosin light chain (MLC) composition. Johnston *et al.* (1975) found myosin ATPase activity in fast muscle from the goldfish to be about three times higher in 1 °C- than 31 °C-acclimated goldfish, when assayed at 1 °C. The increase in ATPase activity at low temperature in cold acclimated fish was associated

with an increased susceptibility to thermal denaturation at 37 °C, implying structural changes in the myosin proteins. Alterations in ATPase activity with thermal acclimation have been a fairly common finding in cyprinids (Heap *et al.* 1985; 1986; Hwang *et al.* 1990; 1991; Crockford and Johnston, 1990; Johnson and Bennett, 1995).

Thermal acclimation can alter the quantity of a specific muscle protein expressed and, in addition, the particular isoform of the protein. MHC isoforms are thought to be expressed by different genes in carp (Gerlach *et al.* 1990; Watabe *et al.* 1995). Ennion *et al.* (1995) found that a gene encoding the carp MHC was only transcribed following an increase in temperature, and similarly, the expression of one RNA isoform was increased in warm-acclimated carp (Gerlach *et al.* 1990; Goldspink *et al.* 1992).

Johnson and Bennett (1995) investigated the physiological properties of fast muscle fibres underlying the acclimatory responses of fast-starts in the goldfish and killifish. Goldfish showed a shift in the expression of MHC isoforms between 10 °C- and 35 °C-acclimated fish. The more eurythermal killifish that exhibited reduced fast-start plasticity, however, showed no change in MHC composition with acclimation. This was reflected in a similarly reduced acclimation response in myofibrillar ATPase activity, compared to the goldfish. Neither of these variables were found to alter with thermal acclimation in the marine teleosts, the short-horn sculpin and the long-spined sea scorpion (*Taurulus bubalis*) (Ball and Johnston, 1996; Nicholas Cole, personal communication).

The myosin light chains, LC1 and LC3, originate from two different genes in fast muscle of the mullet (*Mugil cephalus*) (Dalla Libera *et al.* 1991) and carp (Hirayama *et al.* 1997). Crockford and Johnston (1990) found altered expression of the MLC in the fast muscle from the carp. Cold-acclimated carp exhibited an additional MLC and an increase in the LC1:LC3 ratio. In the short-horn sculpin, Ball and Johnston (1996) found the ratio of LC3:LC1 changed from 0.73 in fast muscle from 15 °C-acclimated sculpin, to 1.66 in 5 °C-acclimated fish.

The MLCs have been found to modulate muscle shortening speed (Lowey *et al.* 1993). Ball and Johnston (1996) found altered MLC expression in the short-horn sculpin correlated with changes in $V_{max}$ of skinned fast muscle fibres. At 20 °C, $V_{max}$ changed

from 5.3 fibre lengths.$s^{-1}$ in 5 °C-acclimated fish to 8.7 fibre lengths.$s^{-1}$ in 15 °C-acclimated fish. $V_{max}$ showed a similar trend in live fast muscle fibres from this species (Beddow and Johnston, 1995). Warm acclimation also significantly reduced the time to development of half maximal force. Furthermore, at 15 °C, maximum force ($P_0$) increased from 78.3 kN.$m^{-2}$ in 5 °C-acclimated fish to 282.0 kN.$m^{-2}$ in 15 °C-acclimated fish. This meant that at 15 °C, the power output calculated from the force-velocity (*P-V*) relationship increased from 33.62 W.$kg^{-1}$ in 5 °C-acclimated fish to 206.3 W.$kg^{-1}$ in 15 °C-acclimated fish. Cold acclimation, on the other hand, was found to significantly alter the shape of the *P-V* curve, such that the change in curvature was sufficient to produce a 40 % increase in relative power output at 5 °C, in cold-acclimated fish (Beddow and Johnston, 1995).

In carp cold acclimation has been found to significantly increase $V_{max}$ (Johnston *et al.* 1985). Moreover, at 7 °C, the maximum tension produced by isolated skinned muscle fibres, was 57 % higher in fast muscle fibres from 7 °C-acclimated, compared to 23 °C-acclimated carp (Johnston *et al.* 1985). Cold-acclimation also increases the rates of force activation and relaxation in cyprinid muscle fibres at low temperature (Fleming *et al.* 1990; Johnson and Bennett, 1995). Penny and Goldspink (1980) reported that fast muscle fibres from cold-acclimated goldfish had a higher density of SR, than warm-acclimated fish. However, Fleming *et al.* (1990) attributed increased contraction kinetics in cold-acclimated common carp to an increase in the SR $Ca^{2+}$-ATPase activity.

The link between changes in the intrinsic muscle properties with thermal acclimation and actual fast-start performance has been provided by work loop experiments (Josephson, 1985). For example, improved fast-start performance with warm acclimation in the short-horn sculpin has been reflected in increased maximum power output of fast muscle undergoing sinusoidal length changes (Johnson and Johnston, 1991). Johnston *et al.* (1995) found that at 15 °C, the average power, during conditions simulating the first tail-beat of a fast-start, increased from 6.3 W.$kg^{-1}$ in 5 °C-acclimated fish to 23.8 W.$kg^{-1}$ in 15 °C-acclimated fish. They also found near-perfect compensation of muscle performance with temperature acclimation, as the average power was similar from 5 °C- and 15 °C-acclimated fish tested at their respective acclimation temperatures.

## 1.5 Aims

The primary aims of this thesis were to examine the plasticity of fast-starts and muscle performance. Particular focus was on the potential for temperature change, over both acute and seasonal time scales, to alter fast-start performance at the whole animal and cellular levels. The studies used two species of marine Cottidae, the short-horn sculpin (*Myoxocephalus scorpius* L.) and the long-spined sea scorpion (*Taurulus bubalis* Euphr.) which are common temperate teleosts in the eastern Atlantic.

A number of important questions were addressed. Does acclimation to high temperature involve trade-offs in whole animal and muscle performance at low temperature, compared to cold-acclimated fish? Is the ability to thermally acclimate constant through development? Does muscle performance mimic that of whole animal performance? Do acclimation abilities differ between the two species? How similar are the kinematics, mechanics and energetics of fast-starts involved in prey capture and escape responses? Can power requirements be reliably predicted from whole animal performance? Are fast-starts a good correlate of fitness?

Answering these various queries required integrating methods in kinematics, muscle mechanics and energetics in the following way. The kinematics of fast-starts were studied using high speed cinématography or video. Muscle strain and activation were measured during fast-starts using implanted sonomicrometry crystals and electromyography wires, respectively. Muscle activation times and strain waveforms were abstracted to create cyclic events for use in work-loop experiments for measurements of power output. The power requirements for fast-start escape responses were also calculated non-invasively using a mathematical modelling approach devised by Dr. James Wakeling. The energetics of fast-start performance were assessed in isolated muscle following work loops, using high performance liquid chromatography (HPLC).

*Chapter 2*

# TESTING HYPOTHESES CONCERNING THE PHENOTYPIC PLASTICITY OF ESCAPE PERFORMANCE IN FISH OF THE FAMILY COTTIDAE.

## 2.1 Introduction

Ectothermic organisms in the temperate zone exhibit phenotypic plasticity in reaction to changes in environmental factors such as temperature, producing what has been termed, a norm of reaction over an environmental gradient (Scheiner, 1993). Seasonal temperature fluctuations have long been known to produce physiological compensatory changes, known as acclimation responses, in a diversity of rate functions and a wide variety of species (see Bullock, 1955, for early review). Fry and Hart (1948) carried out a classic study examining the effect of thermal acclimation on the swimming speed of goldfish (*Carassius auratus* L.). Acclimation extended the thermal range of activity, and there was a shift in the optimum temperature for performance, between high and low temperature acclimation. Increases in speed at low temperature following cold acclimation were at the expense of swimming performance at high temperature, compared to warm-acclimated fish. Following cold acclimation, the mechanisms underlying altered swimming performance include, *inter alia*, hypertrophy of red muscle fibres (Johnston and Lucking, 1978; Sidell, 1980), increases in mitochondrial volume density (Johnston and Maitland, 1980; Egginton and Sidell, 1989; Hubley *et al.* 1997), altered expression of myosin light chain (MLC) isoforms (Crockford and Johnston, 1990; Langfeld *et al.* 1991; Hirayama *et al.* 1997), and altered expression of myosin heavy chain isoforms (MHC) (Gerlach *et al.* 1990; Hwang *et al.* 1991; Johnson and Bennett, 1995; Imai *et al.* 1997) and associated increases in fast muscle myofibrillar ATPase activity (Johnston *et al.* 1975; Heap *et al.* 1986; Crockford and Johnston, 1990).

Not all temperate species of fish exhibit thermal acclimation. In fact, fish from habitats with large, daily temperature fluctuations may exhibit reduced, or no, thermal acclimation. In the killifish (*Fundulus heteroclitus*), rapid daily fluctuations in water temperature of salt marsh habitats, have been associated with a lack of acclimation in myofibrillar ATPase activity over a temperature range of 5 to 25 °C (Sidell *et al.* 1983). Over a broader test range of 10 to 35 °C, ATPase activity showed some acclimation,

although reduced in comparison to goldfish. This was reflected in a similarly reduced acclimation response in escape performance (Johnson and Bennett, 1995). It has been suggested that acclimation modifications are avoided if the contractile complex has a low thermal sensitivity to acute temperature fluctuations experienced by the organism (Sidell *et al.* 1983). Furthermore, the high degree of short-term temperature variation may mask any stable cues for acclimation.

During ontogeny, seasonal shifts in temperature and/or changes in habitat, can profoundly alter the thermal optimum for physiological processes in ectotherms. For example, the thermal optimum for growth increases during ontogeny in larval plaice (*Pleuronectes platessa*) (Hovenkamp and Witte, 1991) and winter flounder (*Pseudo-pleuronectes americanus*) (Buckley, 1982). In the dragonfly (*Libellula pulchella*), maturation is associated with a more defined thermal sensitivity and an increase in optimum thoracic temperature and upper lethal temperature (Marden, 1995), paralleling the increase in thoracic temperature associated with the more active lifestyle of adult stages (Marden *et al.* 1996).

The evolutionary significance of acclimation, has been less rigorously examined than the mechanisms (Huey and Berrigan, 1996). Precht (1958) classified compensatory changes in physiological rate processes with thermal acclimation, as "perfect", "partial", "excess", "inverse" or absent ("no"). Despite a cautionary note by Fisher (1958), that "when acclimations occur it does not follow that these necessarily have obvious survival value", it has often been assumed that acclimation responses are adaptive (Hazel and Prosser, 1974), and fitness is improved following a period of acclimation. This has become known as the "Beneficial Acclimation Hypothesis" (Leroi *et al.* 1994; Huey and Berrigan, 1996) and, until recently, has been proffered as the primary evolutionary explanation of acclimation. However, it has received recent criticism by Huey and Berrigan (1996), since, in reality, *post-hoc* adaptive stories can be created for any of Precht's above-mentioned acclimation responses. According to Leroi *et al.* (1994), an acclimation response is only to be regarded as beneficial if it enhances differential reproduction, also known as Darwinian fitness. Furthermore, when specifically tested, the assumption has often been rejected (Leroi *et al.* 1994; Zamudio *et al.* 1995; Bennett and Lenski, 1997). Huey and Berrigan (1996) suggest examination of the evolutionary

significance of patterns of acclimation should use more rigorous *a priori* hypotheses based on the natural history of the species. Using this rationale, a set of hypotheses concerning thermal acclimation, were tested in two fish species of the family Cottidae.

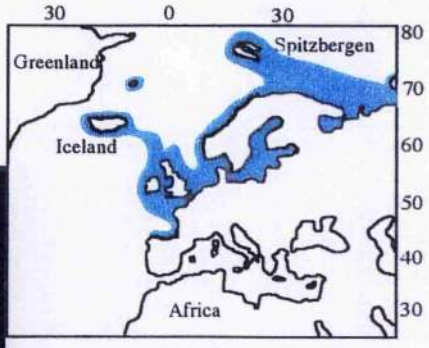
Marine Cottidae are partial residents of the littoral zone (Gibson, 1969). Both short-horn sculpin (*Myoxocephalus scorpius* L.) and long-spined sea scorpion (*Taurulus bubalis* Euphr.) are benthic, marine teleosts of the temperate zone. In the north-east Atlantic, short-horn sculpin have a distribution between 45 and 78 °N, whilst long-spined sea scorpion occupy a somewhat lower clime, between 40 and 68 °N (Unesco, 1986) (Fig. 2.1). Around the British Isles, adult short-horn sculpin are caught offshore (Foster, 1969; King *et al.* 1983) between about 30-50 m (Unesco, 1986). In contrast, juvenile sculpin, and all stages of long-spined sea scorpion are found in rock pools and in the shallow sublittoral zone (Foster, 1969; King and Fives, 1983; Unesco, 1986). Surface water temperatures in St. Andrews Bay, Scotland, range from 3-5 °C in the winter, to 15-18 °C in the summer (J. Murdoch, unpublished observations). Both sea scorpion and juvenile sculpin also experience acute changes in temperature, particularly in the intertidal zone. In the same bay, rock pools where these species are found have mean water temperatures in spring ranging from 5.7 °C to 12.5 °C over a 24 hour period (G. Temple, unpublished observations). Pool temperatures have been found to be even more variable during the summer months (Morris and Taylor, 1983).

The short-horn sculpin and long-spined sea scorpion are "ambush predators", and engage in very little steady swimming. Despite other forms of predator avoidance, such as camouflage and spines, both species commonly employ fast-starts for escape. Measurement of performance has been proposed as a practical method of yielding information on fitness and physiological compensation (Huey and Stevenson, 1979; Arnold, 1983). Several studies have shown or suggested a positive correlation between burst speed and survival, which can have a perceptible influence on fitness (Taylor and McPhail, 1985; Swain, 1992; Watkins, 1996; Andraso, 1997).

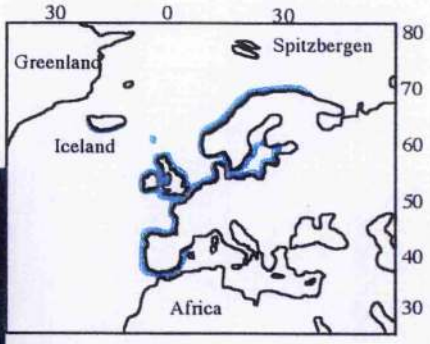
In the present study the following predictions were made, using the escape response, as a correlate of fitness: 1) in the short-horn sculpin, warm acclimation improves escape performance at high temperature, at the expense of performance at low temperature, compared to cold-acclimated fish, 2) escape performance in the intertidal

Figure 2.1. A) Short-horn sculpin (*Myoxocephalus scorpius* L.) and geographic distribution in the north-east Atlantic. B) Long-spined sea scorpion (*Taurulus bubalis* Euphr.) and geographic distribution in the north-east Atlantic. C) High speed video recording of the first two half tail-beats and gliding stage of an escape response typical of both the short-horn sculpin and long-spined sea scorpion. Scale bars, 20 mm.

**A.** Short-horn sculpin (*Myoxocephalus scorpius* L.)




**B.** Long-spined sea scorpion (*Taurulus bubalis* Euphr.)




**C.**

long-spined sea scorpion exhibits reduced thermal sensitivity and therefore does not vary with acclimation temperature over the average range of seasonal temperatures, and 3) in short-horn sculpin, the ability to thermally acclimate maximum speed, is acquired during ontogeny in parallel with the change in thermal habitat due to offshore migration of late juveniles.

## 2.2 Materials and methods

### *2.2.1 Fish*

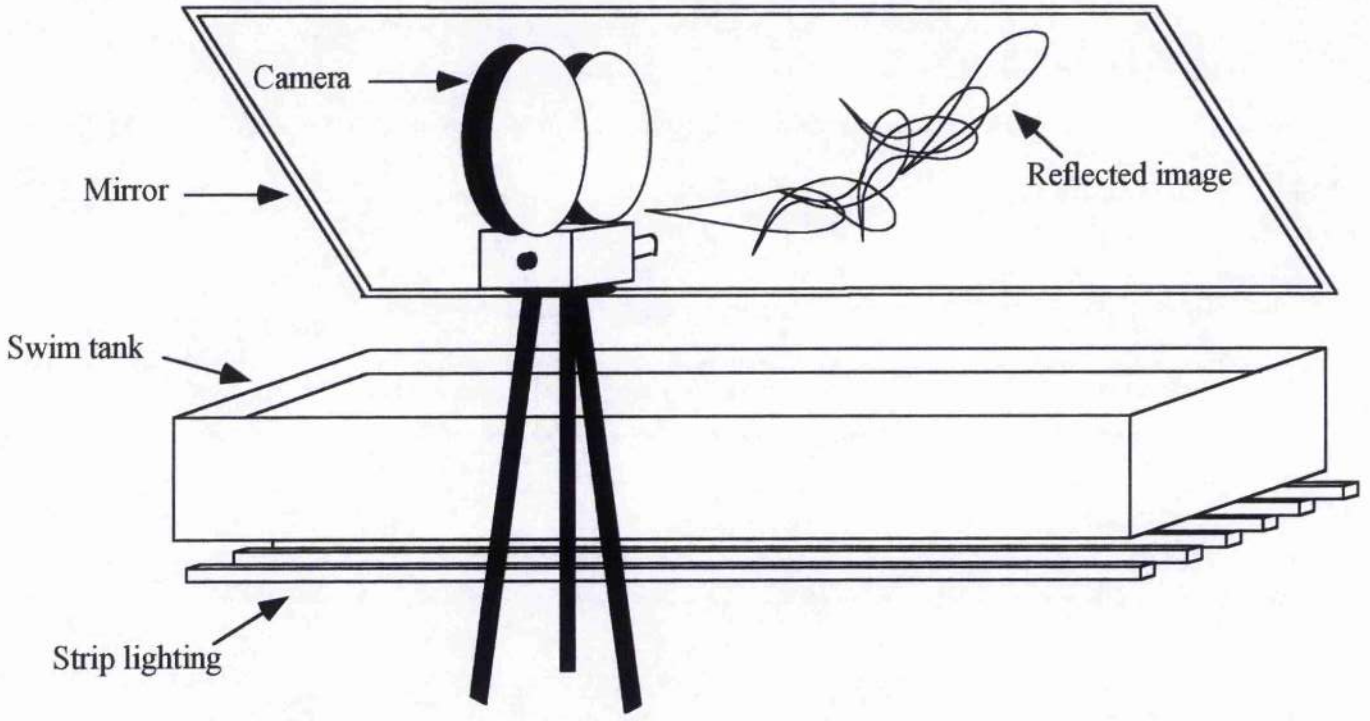
All fish were collected between March 1995 and November 1996. Adult short-horn sculpin (*Myoxocephalus scorpius* L.) were caught using trawls and creels off the Fife coast and Isle of Cumbrae, Scotland. Long-spined sea scorpion (*Taurulus bubalis* Euphr.) and juvenile short-horn sculpin were collected on rocky shores around St. Andrews using hand nets. In one series of experiments, adult fish of a discrete size class (sculpin, 157.2±2.7 mm total length (*L*), 59.2±3.4 g wet mass, *N*=38; sea scorpion, 116.0±1.2 mm *L*, 27.0±1.5 g wet mass, *N*=35: lengths are means ± S.E.M.) were acclimated to one of three temperatures. During winter months (November to March) fish were caught and acclimated to 5±0.5 °C for a minimum of six weeks in a re-circulating sea water aquarium. Fish were kept under a 10 hour light: 14 hour dark photoperiod regime. Similarly, during the summer months (June to September) fish were caught and acclimated to 15 or 20±0.5 °C and kept under a 13 hour light: 11 hour dark photoperiod regime. In both cases fish were fed on prawns and squid twice a week.

To examine the scaling of temperature acclimation responses, fish of a range of lengths (sculpin, 43-270 mm *L*; sea scorpion, 45-160 mm *L*) were either caught in winter and acclimated to 5±0.5 °C, or caught in summer and acclimated to 15±0.5 °C. Acclimation was again carried out for a minimum of six weeks, under the above mentioned photoperiod and feeding regimes. This investigation included the discrete size class of fish above that were acclimated to 5 or 15±0.5 °C.

### *2.2.2 Measurement of fast-start performance*

Following thermal acclimation to 5, 15, or 20±0.5 °C, fish were transferred to a 2.0×0.6×0.25 m static filming arena (Fig. 2.2) at their acclimation temperature. Escape responses were elicited by tactile stimulation. A 4 mm diameter rod was presented randomly from either side of the caudal fin of a stationary fish. Filming was carried out using a high speed ciné camera (NAC-Japan) operating at 500 frames.s$^{-1}$, *via* a mirror set at 45 ° above the tank. Lighting from below the tank enabled sharp silhouettes of the fish to be recorded on film. Adult short-horn sculpin (157.2±2.7 mm *L*) and long-spined sea

Figure 2.2. Schematic diagram of the swim tank and filming setup.


Camera
Mirror
Reflected image
Swim tank
Strip lighting

scorpion (116.0±1.2 mm *L*) acclimated to 5 and 15±0.5 °C, were filmed at 0.8, 5.0, 15.0 and 20.0 °C. Fish acclimated to 20±0.5 °C, were only filmed at their acclimation temperature.

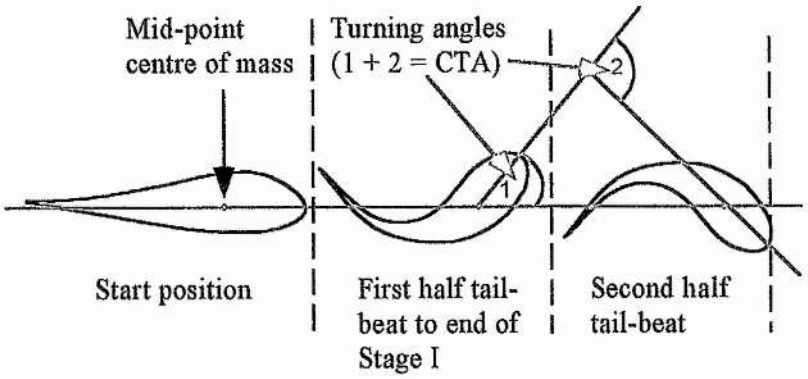
The scaling of fast-start performance was assessed in fish acclimated to 5 and 15±0.5 °C, and tested acutely at 5.0 and 15.0 °C, in a reciprocal experimental design.

### *2.2.3 Kinematic analysis*

Prior to analysis, it was necessary to calculate the position of the mid-point (centre of mass) of the fish relative to the snout. A long pin with a weighted string attached, was placed transversely through the head of a straight, frozen fish. The pin was balanced on two parallel bars. The fish, inclined at an angle of about 45 ° to the horizontal, was photographed. The pin was then removed and placed through the body beneath the first dorsal fin, and the fish photographed. On superimposing one photograph on top of the other, the point at which the strings crossed was found and used as the mid-point. This procedure was carried out on three fish of varying sizes, for each species.

After processing, the films were digitized using MOVIAS-NAC Corp. software. Escape sequences from adult fish of the discrete size classes were analysed by digitizing ten points down the centre of the fish (approximate position of the spine (Wakeling and Johnston, 1998) from snout to tail tip. This was done every fourth frame (8 ms) due to the large number of points being digitized. Thirty frames were digitized before the start of the response and after the end of the second half tail-beat. This guaranteed including the start and end of the response and avoided the problems associated with smoothing the first and last few frames (Harper and Blake, 1989). The end of the second half tail-beat was taken as the maximum angular displacement of the snout to left or right, before myotomal contraction caused bending on the contralateral side of the fish at the start of the third half tail-beat or glide (Figs. 2.1C and 2.3). $x$ and $y$ position data were smoothed using piecewise cubic regressions (Mathmatica, Wolfram Research Inc. USA). Length-specific maximum velocity ($\hat{U}_{max}$, $s^{-1}$) and length-specific maximum acceleration ($\hat{A}_{max}$, $s^{-2}$), of the centre of mass, were calculated from the first and second differentials of each cubic regression. The correct smooth width was established by plotting values of

Figure 2.3. Schematic diagram of a typical escape response. The Cumulative Turning Angle (CTA) is the sum of the absolute values of degrees turned during the first and second half tail-beats. The Response Duration (RD) is taken as the time to the end of the second half-tail-beat.


Mid-point
centre of mass
Turning angles
(1 + 2 = CTA)
1
2
Start position
First half tail-
beat to end of
Stage I
Second half
tail-beat

$\hat{U}_{max}$ calculated using a number of different widths. As the smooth width was increased from three, values for $\hat{U}_{max}$ showed an initial drop, due to the digitizing errors being smoothed. $\hat{U}_{max}$ then reached a plateau and finally dropped a second time due to oversmoothing using smooth widths that were too wide. The correct smooth width was thus taken from the plateau region of graphs of $\hat{U}_{max}$ *versus* smooth width. This resulted in 13 point smoothing for short-horn sculpin and 9 point smoothing for long-spined sea scorpion. The fastest escape sequence from each fish was selected for further analysis. The cumulative turning angle (CTA, °), defined as the sum of the absolute angles of turning of the snout relative to the mid-point throughout the first and second half tail-beats (Fig. 2.3), and the maximum angular velocity ( $\omega_{max}$, rad.s$^{-1}$), were determined in a similar way to the position and velocity. The CTA was only calculated for sequences in which escape responses consisted of two complete half tail-beats.

To examine the scaling of swimming velocity, a second method was used to calculate $\hat{U}_{max}$. This involved digitizing the mid-point of the fish frame-by-frame, using an overhead projector overlay on which the distance of the mid-point from the snout had been marked. This method was used because only maximum velocities and the response durations (RD; the time to the end of the second half tail-beat) were calculated from these sequences; the first method used above enabled further calculations discussed in Chapter 5. Once again digitizing was started before the fish first moved and after the end of the second half tail-beat. $x$ and $y$ position data were smoothed using cubic piecewise regressions (LabView, National Instruments, USA) and $\hat{U}_{max}$ calculated. The width of each piece was set to ensure the standard error of all points from their smoothed positions matched the standard error of digitizing. This resulted in smoothing ranging from 21 points for large fish to 15 points for small fish. The fastest escape response from each fish was again selected. Maximum absolute velocity was also derived from each response. Only sequences in which two half tail-beats were completed were used to measure the response duration.

To check the two methods of calculating $\hat{U}_{max}$ were comparable, fast-starts from the discretely-sized adult fish which had been acclimated to 5 and 15 °C and swum at 5.0

and 15.0 °C, were analysed by both methods and compared using a one-way ANOVA, where there was found to be no significant difference between values from the two procedures ($P$>0.2).

### *2.2.4 Statistics*

A two-way General Linear Model (GLM) ANOVA was used to examine the effects of acclimation (5 and 15 °C) and acute temperature on $\hat{U}_{max}$, $\hat{A}_{max}$, $\omega_{max}$ and CTA. A one-way ANOVA was used to examine the effects of acclimation temperature at all four test temperatures. If fish were unable to swim (scored as zero), Mann-Whitney $U$ tests were used to test for differences between acclimation to 5 and 15 °C at that particular test temperature. All statistical tests were computed using Minitab software (Minitab Inc. USA).

To examine the scaling of $\hat{U}_{max}$, $U_{max}$ and RD with temperature acclimation, linear regressions ($y = aL^b$, where $L$ = total body length) for the following groups: 5@5.0, 5@15.0, 15@15.0 and 15@5.0 (acclimation temperature at test temperature, °C), were calculated on log transformed data, using least squares regression analysis. The significance of all regression lines was assessed by means of the Analysis of Variance (ANOVA) $F$ statistic. To test for differences in acclimation ability between short-horn sculpin from the two different habitats, a two-way GLM Analysis of Covariance (ANCOVA) (Minitab Inc. USA) was used, with habitat in which the fish were caught and temperature group as between subject factors, and length as a covariate. Furthermore, ANCOVA was used to test for differences in regression lines. Slopes (exponent $b$) were compared using the first stage of ANCOVA. Tukey multiple comparison tests were employed to determine between which groups any differences lay. Where no differences were found, the second stage of ANCOVA was used to test for differences in regression elevations (intercepts, proportionality coefficient $a$). Tukey tests were again used to determine where differences lay. These tests were carried out following Zar (1996).

## 2.3 Results

Escape responses fitted the description of Weihs (1973) and were typical "C-starts". Initial contraction of the trunk muscles on one side of the fish (stage 1) bent the fish into a curved shape. Following this was a propulsive stroke or strokes (stage 2), consisting of one or more tail-beats, and then a final gliding or steady swimming stage (stage 3) (Figs. 2.1C and 2.3).

### *2.3.1 Configuration of temperature acclimation responses*

The results for the eight two-way GLM ANOVAs are given in Table 2.1. Test temperature had a significant effect on $\hat{U}_{max}$ and $\hat{A}_{max}$ in both short-horn sculpin ($\hat{U}_{max}$ $F_{(3,59)}$=8.38, $P$<0.0005; $\hat{A}_{max}$ $F_{(3,59)}$=6.76, $P$=0.001) and long-spined sea scorpion ($\hat{U}_{max}$ $F_{(3,58)}$=36.05, $P$<0.0005; $\hat{A}_{max}$ $F_{(3,58)}$=21.07, $P$<0.0005) (Fig. 2.4). In the short-horn sculpin, the adjusted mean $\hat{U}_{max}$ and $\hat{A}_{max}$ for both acclimation groups increased by 54.8 % and 53.1 %, respectively, between 0.8 and 15.0 °C. In long-spined sea scorpion, these values increased by 177.8 % and 161.6 %, respectively, over the same temperature range. Test temperature had a significant effect on $\omega_{max}$ ($F_{(3,58)}$=5.55, $P$=0.002) and the CTA ($F_{(3,54)}$=4.75, $P$=0.005) in long-spined sea scorpion, but not in short-horn sculpin ($P$=0.441, $P$=0.393, respectively) (Fig. 2.5). In sea scorpion the mean $\omega_{max}$ increased by 136.6 % and the mean CTA by 81.3 %, between test temperatures of 0.8 and 5.0 °C.

Acclimation temperatures of 5 and 15 °C, had a significant effect on $\hat{U}_{max}$ ($F_{(1,59)}$=13.52, $P$=0.001) and $\hat{A}_{max}$ ($F_{(1,59)}$=7.68, $P$=0.007) in short-horn sculpin (two-way ANOVA) (Fig. 2.4A and B). However, there was only a significant interaction between test and acclimation temperature for $\hat{U}_{max}$ ($F_{(3,59)}$=6.02, $P$=0.001) (Fig. 2.4A). For 5 °C-acclimated short-horn sculpin, mean $\hat{U}_{max}$ was 3.7 $s^{-1}$ at 0.8 °C and 3.8 $s^{-1}$ at 5.0 °C, a $Q_{10}$ of 1.07 (Table 2.2). In contrast the $Q_{10}$ for the 15 °C-acclimated group was 3.02, with mean $\hat{U}_{max}$ increasing from 2.9 $s^{-1}$ at 0.8 °C to 4.6 $s^{-1}$ at 5.0 °C. Similar

Table 2.1. *Analysis of variance for maximum velocity, maximum acceleration, maximum angular velocity and cumulative turning angle.*

| | | ***Myoxocephalus scorpius*** | | | ***Taurulus bubalis*** | | |
|---|---|---|---|---|---|---|---|
| Variable | Source | d.f. | *F*-ratio | *P* | d.f. | *F*-ratio | *P* |
| $\hat{U}_{max}$, $s^{-1}$ | A | 1 | 13.52 | 0.001 | 1 | 0.10 | 0.751 |
| | B | 3 | 8.38 | 0.000 | 3 | 36.05 | 0.000 |
| | A × B | 3 | 6.02 | 0.001 | 3 | 5.87 | 0.001 |
| | Error | 59 | | | 58 | | |
| $\hat{A}_{max}$, $s^{-2}$ | A | 1 | 7.68 | 0.007 | 1 | 2.29 | 0.136 |
| | B | 3 | 6.76 | 0.001 | 3 | 21.07 | 0.000 |
| | A × B | 3 | 2.04 | 0.118 | 3 | 1.60 | 0.199 |
| | Error | 59 | | | 58 | | |
| CTA, ° | A | 1 | 0.61 | 0.441 | 1 | 3.46 | 0.068 |
| | B | 3 | 1.02 | 0.393 | 3 | 4.75 | 0.005 |
| | A × B | 3 | 0.06 | 0.982 | 3 | 1.13 | 0.344 |
| | Error | 43 | | | 54 | | |
| $\omega_{max}$, $rad.s^{-1}$ | A | 1 | 2.84 | 0.097 | 1 | 8.37 | 0.005 |
| | B | 3 | 0.91 | 0.441 | 3 | 5.55 | 0.002 |
| | A × B | 3 | 0.73 | 0.539 | 3 | 0.76 | 0.521 |
| | Error | 59 | | | 58 | | |

A, acclimation temperature; B, test temperature; d.f., degrees of freedom

Figure 2.4. Maximum length-specific velocity (expressed as $s^{-1}$), $\hat{U}_{max}$ (A and C) and maximum length-specific acceleration (expressed as $s^{-2}$), $\hat{A}_{max}$ (B and D) attained during the first two half tail-beats of escape responses in, i) short-horn sculpin and ii) long-spined sea scorpion. Values represent means ± S.E.M. Asterisks indicate significant differences between acclimation groups at a particular test temperature (short-horn sculpin, Tukey multiple comparison test; long-spined sea scorpion, Mann Whitney *U* tests). For short-horn sculpin groups 5@0.8, 5@5.0, 5@15.0, 5@20.0, 20@20.0, 15@20.0, 15@15.0, 15@5.0, 15@0.8 (acclimation temperature at test temperature, °C), *N*=9, 9, 9, 5, 11, 5, 10, 11, 9, respectively. For long-spined sea scorpion *N*=7, 12, 8, 7, 8, 8, 13, 9, 2, respectively. Sculpin were 157.2±2.6 mm *L*, *N*=38 and sea scorpion were 116.0±1.2 mm *L*, *N*=35.

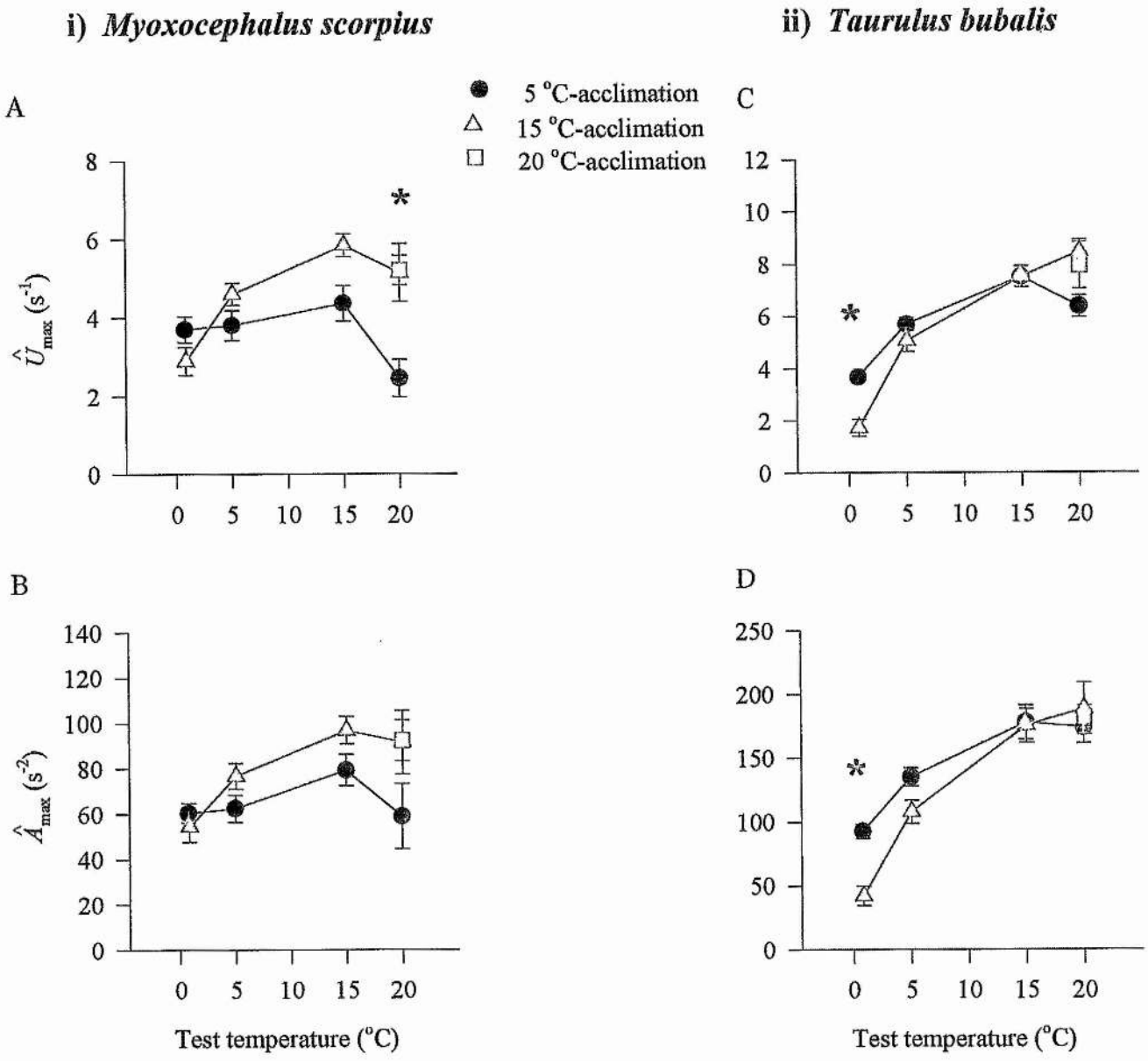
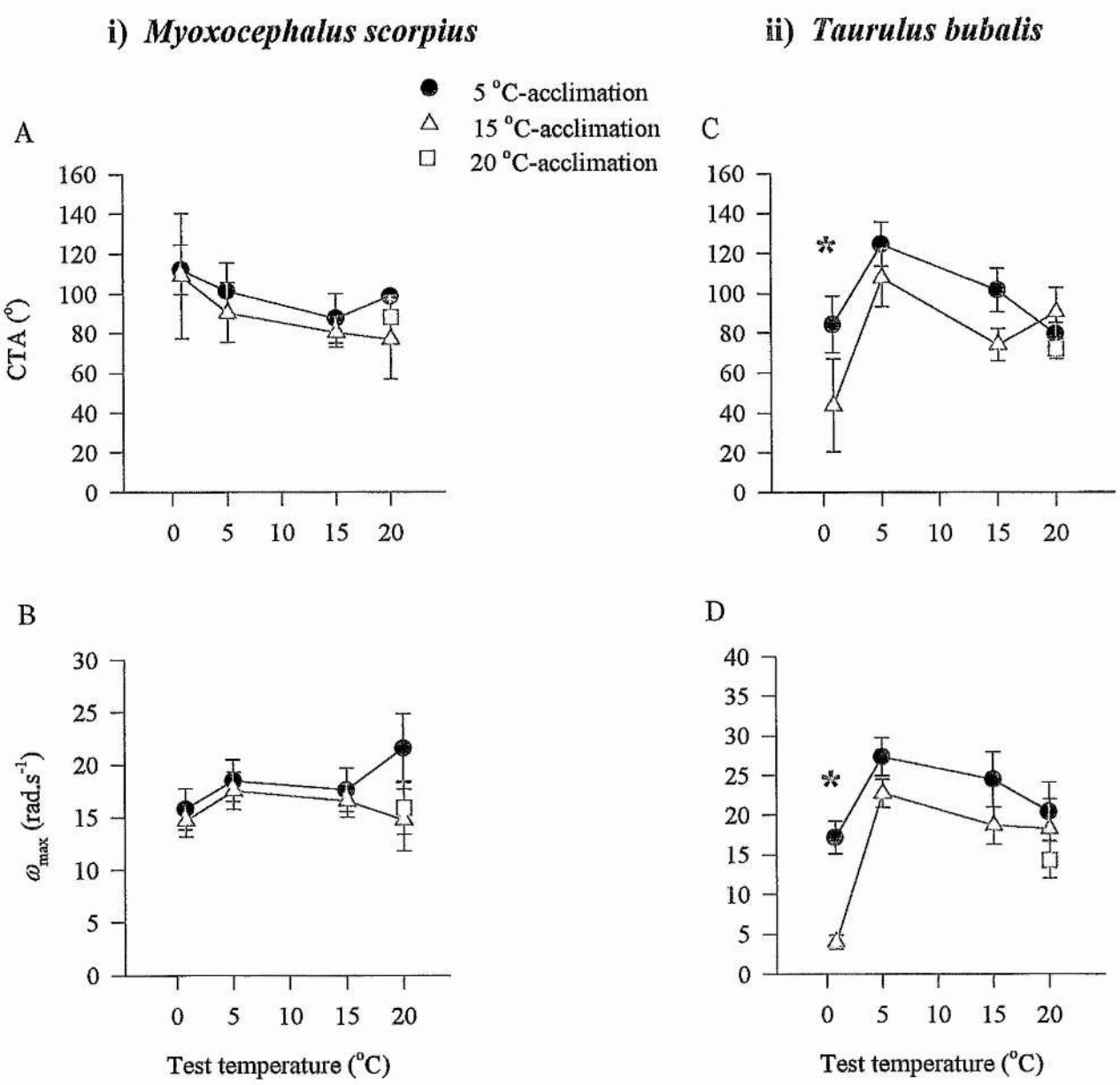

i) Myoxocephalus scorpius
ii) Taurulus bubalis
5 °C-acclimation
15 °C-acclimation
20 °C-acclimation
A
B
C
D
$\hat{U}_{max}$ ($s^{-1}$)
$\hat{A}_{max}$ ($s^{-2}$)
Test temperature (°C)
Test temperature (°C)
*

Figure 2.5. Cumulative turning angle, CTA (A and C) and maximum angular velocity, $\omega_{max}$ (B and D) attained during escape responses in, i) short-horn sculpin and ii) long-spined sea scorpion. Values represent means ± S.E.M. Asterisks indicate significant differences between acclimation groups at a particular test temperature (short-horn sculpin, Tukey multiple comparison test; long-spined sea scorpion, Mann Whitney *U* tests). For short-horn sculpin groups 5@0.8, 5@5.0, 5@15.0, 5@20.0, 20@20.0, 15@20.0, 15@15.0, 15@5.0, 15@0.8 (acclimation temperature at test temperature, °C) CTA $N$=8, 8, 8, 1, 10, 5, 10, 9, 3, respectively and $\omega_{max}$ $N$=9, 9, 9, 5, 11, 5, 10, 11, 9, respectively. For long-spined sea scorpion, CTA, $N$=7, 12, 8, 5, 8, 8, 13, 7, 2, respectively and $\omega_{max}$ $N$=7, 12, 8, 7, 8, 8, 13, 9, 2, respectively. Sculpin were 157.2±2.6 mm *L*, $N$=38 and sea scorpion were 116.0±1.2 mm *L*, $N$=35.


i) Myoxocephalus scorpius
ii) Taurulus bubalis
5 °C-acclimation
15 °C-acclimation
20 °C-acclimation
A
B
C
D
CTA (°)
ω max (rad.s-1)
Test temperature (°C)
Test temperature (°C)
*
*

differences in $Q_{10}$ were found for $\hat{A}_{max}$ (Table 2.2). However, Tukey tests revealed no significant differences in $\hat{U}_{max}$ or $\hat{A}_{max}$ at 0.8 or 5.0 °C between the two acclimation groups (Fig. 2.4A and B). On the other hand, at 20.0 °C, the 20 °C and 15 °C-acclimated fish had significantly higher $\hat{U}_{max}$ (5.2 $s^{-1}$) than the 5 °C-acclimated fish (2.5 $s^{-1}$) ($P<0.001$, $P<0.05$ respectively, Tukey multiple comparison test) (Fig. 2.4A). At this test temperature, only 20 % of 5 °C-acclimated fish completed two half tail-beats, compared to 90.9 % and 80.0 % for 20 and 15 °C-acclimated short-horn sculpin, respectively. At a test temperature of 15.0 °C, the 15 °C-acclimated sculpin had a mean $\hat{U}_{max}$ of 5.8 $s^{-1}$ compared to 4.4 $s^{-1}$ for the 5 °C-acclimated fish ($P<0.1$, Tukey multiple comparison test). In this species, the CTA and $\omega_{max}$ were not influenced by acclimation temperature ($P=0.441$, $P=0.097$, respectively, two-way ANOVA) (Fig. 2.5A and B).

In long-spined sea scorpion, two-way ANOVA revealed that acclimation temperature had no significant effect on $\hat{U}_{max}$, $\hat{A}_{max}$ or CTA ($P=0.751$, $P=0.136$, $P=0.068$, respectively) (Fig. 2.4C and D, Fig. 2.5C) but did significantly affect $\omega_{max}$ ($F_{(1,58)}=8.37$, $P=0.005$), with higher values for 5 °C-acclimated fish (Fig. 2.5D). There was a significant interaction between acclimation and test temperature in $\hat{U}_{max}$ ($F_{(3,58)}=5.87$, $P=0.001$) (Fig. 2.4C). Only two out of five fish acclimated to 15 °C were able to swim at 0.8 °C, although all individuals fully recovered following a rise in temperature. When scoring zero for fish which did not swim, Mann-Whitney $U$ tests revealed significantly higher values for all variables for 5 °C- than 15 °C-acclimated fish at 0.8 °C ($P<0.05$) (Fig. 2.4C and D, Fig. 2.5C and D). Using mean values for fish that swam, $\hat{U}_{max}$ was 3.7 $s^{-1}$ at 0.8 °C and 5.7 $s^{-1}$ at 5.0 °C in 5 °C-acclimated fish ($Q_{10}$ of 2.83) (Table 2.2). For 15 °C-acclimated sea scorpion, $\hat{U}_{max}$ was 1.7 and 5.1 $s^{-1}$ at 0.8 and 5.0 °C, respectively ($Q_{10}$ of 12.88) (Table 2.2). At the other end of the temperature range no variable showed any significant difference between acclimation temperatures ($P>0.05$).

Table 2.2. *$Q_{10}$ values for 5 and 15 °C acclimated fish over the temperature range 0.8 °C to 15.0 °C and 0.8 to 5.0 °C.*

| | ***Myoxocephalus scorpius*** | | ***Taurulus bubalis*** | |
|---|---|---|---|---|
| | Acclimation temperature | | | |
| | 5 °C | 15 °C | 5 °C | 15 °C |
| Variable | Temperature range, 0.8 - 15.0 °C | | | |
| $\hat{U}_{max}$, $s^{-1}$ | 1.13 | 1.64 | 1.65 | 2.81 |
| $\hat{A}_{max}$, $s^{-2}$ | 1.21 | 1.50 | 1.58 | 5.20 |
| | Temperature range, 0.8 - 5.0 °C | | | |
| $\hat{U}_{max}$, $s^{-1}$ | 1.07 | 3.02 | 2.83 | 12.88 |
| $\hat{A}_{max}$, $s^{-2}$ | 1.08 | 2.24 | 2.45 | 9.37 |

### *2.3.2 Scaling of temperature acclimation responses*

In both species of fish, maximum length-specific velocity of the midpoint decreased and response duration increased, with increasing total body length at all temperature regimes (5@5.0, 5@15.0, 15@15.0, 15@5.0 °C; acclimation temperature at test temperature) ($P$<0.01, $F$ statistic) (Fig. 2.6; Table 2.3). Absolute velocity ($U_{max}$, $m.s^{-1}$) increased with increasing total fish length for all groups of short-horn sculpin ($P$<0.01, $F$ statistic) apart from those acclimated to 5 °C and swum at 15.0 °C ($P$=0.865, $F$ statistic; regression coefficient of 0.02) (Table 2.3). In the long-spined sea scorpion, $U_{max}$ increased significantly with total fish length for all groups ($P \leq 0.05$, $F$ statistic) except those acclimated to 5 °C and swum at 5 °C ($P$=0.34, $F$ statistic; regression coefficient of 0.11) (Table 2.3).

Using $\hat{U}_{max}$ of short-horn sculpin as the response variable, the two-way GLM ANCOVA revealed there to be significant interaction between habitat and temperature group ($F_{(3,104)}$=4.41, $P$=0.006), thus indicating differences in the acclimation ability of sculpin from the two localities (Table 2.4). $\hat{U}_{max}$ scaled to a common slope exponent of -0.55 for 5 °C-acclimated fish at a test temperature of 5.0 °C, and 15 °C-acclimated fish at test temperatures of 15.0 °C and 5.0 °C. In the latter acclimation group, there was significant regression elevation, with log values of $\hat{U}_{max}$ being 26.1 % higher in fish swimming at their acclimation temperature (test temperature) of 15.0 °C, than at an acute temperature of 5.0 °C ($P$<0.05, Tukey multiple comparison test) (Fig. 2.6C; Table 2.5). However, escape performance was not significantly different between acclimation groups at a test temperature of 5.0 °C (Fig. 2.7C; Table 2.6). The scaling relationship for $\hat{U}_{max}$ changed to $aL^{-0.98}$, when 5 °C-acclimated short-horn sculpin were swum at an acute temperature of 15.0 °C (Figs. 2.6A, 2.7A). This caused the scaling relationships (exponent $b$) to significant differ ($P$<0.01, Tukey multiple comparison test) between fish swum at an acute temperature of 15.0 °C and those swimming at their acclimation temperature of 15.0 °C (Fig. 2.7A, Table 2.6). The first part of this study revealed $\hat{U}_{max}$ at 15.0 °C to be higher in adult fish acclimated to 15 °C than in those acclimated to 5 °C. However, the scaling of $\hat{U}_{max}$ changed with temperature such that the regression lines

Table 2.3. *Regression data for the scaling of maximum length-specific velocity, maximum absolute velocity and response duration with temperature. Values are given for the slope (exponent b), intercept (proportionality coefficient a), coefficient of determination ($r^2$) and significance of regression (P).*

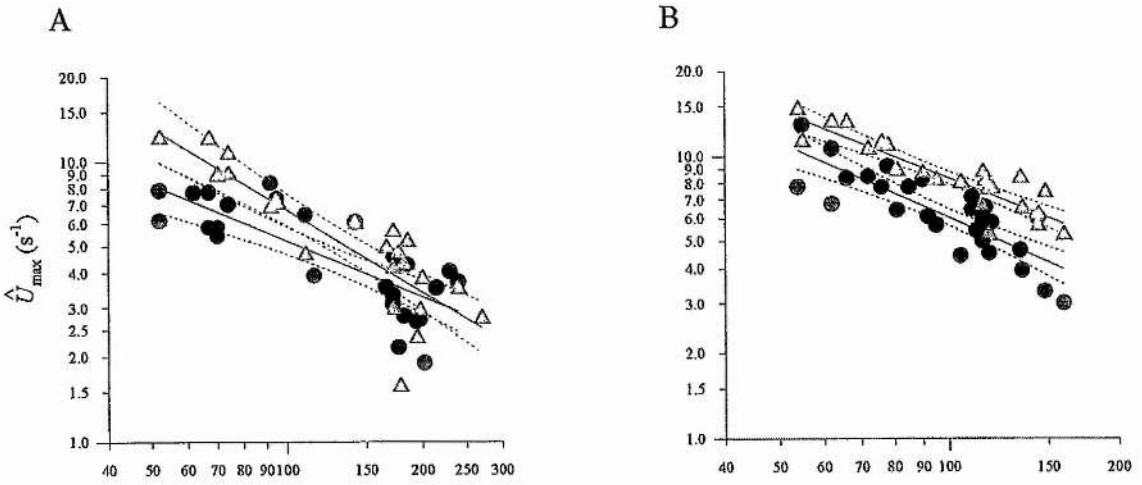
| | | | ***Myoxocephalus scorpius*** | | | | ***Taurulus bubalis*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Temperature | Symbols in Fig. 5 & 6 | *b* | *a* | $r^2$ | *P* | *b* | *a* | $r^2$ | *P* |
| $\hat{U}_{max}$, $s^{-1}$ | 5@5.0 °C | ○ | -0.66 | 2.05 | 0.63 | <0.01 | -0.89 | 2.56 | 0.68 | <0.01 |
| | 5@15.0 °C | △ | -0.98 | 2.79 | 0.75 | <0.01 | -0.79 | 2.51 | 0.81 | <0.01 |
| | 15@15.0 °C | □ | -0.50 | 1.87 | 0.76 | <0.01 | -0.71 | 2.34 | 0.66 | <0.01 |
| | 15@5.0 °C | ▽ | -0.49 | 1.70 | 0.42 | <0.01 | -0.70 | 2.15 | 0.47 | <0.01 |
| $U_{max}$, $m.s^{-1}$ | 5@5.0 °C | | 0.34 | -0.96 | 0.30 | <0.01 | 0.11 | -0.44 | 0.03 | =0.34 |
| | 5@15.0 °C | | 0.02 | -0.22 | 0.001 | =0.87 | 0.21 | -0.49 | 0.22 | =0.02 |
| | 15@15.0 °C | | 0.50 | -1.13 | 0.74 | <0.01 | 0.29 | -0.66 | 0.26 | <0.01 |
| | 15@5.0 °C | | 0.51 | -1.30 | 0.43 | <0.01 | 0.31 | -0.85 | 0.15 | =0.05 |
| RD, ms | 5@5.0 °C | | 0.45 | 1.17 | 0.54 | <0.01 | 0.35 | 1.35 | 0.30 | <0.01 |
| | 5@15.0 °C | | 0.63 | 0.64 | 0.57 | <0.01 | 0.25 | 1.34 | 0.18 | =0.04 |
| | 15@15.0 °C | | 0.33 | 1.24 | 0.35 | <0.01 | 0.27 | 1.28 | 0.26 | <0.01 |
| | 15@5.0 °C | | 0.30 | 1.51 | 0.19 | =0.02 | 0.26 | 1.54 | 0.28 | <0.01 |

Temperature, acclimation temperature at test temperature

Figure 2.6. The effect of test temperature on maximum length-specific velocity attained during the first two half tail-beats of escape responses for i) short-horn sculpin, size range 43-270 mm *L* and ii) long-spined sea scorpion, size range 45-160 mm *L*. 95 % confidence intervals appear as dotted lines. A and B are for fish acclimated to 5 °C; C and D are for fish acclimated to 15 °C.

**i) *Myoxocephalus scorpius*** **ii) *Taurulus bubalis***

5@5.0 °C (●) *vs.* 5@15.0 (△)




15@15.0 °C (□) *vs.* 15@5.0 (▽)

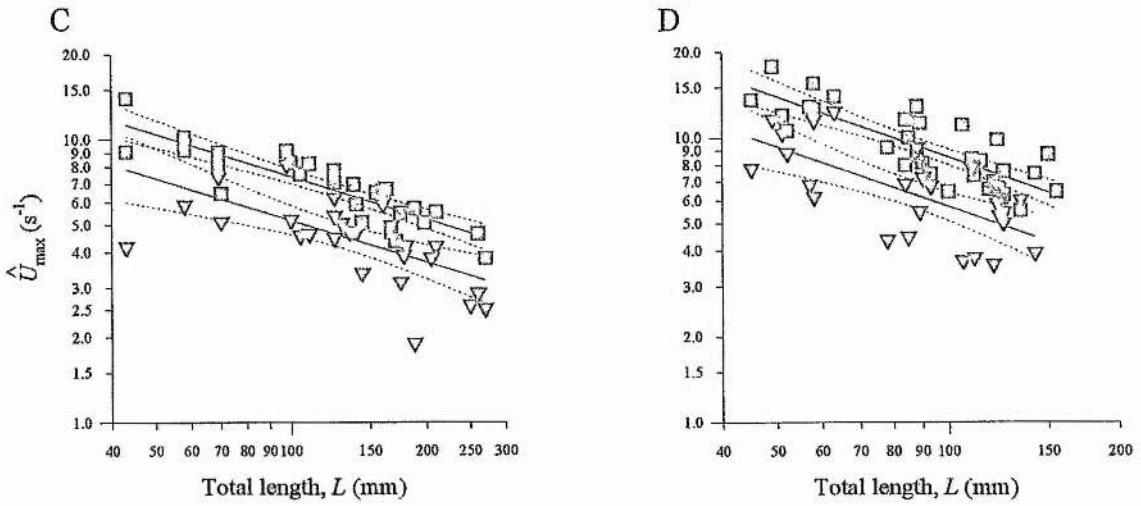
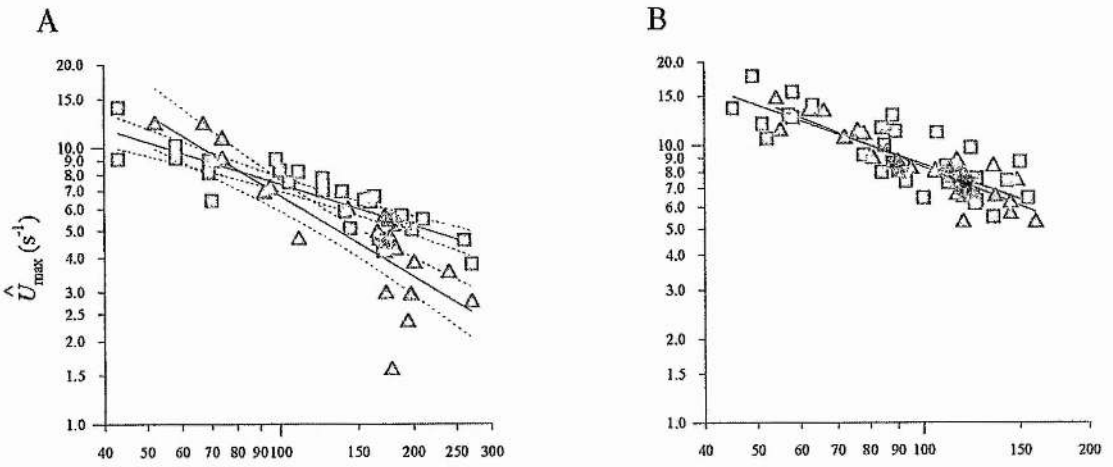


Figure 2.7. The effect of acclimation on maximum length-specific velocity attained during the first two half tail-beats of escape responses for i) short-horn sculpin, size range 43-270 mm *L*, and ii) long-spined sea scorpion, size range 45-160 mm *L*. The data for each group are the same as in Figure 5. 95 % confidence intervals appear as dotted lines in A. Legends are acclimation temperature at test temperature. A and B are for fish tested at 15 °C; C and D are for fish tested at 5 °C.

**i) *Myoxocephalus scorpius*** **ii) *Taurulus bubalis***

5@15.0 (△) *vs.* 15@15.0 °C (□)




15@5.0 (▽) *vs.* 5@5.0 °C (●)

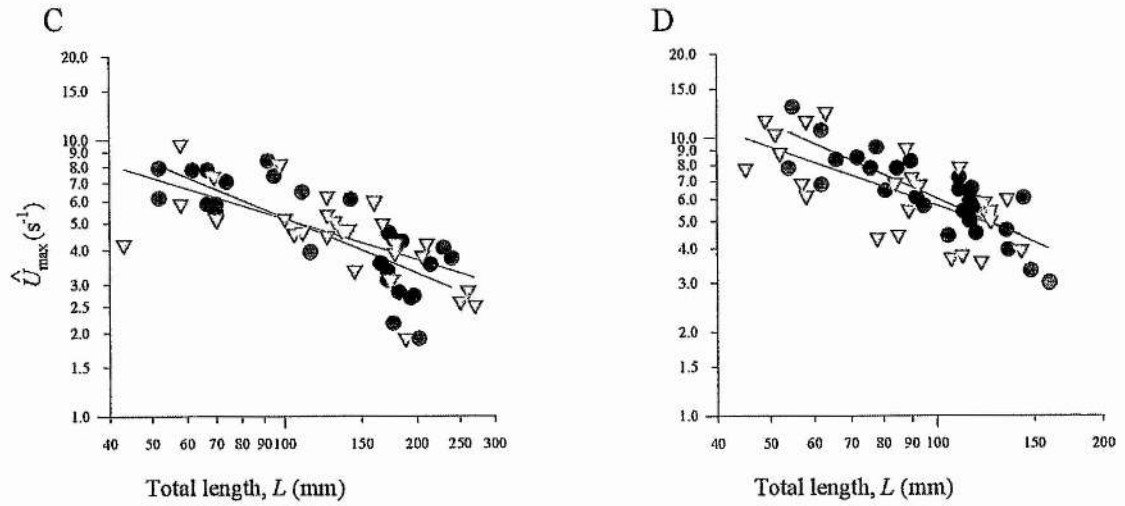


Table 2.4. *Analysis of covariance for maximum length-specific velocity of short-horn sculpin (size range 43-270 mm* L*).*

| Source | d.f. | Sequential sum of squares | Adjusted sum of squares | Adjusted mean square | *F*-ratio | *P* |
|---|---|---|---|---|---|---|
| Length | 1 | 337.008 | 58.486 | 58.486 | 35.36 | 0.000 |
| A | 3 | 83.178 | 87.540 | 29.180 | 17.64 | 0.000 |
| B | 1 | 3.424 | 5.316 | 5.316 | 3.21 | 0.076 |
| A*B | 3 | 21.906 | 21.906 | 7.302 | 4.41 | 0.006 |
| Error | 104 | 172.008 | 172.008 | 1.654 | | |
| Total | 112 | 617.523 | | | | |

A, temperature group; B, habitat; d.f., degrees of freedom

Table 2.5. *Regression analysis of the effects of acute temperature on the scaling of maximum length-specific velocity and response duration for the groups, 5@5.0, 5@15.0, 15@15.0 and 15@5.0 °C (acclimation temperature at test temperature). All regressions were significant (P<0.05, ANOVA). All results refer to differences in slope elevations (stage 2 ANCOVA) with the most elevated groups in parentheses. For* $\hat{U}_{max}$ *of short-horn sculpin this was assessed between groups 15@15.0 and 15@5.0 °C.*

| | ***Myoxocephalus scorpius*** | | ***Taurulus bubalis*** | |
|---|---|---|---|---|
| | Acute temperature effects | | | |
| Variable | 5@5.0 *vs.* 5@15.0 °C | 15@15.0 *vs.* 15@5.0 °C | 5@5.0 *vs.* 5@15.0 °C | 15@15.0 *vs.* 15@5.0 °C |
| $\hat{U}_{max}$, $s^{-1}$ | ----- | $P<0.01$ (15@15.0 °C) | $P<0.01$ (5@15.0 °C) | $P<0.01$ (15@15.0 °C) |
| RD, ms | $P<0.01$ (5@5.0 °C) | $P<0.01$ (15@5.0 °C) | $P<0.01$ (5@5.0 °C) | $P<0.01$ (15@5.0 °C) |

Regression 5@15 °C of *M. scorpius* caused significant differences in stage 1 ANCOVA and therefore was omitted from second stage ANCOVA analysis.

Table 2.6. *Regression analysis of the effects of acclimation on the scaling of maximum length-specific velocity and response duration for the groups, 5@5.0, 5@15.0, 15@15.0 and 15@5.0 °C (acclimation temperature at test temperature). All regressions were significant (P<0.05, ANOVA). Unless stated otherwise, all results refer to differences in slope elevations (stage 2 ANCOVA).*

| | ***Myoxocephalus scorpius*** | | ***Taurulus bubalis*** | |
|---|---|---|---|---|
| | Effect of acclimation temperature | | | |
| Variable | Improvement in performance after acclimation to 15 °C (5@15.0 *vs.* 15@15.0 °C) | Improvement in performance after acclimation to 5 °C (15@5.0 *vs.* 5@5.0 °C) | Improvement in performance after acclimation to 15 °C (5@15.0 *vs.* 15@15.0 °C) | Improvement in performance after acclimation to 5 °C (15@5.0 *vs.* 5@5.0 °C) |
| $\hat{U}_{max}$, $s^{-1}$ | significant difference in slope exponent *b* (stage 1 ANCOVA, *P*<0.01) | n.s. | n.s. | n.s. |
| RD, ms | n.s. | n.s. | n.s. | n.s. |

n.s., not significant

for these two groups converged at the small size range of fish indicating that this was not true for the smaller fish; $\hat{U}_{max}$ at 15.0 °C was not higher in sculpin acclimated to 15 °C compared with those acclimated to 5 °C.

Using RD as the response variable, the two-way GLM ANCOVA revealed there to be no significant interaction between habitat and temperature group. Thus there was no significant change in the scaling of RD in short-horn sculpin with acclimation temperature, the slope exponent being 0.41. However, there was significant regression elevation (Table 2.5; $P<0.01$, Tukey multiple comparison test), with fish swimming at 15.0 °C having about a 9 % shorter RD than fish swimming at 5.0 °C, irrespective of previous acclimation temperature. This was also the pattern found in long-spined sea scorpion (Tables 2.5 and 2.6). RD scaled to a common regression coefficient of 0.28, with fish swimming at 15.0 °C having significantly shorter RDs (c. 11 %) than fish swimming at 5.0 °C, regardless of thermal acclimation temperature ($P<0.01$, Tukey multiple comparison test). Furthermore, $\hat{U}_{max}$ of sea scorpion scaled to a common slope exponent of -0.76. Regardless of acclimation temperature, fish swimming at 15.0 °C had significantly higher $\hat{U}_{max}$ than fish swimming at 5.0 °C (c. 18 %) (Tables 2.5 and 2.6; Fig. 2.6B and D; Fig. 2.7B and D).

### *2.3.3 Summary of results*

At a test temperature of 15.0 °C, adult short-horn sculpin achieved maximum velocities and accelerations during the escape response that were 34 % and 22 % higher, respectively, in 15 °C-acclimated, than 5 °C-acclimated fish. At a test temperature of 20.0 °C, these values increased to 110 % and 55 %, respectively. However, at cold temperatures of 5.0 and 0.8 °C, 5 °C-acclimated fish did not have significantly improved escape performance over 15 °C-acclimated fish.

Over the test range 5.0-15.0 °C, adult long-spined sea scorpion only showed acclimation responses in maximum angular velocity, this being greater in 5 °C- than 15 °C-acclimated fish. Over the extended temperature range of 0.8-20.0 °C, long-spined sea scorpion exhibited acclimation responses in all variables at low temperature (0.8 °C). For example, maximum velocity and acceleration were 111 % and 120 % greater,

respectively, in cold- than warm-acclimated fish. At 20.0 °C, there was no significant difference in any variable between acclimation temperatures.

In short-horn sculpin, the scaling of maximum velocity at 15 °C was significantly different between the two acclimation groups. Juvenile short-horn sculpin found on the shore and all stages of long-spined sea scorpion showed increased speeds upon acute exposure to 15.0 °C, whereas adult short-horn sculpin showed enhanced velocity only following a period of thermal acclimation to 15 °C.

## 2.4 Discussion

### *2.4.1 Hypotheses testing: Hypothesis 1*

The first hypothesis stated that acclimation in short-horn sculpin improves escape performance at high temperature, but at the expense of performance at low temperature, compared to cold-acclimated fish. Consistent with this prediction, it was found that acclimation to 15 °C and 20 °C clearly improved swimming speed and acceleration at 15.0 and 20.0 °C, compared to 5 °C-acclimated fish acutely exposed to high temperature. These results echo those of Beddow *et al.* (1995) who examined the effect of acclimation to a summer temperature of 15 °C on the prey capture response in the same species. In their study, maximum velocity at 15 °C was 33 % higher in summer- than winter-acclimated sculpin. This phenomenon is reflected in the power output of fast muscle fibres. Johnson and Johnston (1991) used the work loop technique to examine maximum power output of fast muscle fibres performing oscillatory work, in summer- and winter-acclimatised short-horn sculpin. At 15 °C, summer-acclimatised fish produced three times the power output of winter-acclimatised fish. Similarly, Johnston *et al.* (1995) used the work loop technique under conditions simulating the first tail-beat of a fast-start. At 15 °C, power output increased from 6.3 $W.kg^{-1}$ in 5 °C-acclimated fish, to 23.8 °C $W.kg^{-1}$ in 15 °C-acclimated fish. Examination of the power output from the force-velocity (*P-V*) relationship, which gives a good estimate of the maximum instantaneous power output (Josephson, 1993), revealed that power was about six times higher in 15 °C- than 5 °C-acclimated short-horn sculpin at 15 °C (Beddow and Johnston 1995).

Contrary to the first hypothesis, however, it was found that acclimation to high temperature did not significantly compromise escape performance at low temperature (Fig. 2.4A and B). Work loop studies using imposed sinusoidal length changes about resting muscle fibre length have found that maximum power output at 4 °C showed little variation between summer- and winter-caught short-horn sculpin (Johnson and Johnston, 1991). The relaxation rates of fast muscle fibres in the short-horn sculpin were also found to be independent of acclimation temperature (Beddow and Johnston, 1995). However, these authors found that the *P-V* relationship was less curved at 5 °C in fast muscle fibres from 5 °C- than 15 °C-acclimated short-horn sculpin. After normalising the curves for maximum force ($P_0$) and velocity ($V_{max}$), this change in curvature produced a 40 %

increase in relative power output in 5 °C-acclimated fish. It was concluded that the contractile properties of fibres are modified at low, as well as high, temperatures. $Q_{10}$ values in the present study did indicate a less thermally sensitive performance by 5 °C- than 15 °C-acclimated fish. Between 0.8 and 5.0 °C, $\hat{U}_{max}$ and $\hat{A}_{max}$, in the 5 °C-acclimated fish were virtually unchanged, whereas $Q_{10}$s were 3.02 and 2.24 respectively in the 15 °C-acclimated fish (Table 2.2).

On the basis of these results, only part of the first hypothesis can be accepted. Seasonal changes in fast-start performance occurred predominantly at high temperature. Following warm acclimation, escape performance was improved. The emphasis of the hypothesis was with the trade-off in performance between high and low temperature acclimation. At low temperatures, acclimation was not detrimental but it did not improve performance and therefore there was no trade-off. Consequently, this part of the prediction must be rejected. Likewise, in terms of the Beneficial Acclimation Hypothesis, only warm acclimation was beneficial (as regards fast-start performance). Bennett and Lenski (1997) similarly found acclimation in bacteria to be beneficial in some cases but not in others. In their experiments, fitness was measured directly; in this study it can only be speculated that improved fast-start performance with warm acclimation may enhance individual survival and ultimately fitness.

### *2.4.2 Hypothesis 2*

The second hypothesis concerned the inability of long-spined sea scorpion to acclimate, over the average range of seasonal sea water temperatures (test temperatures, 5.0-15.0 °C). The rationale was that temperature sensitivity is lower in organisms subjected to a high degree of short-term temperature variation, and that this variation would not provide a stable cue for acclimation. An increase in temperature accelerates most processes (Schmidt-Nielsen, 1990) and this did occur with the escape response, but it was irrespective of previous thermal history. At test temperatures of 5.0 and 15.0 °C, the values for $\hat{U}_{max}$ and $\hat{A}_{max}$ were not significantly different between acclimation groups (Fig. 2.4C and D). Furthermore, the study on the scaling of escape performance revealed there to be no significant differences in $\hat{U}_{max}$ and RD between acclimation

groups; $\hat{U}_{max}$ was always lower and RD always longer at 5.0 than 15.0 °C (Fig. 2.6B and D, Fig. 2.7B and D, Tables 2.5 and 2.6). Therefore no effect of acclimation at these two test temperatures was found in these particular kinematic variables.

Acclimation temperature did affect $\omega_{max}$. The $\omega_{max}$ is calculated from angle and time components, and therefore reflects the CTA and RD. As RD's were not significantly different between the two main acclimation temperatures the $\omega_{max}$ consequently mirrors the CTA (although this was not significantly affected by acclimation temperature, $P$=0.068), both being greater in 5 °C- than 15 °C-acclimated sea scorpion over the 5.0-15.0 °C test range (Fig. 2.5C and D). The functional significance of this is not known. However, greater body curvature during fast-starts has been found in fish from cold habitats compared to those from warm habitats (Wakeling and Johnston, 1998). In the present study, the fact that one measure of fast-start performance changed with acclimation between 5.0 and 15.0 °C, encourages a rejection of our second hypothesis. However, the existence of acclimation in this sole variable, could still be an indication of a reduced acclimation response in long-spined sea scorpion over this temperature range.

With regards to test temperature, the CTA was greater at a test temperature of 5.0 °C, than 15.0 °C, in both acclimation groups (Fig. 2.5C). A greater degree of turning has been associated with sub-maximal responses (Domenici and Blake, 1991). Therefore the high CTA may be a result of the lower values of $\hat{U}_{max}$ and $\hat{A}_{max}$ at test temperatures of 5.0 °C, compared to 15.0 °C. In addition, long-spined sea scorpion at 5.0 °C may undertake more turning throughout the first two half tail-beats, as an avoidance mechanism, used to confuse predators, when higher values of velocity and acceleration are not attainable.

Over the entire thermal test range, acclimation responses were evident in $\hat{U}_{max}$, $\hat{A}_{max}$, CTA and $\omega_{max}$ of adult long-spined sea scorpion, with 5 °C-acclimated fish having significant greater values at 0.8 °C, than 15 °C-acclimated fish. In the strict sense Beneficial Acclimation has been defined as conferring an advantage at the temperature that produced the phenotypic response (Leroi *et al.* 1994). However, 5 °C-acclimation was clearly advantageous to the fish at 0.8 °C, as only 40 % of 15 °C-acclimated sea

scorpion could swim at this temperature. I therefore believe cold acclimation to be beneficial in this species. There was little evidence for a trade-off in performance optimum temperature with thermal acclimation. This may have been manifest at greater test temperatures. However, higher temperatures would have been above those normally experienced by these fish and would therefore have been ecologically irrelevant. Therefore, in contrast to the short-horn sculpin, performance curves crossed at high temperature in the sea scorpion, as seasonal changes in swimming performance, were principally manifest at low temperature.

It is possible that acclimation ability is retained in this species, despite diurnal temperature fluctuations, because the seasonal differences in water temperatures are so large. The general seasonal change in temperatures may be sufficient to cue acclimation, although other factors are also known to have some control. For example, photoperiod is known to influence acclimation in some species (Kleckner and Sidell, 1985; Kolok, 1991). In the pupfish (*Cyprinodon nevadensis amargosae*), a eurythermal desert stream fish, acclimation has furthermore been achieved using a cycling thermal regime. The fish adapted their metabolic rate to both high and low daily temperatures, increasing their thermal tolerance by 2 °C (Feldmeth *et al.* 1974).

### *2.4.3 Hypothesis 3*

The third hypothesis was accepted over the temperature range tested. In short-horn sculpin, the ability to thermally acclimate maximum speed $\hat{U}_{max}$, was acquired during ontogeny. Sculpin exhibited differential responses in reaction to temperature change, depending on acclimation temperature, and stage of development. In contrast to adults found offshore, juveniles from the intertidal and shallow subtidal, increased $\hat{U}_{max}$ upon acute exposure to 15.0 °C (Fig. 2.6A) but did not increase $\hat{U}_{max}$ any further following acclimation to 15 °C (Fig. 2.7A). At a test temperature of 5.0 °C, all sizes of sculpin failed to improve $\hat{U}_{max}$ following acclimation to 5 °C (Fig. 2.7C). All stages of long-spined sea scorpion lacked compensation in $\hat{U}_{max}$ following acclimation to 5 or 15 °C. I conclude that thermal sensitivity in the short-horn sculpin changes during ontogeny,

such that the potential to exhibit acclimation responses to these temperatures is acquired, concomitant with a migration to deeper more thermally stable, but seasonally fluctuating, water mass.

### *2.4.4 Scaling of burst swimming speed*

Maximum length-specific velocities decreased with increasing total fish length in both species of Cottidae, with absolute velocities increasing with length for the majority of temperature groups. Bainbridge (1958) measured swimming speed in dace (*Leuciscus leuciscus*), trout (*Salmo irideus*) and goldfish (*Carassius auratus*) and found absolute speed increased with length, at any particular tail-beat frequency. Size similarly affected maximum absolute speed during escape responses in rainbow trout (*Salmo gairdneri*) (Webb 1976) and turbot (*Scophthalmus maximus*) larvae (Gibson and Johnston, 1995), but not in angelfish (*Pterophyllum eimekei*) (Domenici and Blake, 1993*b*). On examination of the literature, Wardle and He (1988), found maximum velocity scaled to an average $aL^{0.85}$. In turbot larvae, Gibson and Johnston (1995) found that the maximum length-specific velocity scaled to $aL^{-0.69}$. Maximum length-specific velocity scaled to an average $aL^{-0.55}$ in short-horn sculpin (all tests except 5@15.0 °C), and to $aL^{-0.76}$ in long-spined sea scorpion. Response durations were found to increase with size, as was also found in rainbow trout (Webb, 1976) and angelfish fast-starts (Domenici and Blake, 1993*b*). Archer and Johnston (1989) found length-specific tail-beat amplitude, in the Antarctic fish *Notothenia coriiceps,* was also dependent on size, and decreased with increasing fish length.

Changes in swimming performance with increasing size can be related to increases in myotomal area, mass and length, and fibre length (Webb, 1978*b*; Webb and Johnstrude, 1988; Archer *et al.* 1990). Isotonic contraction (Wardle, 1975) and isometric twitch contraction times of fast muscle fibres increase with size (Archer *et al.* 1990), in part reflecting tail-beat duration, and hence maximum speed (Wardle, 1975). James and co-workers (1998) have recently examined scaling in the short-horn sculpin and found myofibrillar ATPase activity of fast muscle to decline with length, a situation found in a number of marine teleosts (Witthames and Greer-Walker, 1982). However, this was not

related to changes in MHC or MLC ratios with increasing size, but accompanied changes in Troponin I isoforms (James *et al.* 1998).

Swimming performance also alters with size due to the change in the ratio of inertial to viscous forces (Reynold's number, *Re*) during growth. However, the *Re* is most relevant to larvae where viscous forces dominate swimming performance. Although the viscous hydrodynamic regime has recently been found to extend to a higher *Re* than previously thought (Fuiman and Batty, 1997) the fish used in our study were juveniles whose swimming performance would have been dominated by inertial forces.

### *2.4.5 Evolutionary significance of temperature acclimation responses*

Phenotypic plasticity has evolutionary significance only if it enhances fitness (Leroi *et al.* 1994). The most compelling studies of the Beneficial Acclimation Hypothesis have used organisms with short generation times, such as bacteria (Leroi *et al.* 1994; Bennett and Lenski, 1997) on which fitness could be assessed directly. In order to study any organisms with longer generation times, correlates usually have to be used. In this study fitness was not measured directly but instead the escape response was used as a correlate. Warm acclimation was found to be beneficial and improve fast-start performance in adult short-horn, whereas cold acclimation appeared to offer no benefit. In the long-spined sea scorpion, these results were reversed. If fitness had been measured *per se* and similar results obtained, "the generality of the beneficial acclimation assumption," (Leroi *et al.* 1994) would have been rejected, since acclimation did not always confer an advantage in either species.

The influence of fast-start performance on fitness, however, is not known in these species and perhaps the assumption that this performance parameter is a good indicator of fitness should be questioned. The short-horn sculpin extends to a high latitude and is a cold tolerant species which excretes a polypeptide antifreeze from the liver during the winter months (Fletcher *et al.* 1989). This perhaps indicates an obvious adaptation to its environment, but not one that is manifest in a significantly improved escape performance following cold acclimation. That cold temperatures restrict locomotor capacity, is not uncommon (see Bennett, 1990, for review). A combination of lower predation pressure in the winter and a preferential investment in cold tolerance rather than fast-start capability,

may perhaps explain why locomotor and muscle contractile compensations (Johnson and Johnston, 1991) are lacking at low temperature in this species. Only further studies including field experiments could adequately address this question.

It should be noted that these experiments were conducted at different times of the year that corresponded to a particular acclimation group. This was to avoid disrupting the natural seasonal rhythms of the fish too greatly and to thereby enhance detection of any acclimation responses. Although gravid fish were not used, the results may have been influenced by some factor other than temperature, such as hormonal status. However, in a previous study, Beddow and Johnston (1995) found fast muscle contractile properties in short-horn sculpin to be similar between 15 °C- and 5 °C-laboratory acclimated short-horn sculpin and summer- and winter-caught fish, respectively. The thermal acclimation conditions used in this study are therefore believed to be relevant to the field situation.

In conclusion, escape performance was significantly altered by acclimation temperature in both the short-horn sculpin and the long-spined sea scorpion. Walsh *et al.* (1997) examined the temperature tolerance of a number of different species and populations of freshwater sculpin, in order to explain habitat distributions. Stream dwelling species and populations were expected to be more stenothermal than those inhabiting thermally labile springs. Although they found this in a number of cases, they did not find support for the theory that temperature alone accounts for the distribution patterns observed. In the present study, acclimation responses were exhibited in both species of marine Cottidae, which differed from predicted patterns. However, there were interspecific and intraspecific differences in these patterns which may reflect local niche distributions, although undoubtedly other factors are important.

*Chapter 3*

# THE THERMAL ACCLIMATION OF MUSCLE PERFORMANCE DURING ESCAPE RESPONSES IN THE SHORT-HORN SCULPIN.

## 3.1 Introduction

Since Fry and Hart's classic study of 1948, which revealed the cruising speed of goldfish (*Carassius auratus*) to be dependent on acute and acclimation temperature, a number of studies have brought to light the plastic nature of fish locomotion during unsteady swimming (e.g. Webb, 1978*a*; Batty *et al.* 1993; Beddow *et al.* 1995; Gibson and Johnston, 1995; Johnson and Bennett, 1995; Chapter 2). Fish swim unsteadily during fast-starts which are used to capture prey or avoid predators (see Domenici and Blake, 1997, for review). Johnson and Bennett (1995) found that maximum speed during escape responses in goldfish acclimated to 35 °C had a $Q_{10}$ of 2.0 over the range 10 to 35 °C and at 10 °C only 30 % of fish could swim. However, after a period of thermal acclimation to 10 °C goldfish could compensate for these acute changes in temperature; maximum speed at 10 °C increased by 35 % and all fish could elicit escape responses. In the marine fish the short-horn sculpin (*Myoxocephalus scorpius*), Beddow *et al.* (1995) discovered that thermal acclimation to a summer temperature of 15 °C, increased the success of prey capture at 15 °C by 74 %, compared to 5 °C-acclimated fish.

Until recently, few investigations had calculated the power output of fast-starts or the effects of temperature on these estimates, due mainly to the absence of a suitable method. However, in insects and birds the power requirements for flight have often been estimated using aerodynamic models (e.g. Weis-Fogh, 1973; Ellington, 1984; 1985; Spedding, 1992). Frith and Blake (1995) applied a hydrodynamic model in order to estimate the power requirements of fast-start swimming during prey capture and escape responses in pike. In the same year, Johnston *et al.* (1995) used an alternative method to evaluate the power output of the fast muscle during prey capture responses in the short-horn sculpin. Their method applied the work loop technique which, to recap on Chapter 1, measures the power output of muscle fibres during cycles of lengthening and shortening with imposed periods of stimulation (Josephson, 1993). Most applications of the work loop technique have used muscle length (strain) fluctuations of equal amplitude

from cycle to cycle, as is assumed during steady cyclic locomotion (e.g. Altringham and Johnston, 1990*b*; Altringham *et al.* 1993; Johnson and Johnston, 1991; Josephson, 1985; Josephson and Stokes, 1989; Rome and Swank, 1992). Johnston *et al.* (1995) further refined the technique to model unsteady swimming during fast-starts. Using information on backbone curvature and the geometric arrangement of the fast muscle fibres, they predicted muscle strains of varying amplitude from tail-beat to tail-beat during prey capture sequences of the short-horn sculpin.

Complex strain waveforms have also been determined using sonomicrometry in swimming fish. Sonomicrometry records alterations in the transit time of ultrasound between two piezoelectric crystals during lengthening and shortening of the muscle. Rushmer *et al.* (1956) first used this method to measure muscle strain in the heart. Covell *et al.* (1991) inserted piezoelectric crystals into the skeletal muscle of rainbow trout (*Oncorhynchus mykiss*) and presented some preliminary results on muscle length changes during fast-starts. Coughlin *et al.* (1996) found that red muscle strain, determined by sonomicrometry during steady swimming in scup (*Stenotomus chrysops*), matched predictions made from anatomical considerations and high speed cinématography. Recent studies in our laboratory have used sonomicrometry to record fast muscle strain during fast-starts and then applied the same muscle length fluctuations to isolated muscle *in vitro* during work loop experiments (Franklin and Johnston, 1997; James and Johnston, 1998). Muscle stimulation patterns during the work loops were similarly abstracted from *in vivo* recordings made using implanted electromyography (EMG) electrodes in adjacent myotomes.

By integrating methods that have become available in the last 20 years, the present investigation aimed to quantify temperature effects on the *in vivo* strain and power output of fast muscle fibres, during escape responses in short-horn sculpin. Escape responses in this species have been described as typical C-starts (James and Johnston, 1998; Chapter 2). Fish were acclimated to summer or winter water temperatures and the power outputs of the C-bend and contralateral contraction were measured at both temperatures in both acclimation groups.

## 3.2 Materials and Methods

### *3.2.1 Fish*

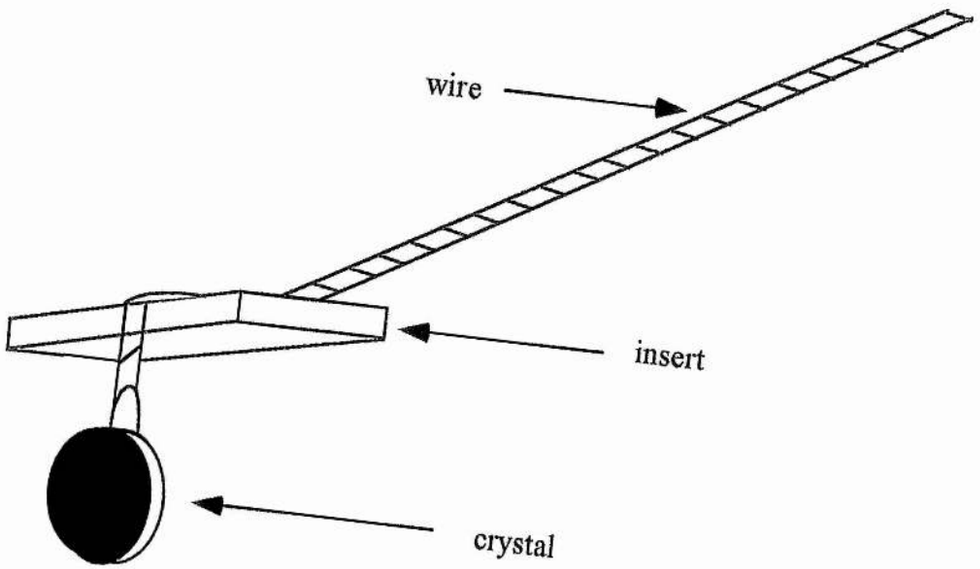
Short-horn sculpin (*Myoxocephalus scorpius* L.) (161.74 ± 9.14 g wet mass, 215.71 ± 3.37 mm total body length (*L*); mean ± S.E.M., *N*=14) were collected between March 1996 and March 1997, using bottom trawls and creels off the Fife coast, Scotland. Fish were held in a flow-though sea water aquarium at the Gatty Marine Laboratory, St. Andrews, Scotland, and fed twice weekly with chopped squid or live *Crangon*. During the winter months (November to April), fish were acclimated to 5±0.5 °C, for a minimum of six weeks, and kept under a constant photoperiod regime of 10 hours light: 14 hours dark. During the summer months (June to September) fish were held at a constant temperature of 15±0.5 °C for the same minimum time, and kept under a constant photoperiod regime of 13 hours light: 11 hours dark.

### *3.2.2 Preparation of sonomicrometry crystals and EMG wires*

Muscle strain was measured using sonomicrometry. Sonomicrometry (piezoelectric) crystals (Triton Technology Inc., San Diego, USA) of 1 mm diameter were attached to perplex inserts (2.0×3.0×0.5 mm) (Fig. 3.1) to ensure the crystals were inserted to the correct depth and maintained a stable orientation in the muscle. The inserts were glued onto the sonomicrometry wires about 2 mm behind the crystals. The sonomicrometry wires were bent at 90 ° so the crystals were suspended just below the inserts. Two crystals, one an emitter and one a receiver, were required for each side of a fish.

EMG electrodes (Teflon coated silver wire; A-M Systems, Everett, USA) were prepared by twisting together two wires of 150 μm diameter and approximately 1.5 m in length. About 2 mm of Teflon was then stripped off each wire at both ends. The wires were inserted through a 21 gauge syringe needle and part of the exposed wires at one end bent back to form a hook, to maintain position in the muscle. One pair of bipolar electrodes were required for insertion into each side of the fish. The total mass of two pairs of sonomicrometry crystals and two EMG wires was less than 8 g.

Figure 3.1. Diagrammatic representation of a sonomicrometry crystal attached to a perplex insert.


wire
insert
crystal

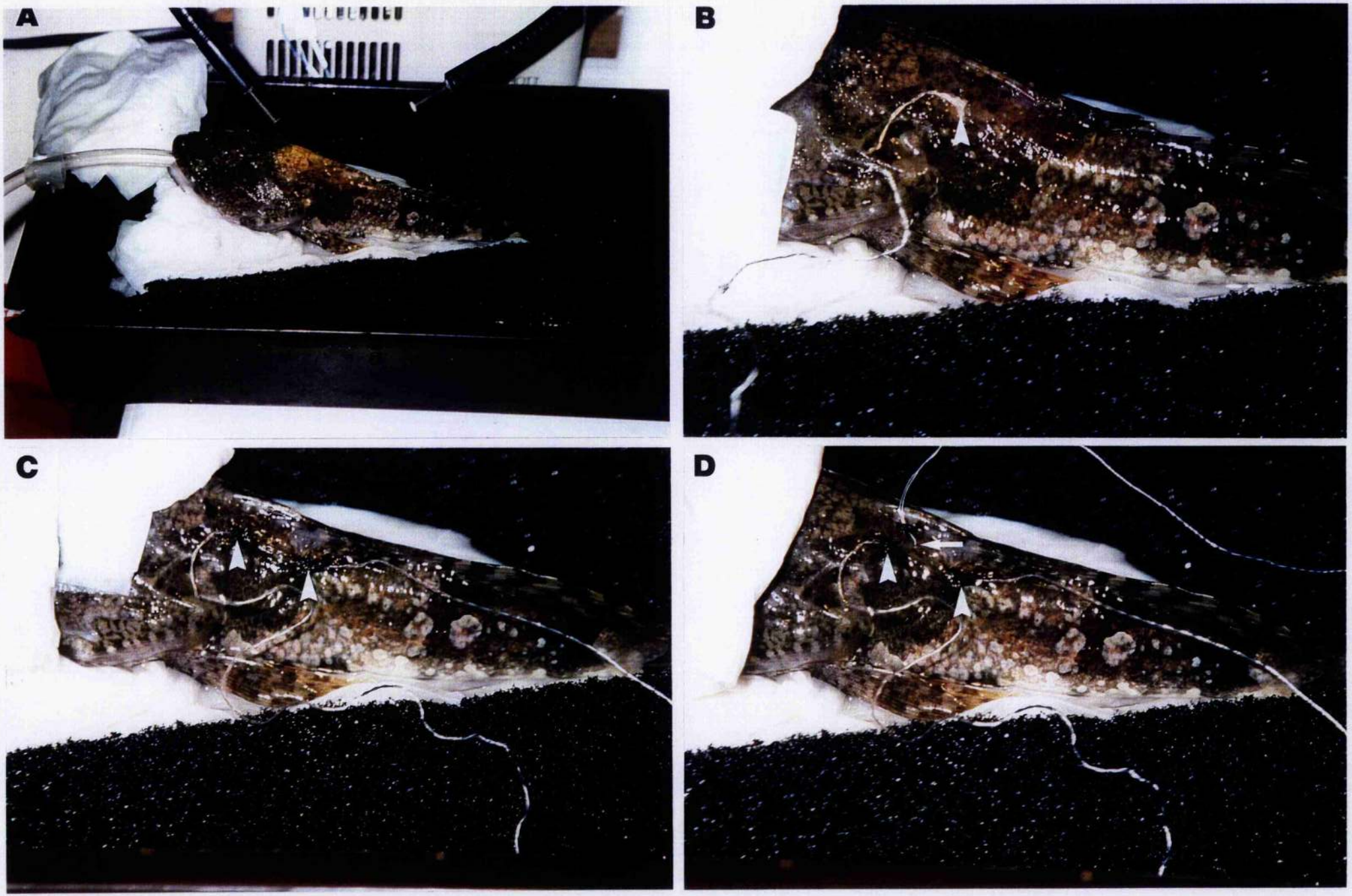
### *3.2.3 Surgical procedure*

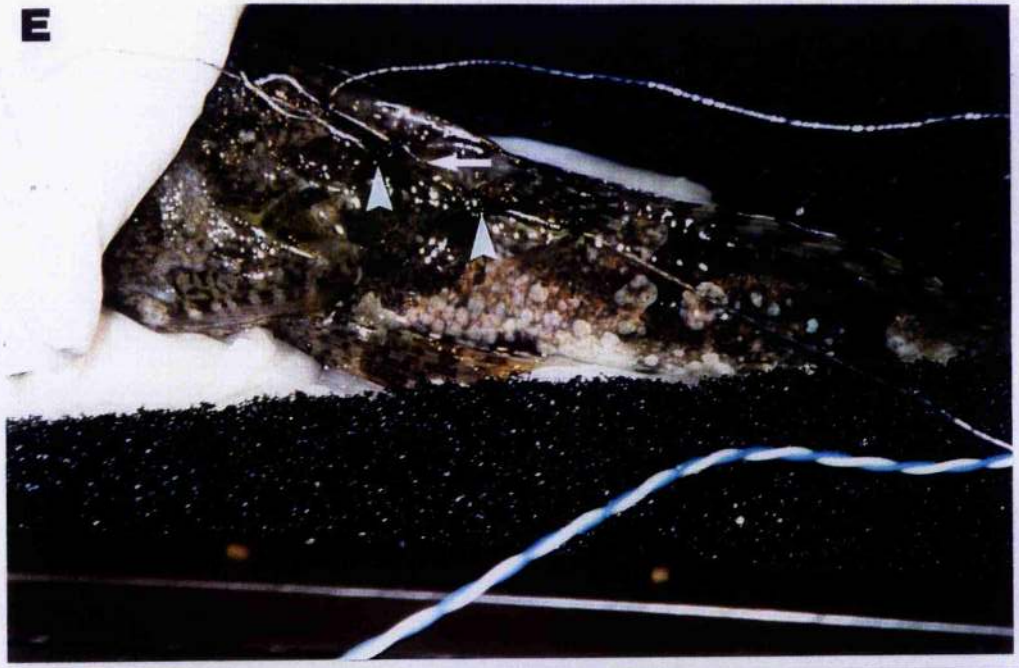
Anaesthesia was induced with a 1:5000 (m/v) solution of tricaine methane sulphonate (MS222), buffered with 0.7 mmol.$l^{-1}$ of sodium hydrogencarbonate at the acclimation temperature. Approximately ten minutes exposure to MS222 solution induced unconsciousness and eliminated any nervous response (assessed by pinching the fish just anterior to the caudal fin). The fish was then placed in a moist, V-shaped foam mattress and a wet cloth was used to cover the eyes and keep the fish moist. MS222 at a concentration of 1:7000 (m/v) was pumped into the mouth *via* 5 mm diameter tubing and a T-connector, in order to aerate the gills (Fig. 3.2A). The head of the fish was kept higher than the tail to ensure gravity maintained the flow of water through the mouth and across the gills.

An incision was made in the skin just posterior to the start of the first dorsal fin (about 0.35 body lengths from the snout of the fish) and at about 4-5 mm ventral to the fin, which exposed the dermis layer underneath. A further cut was made in this layer to expose the myotomal muscle beneath. Using a pair of forceps the dermis was gently pulled away exposing about 4 $mm^2$ of muscle. The superficial fast muscle fibres in this part of the fish run parallel to the long axis of the fish (James and Johnston, 1998). A 19 gauge syringe needle was inserted to a depth of 1-1.5 mm, perpendicular to the orientation of the muscle fibres. The needle was removed and the first sonomicrometry crystal inserted into the hole, with the lens facing the tail (Fig. 3.2B). This procedure was repeated with the second sonomicrometry crystal, about 8-10 mm posterior to the first crystal with the lens facing the first crystal (Fig. 3.2C). The ends of the sonomicrometry wires were connected up to a sonomicrometer (Triton Technology Model 120) and the alignment of the crystals was checked using an oscilloscope. The crystals were anchored in the muscle by suturing the skin and connective tissue using surgical silk in a cross formation over the inserts. The wires were sutured to the skin some distance from the crystal insertions allowing some slack, in order to avoid the crystals being dislodged during swimming.

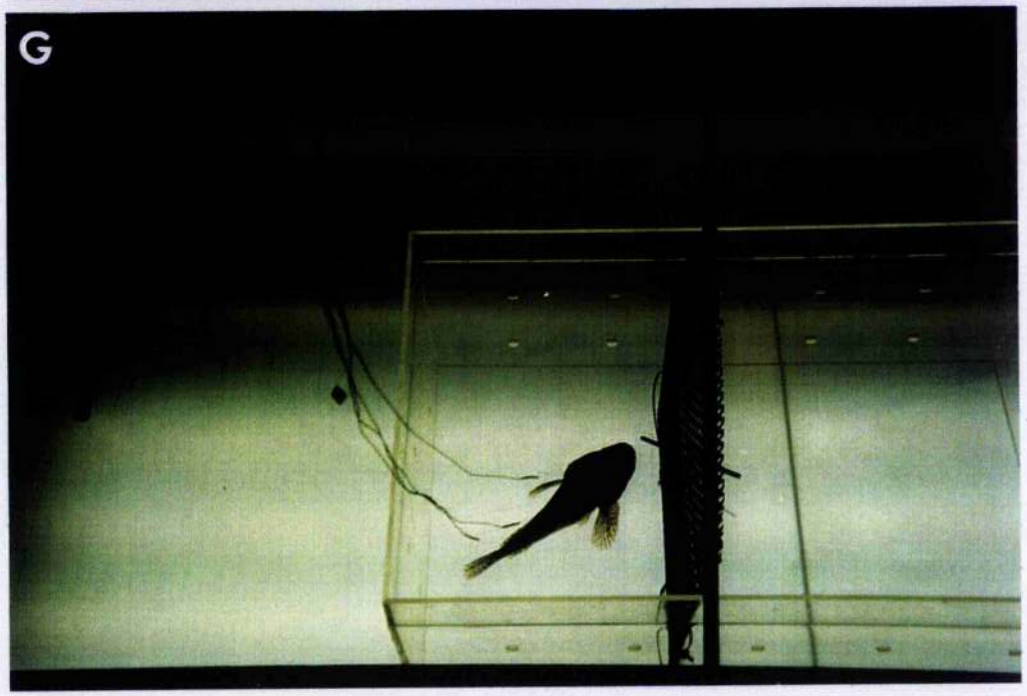
An EMG electrode was inserted to a depth of 1-1.5 mm, about 3 mm dorsally to the midpoint of the two sonomicrometry crystals (Fig. 3.2D), using the 21 gauge syringe needle which was then carefully withdrawn. The EMG wires were sutured near the point

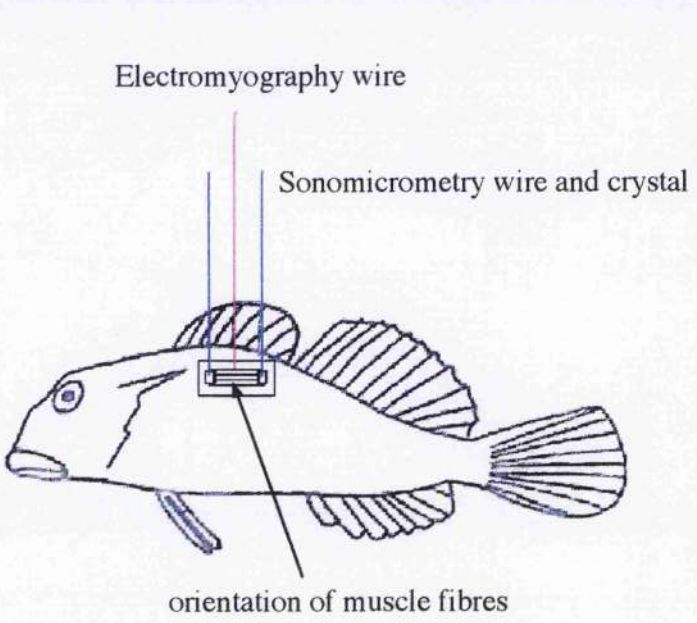
Figure 3.2. Demonstration of the surgical procedure for the implantation of sonomicrometry crystals and EMG wires. Upwards-pointing arrowheads indicate insertion points of the sonomicrometry crystals. Left-pointing arrows indicate insertion points of the EMG wires. G) shows the recovery area with wires secured above the fish. H) shows a diagrammatic representation of the implanted EMG and sonomicrometry wires.


A
B
C
D


E


F


G


H
Electromyography wire
Sonomicrometry wire and crystal
orientation of muscle fibres

of insertion. A loop was then made in the wires and this sutured just behind the head, again to avoid the wires being dislodged during activity (Fig. 3.2E).

Sonomicrometry crystals and EMG electrodes were also inserted on the other side of the fish (Fig. 3.2F). Following surgery which lasted 1-1.5 hours, the fish was placed in a swimming arena (2.0×0.6×0.25 m) at its acclimation temperature and gently moved through the water a few times to allow fresh water to flow over the gills. The fish was then confined to a small area to restrict swimming activity. All wires were secured to a length of elastic above the fish, leaving some slack (Fig. 3.2G). Fish were allowed to recover for 24 hours before experimenting.

### *3.2.4* In vivo *measurements of muscle strain and activity*

Escape responses were elicited by tactile stimulation on the caudal fin of a stationary fish (see Chapter 2). Muscle activity, amplified by a differential amplifier (A-M Systems, Everett, USA) was recorded together with the sonomicrometry signals on a thermal array recorder (Summagraphics Ltd.). The sonomicrometry trace was retarded due to the four-pole Bessel filter in the sonomicrometer and therefore the time was adjusted by 5 ms. Occasionally traces showed extremely rapid changes in muscle strain. However, these were discarded as artifacts due to rotation of the sonomicrometry crystals (Kirkpatrick *et al.* 1972). The errors in sonomicrometry readings, due to the change in the velocity of sound through the crystal lens and to the changes in muscle stiffness (Tamura *et al.* 1982) were less than 4 % of the measured strain (James and Johnston, 1998).

Muscle strain and activation were recorded simultaneously on both sides of the fish. Escape responses were elicited in 5 °C-acclimated fish at an experimental temperature of 5.0 °C. The temperature of the swim tank was then changed at a rate of 1 °C every half hour, until 15.0 °C. Fish were left for an hour before escape responses were again recorded. Escape responses of 15 °C-acclimated fish were similarly recorded at 15.0 and 5.0 °C. The sonomicrometer was re-calibrated prior to each new set of recordings. Muscle activation onset and duration, muscle strain, and cycle duration, were measured from the chart recordings. The *in vivo* shortening velocity ($V$) was calculated as the slope of the tangent fitted to the strain data and was expressed as fibre lengths per second ($s^{-1}$).

### *3.2.5 Isolation of muscle fibre preparations*

Muscle fibre preparations were isolated from the anterior abdominal myotomes, which have been found to provide consistent, good quality fibre bundles. Abdominal fibre preparations are easier to dissect than dorsal fibre preparations from the region of the EMG and sonomicrometry insertion, but both exhibit the same contractile properties (compare Altringham and Johnston, 1990*a* and Johnston *et al.* 1993). Fish were killed by a blow to the head followed by pithing of the spinal chord. An incision was made running anteriorly from the anus to the pectoral gill arch. A segment of anterior abdominal muscle was removed and placed in an ice-cooled sylgard dish containing Ringer solution. The Ringer consisted of (in $mmol.l^{-1}$): NaCl, 143; sodium pyruvate, 10, as an energy source (Altringham and Johnston, 1988); KCl, 2.6; $MgCl_2$, 1.0; $NaHCO_3$, 6.18; $NaH_2PO_4.2H_20$, 3.2; Hepes sodium salt, 3.2; Hepes, 0.97; $CaCl_2$, 2.6; pH 7.4 (James and Johnston, 1998). The muscle was bound on one side by abdominal peritoneum and on the other side by skin layers. The muscle was trimmed down to a workable size of about 5×40 mm (width×length). Using a binocular microscope the skin was removed thus exposing the muscle fibres and myosepta. The muscle was cut so there were fibres running the whole length of each of three myotomes. The peritoneum attached to the fibres of the middle myotome was carefully removed. Bundles of 10-20 fibres from this myotome were isolated by removing fibres from the skin side of the preparation. The fibres at each end of the preparation were removed exposing the peritoneum underneath, and aluminium T-clips were wrapped firmly around the peritoneum. The Ringer was changed regularly throughout the dissection. The aluminium clip at one end of the preparation was attached to a servo motor (MFE Model R4077, Emerson Electronics, England) and the other to a strain gauge (AME 801, Senso-Nor, Horten, Norway). The preparation was held in a temperature controlled chamber through which Ringer solution circulated at the acclimation temperature.

### *3.2.6 Contractile properties*

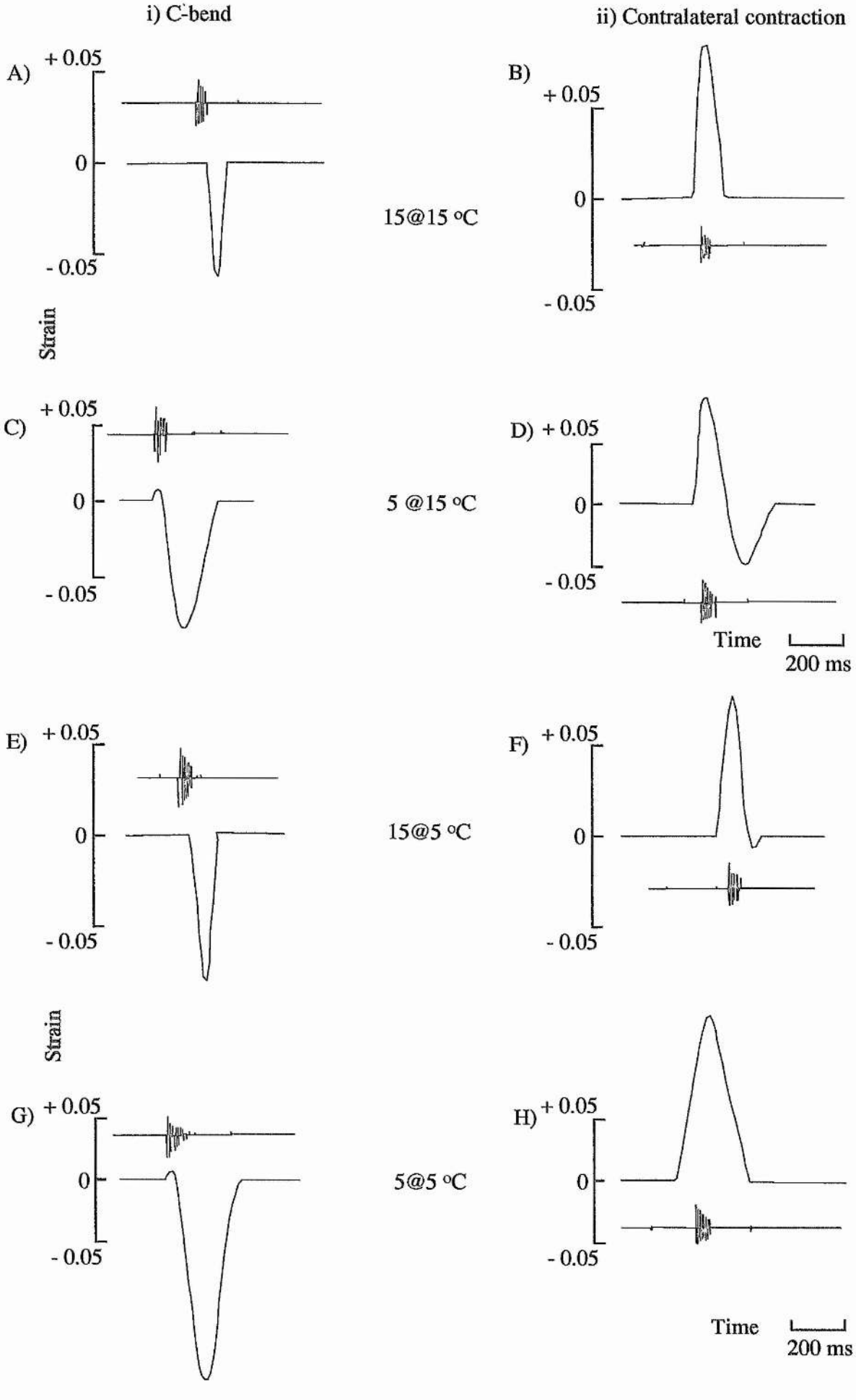
The stimulus amplitude and width (6-16 V; 0.8-2.0 ms respectively), and the length of the muscle preparation were adjusted to give a maximum isometric twitch. The latter corresponded to a sarcomere length of 2.3-2.4 μm, as measured by laser diffraction

using a HeNe laser. The method involved shining the laser through the muscle preparation such that diffraction bands could be seen on a paper screen at a known distance above the muscle. The distance between the zero and first order diffraction bands was measured. Using the two distance measurements, the angle ($\sin\phi$) subtended by the diffraction bands at the level of the muscle, could be calculated. Sarcomere length was calculated as $\lambda$ (the wavelength of the laser radiation, 0.6328 $\mu$m) divided by $\sin\phi$.

The time from the stimulus to half twitch activation (T50%a), the time from the stimulus to peak twitch activation (TPTw), the time from maximum twitch activation to half twitch relaxation (T50%r) and the time from the stimulus to 90 % twitch relaxation (T90%r), were measured. The stimulus frequency was also optimised (77-132 Hz) to maximise isometric tetanic force and the following measurements were made: the time from stimulus onset to half tetanic activation (T50%a), the time from stimulus onset to peak tetanus (TPTet) and the time from the last stimulus to half relaxation (LS50%r).

Work loop experiments were carried out using representative strain waveforms and activation patterns recorded *in vivo* during the first two half tail-beats of escape responses. Strain patterns were abstracted to create a cyclic event for the work loop experiments and thus length fluctuations started and finished at the resting length of the muscle (Fig. 3.3). The cyclic events corresponded to the C-bend and contralateral contraction. The waveforms were digitized (Sigmascan, Jandel Scientific, USA) and smoothed using cubic piecewise regressions (LabView, National Instruments, USA) as described to calculate velocity in Chapter 2. The smooth width was set to fit about 10 % of the data of each cycle. Work loop experiments were carried out *via* a LabView programme (National Instruments, USA) connected to the muscle apparatus. The servomotor subjected muscle preparations to the abstracted length-change waveforms and the muscle was stimulated by electrodes at the same phase and for the same duration as indicated by the EMG recordings. Stimulus phase, stimulus duration, strain and cycle duration were individually altered to maximise power output. Muscle preparations were allowed to recover for 6 minutes between each work loop cycle. Passive work loops were performed before and following each new set of stimulated work loops. The passive work is that done on the muscle to lengthen it. Work loops were constructed by plotting the stress (force per cross-sectional area of muscle) against muscle length (see Fig. 3.4). The

Figure 3.3. Representative *in vivo* muscle strain waveforms and activation patterns obtained for the C-bend and contralateral contraction during escape responses of short-horn sculpin. These patterns have already been abstracted to create cyclic waveforms for use on bundles of fast muscle fibres *in vitro*. Legends represent acclimation temperature at test temperature.


i) C-bend
ii) Contralateral contraction
A)
B)
C)
D)
E)
F)
G)
H)
+ 0.05
0
- 0.05
Strain
15@15 °C
5 @15 °C
15@5 °C
5@5 °C
Time
200 ms

area of each loop represented the net work done by the muscle over the cycle (Josephson, 1985).

At the acclimation temperature of the fish, a muscle preparation would do four different types of work loops. The patterns of muscle action used corresponded to recordings of the C-bend and contralateral contraction at both test temperatures from fish acclimated to the same temperature as that of the preparation. The rig temperature was changed over a period of 1-2 hours to an acute temperature of 5 or 15 °C. The fibre preparation was then subjected to the same four patterns of muscle strain and activation. Maximum isometric twitches and tetani were recorded prior to and following experiments at each temperature. Stimulation amplitude, width and frequency were optimised at the beginning of experiments at a new temperature.

### *3.2.7 Determination of fibre bundle cross-sectional area*

At the end of each experiment, which typically lasted 12 hours, the muscle preparation was removed from the experimental apparatus and pinned out at its resting length. The preparation was immersed in liquid isopentane, cooled to -159 °C in liquid nitrogen. It was then covered with cryomatrix and frozen again in liquid isopentane ready for subsequent sectioning. Transverse sections of 10 μm were cut at several points along the preparation, transferred to coverslips and stained for myosin ATPase activity following the method of Johnston *et al.* (1974). The cross-sectional area of each muscle preparation was determined by digitizing all live muscle fibres (VideoPlan, Kontron, Basel, Switzerland). The cross-sectional area was used to calculate muscle stress. Mass-specific power output was calculated using the measured cross-sectional area, the resting fibre length and by assuming a muscle density of 1060 $kg.m^{-3}$ (Méndez and Keyes, 1960).

### *3.2.8 Statistics*

The effects of thermal acclimation and acute temperature on the muscle parameters of the C-bend and contralateral contraction were analysed separately using one-way analysis of variance (ANOVA) and Tukey multiple comparison tests. Where data were found not to be normally distributed, the Kruskal-Wallis non-parametric test

was used. The effect of temperature on rate parameters was assessed using $Q_{10}$s. $R_{10}$s were used to indicate the ratio of two quantities measured over a 10 °C range (Bennett, 1984). All values are presented as mean ± S.E.M. unless otherwise stated. *N* represents the number of observations.

## 3.3 Results

### *3.3.1* In vivo *measurements of muscle strain and activation*

Strain and activation patterns were measured in fast muscle fibres at 0.35 body lengths for a series of fast-starts. Recordings were abstracted so that cyclic strain patterns were created for the C-bend and the contralateral contraction. Representative muscle strain and activation patterns (the representative recordings were not necessarily from the same escape response) which were used in *in vitro* experiments, are shown in Figure 3.3 (length against cycle time also shown in Fig. 3.4A, D, G, J, M, P, S, V). The abstracted strain trajectory for the contralateral contraction consisted of either a full half tail-beat after the C-bend before returning to the straight position (resting muscle length) (e.g. Fig. 3.3D) or simply a return to resting muscle fibre length following the C-bend (e.g. Fig. 3.3B and H). Muscle strain during the C-bend sometimes included a small pre-stretch of about 1 % of muscle fibre length (Fig. 3.3C and G). Muscle strain ranged from 0.03 to 0.16 during both the C-bend and contralateral contraction and was the only measurement of muscle action found not to significantly differ with either acute or acclimation temperature.

The effects of acute and acclimation temperature on the *in vivo* parameters of muscle action are shown in Table 3.1. Only the *in vivo* cycle and shortening durations of 5 °C-acclimated fish were significantly influenced by acute temperature. However, the trends differed for the two stages of the escape response, with the shortening duration significantly decreasing between 5 and 15 °C in the C-bend ($P<0.05$), but the cycle and shortening durations significantly increasing between these two temperatures in the contralateral contraction ($P<0.05$).

Acclimation to 15 °C significantly reduced the cycle duration for both the C-bend and contralateral contraction at both test temperatures ($P<0.05$). The shortening durations were also significantly shorter, consequently increasing the shortening velocities (Table 3.1). At 15 °C, shortening velocity was 60 and 154 % higher, for the C-bend and contralateral contraction, respectively, in 15 °C- than 5 °C-acclimated fish ($P<0.05$).

Table 3.1. In vivo *parameters of muscle action during escape responses in short-horn sculpin acclimated to 5 or 15 °C.*

| | 5 °C-acclimated | | 15 °C-acclimated | |
|---|---|---|---|---|
| | Test temperature (°C) | | | |
| | 5 | 15 | 5 | 15 |
| C-bend | $N$=5 | $N$=5 | $N$=5 | $N$=5 |
| Cycle duration (ms) | 247.6 ± 12.3 * | 233.4 ± 8.8 * | 107.8 ± 14.1 | 69.5 ± 6.1 |
| Strain | 0.111 ± 0.014 | 0.076 ± 0.006 | 0.084 ± 0.010 | 0.085 ± 0.016 |
| Shortening duration (ms) | 115.6 ± 7.8 * * | 81.1 ± 2.5 * | 56.0 ± 9.1 | 46.8 ± 8.3 |
| Shortening velocity ($s^{-1}$) | 1.50 ± 0.205 | 1.56 ± 0.11 * | 2.34 ± 0.19 | 2.49 ± 0.28 |
| $V/V_{max}$ | 0.32 * | 0.30 | 0.50 * | 0.30 |
| Contralateral contraction | $N$=5 | $N$=5 | $N$=7 | $N$=6 |
| Cycle duration (ms) | 223.4 ± 9.3 * * | 280.0 ± 11.1 * | 144.8 ± 14.6 | 126.4 ± 9.6 |
| Strain | 0.098 ± 0.013 | 0.100 ± 0.011 | 0.103 ± 0.015 | 0.103 ± 0.008 |
| Shortening duration (ms) | 76.8 ± 9.6 * | 118.9 ± 4.0 * | 60.5 ± 6.4 | 45.1 ± 3.6 |
| Shortening velocity ($s^{-1}$) | 1.57 ± 0.15 | 1.27 ± 0.11 * | 2.24 ± 0.29 | 3.23 ± 0.35 |
| $V/V_{max}$ | 0.33 | 0.24 | 0.48 | 0.39 |

* denotes a significant difference ($P$<0.05) between test temperatures in one acclimation group (an acute temperature effect).

* denotes a significant difference ($P$<0.05) between acclimation groups at the same test temperature (an acclimation effect).

$V/V_{max}$ estimated using values of $V_{max}$ taken from Beddow and Johnston (1995).

### *3.3.2* In vitro *isometric studies*

Maximum tetanic stress was not significantly different between 5 and 15 °C and was independent of acclimation temperature (Table 3.2). However, maximum twitch stress, was affected by both acute and acclimation temperature; in 5 °C-acclimated fish, twitch stress had a $R_{10}$ of 1.9. In these fish the twitch:tetanus ratio also declined by 33 % between 5 and 15 °C. At a test temperature of 15 °C twitch stress increased by 96 % between fibres from 5 °C- and 15 °C-acclimated fish. The only other parameter to show a significant effect of acclimation temperature was the time to half twitch activation; at 5 °C, fibres from 5 °C-acclimated fish took 21 % longer to develop half-maximal tension than fibres from 15 °C-acclimated fish.

All times for twitch and tetanus force development were shorter at 15 °C than at 5 °C in both acclimation groups (Table 3.2). When taking into consideration force development, however, $Q_{10}$s for the rate of twitch force development were low (5 °C-acclimated fish, 0.9-1.0 and 15 °C-acclimated fish, 1.3-1.5); the highest $Q_{10}$ was 1.6 for the rate of force development in tetani in fibres from 15 °C-acclimated sculpin. Rates of force development were affected by acclimation temperature. At 5 °C, the rates of twitch and tetanic activation were 36 and 27 % higher, respectively, in 15 °C- than 5 °C-acclimated fish. However, at 15 °C, the rates of twitch and tetanic activation were 111 and 72 % higher in 15 °C- than 5 °C-acclimated fish.

Relaxation times were significantly shorter at 15 °C than 5 °C in both acclimation groups. $Q_{10}$s for half twitch relaxation times were 2.0 and 1.9 for 5 and 15 °C-acclimated fish, respectively. Tetanus relaxation times were 2.1 and 1.9 for 5 and 15 °C-acclimated fish, respectively. The $Q_{10}$ for the rate of twitch relaxation was 1.1 in fibres from 5 °C-acclimated fish, but was 1.7 for fibres from 15 °C-acclimated fish. Tetanic relaxation rates had $Q_{10}$ values of 1.6 and 1.9 for fibres from 5 and 15 °C-acclimated fish, respectively. Twitch relaxation rates were affected by acclimation temperature, being 102 % higher at 15 °C in 15 °C- than 5 °C-acclimated fish.

Table 3.2. *Influence of acute temperature and temperature acclimation on the isometric contractile properties of the fast myotomal muscle fibres of short-horn sculpin (N=7 in each acclimation group).*

| | 5 °C-acclimated | | 15 °C-acclimated | |
|---|---|---|---|---|
| | Test temperature (°C) | | | |
| | 5 | 15 | 5 | 15 |
| Twitch | | | | |
| Stress ($kN.m^{-2}$) | 92.6 ± 7. 5 * | 48.4 ± 6.6 * | 106.6 ± 10.7 | 94.8 ± 12.1 |
| TPTw (ms) | 53.4 ± 2.0 * * | 27.6 ± 0.9 | 44.2 ± 2.7 * | 25.5 ± 1.1 |
| T50%a (ms) | 23.8 ± 0.9 * * | 13.5 ± 0.5 | 19.7 ± 1.4 * | 13.6 ± 0.7 |
| T50%r (ms) | 39.8 ± 3.4 * | 19.7 ± 1.3 | 35.5 ± 4.7 * | 19.0 ± 1.7 |
| T90 %r (ms) | 127.8 ± 8.6 * | 65.8 ± 3.7 | 115.5 ± 9.7 * | 71.0 ± 7.5 |
| Tetanus | | | | |
| Stress ($kN.m^{-2}$) | 194.7 ± 9.0 | 150.0 ± 12.8 | 181.4 ± 17.7 | 176.7 ± 10.1 |
| T50%a (ms) | 39.5 ± 3.1 * | 26.7 ± 1.8 | 30.4 ± 3.2 * | 19.9 ± 1.4 |
| TPTet (ms) | 128.1 ± 13.0 * | 86.4 ± 10.3 | 94.2 ± 11.3 | 58.6 ± 5.7 |
| LS50%r (ms) | 126.7 ± 7.4 * | 60.2 ± 2.5 | 123.2 ± 17.7 | 64.6 ± 5.4 |
| Twitch : tetanus ratio | 0.48 | 0.32 | 0.59 | 0.54 |

Half twitch relaxation time and time from last stimulus to half tetanic relaxation were tested using the Kruskal-Wallis nonparametric ANOVA test; all others were tested using one-way ANOVA.

* denotes significant difference ($P<0.05$) between test temperatures in one acclimation group (an acute temperature effect).

* denotes significant difference ($P<0.05$) between acclimation groups at the same test temperature (an acclimation effect).

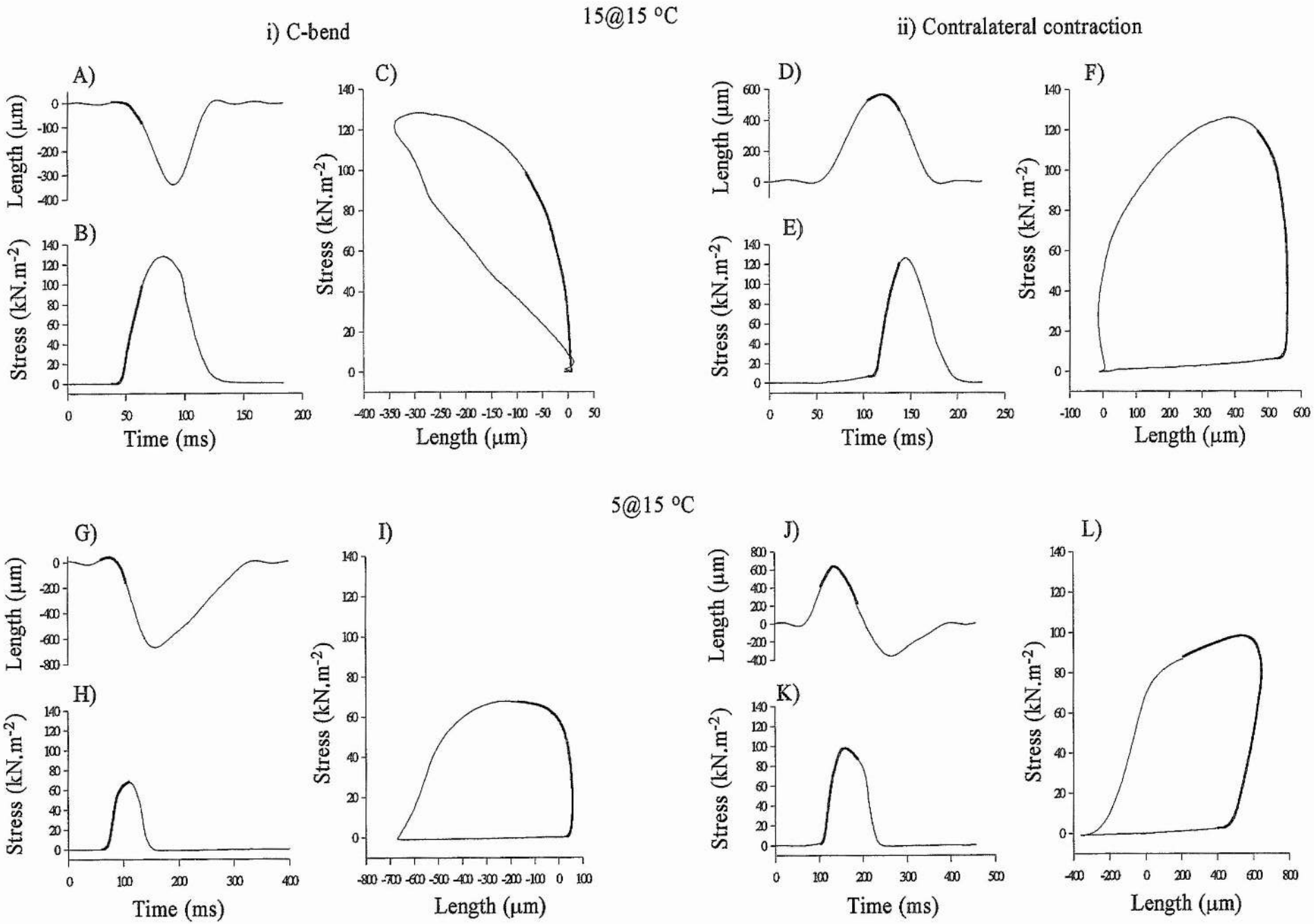
### *3.3.3 Determination of* in vivo *work and power*

Bundles of fast muscle fibres were subjected to the muscle strain and activation patterns shown in Figure 3.3 (length and stimulation patterns also shown in Fig. 3.4A, D, G, J, M, P, S, V). Representative plots of stress against cycle time are shown Figure 3.4 (B, E, H, K, N, Q, T, W). There was no significant variation in stress with acute or acclimation temperature for either stage ($P>0.05$). The mean stress values for the C-bend and contralateral contraction were 98.97 $kN.m^{-2}$ and 122.82 $kN.m^{-2}$, respectively. Plots of stress against muscle length produced work loops (Fig. 3.4C, F, I, L, O, R, U, X), the area of which represents the net work produced during the cycle (Josephson, 1985). The only work loops that contained a substantial negative component (clockwise loop) were those constructed under conditions simulating the C-bend of 15 °C-acclimated fish escaping at an acute temperature of 5 °C (Fig. 3.4 'O').

The power output is the net work done by the muscle per unit time. Representative plots of the instantaneous power output during each cycle are shown in Figure 3.5. In 15 °C-acclimated fish, maximum instantaneous power output decreased with an acute temperature decrease from 15 °C to 5 °C; in the contralateral contraction this was significant, decreasing by 73.0 % (276-160 $W.kg^{-1}$; $P<0.001$). Acclimation temperature significantly affected power output in both stages. Under escape conditions from both stages, maximum instantaneous power output at 15 °C was about 54 % higher in fibres from 15 °C-acclimated than 5 °C-acclimated fish ($P<0.05$) (Table 3.3).

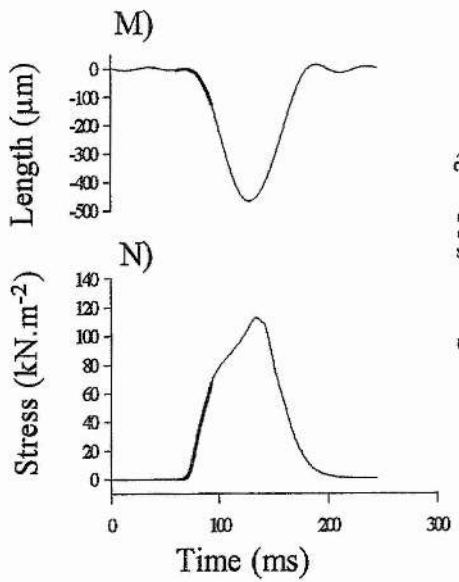
Mean muscle mass-specific power output showed a similar trend with acute and acclimation temperature to that of maximum instantaneous power output. Figure 3.6 shows the power output integrated over time for each cycle from the C-bend and contralateral contraction. Acute temperature effects were evident in the power output produced during each stage. In 15 °C-acclimated fish, the mean muscle mass-specific power outputs at 5 °C were 10.47 and 33.81 $W.kg^{-1}$ for the C-bend and contralateral contraction, respectively, increasing to 61.65 and 69.26 $W.kg^{-1}$, respectively, at 15 °C ($P<0.001$). At 15 °C, power output was around 150 % higher during both stages of the escape response in 15 °C- than in 5 °C-acclimated fish ($P<0.001$). There was also some

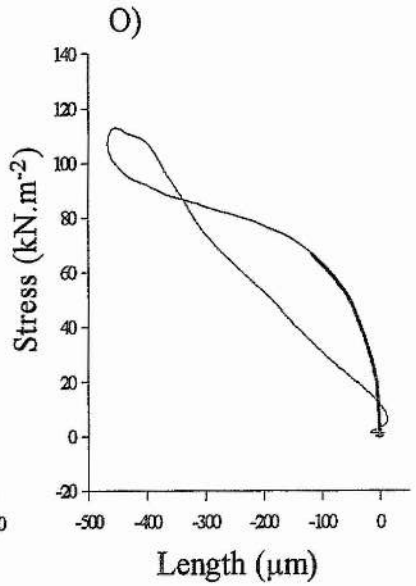
Figure 3.4. Representative plots of muscle length and stress (force per cross-sectional area of muscle) *versus* cycle time, and work loops (stress *versus* length) for sculpin fast muscle fibres. Bold lines represent the stimulation and legends represent acclimation temperature at test temperature.


15@15 °C
i) C-bend
ii) Contralateral contraction
A)
B)
C)
D)
E)
F)
Length (µm)
Stress (kN.m-2)
Time (ms)
Length (µm)
5@15 °C
G)
H)
I)
J)
K)
L)

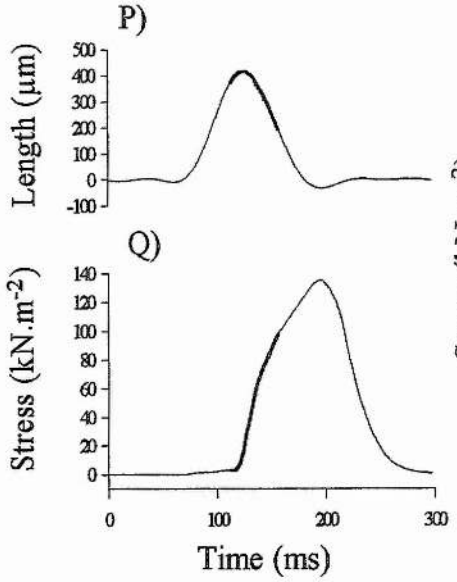
15@5 °C

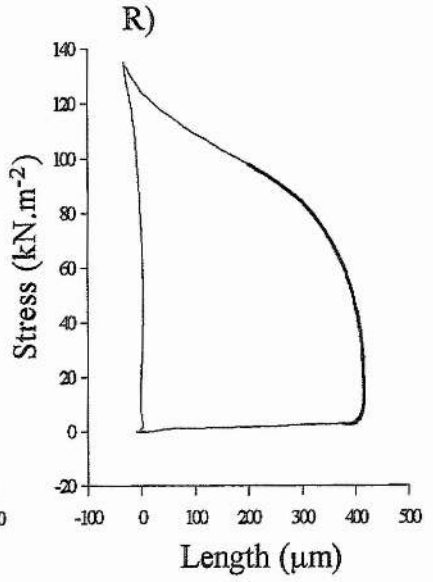
i) C-bend



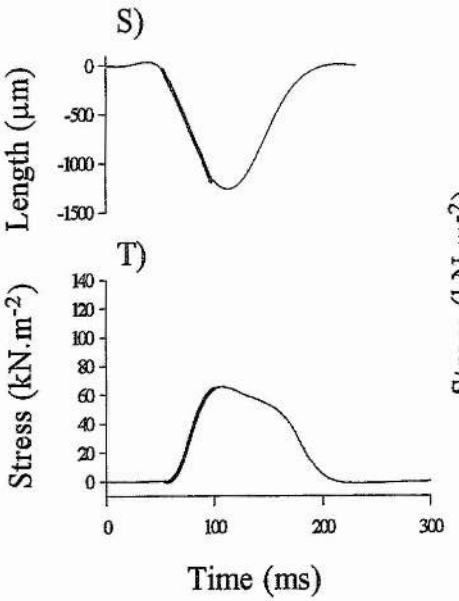



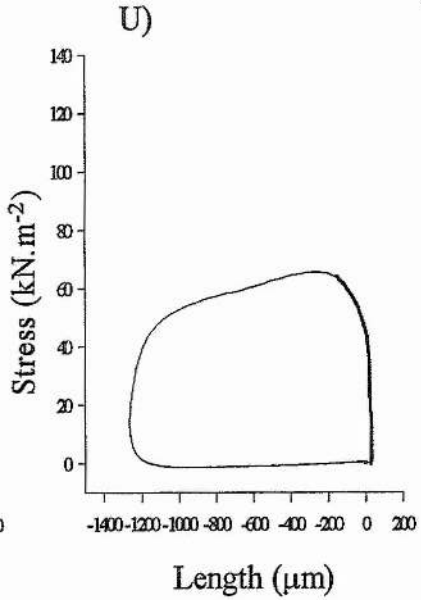

i) Contralateral contraction

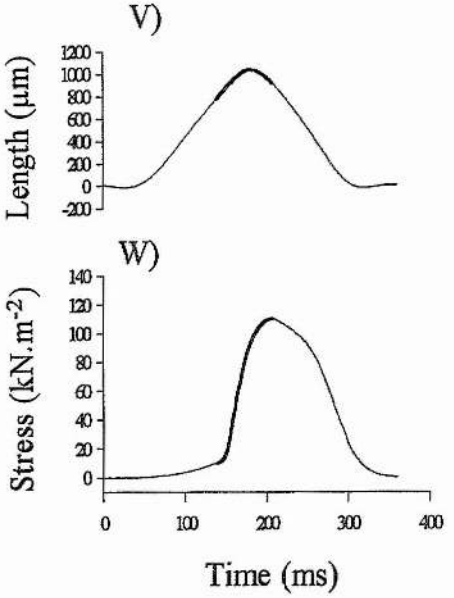



5@5 °C



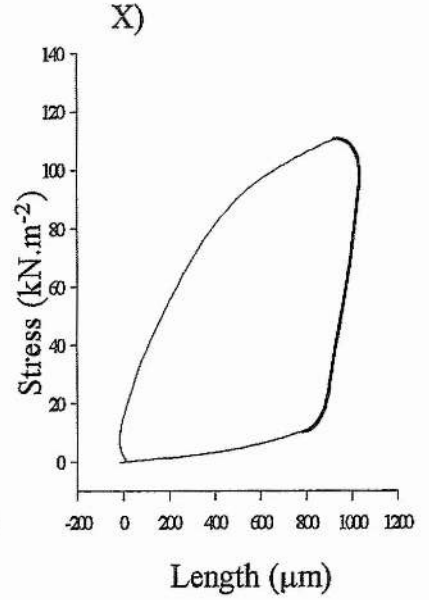



Figure 3.5. Representative plots of instantaneous power output for fast muscle fibres under conditions shown in Figure 3. Bold lines represent stimulation. Legends represent acclimation temperature at test temperature.

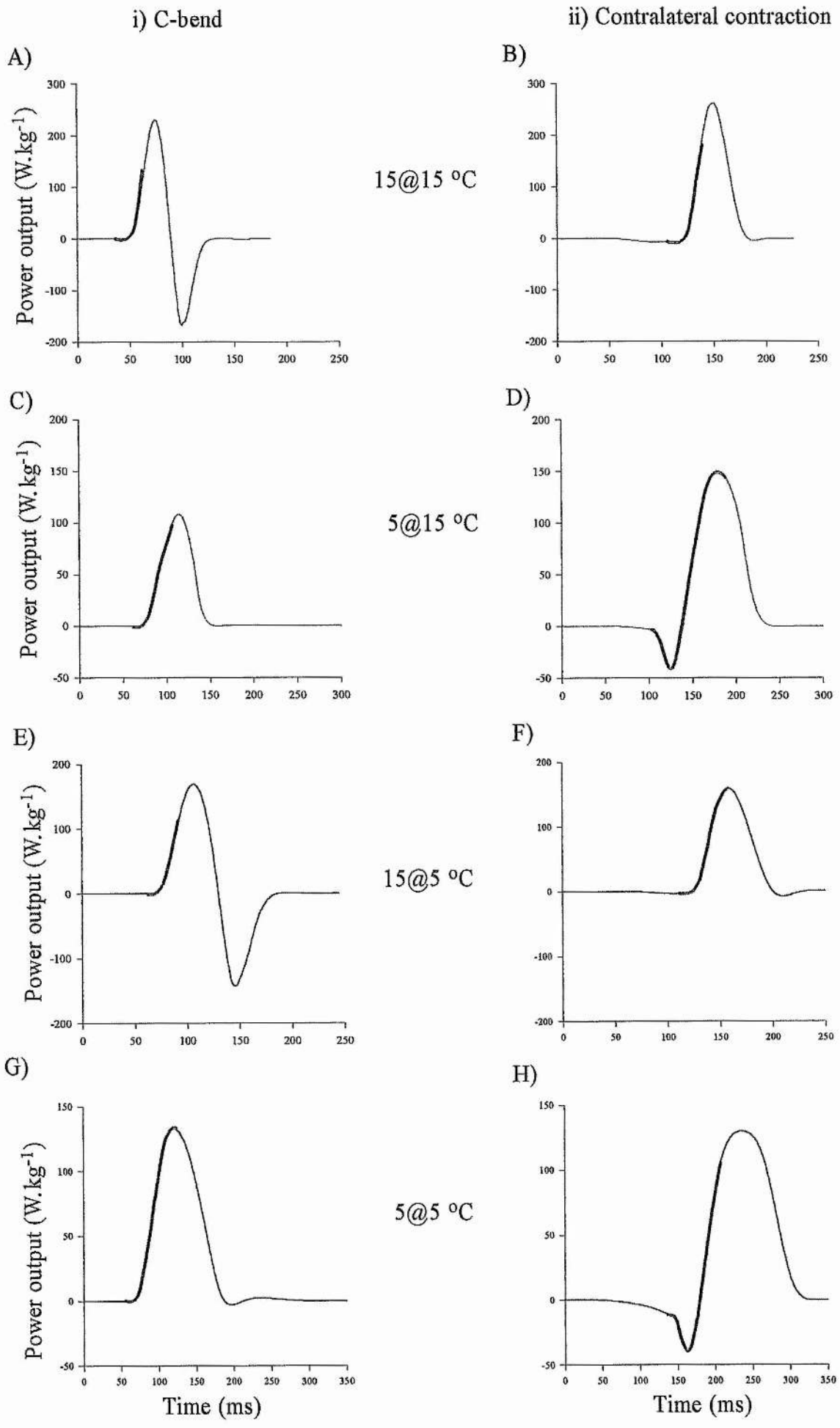

i) C-bend
ii) Contralateral contraction
A)
B)
C)
D)
E)
F)
G)
H)
15@15 °C
5@15 °C
15@5 °C
5@5 °C
Power output (W.kg-1)
Time (ms)

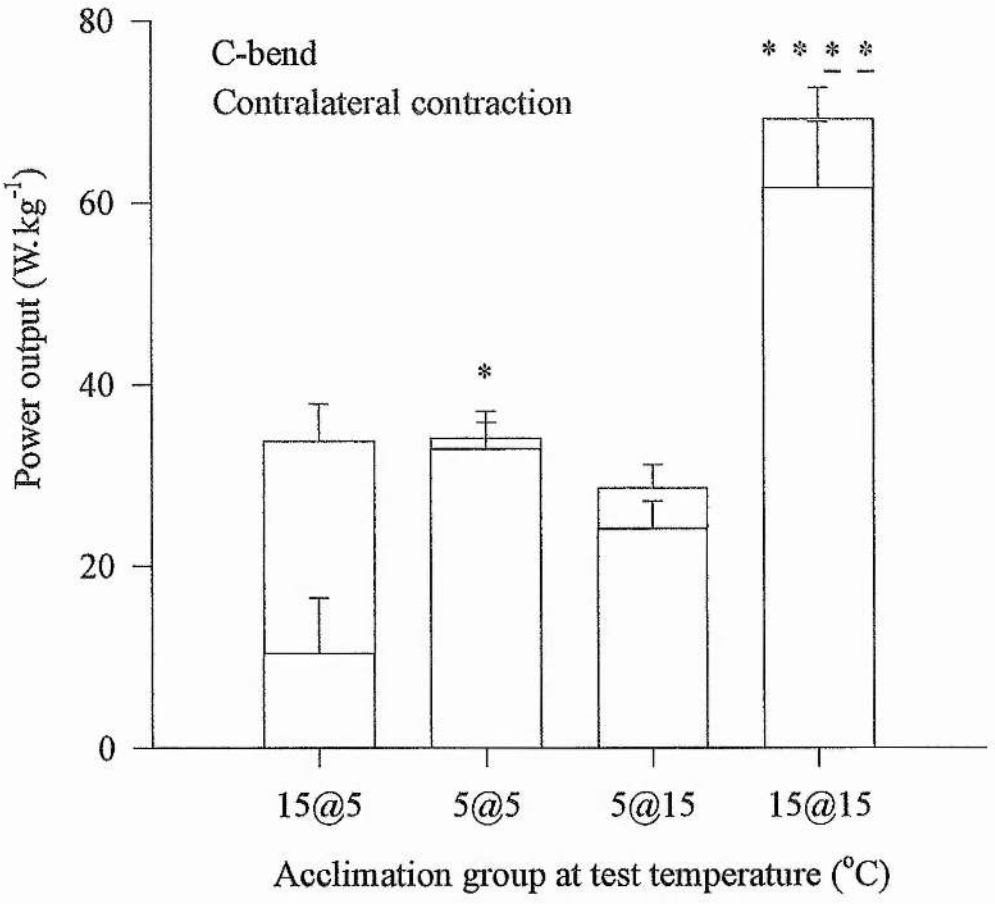
Table 3.3. *Influence of acute and acclimation temperature on the maximum instantaneous power output ($W.kg^{-1}$) of sculpin fast muscle fibres. Isolated muscle bundles* in vitro *were subjected to abstracted muscle strain and activation patterns that were recorded* in vivo *under the same thermal conditions.*

| | 5 °C-acclimated | | 15 °C-acclimated | |
|---|---|---|---|---|
| | Test temperature (°C) | | | |
| | 5 | 15 | 5 | 15 |
| C-bend | 152.62 ± 19.21 | 149.03 ± 21.37 * | 162.14 ± 18.40 | 228.93 ± 21.24 |
| Contralateral contraction | 146.49 ± 12.34 | 178.53 ± 12.49 * | 159.30 ± 14.98 * | 275.64 ± 14.19 |

* denotes a significant difference ($P<0.001$) between test temperatures in muscle fibres from 15 °C-acclimated fish (an acute temperature effect).

* denotes a significant difference ($P<0.05$) between acclimation groups at 15 °C (an acclimation effect).

Figure 3.6. Mean power output of sculpin fast muscle fibres during the C-bend and contralateral contraction calculated using the *in vivo* strain waveforms and activation patterns shown in Figure 3. $N$=6 for 5 °C-acclimated fish; $N$=7 for 15 °C-acclimated fish. $\underline{*}$ denotes a significant difference ($P$<0.05) between test temperatures within the same acclimation group (an acute temperature effect). * denotes a significant difference ($P$<0.05) between acclimation groups at the same test temperature (an acclimation effect).


C-bend
Contralateral contraction
Power output (W.kg-1)
80
60
40
20
0
*
* * * *
15@5
5@5
5@15
15@15
Acclimation group at test temperature (°C)

compensation in power output following 5 °C-acclimation. At 5 °C, the power output during the C-bend was 204 % higher in 5 °C- than in 15 °C- acclimated fish ($P<0.05$).

### *3.3.4* In vivo *improvement in power output at acute temperatures*

In each acclimation group, bundles of fast muscle fibres were subjected to 4 strain waveforms and activation patterns at each experimental temperature. The 4 patterns corresponded to recordings during both stages of the escape response at test temperatures of 5 and 15 °C. The purpose of replaying all waveforms was to investigate the improvement in power output afforded by the *in vivo* patterns of muscle action. The results of this investigation can be seen in Tables 3.4 and 3.5.

In fibre bundles from 15 °C-acclimated fish, power output was always significantly higher at a test temperature of 15 °C irrespective of the temperature at which the waveforms were originally recorded ($P<0.05$; Table 3.4). In 5 °C-acclimated fish, power output did not differ as dramatically between temperatures, but was significantly greater at 5 °C in the contralateral contraction when the waveforms had originally been recorded at 5 °C (Table 3.5).

Although the power output of the fast muscle fibres of 15 °C-acclimated fish was low at test temperatures of 5 °C, there was significant improvement in the mean power output of the C-bend when the *in vivo* patterns had originally been recorded at 5 °C (-18.30 compared to 10.47 $W.kg^{-1}$, $P<0.05$; Table 3.4).

Similarly, during the contralateral contraction of 5 °C-acclimated fish, power output was lower at test temperatures of 15 °C than 5 °C. However, power output at this test temperature was significantly higher in those fibre bundles that were subjected to the *in vivo* patterns originally recorded at 15 °C rather than 5 °C (Table 3.5).

Table 3.4. *The mean power output ($W.kg^{-1}$) of fast muscle fibres from 15 °C-acclimated fish (N=7) under conditions simulating the C-bend and the contralateral contraction at different combinations of original recording temperature and rig temperature.*

| | Temperature at which waveforms recorded (°C) | | | |
|---|---|---|---|---|
| | 15 | | 5 | |
| | Temperature at which waveforms replayed (rig temperature, °C) | | | |
| | 15 | 5 | 15 | 5 |
| C-bend | 61.65 ± 7.37 * | -18.30 ± 5.57 * | 64.14 ± 7.94 * | 10.47 ± 6.04 |
| Contralateral contraction | 69.26 ± 3.54 * | 48.66 ± 5.08 | 53.86 ± 2.98 * | 33.81 ± 4.08 |

* denotes a significant difference ($P<0.05$; one-way ANOVA) in power output between the two rig temperatures when the waveforms were originally recorded at the same temperature (an acute temperature effect).

* denotes a significant difference ($P<0.05$; one-way ANOVA) in power output between the two recording temperatures, at a rig temperature of 5 °C.

Table 3.5. *The mean power output ($W.kg^{-1}$) of fast muscle fibres from 5 °C-acclimated fish (N=6) under conditions simulating the C-bend and the contralateral contraction at different combinations of original recording temperature and rig temperature.*

| | Temperature at which waveforms recorded (°C) | | | |
|---|---|---|---|---|
| | 15 | | 5 | |
| | Temperature at which waveforms replayed (rig temperature, °C) | | | |
| | 15 | 5 | 15 | 5 |
| C-bend | 24.12 ± 3.04 | 23.07 ± 3.33 | 32.25 ± 3.38 | 32.88 ± 4.18 |
| Contralateral contraction | 28.63 ± 2.61 * | 34.98 ± 3.52 | 18.43 ± 2.05 * | 34.11 ± 4.33 |

* denotes a significant difference ($P<0.05$; one-way ANOVA) in power output between the two rig temperatures when the waveform was originally recorded at 5 °C (an acute temperature effect).

* denotes a significant difference ($P<0.05$; one-way ANOVA) in power output between the two recording temperatures, at a rig temperature of 15 °C.

## 3.4 Discussion

### *3.4.1 Changes in the strain cycle parameters with temperature*

Temperature acclimation significantly altered the *in vivo* parameters of the muscle strain cycle during escape responses in the short-horn sculpin (Table 3.1). Differences between acclimation groups occurred primarily at a test temperature of 15 °C. In both the C-bend and contralateral contraction, the cycle and shortening durations were shorter although at the whole animal level, Chapter 2 revealed no acclimation effect on the duration of the escape response. It was found that the swimming response duration up to the end of the contralateral contraction was shorter at 15 °C than at 5 °C, irrespective of previous acclimation temperature.

The present study revealed the muscle shortening velocity to be greater at 15 °C, in fish acclimated to 15 °C- than in those acclimated to 5 °C. In Chapter 2, it was found that the maximum swimming velocity of short-horn sculpin during escape responses was 33 % higher at a test temperature of 15 °C, in 15 °C- than 5 °C-acclimated fish, a result additionally reported during prey capture responses in this species (Beddow *et al.* 1995). Acclimation to 15 °C, however, did not compromise the maximum velocity of fast-starts at low temperature (Chapter 2), a result contrary to that found in goldfish (*Carassius auratus*) and killifish (*Fundulus heteroclitus*) during escape responses (Johnson and Bennett, 1995). Acclimation of the *in vivo* muscle shortening velocity in the short-horn sculpin was only manifest at high temperature and therefore mimics that of whole animal performance.

The relationship between the thermal acclimation of *in vivo* muscle shortening velocity and the thermal acclimation of maximum swimming velocity could be due to two factors: increases in the maximum shortening velocity ($V_{max}$) of the fast muscle fibres and/or a shift along the steady-state force-velocity (*P-V*) curve due to reduced force with acclimation to 15 °C. Beddow and Johnston (1995) found reduced tetanic force at 15 °C in fast muscle fibres from 5 °C- compared to 15 °C-acclimated sculpin. They attributed this to a failure in excitation-contraction coupling and cross-bridge function. The results of the isometric experiments in the present study found maximum force during tetanus was not significantly changed with either acclimation or acute temperature, therefore

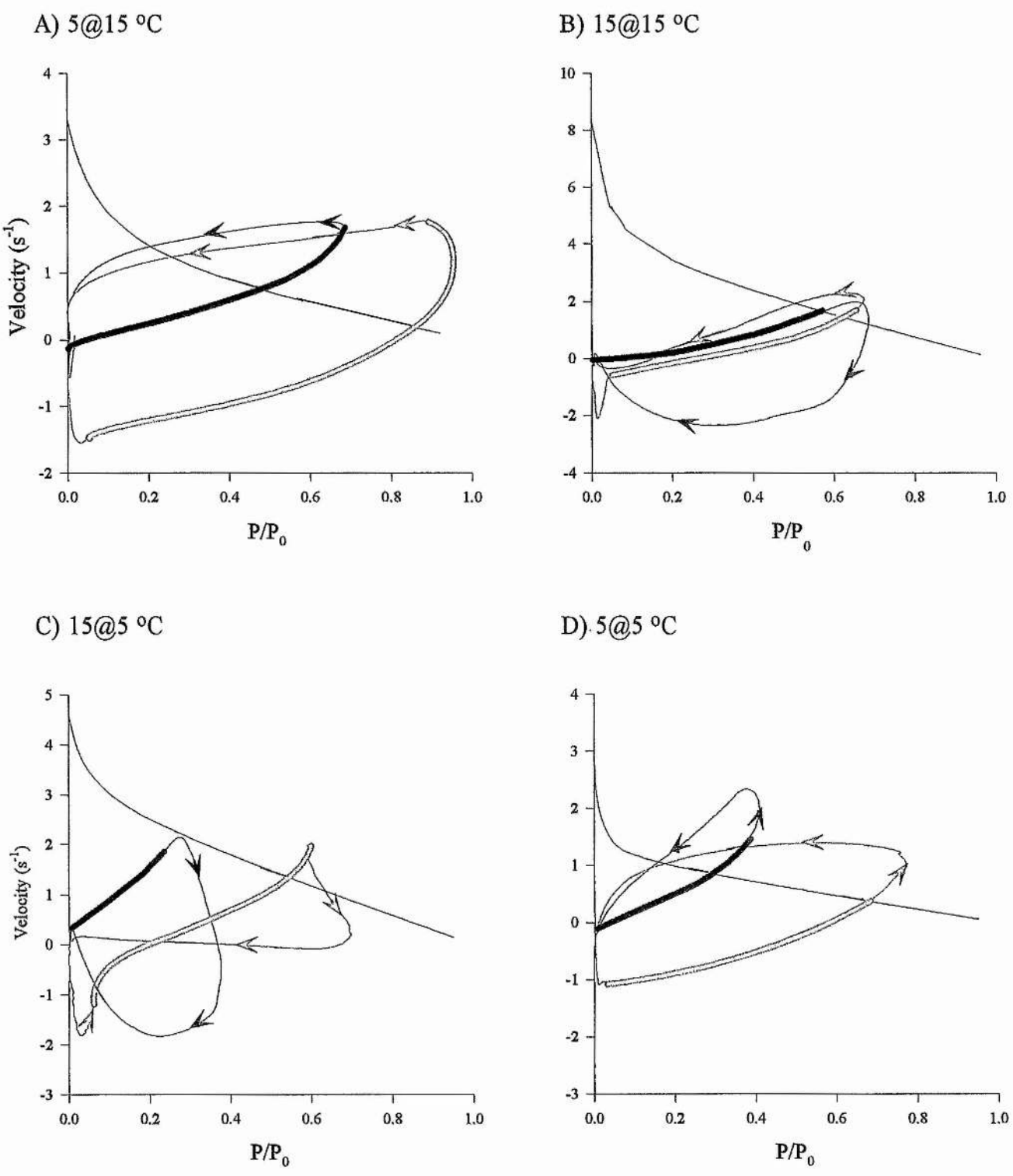
ruling out the possibility of a shift along the *P-V* curve. This is not an uncommon phenomenon and has been reported in other phyla (Bennett, 1985). Reasons for the discrepancy between the present results and those of Beddow and Johnston (1995), however, are unclear but could include differences in the acclimation state or physiological condition of the fish used.

With regards to alterations in $V_{max}$, Beddow and Johnston (1995) reported a 2.4 fold increase in $V_{max}$ at 15 °C, in 15 °C- compared to 5 °C-acclimated short-horn sculpin, and once again no changes were manifest at low temperature. Muscle fibres in several fish species have been found to contract over a narrow range of $V/V_{max}$ (where $V$ is the velocity at which the muscle shortens *in vivo*) of around 0.2-0.4, such that power and efficiency were close to optimal (Rome *et al.* 1988, 1990, 1992; Rome and Sosnicki, 1990, 1991; James and Johnston, 1998). James and Johnston found $V/V_{max}$ to fall between 0.17-0.42 during the C-bend of escape responses in short-horn sculpin of a range of body sizes. In the present study, $V/V_{max}$ values were calculated using $V_{max}$ data from Beddow and Johnston (1995; see Table 3) (Table 3.2). $V/V_{max}$ values fell between 0.2 and 0.5 for all acclimation groups at all test temperatures, thus confirming previous findings that $V/V_{max}$ is an important design constraint for locomotor capacity. Therefore in the present study, it would appear that a higher *in vivo* shortening velocity of fast fibres in 15 °C- than 5 °C-acclimated fish at a test temperature of 15 °C, must be attributed to increases in the maximum shortening velocity of the muscle fibres.

### *3.4.2 Power loops*

In Figure 3.7 the instantaneous force and velocity during both stages of the escape response have been plotted against representative *P-V* curves from Beddow and Johnston (1995) to produce power loops (Stevens, 1993). Although some caution should be exercised in the interpretation of these plots, as the *P-V* studies and work loop experiments were not carried out on the same muscle preparations, trends similar to those found in other studies can be seen. For example, the *in vivo* velocity can be seen to exceed that predicted from the *P-V* relationship (Franklin and Johnston, 1997; James and Johnston, 1998). This is most evident in fish which were not swimming at their acclimation temperatures (Fig. 3.7A, C). Furthermore, as the muscle is not maximally

Figure 3.7. Power loops imposed upon steady-state force-velocity data from Beddow and Johnston (1995). Velocity is in fibre lengths per second, expressed as $s^{-1}$. Muscle strain waveforms and activation patterns were those shown in Figure 3. Bold lines indicate stimulation. Legends represent acclimation temperature at test temperature.


C-bend
Contralateral contraction
A) 5@15 °C
B) 15@15 °C
C) 15@5 °C
D) 5@5 °C
Velocity (s-1)
P/P0

activated *in vivo*, the force production under *in vivo* conditions is generally less than that under steady-state conditions, a result that has been reported in this species at 12 °C (James and Johnston, 1998) and in the Antarctic rock cod (*Notothenia coriiceps*) (Franklin and Johnston, 1997) at 0 °C. In some cases however force exceeds that produced during *P-V* conditions. This tends to occur during the contralateral contraction and in the present study was most noticeable in fibres from 5 °C-acclimated fish tested at 15 °C. In this group the muscle fibres were stretched substantially and at fairly high velocity whilst active which improves performance by force enhancement (Edman *et al.* 1978; Stevens, 1993). It is thought that force enhancement may be caused by an altered interaction between the cross-bridges and the actin filament (Edman *et al.* 1978). Although small active pre-stretches do occur during the C-bend (Fig. 3.3C and G) due to shortening of more anterior myotomes or changes in muscle stiffness (Franklin and Johnston, 1997), they are unlikely to cause force enhancement as the stretch and velocity of stretch are low.

### *3.4.3 Acute temperature effects*

This study revealed $Q_{10}$ values for maximum instantaneous power output and mean power output during the contralateral contraction of 1.7 and 2.0, respectively. Beddow and Johnston (1995) reported a $Q_{10}$ of 2.0 for $V_{max}$ in 15 °C-acclimated sculpin over the temperature range 5-15 °C. In contrast, the maximum swimming velocity during fast-starts of the short-horn sculpin was found to only have a $Q_{10}$ of 1.2 over the temperature range 5-15 °C (Chapter 2; Beddow *et al.* 1995). Similarly, Marsh and Bennett (1985) reported that the $Q_{10}$ values for the thermal dependence of rate functions in the muscle were higher than those for locomotor performance in the lizard (*Dipsosaurus dorsalis*). Over a temperature range of 25-40 °C, maximum running velocity had a $Q_{10}$ of 1.3 whereas $V_{max}$ had a value of 1.7. Differences in the thermal dependence between rate processes of the muscle and whole-animal locomotion have been attributed to the involvement of elastic structures with a low thermal sensitivity (Marsh and Bennett, 1985). Muscle i*n vivo* is associated with elastic structures in series and in parallel, whereas muscle preparations used *in vitro* have no series elastic components and reduced parallel elastic components.

### *3.4.4 Changes in muscle power output with thermal acclimation*

Both instantaneous and mean power outputs showed significant temperature acclimation effects, again particularly following acclimation to 15 °C. As stated earlier, this is mainly attributable to increases in the shortening velocity component. Power output was around 150 % higher at 15 °C during escape responses in 15 °C- than in 5 °C-acclimated fish. Johnson and Johnston (1991) carried out work loop experiments using sinusoidal strain waveforms on isolated fast muscle fibres from summer- and winter-acclimatised short-horn sculpin. In agreement with the present study these authors also found that significant improvements in power output were principally manifest at high temperature. Johnston *et al.* (1995) similarly found dramatic increases (277 %) in the average power output under conditions representing the first tail-beat during prey capture responses in this species. However, their values of power output were lower (23.8 $W.kg^{-1}$ for 15 °C-acclimated fish at 15 °C) than measured in the present study (61.7 $W.kg^{-1}$) perhaps due to the different methods used in determining muscle strain. The values reported in this study are more in accord with those of James and Johnston (1998) who calculated power output in the short-horn sculpin using the same methods as used in this study. They found that at an acclimation and test temperature of 12 °C, mean power output for both the C-bend and contralateral contraction was 41-42 $W.kg^{-1}$ for fish of a range of sizes. This falls between our values of 33-34 for 5 °C-acclimated fish and 62-70 $W.kg^{-1}$ for 15 °C-acclimated fish each tested at their acclimation temperatures.

The C-bend produced mean power outputs that were always lower than those of the contralateral contraction, probably due to the reduced active pre-stretch. In most groups the reduction in power output was not significant, the exception being the significantly lower mean power output during the C-bend of the group acclimated to 15 °C and tested at 5 °C. There was therefore an acclimation effect for the C-bend at a test temperature of 5 °C with power output being higher in the 5 °C- than the 15 °C-acclimated group.

The dramatic difference between the C-bend and contralateral contraction of the 15 °C-acclimation group tested at 5 °C was due to the chosen strain and activation patterns which produced work loops with substantial negative components (Fig. 3.4 'O').

In both the C-bend and contralateral contraction, force continued to rise following stimulation, which is indicative of a low shortening velocity. In comparison to fibres from 5 °C-acclimated fish at 5 °C (Fig. 3.4S, V), fibres from 15 °C-acclimated fish at 5 °C had lower muscle strain in both stages. Cycle durations were not greatly different and therefore shortening velocities were lower. In fact, the muscle shortening velocities of the representative waveforms (Fig. 3.4 'O', R) from 15 °C-acclimated fish at 5 °C, were lower than the average values for the group (2.0 $s^{-1}$ instead of 2.3 $s^{-1}$ and 1.6 $s^{-1}$ instead of 2.2 $s^{-1}$ for the C-bend and contralateral contraction, respectively). The $V/V_{max}$ values were 0.4 and 0.3 for the C-bend and contralateral contraction, respectively, whereas the mean $V/V_{max}$ for both sides was 0.5. Had shortening velocity been higher in both waveforms either as a result of a higher strain (Josephson and Stokes, 1989) or a shorter cycle duration (James *et al.* 1996), shortening-induced deactivation could have aided muscle relaxation prior to relengthening *via* decreasing the force producing ability of the muscle (Edman, 1980). When stimulation phase, stimulation duration, strain and cycle duration were individually altered it was found that the *in vivo* conditions produced between 81-100 % of the maximum power output in all groups except during the C-bend for fibres from 15 °C-acclimated fish at a test temperature of 5 °C. In this latter group the maximum power output was 33.6 $W.kg^{-1}$ but the power output under *in vivo* conditions was 10.5 $W.kg^{-1}$, thus indicating that the response was not maximal.

The complex effects of temperature on *in vivo* power output can be seen in Tables 3.4 and 3.5. In the fibres from 15 °C-acclimated fish, power output was always greatest at 15 °C irrespective of original recording temperature of the waveforms (Table 3.4). However, when the C-bend strain was recorded at an acute temperature of 5 °C and used in work loop experiments at 5 °C, power output was higher than when the strain was recorded at the acclimation temperature of 15 °C and used *in vitro* at 5 °C. At 5 °C, the cycle and stimulus durations of the 15 °C-waveform would have been too short for maximal power generation. In addition, $V/V_{max}$ increased marginally from 0.50 using the waveform recorded at 5 °C to 0.53 using the waveform recorded at 15 °C. In 5 °C-acclimated fish, the contralateral contraction showed significant differences depending on original recording temperature and the temperature during work loop experiments. When

the waveform had been recorded at 15 °C and used in work loop studies at 15 °C, power output was higher than when the waveform had been recorded at the acclimation temperature of 5 °C and used *in vitro* at 15 °C. At 15 °C, $V_{max}$ would be high, activation and relaxation times low, but the stimulation and cycle durations of the waveform from the 5 °C-acclimated fish escaping at its acclimation temperature would be too long for maximal power output at a test temperature of 15 °C.

*Chapter 4*

# THE ENERGETICS OF MUSCLE CONTRACTION DURING PREY CAPTURE AND ESCAPE RESPONSES IN THE SHORT-HORN SCULPIN

## 4.1 Introduction

Fast-starts are most commonly used by fish during prey capture or escape manoeuvres. The two functionally distinct fast-starts have generally been recognised as being kinematically different. Escape responses involve C-starts which are triggered by Mauthner cells, a bilateral pair of brainstem neurons (Eaton *et al.* 1977). The direction of escape is variable as the fish may undergo highly changeable turning angles (Domenici and Blake, 1993*a*) perhaps as a means of confusing predators (James and Johnston, 1998; Chapter 2). In contrast, prey capture responses often conform to what have been termed S-starts (Hoogland *et al.* 1956). It is thought that prey capture does not rely on the Mauthner neurons as these have only been found to fire in the latter stages of an attack (Canfield and Rose, 1993). S-starts involve little change in direction with direct movement of the head towards the prey (Harper and Blake, 1991; Beddow *et al.* 1995). Unlike escape responses, prey capture does not simply involve swimming; ram feeding, suction feeding and biting have varying degrees of importance (Liem, 1980). In ram feeding, prey capture is achieved by rapid acceleration of the body and protrusion of the premaxilla. Suction feeding involves rapid expansion of the buccal and opercular cavities thus drawing water into the mouth (Osse and Muller, 1980; Lauder, 1983). Van Leeuwen (1983) found suction feeding significantly increased the velocity of the prey towards the predator. The strict division between C and S-starts has recently been challenged by Wakeling and Johnston (1998), who studied escape responses in six species of fish ranging from tropical to Antarctic. By quantifying the curvature down the fish they found body shape to vary along a continuum between the classic S and C forms.

During fast-starts a wave of curvature passes from head to tail down the body of the fish (Wakeling and Johnston, 1998) similar to that found in steady swimming (Gray, 1933). Muscle activation as determined using electromyography (EMG) has been found to occur simultaneously down the length of the fish during the C-bend of escape responses, but as a wave of activity during steady swimming (Johnston *et al.* 1993; Jayne

and Lauder, 1993; 1995). In the Antarctic rock cod (*Notothenia coriiceps*) and the short-horn sculpin, muscle length (strain) changes have been measured directly during escape responses using sonomicrometry (Franklin and Johnston, 1997; James and Johnston, 1998). During steady swimming the amplitude of muscle shortening and lengthening is often assumed to be equal. However, during fast-starts the fast muscle fibres were observed to undergo variable length changes from tail-beat to tail-beat (Johnston *et al.* 1995; Franklin and Johnston, 1997; James and Johnston, 1998).

The work loop technique (Josephson, 1985) has been used to subject isolated muscle to the *in vivo* muscle strain and activation patterns to determine the likely force, work and power produced during locomotion (Altringham *et al.* 1993; Johnston *et al.* 1995; Franklin and Johnston, 1997; James and Johnston, 1998). When strain and activation parameters were determined from sonomicrometry and EMG during escape responses, isolated muscle yielded 83 - 100 % of maximal power output (Franklin and Johnston, 1997; James and Johnston, 1998).

The energetic performance of fish muscle has been determined *in vitro* using one of two approaches: 1) by heat production measurements in which a thermopile is used to directly record muscle temperature whilst work is measured during imposed cycles of shortening and lengthening (Curtin and Woledge, 1993, 1996); and 2) by high performance liquid chromatography (HPLC) to measure changes in the concentration of high energy phosphate compounds (Johnson *et al.* 1991; Moon *et al.* 1991). All previous studies have used sinusoidal muscle length changes to simulate steady swimming movements and have optimised stimulation patterns to maximise muscle power output. Energetic costs were defined in terms of the economy of contraction, which equates to the net positive work per unit energy expended (Woledge, 1989; Johnson *et al.* 1991; Moon *et al.* 1991). When using the HPLC method, muscle efficiency, defined as the ratio of work done to energy used (Curtin and Woledge, 1996) was estimated assuming a Gibb's free energy change for the hydrolysis of PCr of 55 $kJ.mol^{-1}$ (Woledge and Reilly, 1988; Johnson *et al.* 1991; Moon *et al.* 1991).

The aim of the present study was to measure muscle power and economy during prey capture and escape responses in the marine cottid, short-horn sculpin, and thus

compare these two functionally distinct behaviours. The energetic cost of these swimming behaviours was examined by determining changes in metabolites using HPLC.

## 4.2 Materials and methods

### *4.2.1 Fish*

Short-horn sculpin ($N$=15) were caught by local fisherman in lobster creels or by trawling in the Firth of Forth in May/June and October/November 1996. Fish were acclimated to 5 °C for 6 - 8 weeks in re-circulating sea water tanks (photoperiod 11 h light : 13 h dark). Fish were fed twice a week on live shrimps (*Crangon crangon*: caught by dredging in St. Andrews Bay) and chopped squid. Fish caught in October/November were used for energetic studies whereas all other experiments were conducted with fish caught in May/June. The sea water temperature varied between 7-10 °C at these times of the year (J. Murdoch, unpublished observations).

### *4.2.2 Surgical procedure*

The methods were carried out as described fully in Chapter 3. A brief description is given below. Six fish (159 ± 13 g wet weight, 21.8 ± 0.7 cm total length ($L$)) were used for EMG and sonomicrometry experiments. Individuals were anaesthetised using a 1:5000 (m/v) solution of tricaine methane sulphonate (MS222) containing 0.70 mmol.$l^{-1}$ sodium hydrogen carbonate. Anaesthesia was maintained during surgery by irrigation of the gills with a 1:7000 (m/v) concentration of MS222.

Pairs of sonomicrometry crystals (1 mm diameter, lensed crystal, Triton Technology Inc, San Diego, USA) were implanted to a depth of 1 to 1.5 mm below the skin and 0.35 body lengths from the snout of the fish, with 8 - 12 mm between the emitter and receiver crystal. EMG wires (A-M Systems, Everett, USA) were implanted in the same region as the sonomicrometry wires. All wires were sutured to the skin allowing some slack to avoid rapid movements by the fish dislodging the crystals or wire.

### *4.2.3 Kinematic performance and* in vivo *muscle length and activity patterns*

Fish were filmed in a static tank (2.0×0.62×0.26 m: length×width×depth) of circulating sea water maintained at 5 °C. The tank had a perspex base and was lit from underneath by five 70 W fluorescent strip lights.

Escape responses were elicited by touching the tail of the fish with a metal rod presented from behind the fish at an angle of 30 - 45 ° to the midline of the tail. Prey

capture responses were elicited by the introduction of live shrimps (*Crangon crangon*) into the tank. All resultant fast-starts was filmed in silhouette using a high speed video camera (NAC, Japan) operating at 200 frames per second, aimed at a mirror set at 45 ° above the tank.

Video recordings of fish and EMG and sonomicrometry outputs were synchronised with a pulsed light source in the filming arena. The error in matching a frame of video to the sonomicrometry and electromyography data was estimated to be less than 2 ms. EMG recordings were amplified using a differential A-C amplifier (AM Systems, Everett, USA) with low and high cut off settings of 100 Hz and 5000 Hz respectively.

A correction of 5 ms was used for the lag in sonomicrometry readings due to the data filter in the Triton Technology Model 120 sonomicrometer. The *in vivo* muscle shortening velocity ($V$) was determined by fitting a tangent to the strain records obtained by sonomicrometry.

### *4.2.4 Kinematic analysis*

The position of the centre of mass (midpoint) of the fish was determined as described in Chapter 2. The midpoint of each fish was digitized for each video frame using a motion analysis system (MOVIAS, NAC, Japan). An object of known length was digitized on the first frame of each sequence to act as a distance calibration. For escape responses digitizing was carried out up until the end of the contralateral contraction and for prey capture responses until the mouth closed again following capture. Errors in digitizing the midpoint caused less than a ± 1.5 % error in both the $x$ and $y$ position co-ordinates. Smoothing was carried out using cubic piecewise regressions (LabView, National Instruments, USA) as described in Chapter 2, with a smooth width of 20. The smoothed position data were used to calculate the velocity ($U$) of the fish. For each fish only the response with the highest maximum velocity ($U_{max}$) was selected for further analysis. The end of the C-bend and the end of the contralateral contraction were determined as outlined in Chapter 2. The turning angle was calculated using MOVIAS by drawing a line from the snout to the centre of mass of the fish and measuring the angle of rotation of this line during the C-bend.

### *4.2.5 Contractile properties*

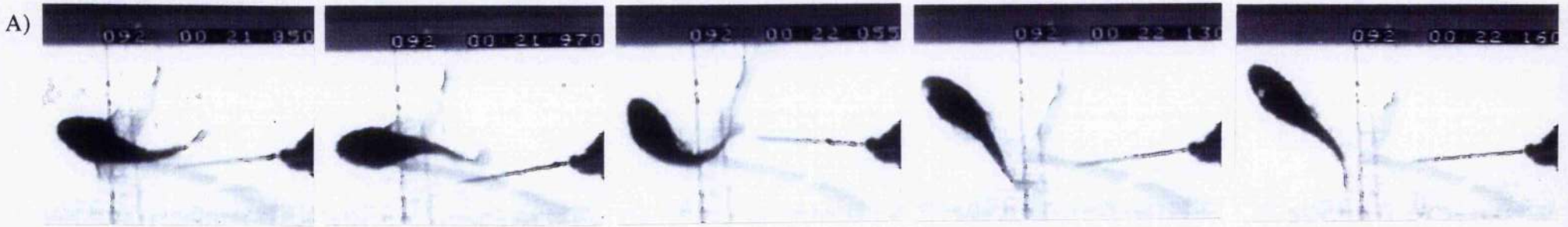
Fuller methods are described in Chapter 3. The contractile properties of isolated muscle fibres were determined on the same individuals used for swimming experiments. Fish were stunned by a blow to the head and killed by pithing to destroy the central nervous system. An incision was made from the anus to the pectoral gill arch and the anterior abdominal myotomes (0.35 body lengths from the snout) were removed. Small bundles of approximately 10-20 fibres were dissected from anterior abdominal myotomes in Ringer solution maintained at 4 °C. The Ringer solution contained (in $mmol.l^{-1}$): NaCl, 143; sodium pyruvate, 10; KCl, 2.6; $MgCl_2$, 1.0; $NaHCO_3$, 6.18; $NaH_2PO_4$, 3.2; $CaCl_2$, 2.6; HEPES- sodium salt, 3.2; HEPES, 0.97 (James and Johnston, 1998). An aluminium foil T-shaped clip was folded over the peritoneum at each end of the muscle fibre bundle preparation. The preparation was then transferred to a flow-through chamber of Ringer maintained at 5 °C. The foil clips were used to attach the preparation to a force transducer at one end (AME 801, Senso-Nor, Horten, Norway) and a servo arm (MFE Model R4077, Emerson Electronics, England) at the other.

Muscle fibre preparations were held at constant length. Stimulus amplitude, pulse width (6 - 10 V; 1.0 - 1.4 ms respectively) and fibre length were adjusted to maximise twitch force. Stimulation frequency was adjusted to maximise tetanus height (90 - 110 Hz). Time to peak twitch force, time from stimulus to 90 % twitch relaxation, time to peak tetanic force and time from last stimulus to 50 % tetanic relaxation were all measured.

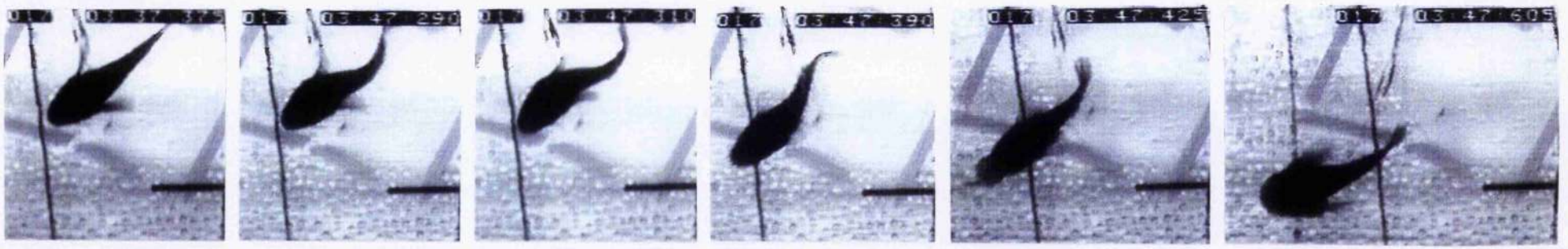
In order to produce the cyclical events needed for work loop experiments, segments of representative strain records recorded from one side of the fish were abstracted (Fig. 4.1C and F). The abstracted strain wave-forms for escape responses started at resting muscle fibre length at the beginning of the response and continued for three half tail-beats before returning to the resting muscle fibre length. Therefore there were 2 periods of muscle shortening on the side of the fish that initially went into the C-shape. Prey capture waveforms included either one or two periods of muscle shortening, depending on the number of tail-beats taken to catch the prey. The abstracted *in vivo* muscle strain wave-forms were digitized then smoothed as described in Chapter 2. The isolated muscle preparations were subjected to the abstracted strain wave-forms and

Figure 4.1. Video (A, D), EMG (B, E) and sonomicrometry (C, F) recordings of i) an escape response and ii) a prey capture response in the short-horn sculpin.

i) Escape response




ii) Prey capture



stimulated at the appropriate point in the strain cycle and for the same duration as found *in vivo* using EMG. Each muscle preparation was subjected to both prey capture and escape response wave-forms measured in that fish. Muscle force was measured and plotted against fibre length to enable work, instantaneous power output and stress to be calculated (Johnston *et al.* 1995). The strain, strain duration, stimulation duration and timing were all altered individually to determine the maximum power output that could be achieved by optimising each parameter.

### *4.2.6. Determination of fibre bundle cross-sectional area*

On completion of the *in vitro* experiments each muscle preparation was rapidly frozen in isopentane cooled to -159 °C by liquid nitrogen. Transverse sections, 10 μm thick, were cut and stained for myosin ATPase activity at pH 9.4 using the method of Johnston *et al.* (1974). A microscope drawing arm was used to draw the outline of each fibre in cross-section. The cross-sectional area of the muscle fibre bundle was then determined *via* a digital planimeter interfaced to a microcomputer using image analysis software (Videoplan, Kontron, Germany). Muscle mass was calculated from muscle length and cross-sectional area assuming a density of 1060 $kg.m^{-3}$ (Méndez and Keys, 1960).

### *4.2.7 Energetics*

Muscle fibre preparations were dissected from 7 fish (166 ± 8 g wet weight, 21.8 ± 0.3 cm *L*) as described above. Preliminary experiments indicated that muscle preparations needed to consist of about 45-60 muscle fibres in order to produce reliable estimates of AMP and IMP concentrations. Muscle fibre preparations were kept in Ringer containing 0.5 mM iodoacetic acid (IAA) bubbled with nitrogen gas ($N_2$) for 1-2 hours. The combination of IAA and $N_2$ were used to block glycolysis and aerobic metabolism to ensure that the only free energy available for contraction was from adenosine triphosphate (ATP) and phosphocreatine (PCr) (Carlson and Siger, 1959). Johnson *et al.* (1991) have previously shown that IAA and $N_2$ have no significant effects on the contraction kinetics of short-horn sculpin muscle. Representative sonomicrometry and EMG recordings from one escape response and one prey capture response were chosen. The two fast-start waveforms differed in the number of shortening periods; the prey

capture and escape response waveforms consisted of 1 and 2 shortening periods per cycle, respectively. Each muscle fibre preparation was subjected to a series of 4 cycles of the escape response waveform or a series of 8 cycles of the prey capture waveform, as preliminary experiments had indicated that these cycle numbers produced measurable changes in energetic substrates. At the end of the last cycle the Ringer was rapidly drained from the chamber and the muscle preparation fast frozen with isopentane cooled to -159 °C. Control muscle fibre preparations were kept in poisoned Ringer as above then fast frozen without being subjected to stimulation.

Frozen muscle fibre preparations were stored in liquid nitrogen, then freeze dried for 12-15 h. The following methods for HPLC and lactate analysis were carried out by Dr. Rob James (Gatty Marine Laboratory, University of St. Andrews). Freeze dried preparations were weighed to the nearest 10 μg using an Oertling microbalance (R52, Oertling, Orpington, Kent, UK) and homogenised at 2 °C in 100 - 150 volumes of 0.3 M perchloric acid for 15 min. 50 μl of supernatant was removed for subsequent lactate analysis. 150 μl of homogenate was removed and spun at 13,000 rpm for 3 minutes in a Microcentaur centrifuge (MSE, Crawley, UK). 100 μl of supernatant was removed, added to 100 μl of deionised distilled water and the pH adjusted to 6.25 using 1 M $K_2HPO_4$. The neutralised solution was spun at 13,000 rpm for 3 min then stored on ice for up to 3 h prior to assay for high energy phosphates. A high performance liquid chromatography (HPLC) method was used to assay for PCr, Cr, IMP, AMP, ADP and ATP as outlined in Franklin *et al.* (1996). Lactate concentration was measured by using Sigma kit 735 (Sigma Chemicals, Dorset, England).

The *in vitro* economy of muscle preparations subjected to *in vivo* strain and activation patterns was calculated as follows:

Economy = work done in 1st work loop × $N$ cycles / total energetic cost for $N$ cycles,

where the total energetic cost was calculated as:

$$\text{the change in } ([\text{PCr}]+3[ATP]+2[ADP]+[AMP]+[IMP]).$$

Muscle efficiency was estimated using a value of 55 mJ . $\mu mol^{-1}$ for the Gibb's free energy change for splitting ATP (Woledge, 1989).

### *4.2.8 Statistics*

Prey capture and escape response parameters were tested for statistical differences either using a two-sided unpaired student t test or, in cases of unequal variance (tested using an $F$ test), a Mann Whitney U test. The latter was used for maximum swimming velocity, the *in vivo* muscle strain cycle duration and the *in vitro* mean muscle power output. Analysis of variance (ANOVA) was used to analyse the results of high energy phosphate compounds. The term 'significant' has been used to signify a $P$ value of less than 0.05. Values are presented as mean ± S.E.M.

## 4.3 Results

### *4.3.1 Kinematics of fast-starts*

All escape responses were C-starts with maximum velocity of the centre of mass occurring during the contralateral contraction. Escape responses involved large degrees of turning to the right and left. Thus the angle of rotation during the C-bend was significantly higher during escape than prey capture responses (Table 4.1). Of fourteen prey capture responses which were captured on film only 57 % of these were S-starts. In 75 % of fish the fastest prey capture responses were yielded from S-starts. S-start prey capture responses consisted of an S-bend followed by a contralateral contraction, then sometimes a further half tail-beat before a glide, with maximum velocity and prey capture occurring during the glide. C-start prey capture responses consisted of an initial C-bend followed by a contralateral contraction and a capture-glide with maximum velocity and prey capture again occurring during the gliding phase. There was no significant difference between prey capture and escape responses in the time to the end of the contralateral contraction and therefore the time to maximum swimming velocity was significantly greater in prey capture than escape responses (Table 4.1). The mean maximum velocity attained during the two kinds of fast-starts was not significantly different although values were higher during prey capture (Table 4.1).

### *4.3.2* In vivo *muscle length and activity patterns*

Figure 4.1 shows video sequences of a C-start escape response and an S-start prey capture response with corresponding muscle strain and EMG activity data traces. Total muscle strain and muscle shortening duration were greater (25 and 67 %, respectively) and total strain cycle duration was significantly greater (156 %) in prey capture than escape behaviour (Table 4.1). However, muscle shortening velocities were comparable, 3.2 and 2.9 $s^{-1}$ for prey capture and escape responses, respectively (Table 4.1).

### *4.3.3 Contractile properties*

In fast muscle fibres from 5 °C-acclimated sculpin, the time to peak twitch and time to 90 % twitch relaxation were 52.4±0.9 and 113.0±9.0 ms respectively. The time to peak tetanus and time from last stimulus to 50 % tetanus relaxation were 115.0±5.0 and

Table 4.1. *Kinematic performance and* in vivo *muscle strain parameters measured during escape and prey capture responses and corresponding* in vitro *contractile performance of muscle preparations subjected to* in vivo *strain and EMG patterns.*

| Variable | Escape response | Prey capture |
|---|---|---|
| Kinematic parameter | *N*=6 | *N*=4 |
| Turning angle used during C-bend/S-bend | 54.7 ± 5.8 | 19.1 ± 5.8 ** |
| Time to end of contralateral contraction (ms) | 174 ± 15 | 166 ± 34 |
| Time to maximum swimming velocity (ms) | 147 ± 0.07 | 231 ± 31 * |
| Maximum swimming velocity ($m.s^{-1}$) | 0.74 ± 0.07 | 0.82 ± 0.13 |
| *In vivo* muscle strain parameters | *N*=6 | *N*=4 |
| Total strain | 0.16 ± 0.02 | 0.20 ± 0.02 |
| Shortening duration (ms) | 59 ± 12.3 | 98 ± 14.5 |
| Cycle duration (ms) | 154 ± 16.5 | 395 ± 84.1 * |
| Shortening velocity ($s^{-1}$) | 2.94 ± 0.22 | 3.18 ± 0.48 |
| *In vitro* contractile performance | *N*=5 | *N*=5 |
| Maximum work loop stress ($kN.m^{-2}$) | 111.8 ± 15.7 | 80.6 ± 8.9 |
| Maximum instantaneous power output ($W.kg^{-1}$) | 127.0 ± 17.7 | 151.0 ± 24.6 |
| Mean power output ($W.kg^{-1}$) | 16.6 ± 3.9 | 11.1 ± 1.5 |

Values represent means ± S.E.M. * signifies $P<0.05$, ** signifies $P<0.01$.

107.0±7.0 ms respectively. These values are comparable to those measured in previous studies (Beddow and Johnston, 1995; Chapter 3).

Each set of work loop experiments used representative strain and EMG data from the same fish as the bundle of fast muscle fibres was isolated from. Isolated muscle preparations subjected to representative *in vivo* muscle strain and activation patterns (Fig. 4.1C and F, 4.2A and B) produced force and velocity traces shown in Figure 4.2C, D, E and F. Figure 4.3 shows work loops and instantaneous power output against time using the same muscle strain and activation patterns. Maximum stress, maximum instantaneous power output and mean power output achieved were not significantly different between the two behaviours (Table 4.1). Optimisation of individual strain, strain duration, stimulation duration and stimulation phase resulted in mean improvements of power output of 0.01-26 %.

### *4.3.4 Energetics*

The economy values calculated (as total work/total energy change) were not quite significantly different at the $P$=0.05 level between prey capture and escape response behaviours (3.45±0.72 mJ.μmol$^{-1}$ for 4 escape response cycles and 5.43±0.53 mJ.μmol$^{-1}$ for 8 prey capture cycles, $P$=0.07). Figure 4.4 shows an HPLC trace following 4 escape response cycles and Figure 4.5, a trace following 8 cycles of prey capture. As with previous studies (Johnson *et al.* 1991) there were no significant changes in IMP, AMP, ADP, ATP or lactate concentrations during either of these stimulation/work regimes ($P$>0.20). The efficiency of muscle during prey capture was 9.9 % in comparison with 6.3 % during escape responses.

Figure 4.2. Representative plots of strain against cycle time for A) an escape response, and B) a prey capture response. C and D, corresponding plots of $P/P_0$ (here written as $P/P_{max}$) and E and F, $V/V_{max}$.

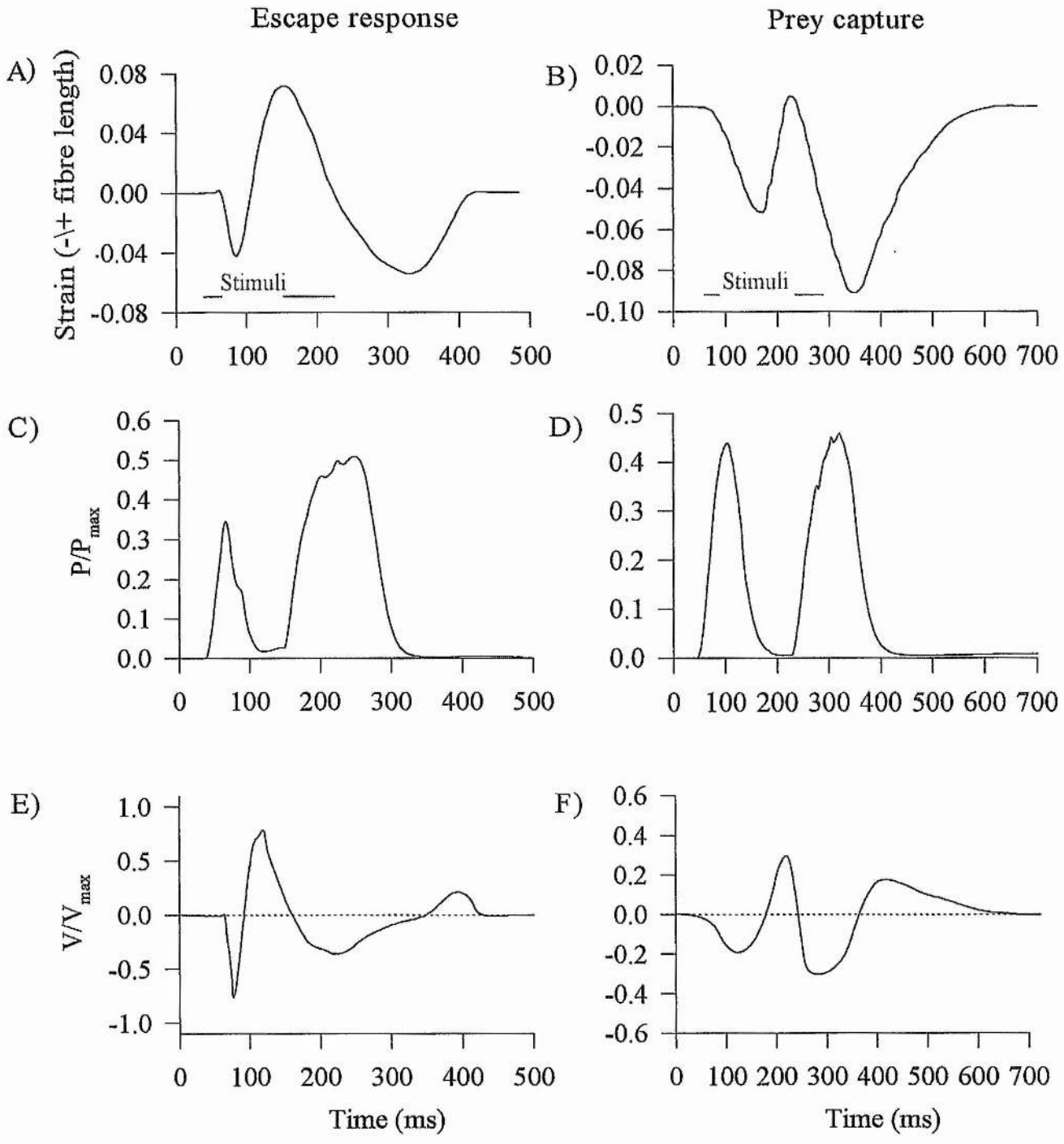

Escape response
Prey capture
A)
B)
C)
D)
E)
F)
Strain (-\+ fibre length)
Stimuli
Stimuli
P/P max
V/V max
Time (ms)
Time (ms)

Figure 4.3. Work loops for A) an escape response, and B) a prey capture response in the short-horn sculpin. C is the corresponding plot of instantaneous power output for the escape response. D is the corresponding plot of instantaneous power output for the prey capture response.

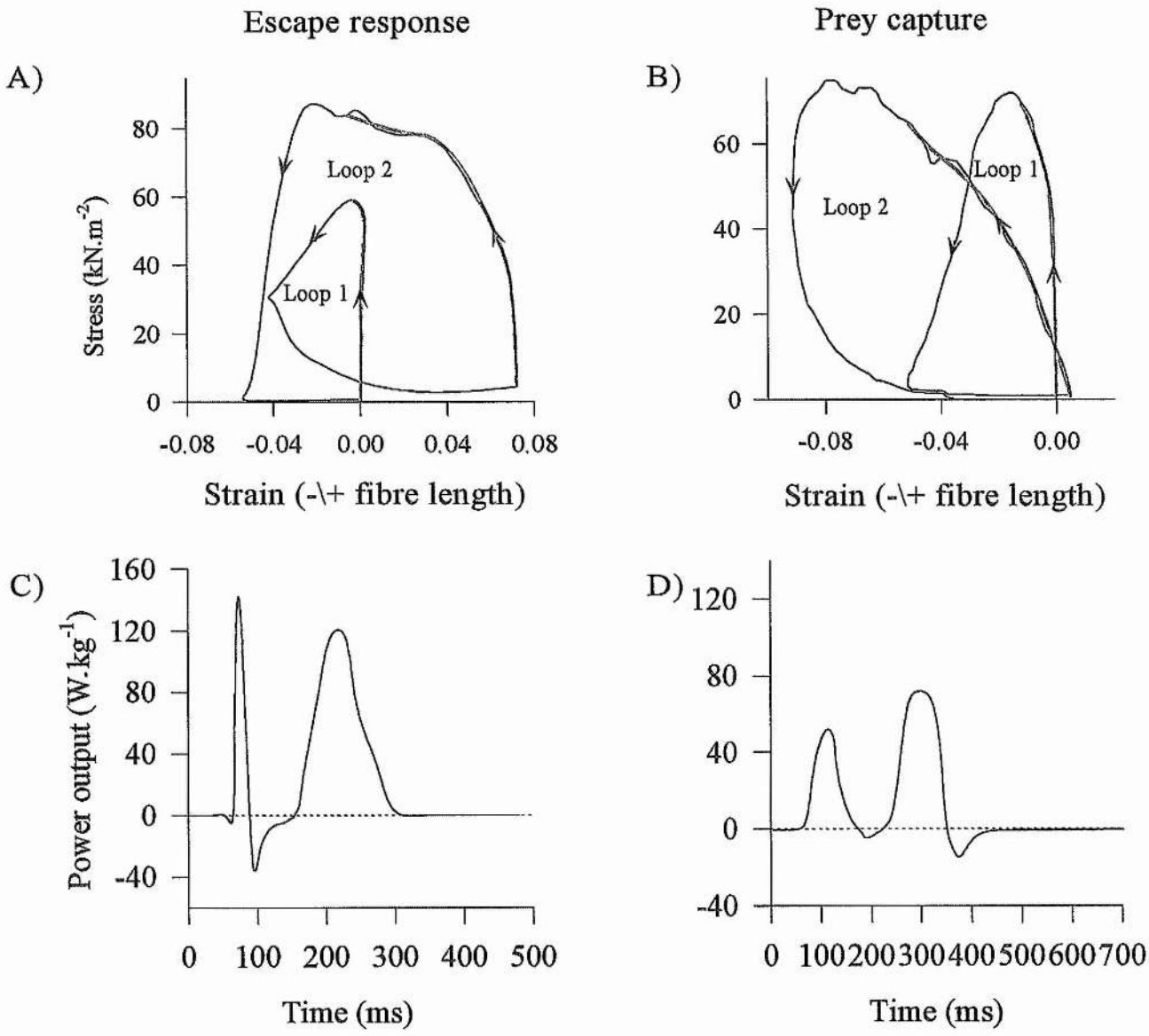

Escape response
Prey capture
A)
B)
Stress (kN.m-2)
Loop 2
Loop 1
Strain (-\+ fibre length)
Strain (-\+ fibre length)
C)
D)
Power output (W.kg-1)
Time (ms)
Time (ms)

Figure 4.4. A) Fibre length and B) force plotted against time for a sequence of 4 escape response cycles. Force *versus* fibre length produces work loops, shown for the 1st (C) and 4th (D) cycles. E) Representative HPLC trace following 4 escape response cycles. Inset is a control HPLC trace.

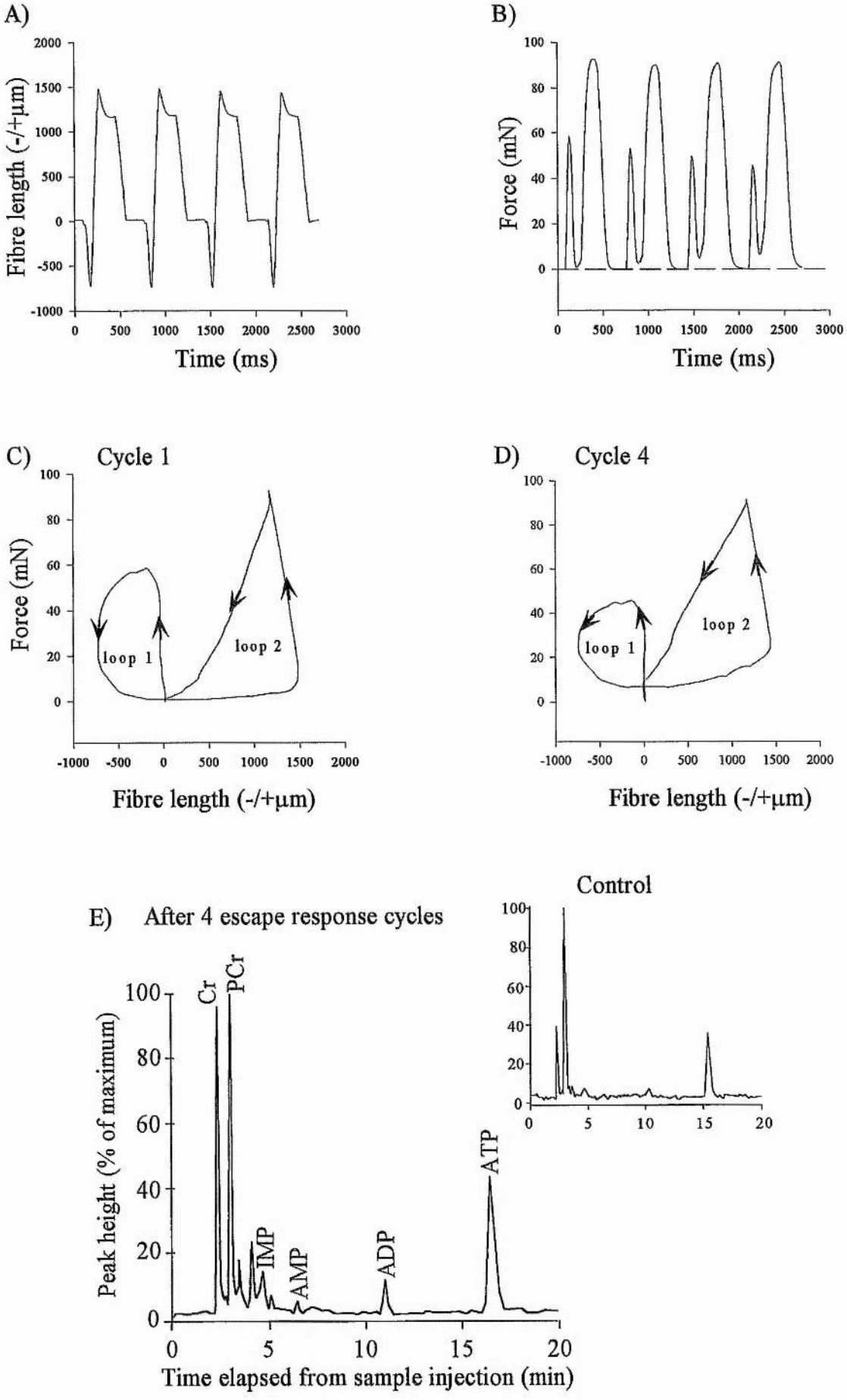
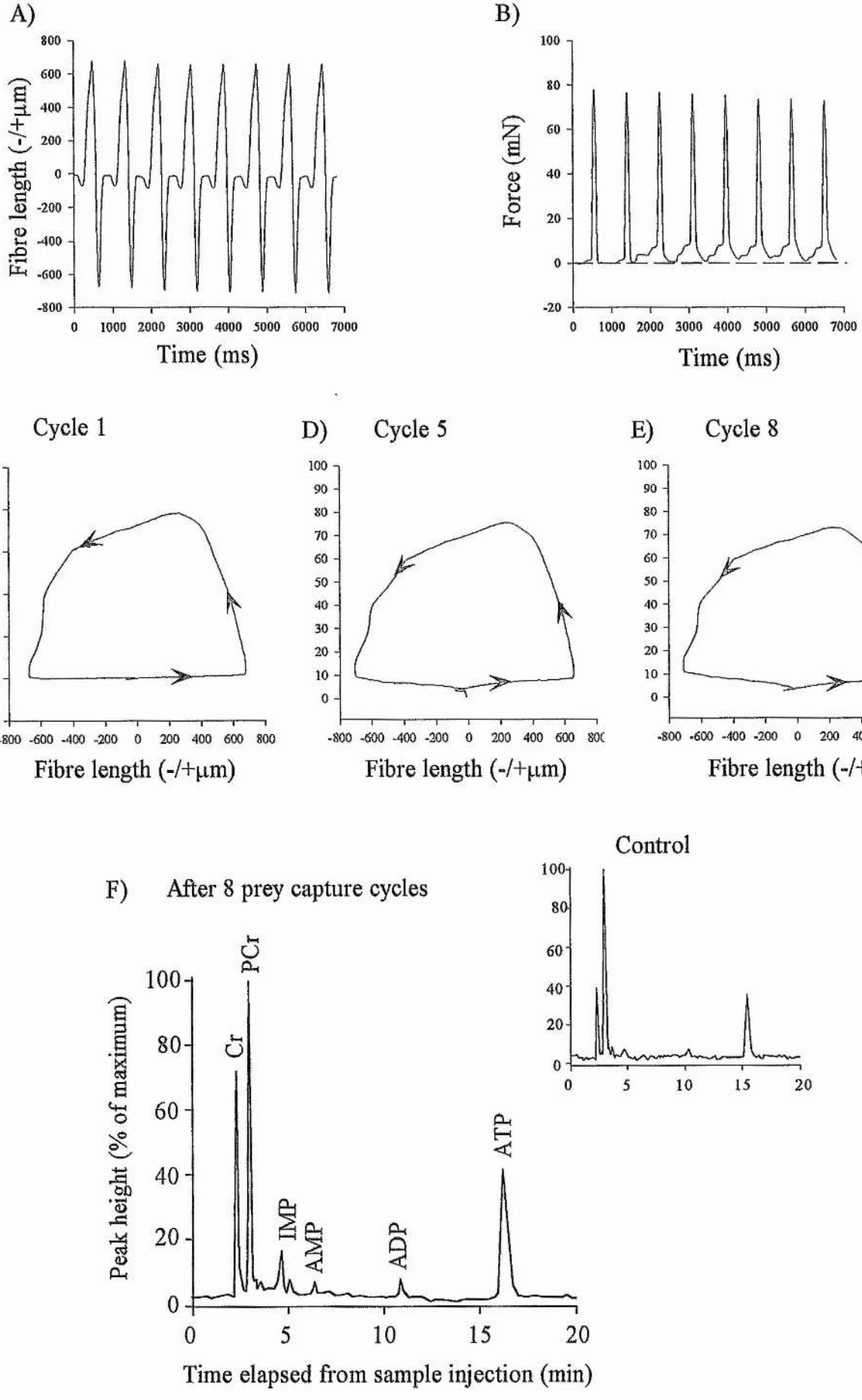

A)
Fibre length (-/+µm)
Time (ms)
B)
Force (mN)
Time (ms)
C)
Cycle 1
Force (mN)
loop 1
loop 2
Fibre length (-/+µm)
D)
Cycle 4
loop 1
loop 2
Fibre length (-/+µm)
E)
After 4 escape response cycles
Control
Peak height (% of maximum)
Cr
PCr
IMP
AMP
ADP
ATP
Time elapsed from sample injection (min)

Figure 4.5. A) Fibre length and B) force plotted against time for a sequence of 8 prey capture response cycles. Force *versus* fibre length produces work loops, shown for the 1st (C), 5th (D) and 8th (E) cycles. F) Representative HPLC trace following 8 prey capture response cycles. Inset is a control HPLC trace.


A)
Fibre length (-/+µm)
Time (ms)
B)
Force (mN)
Time (ms)
C) Cycle 1
Force (mN)
Fibre length (-/+µm)
D) Cycle 5
Fibre length (-/+µm)
E) Cycle 8
Fibre length (-/+µm)
F) After 8 prey capture cycles
Peak height (% of maximum)
Cr
PCr
IMP
AMP
ADP
ATP
Time elapsed from sample injection (min)
Control

## 4.4 Discussion

### *4.4.1 Kinematics*

This main conclusions of this study are that fast-starts used for prey capture and escape response behaviours in the short-horn sculpin are energetically very similar. Maximum swimming velocity, maximum stress, muscle power output and muscle economy and efficiency are not significantly different between the two responses. This is in spite of the dramatically different kinematics of the two fast-starts.

Webb (1984) predicated that the locomotory features of the cottids would enable high accelerations and velocities. Norton (1991) studied the dependence of capture success of specific prey species on the mouth morphology of various cottid predators. Prey capture techniques in cottids were found to lie on a continuum between ram feeding and suction feeding. Elusive prey such as shrimp were caught successfully by large mouthed cottid species using ram feeding. Species with smaller mouth morphologies relied more heavily on suction feeding which lead to a lower capture success of shrimp. The short-horn sculpin used in the present study is a large mouthed cottid which uses whole body accelerations in addition to suction for prey capture of *Crangon crangon* (Beddow *et al.* 1995).

In the present study mean maximum velocities and response durations were similar for both prey capture and escape fast-starts in the short-horn sculpin. In contrast, in the pike (*Esox lucius*) prey capture responses had significantly lower velocities and accelerations and longer mean durations, than for escape responses (Harper and Blake, 1991; Frith and Blake, 1995). Depending on the prey species, some fish predators have been observed to modulate their attack behaviour (Norton, 1991; Norton and Brainerd, 1993; Nemeth, 1997*a*, *b*). In the kelp greenling (*Hexagrammos decagrammus*), ram feeding repertoires with high attack velocities were elicited in response to elusive shrimp species, whereas crabs and pieces of shrimp evoked suction feeding patterns with lower attack velocities (Nemeth, 1997*a*). Pike have also been observed to modulate their attack behaviour into four types of feeding fast-start with differing durations, displacements and accelerations (Harper and Blake, 1991). However, these were all elicited in reaction to the same prey type, goldfish, which rarely escaped. Perhaps a more elusive prey would

have encouraged higher attack speeds and accelerations approaching those of escape fast-starts.

### *4.4.2 Variability of fast-starts*

In the present study, the kinematics and parameters of the muscle strain cycle were found to be highly variable during fast-starts, which contributed to the high error bars in the analysis. In escape responses, turning angles varied from tail-beat to tail-beat, as has been found in angelfish (*Pterophyllum eimekei*) (Domenici and Blake, 1993*a*). Muscle strain fluctuations were also changeable. Franklin and Johnston (1997) found the magnitude of the muscle strain during escape responses of the Antarctic fish, *Notothenia coriiceps*, was dependent on the final direction of travel. However, in the present study, the significantly greater turning angles achieved during escape than prey capture responses were not matched by greater values of muscle strain. In fact strain was greater, although not significantly so, in prey capture than escape responses. Since the total strain cycle duration was also greater in prey capture than escape fast-starts muscle shortening velocities were comparable between the two behaviours (Table 4.1).

Prey capture responses consisted of either one or two tail-beats. The amount of muscle strain during a response again varied from tail-beat to tail-beat despite the fish maintaining a straight trajectory towards the prey. If the original stationary position of the trunk of the fish was not directly in line with the prey, then unequal modulations in muscle strain were required to take the fish towards the prey whilst maintaining the head-on position of the snout.

### *4.4.3 Contractile properties*

Using the *in vivo* parameters of muscle strain and activation recorded from sonomicrometry and electromyography, respectively, work loop experiments demonstrated that maximum instantaneous power output was similar between the two behaviours (127 and 151 W.kg$^{-1}$ for escape and prey capture, respectively). These values were comparable to those measured in Chapter 3, where power output was about 150 W. kg$^{-1}$ for the C-bend and contralateral contraction of sculpin escape responses at 5 °C. On the other hand values of mean power output were lower than those previously reported

for this species (Chapter 3). Values of mean power output were about half that reported for the C-bend and contralateral contraction individually. Undoubtedly variations in strain and activation parameters were a contributing factor to this discrepancy. Differences may also be due to acclimation state or health of the fish. In addition, errors could have arisen in the determination of fibre bundle cross-sectional area.

### *4.4.4 Energetics*

The economy values calculated for the two types of fast-starts were not significantly different. However, variation in the results was high and values for prey capture were actually 57 % higher than for escape. The values of the present study are lower than reported previously for fish muscle. For sculpin fast muscle subjected to sinusoidal length changes, Johnson *et al.* (1991) reported values of 12-15 mJ. $\mu mol^{-1}$ at 4 °C and 3-6 mJ. $\mu mol^{-1}$ at 15 °C. Efficiency values in the present study were also lower than those previously reported. During sinusoidal movement of fast muscle fibres of dogfish (*Scyliorhinus canicula*), Curtin and Woledge (1993) reported a value of 41 %. In cod (*Gadus morhua*), Moon *et al.* (1991) reported efficiency values of 12 and 23 % for 8 and 16 cycles, respectively. For sculpin fast muscle fibres, again under sinusoidal length fluctuations representing steady swimming, efficiency values ranged from 21-26 % at 4 °C to 6-10 % at 15 °C (Johnson *et al.* 1991). These fish had been kept at ambient sea water temperature and were acclimated to summer water temperatures. In general muscles contract over a narrow range of $V/V_{max}$ such that power and efficiency are close to maximal (Rome *et al.* 1988, 1990, 1992; Rome and Sosnicki, 1990, 1991; James and Johnston, 1998; Chapter 3). However, it was proposed that the efficiency of sculpin muscle was partially sacrificed at 15 °C in exchange for a higher power output that would enable higher tail-beat frequencies. In the current investigation the efficiency of 5 °C-acclimated sculpin muscle under escape and prey capture parameters was 6.3 and 9.9 %, respectively. It is known that the compensation of kinematic performance (Chapter 2) and power output (Chapter 3) is not perfect between 5 and 15 °C-acclimated fish. The similar values of efficiency in 5 °C-acclimated fish of the present study and 15 °C-acclimated fish of the study of Johnson *et al.* (1991) can therefore not be explained on thermal grounds alone. Curtin and Woledge (1996) subjected dogfish fast muscle fibres to

sinusoidal length changes and also found that high power was achieved at the expense of efficiency and was dependent on the stimulus duty cycle. The present investigation used strain and activation patterns from fast-starts which require high power output of the muscle fibres once again apparently at the expense of efficiency.

Different species of animals have been found to have different efficiencies of muscular contraction (see Table 1, Curtin and Woledge, 1991). Dogfish muscle has an efficiency during continuous tetanic stimulation of 0.33 (Curtin and Woledge, 1991), whereas frog muscle has an efficiency of 0.45 (Hill, 1964). Lou *et al.* (1997) recently used heat measurements and examined the energetic cost of activation of the fast muscle fibres from the dogfish and found it to be similar to that of frog muscle. It was therefore suggested that the lower efficiency of the dogfish muscle was due to less efficient conversion of chemical energy into mechanical work by the crossbridges, rather than a larger expenditure of energy by activation processes cycling calcium. It has been suggested that high activation costs may be partly responsible for the low efficiency of insect flight muscle (Josephson and Stevenson, 1991). Locust flight muscle has an efficiency of about 6 %. Ellington (1985) predicted muscle efficiency of less than 10 % from free hovering flight in several insects. Higher costs of calcium cycling have been thought to result from the high frequency at which insect muscle operates.

Higher values of efficiency may be attributable to active pre-stretch. In muscles from mammals (Heglund and Cavagna, 1985; 1987) and amphibians (Heglund and Cavagna, 1987) the values of efficiency when the muscle was actively stretched were in agreement with those measured *in vivo* during running (Heglund and Cavagna, 1987). Curtin and Woledge (1993) suggest that for stretch to enhance efficiency, energy conversion by the crossbridges must be more efficient. Active pre-stretch may partially explain the higher values of efficiency during prey capture than during escape responses in the present study.

Harper and Blake (1988) modelled the energetics of prey capture and predicted that the strike accounts for up to 80 % of the total energy expended by the predator. Energetic benefit, i.e. the ratio of the energy gained from the prey to the energy expended in catching the prey, was predicted to be high when predator length and the angle at which the attack was initiated was small and the prey was naive. In the present study,

values of energy content for *Crangon* prey from Johnston and Battram (1993) were used to roughly estimate how many fast-starts one prey capture would fuel. Energetic benefit appeared to be high. Using a low value for the efficiency of utilization of 10 % would still enable about 250 fast-starts. This value should certainly cover escape responses and unsuccessful prey captures until the next *Crangon* was consumed. However, these fast-starts are not the complete behaviours but only the first two tail-beats at most. For escape responses of longer duration, the energy cost of further tail-beats would have to be included and in the case of prey capture, the additional energetic cost of consumption.

*Chapter 5*

# THE THERMAL ACCLIMATION OF MUSCLE PERFORMANCE, AS PREDICTED FROM WHOLE ANIMAL PERFORMANCE, IN THE SHORT-HORN SCULPIN AND LONG-SPINED SEA SCORPION

## 5.1 Introduction

Fish with deep bodies and wide, deep caudal fins are adapted for rapid accelerations as used during fast-starts. Thrust is generated by the combined action of the median fins and locomotor waves passing back along the body (Weihs, 1973; Frith and Blake, 1991; Bone *et al.* 1995). Benthic fish rely on accelerations for fast-starts employed from rest during critical predator-prey encounters (Webb, 1990, 1993). For example, as has been seen in the previous chapters and other studies, the short-horn sculpin (*Myoxocephalus scorpius*), a benthic, marine cottid, uses fast-starts powered by the fast muscle fibres for prey capture (Beddow *et al.* 1995; Chapter 4) and escape responses (Chapter 2, 3, 4; James and Johnston, 1998).

To date all escape responses have been called C-starts (Domenici and Blake, 1997), 'C' being the shape of the fish after the first contraction of the myotomal musculature (Weihs, 1973; Webb, 1978*a*). Many aspects of C-start escape behaviour of adult fish have been extensively studied in a number of teleost species (see Domenici and Blake, 1997, for review), particularly rainbow trout (*Oncorhynchus mykiss*) (Covell *et al.* 1991; Harper and Blake, 1990; Webb, 1975, 1976, 1977, 1978*a*, *b*; Weihs, 1973) and goldfish (*Carassius auratus*) (e.g. Eaton *et al.* 1988; Eaton and Emberly, 1991; Foreman and Eaton, 1993; Johnson and Bennett, 1995; Johnson *et al.* 1993). However, relatively few studies have sought to calculate power output which is thought to be a limiting factor to fast-start performance (Frith and Blake, 1995; Wakeling and Johnston, 1998). Frith and Blake (1995) used a modification of the Weihs (1973) hydrodynamic model to estimate mechanical power output and hydrodynamic efficiency for northern pike (*Esox lucius*) fast-starts. They estimated the useful power as that required to accelerate the mass of the fish and the added mass of water associated with the fish in its direction of motion. In addition, hydrodynamic power is also expended in a direction perpendicular to the velocity of motion and thus a total power was calculated as the resultant of these normal

and tangential components. The hydrodynamic efficiency was the ratio of useful to total power. Efficiency was found to be lower than that reported for burst-and-glide and steady swimming behaviours. The authors state that this could result from the higher cost of decelerating fins and sections of the body during fast-starts which use high tail-beat frequencies and amplitudes. The power output of pike escape responses was estimated at approximately 300 $W.kg^{-1}$ muscle at a temperature of about 10 °C (Frith and Blake, 1995).

Several recent studies have measured power output during escape responses in a more direct way. Muscle strain and activation patterns during escape responses were measured using sonomicrometry and electromyography (EMG), respectively, and these *in vivo* muscle performance patterns were then used in work loop experiments (Josephson, 1985) *in vitro* (Franklin and Johnston, 1997; James and Johnston, 1998; Chapter 3, 4). For a particular muscle shortening cycle, i.e. C-bend or contralateral contraction, a plot of muscle force by muscle strain gave the work over the cycle, and the work per unit time, the power output. In the Antarctic fish, *Notothenia coriiceps*, Franklin and Johnston (1997) measured the mean mass-specific power to be about 16 $W.kg^{-1}$ at 0 °C for rostral fast muscle fibres during both the C-bend and contralateral contraction. Using the same methods, James and Johnston (1998) calculated mean power output to be around 41 $W.kg^{-1}$ at 12 °C for both contractions in the short-horn sculpin. These values are significantly lower than those estimated by Frith and Blake (1995) for pike. Although pike are acceleration specialists and achieved velocities and accelerations that are almost twice that of sculpin, this can not explain the entire difference. Indeed the power requirements for pike escape responses are about two times the power output of the fast twitch muscle fibres of the lizard *Dipsosaurus dorsalis*, one of the highest values measured using work loop experiments (Swoap *et al.* 1993).

Wakeling and Johnston (1998) have employed a method for estimating power from whole body performance that correlated well with values obtained using the *in vivo* and work loop methods outlined above. The methods were similar in principle to Frith and Blake (1995) and involved estimating useful power from velocity and acceleration data of the centre of mass. This required defining the mass distribution down the length

of the fish. Total power was calculated by applying a value of hydrodynamic efficiency based on Frith and Blake's study (1995).

The aim of this study was to apply the methods of Wakeling and Johnston (1998) to investigate the hydrodynamic power requirements during fast-starts of the short-horn sculpin and long-spined sea scorpion (*Taurulus bubalis*) acclimated to summer and winter water temperatures. The results were compared with measurements of muscle power output during the same behaviours obtained from work loop experiments (Chapter 3).

## 5.2 Materials and methods

### *5.2.1 Fish*

Short-horn sculpin (*Myoxocephalus scorpius* L.) and long-spined sea scorpion (*Taurulus bubalis* Euphr.) were collected between March 1995 and November 1996. Short-horn sculpin were caught using trawls and creels off the Fife coast and Isle of Cumbrae, Scotland and long-spined sea scorpion were collected on rocky shores around St. Andrews, Scotland, using hand nets. Fish (short-horn sculpin, 161.8±3.0 mm total length ($L$), 59.4±3.2 g wet mass, $N$=27; sea scorpion, 115.1±1.3 mm $L$, 27.7±1.8 g wet mass, $N$=27: lengths are means ± S.E.M.) were acclimated to one of two temperatures. During winter months (November to March) fish were caught and acclimated to 5±0.5 °C for a minimum of six weeks in a re-circulating sea water aquarium. Fish were kept under a 10 hour light: 14 hour dark photoperiod regime. Similarly, during the summer months (June to September) fish were caught and acclimated to 15±0.5 °C and kept under a 13 hour light: 11 hour dark photoperiod regime. In both cases fish were fed on prawns and squid twice a week.

### *5.2.2 Measurement of fast-start performance*

Following thermal acclimation to 5 or 15±0.5 °C, fish were transferred to a 2.0×0.6×0.25 m static filming arena at their acclimation temperature. Escape responses were elicited by tactile stimulation (see Chapter 2) and filming was carried out using a high speed ciné camera (NAC-Japan) operating at 500 frames $s^{-1}$, *via* a mirror set at 45 ° above the tank. 70 W fluorescent strip lights beneath the swim tank enabled sharp silhouettes of the fish to be recorded on ciné film. All fish were filmed at 0.8, 5.0, 15.0 and 20.0 °C. To achieve this the tank temperature was changed by 1 °C every half hour and the fish left for one hour prior to filming at the new temperature.

### *5.2.3 Kinematic analysis*

The methods used in this study were after Wakeling and Johnston (1998).

Prior to analysing the films, it was necessary to establish the mass distribution along the fish. The method used assumes the fish to have an elliptical profile in cross section and the volume distribution to approximate the mass distribution. Planform and

longitudinal images of 11 straight-stretched fish of each species were scanned into a Power MacIntosh computer. The fish chord of these outlines represented the minor and major axis of the ellipse, respectively. The mass of ten equally spaced segments was calculated from the width, depth and longitudinal dimensions of each segment and expressed as non-dimensional mass, i.e. the mass of a segment divided by the total mass of the fish. The non-dimensional mass of each segment was averaged from all fish of a species.

After processing, the films were digitized using MOVIAS-NAC Corp. software. Ten points were digitized down the centre of the fish from snout to tail tip every fourth frame (8 ms) (see Chapter 2). These points are assumed to lie on the spine. Measurements of fast-start performance included calculating the velocity and acceleration of the centre of mass. For these calculations, the centre of mass was not taken as that lying permanently on the spine of the fish. During bending, as for example when a fish goes into the C-bend, the centre of mass of the fish will lie within the 'C'; this has been called the true centre of mass and has position vector $\mathbf{q}$. During swimming the course of this centre of mass is much straighter than that of the centre of mass positioned on the spine and consequently velocity and acceleration calculated using this point are less than the values calculated using the centre of mass on the spine.

To estimate $\mathbf{q}$ it was necessary to calculate the mean position of the centre of ten equal segments down the length of the fish. For each digitized frame of an escape response, the spine data was fitted using a cubic spline and the positions on the spine for the centre of ten equally spaced segments (position vectors for the centres of each segment) were calculated. Therefore,

$$\mathbf{q} = \frac{\sum \text{the position vector for each segment} \times \text{the mass of each segment}}{\text{total body mass}}$$

Thirty frames were digitized before and after the response which guaranteed including the start and end of the fast-start and avoided the problems associated with smoothing the first and last few frames (Harper and Blake, 1989). $x$ and $y$ position data were smoothed using piecewise cubic regressions (Mathmatica, Wolfram Research Inc. USA) as described in Chapter 2.

### *5.2.4 Estimation of power requirements*

Force is the product of acceleration and mass. Since power is the product of force and velocity,

$$\text{power} = \text{mass} \times \text{velocity} \times \text{acceleration}.$$

Fast-starts involve rapid accelerations and therefore inertial power, $P_i$, is a good estimate of the hydrodynamic power of fish fast-starts (Wakeling and Johnston, 1998). Fish motion also causes the acceleration of water adjacent to the body (Weihs, 1973). Therefore in order to calculate $P_i$, the mass component must include this added mass of water that is accelerated with the fish during the escape response. The added mass of water for fast-starting fish was taken as 0.2 mass ($m$), after Webb (1982). Using the values of velocity, acceleration and mass obtained above, mean inertial power was estimated for the first half tail-beat in which the wave of body curvature could be seen to travel the length of the fish (see Fig. 5.2). This equated to the contralateral contraction, the propulsive stage of the escape response (Weihs, 1973).

The hydrodynamic efficiency is the ratio of inertial power to the total hydrodynamic power. The total hydrodynamic power is the power expenditure of both forward and lateral movement, whereas the useful hydrodynamic power is the component which propels the fish in the direction of travel. Frith and Blake (1995) calculated the hydrodynamic efficiency of pike fast-starts. Wakeling and Johnston (1998) used their data to estimate a value of 0.31 for efficiency in sculpin. In order to calculate the total hydrodynamic power ($P_t$) of the fast-starts in our study, the useful inertial power was divided by this value of hydrodynamic efficiency.

To convert values of power into $W.kg^{-1}$ of muscle, power was divided by the muscle mass; in the short-horn sculpin the muscle mass is 0.298 of the total mass (Wakeling and Johnston, 1998). It was decided that this value could be used for both species as they are closely related members of the family Cottidae and are morphologically very similar.

### *5.2.5 Velocity of the wave of curvature*

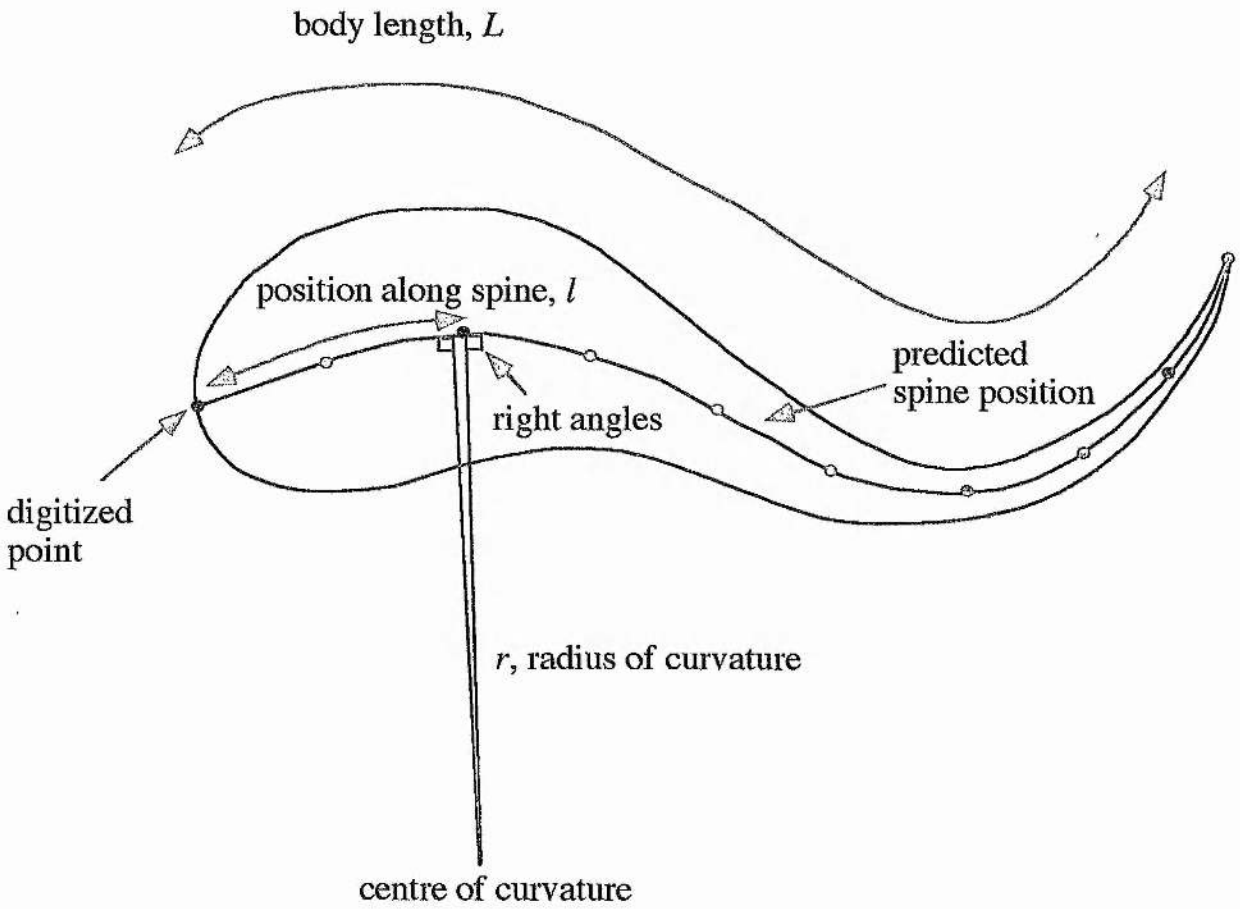
During fast-starts, waves of curvature travel from head to tail along the length of the fish. Non-dimensional curvature, $\hat{c}$, was used as an index of the amount of bend in

the spine. This was estimated for each analysed frame of the fast-start. Figure 5.1 is a diagrammatic representation of the methods involved. The cubic spline fitted to the spine data was used to calculate the radius of curvature of the spine at all positions along its length; length-specific position, $\hat{l}$, is the distance along the spine from the snout divided by the total fish length, $L$. Non-dimensional curvature was calculated by dividing length by the radius of curvature, $r$. Curvature values of 0 signify a straight spine, whilst positive and negative values represent bending to the left and right respectively. $\hat{c}$ was plotted against length-specific body position and time to produce a contour plot (Fig. 5.2). A straight line was drawn through the first full wave of curvature travelling down the fish. The slope of this line was inversely proportional to the rate of the wave of curvature, $\hat{U}$ (see Fig. 5.2).

### *5.2.6 Statistics*

A two-way General Linear Model (GLM) ANOVA was used to examine the effects of acclimation and acute temperature on power and the velocity of the curvature wave. A one-way ANOVA was employed to examine the effects of acclimation temperature at all four test temperatures. If fish were unable to swim (scored as zero), Mann-Whitney $U$ tests were used to test for differences between acclimation to 5 and 15 °C at that particular test temperature. All statistical tests were computed using Minitab software (Minitab Inc. USA).

Figure 5.1. Methods for calculating non-dimensional curvature ($\hat{c}$) along the fish during a fast-start. See text for further details.




Length specific position $\hat{l} = \frac{l}{L}$

Non-dimensional curvature $\hat{c} = \frac{L}{r}$, and is calculated for all locations along the spine

During fast-starts, waves of curvature travel from head to tail along the spine with length-specific velocity $\hat{U}$.

## 5.3 Results

Plots of non-dimensional curvature against time and length-specific body position are shown for the long-spined sea scorpion in Figure 5.2. The slope of the straight line passing through the crest of one full travelling wave is the inverse of the velocity of the curvature wave, $\hat{U}$. There was a clear acclimation effect on $\hat{U}$ in long-spined sea scorpion in that fish swimming at 20.0 °C had greater values of $\hat{U}$ when they were acclimated to 15 °C than 5 °C whereas fish swimming at 0.8 °C had greater $\hat{U}$ when they were acclimated to 5 °C than 15 °C. Only 40 % of fish acclimated to 15 °C could swim at 0.8 °C. Tukey and Mann-Whitney U tests revealed these acclimation effects to be significant ($P$<0.05). The two-way ANOVA revealed mean $\hat{U}$ to be similar between the two acclimation temperatures ($P$=0.329; Fig. 5.3C, Table 5.1). Adjusted means were 10.66 and 9.8 for the 5 and 15 °C-acclimated groups, respectively. However, acclimation temperature did effect the mean power; power was 47 % higher in the 15 °C- than 5 °C-acclimated fish. Tukey tests revealed power to be significantly higher at 20 °C in the 15 °C- (98 $W.kg^{-1}$) than the 5 °C-acclimated group (42 $W.kg^{-1}$) ($P$<0.05). Mann-Whitney U tests revealed significantly higher values for power for 5 °C- than 15 °C-acclimated fish at 0.8 °C (11.26 and 2.7 $W.kg^{-1}$, respectively) ($P$<0.05) (Fig. 5.3D).

Acclimation temperature had a significant effect on both the $\hat{U}$ ($F_{(3,57)}$=11.12, $P$<0.002) and power requirements ($F_{(3,57)}$=5.72, $P$<0.02) of short-horn sculpin and there was significant interaction between acclimation and test temperature in both of these parameters ($\hat{U}$, $F_{(3,57)}$=7.16, $P$<0.001; power, $F_{(3,57)}$ =3.49, $P$<0.019) (Fig. 5.3A and B; Table 5.1). At 20.0 °C there was a 130 % increase in $\hat{U}$ following acclimation to 15 °C compared to 5 °C ($\hat{U}$=11.1 and 4.8, respectively). However, there was no trade-off in this performance parameter at low temperature. Mean power was 37 % higher for the 15 °C than the 5 °C-acclimated group but Tukey tests did not reveal there to be any significant differences between the two acclimation groups at specific test temperatures. Despite this the $Q_{10}$s were quite different for the two acclimation groups between 0.8 and 5.0 °C; the $Q_{10}$ for power in 5 °C-acclimated fish was 1.53 in comparison to 16.76 for the 15 °C-acclimated fish (Table 5.2).

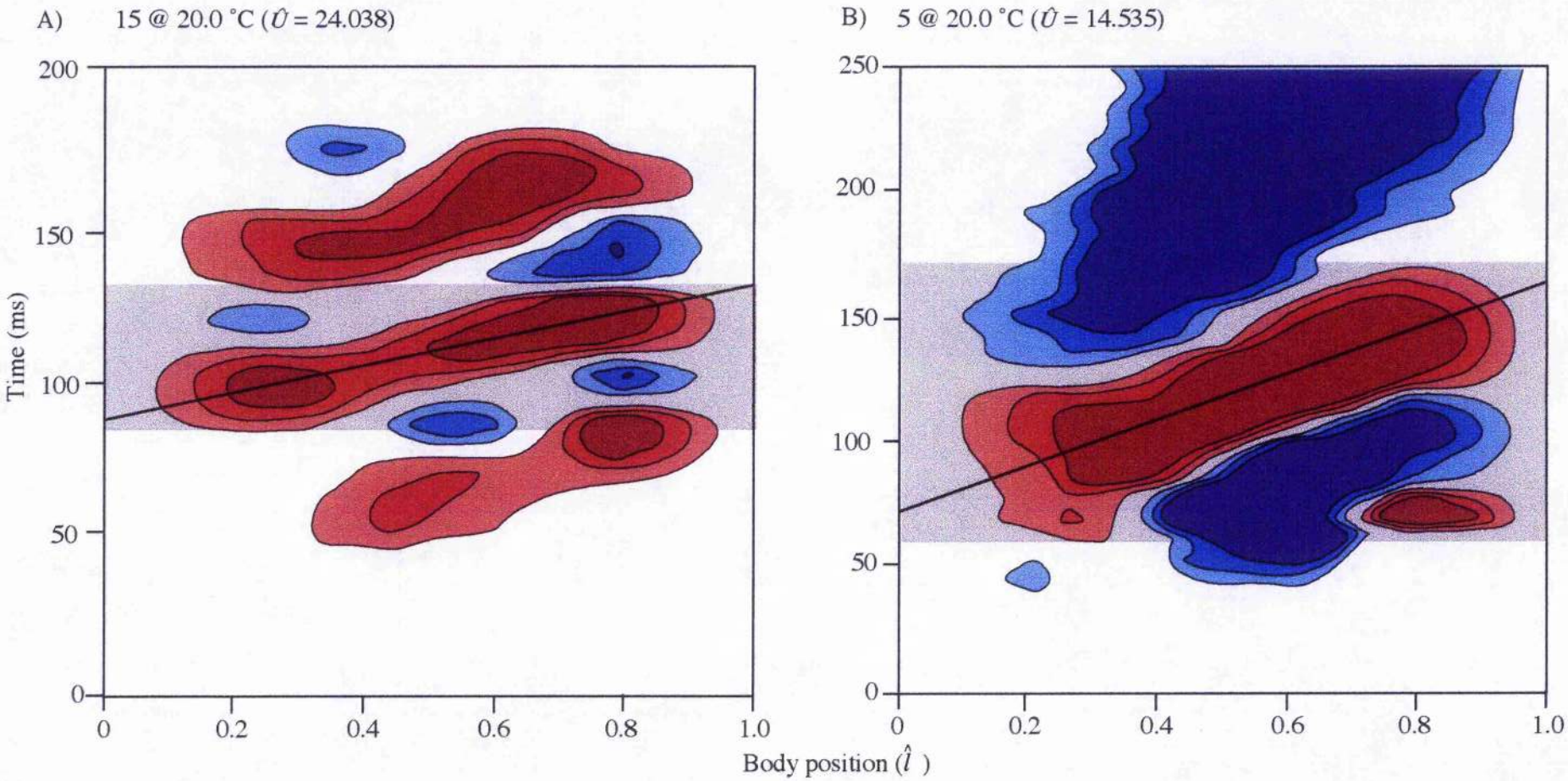
Test temperature had a significant effect on $\hat{U}$ in both species (short-horn sculpin $F_{(3,57)}$=5.93, $P$<0.001; long-spined sea scorpion $F_{(3,57)}$=21.65, $P$<0.0005) (Fig. 5.3A and C; Table 5.1). Between test temperatures of 0.8 and 15.0 °C, the adjusted mean $\hat{U}$ increased by 38 % in the short-horn sculpin and by 158 % in the long-spined sea scorpion (Fig. 5.3A and C). Test temperature furthermore affected the power in both species (short-horn sculpin $F_{(3,57)}$=21.07, $P$<0.0005; long-spined sea scorpion $F_{(3,57)}$= 19.84, $P$<0.005) (Fig. 5.3B and D; Table 5.1). Tukey tests revealed that in 15 °C-acclimated fish of both species, power increased significantly between 5.0 and 15.0 °C ($P$<0.05) and also between 15 and 20 °C for long-spined sea scorpion ($P$<0.05). Power increased by a much greater percentage between test temperatures of 0.8 and 15.0 °C than $\hat{U}$; in short-horn sculpin and long-spined sea scorpion the percentage increases were 310 % and 762 %, respectively (Fig. 5.3B and D).

Table 5.1. *Analysis of variance for the velocity of the curvature wave passing down the length of the fish and the power requirements during the second half tail-beat of escape responses.*

| | | ***Myoxocephalus scorpius*** | | | ***Taurulus bubalis*** | | |
|---|---|---|---|---|---|---|---|
| Variable | Source | d.f. | *F*-ratio | *P* | d.f. | *F*-ratio | *P* |
| $\hat{U}$, $s^{-1}$ | A | 1 | 11.12 | 0.002 | 1 | 0.97 | 0.329 |
| | B | 3 | 5.93 | 0.001 | 3 | 21.65 | 0.000 |
| | A × B | 3 | 7.16 | 0.000 | 3 | 8.62 | 0.000 |
| | Error | 57 | | | 57 | | |
| Power, $W.kg^{-1}$ | A | 1 | 5.72 | 0.020 | 1 | 6.09 | 0.017 |
| | B | 3 | 21.03 | 0.000 | 3 | 19.84 | 0.000 |
| | A × B | 3 | 3.59 | 0.019 | 3 | 5.51 | 0.002 |
| | Error | 57 | | | 57 | | |

A, acclimation temperature; B, test temperature; d.f., degrees of freedom

Figure 5.2. Contour plots showing how the non-dimensional body curvature ($\hat{c}$) changes with time, body position and temperature during fast-starts of the long-spined sea scorpion. The slope of the solid line is inversely proportional to the rate the wave of curvature ($\hat{U}$) travels down the fish. The grey area indicates the period over which the mean inertial power requirements were calculated.


A) 15 @ 20.0 °C ($\hat{U}$ = 24.038)
B) 5 @ 20.0 °C ($\hat{U}$ = 14.535)
Time (ms)
Body position ($\hat{l}$ )
0
50
100
150
200
250
0.2
0.4
0.6
0.8
1.0

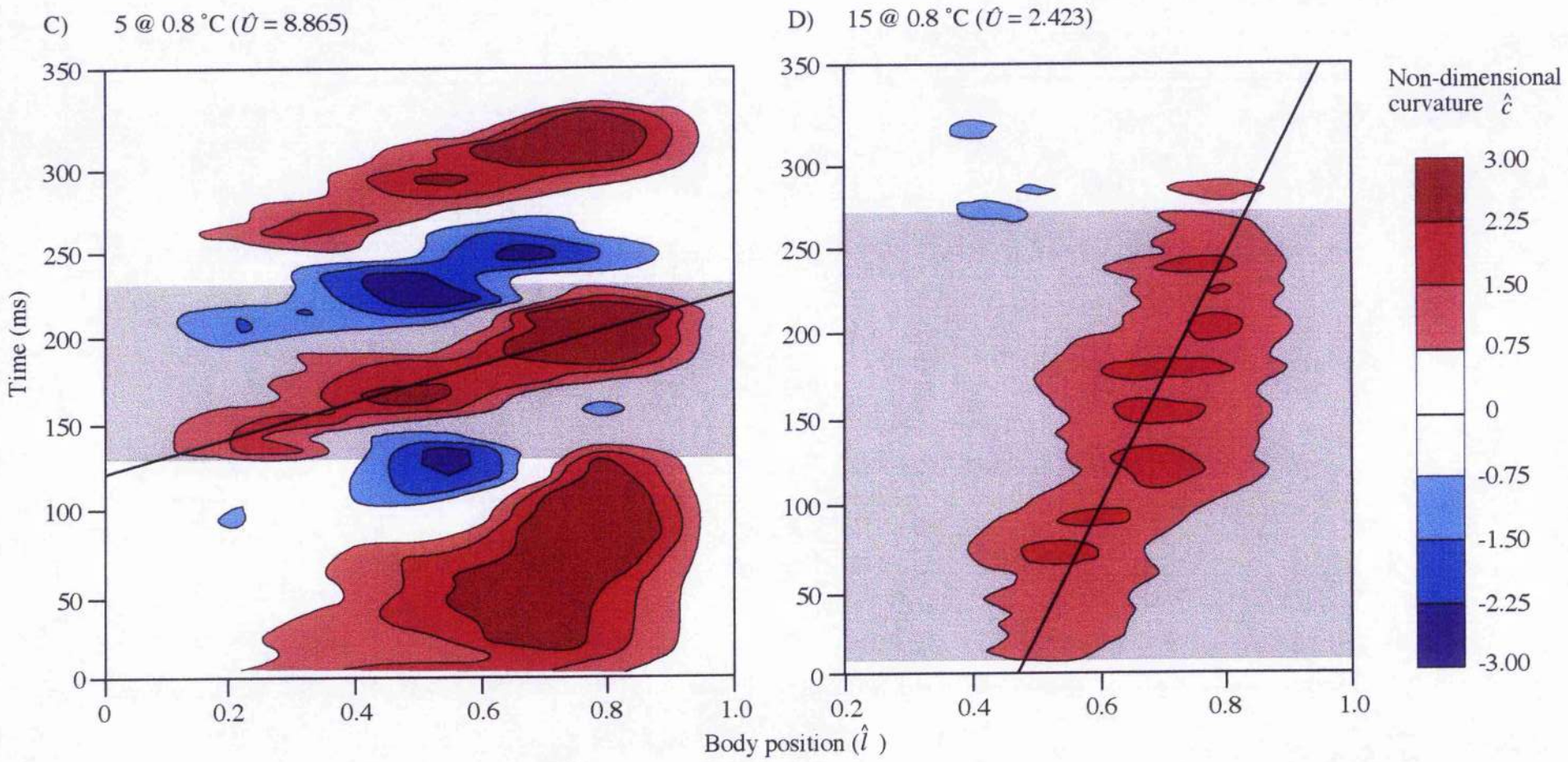
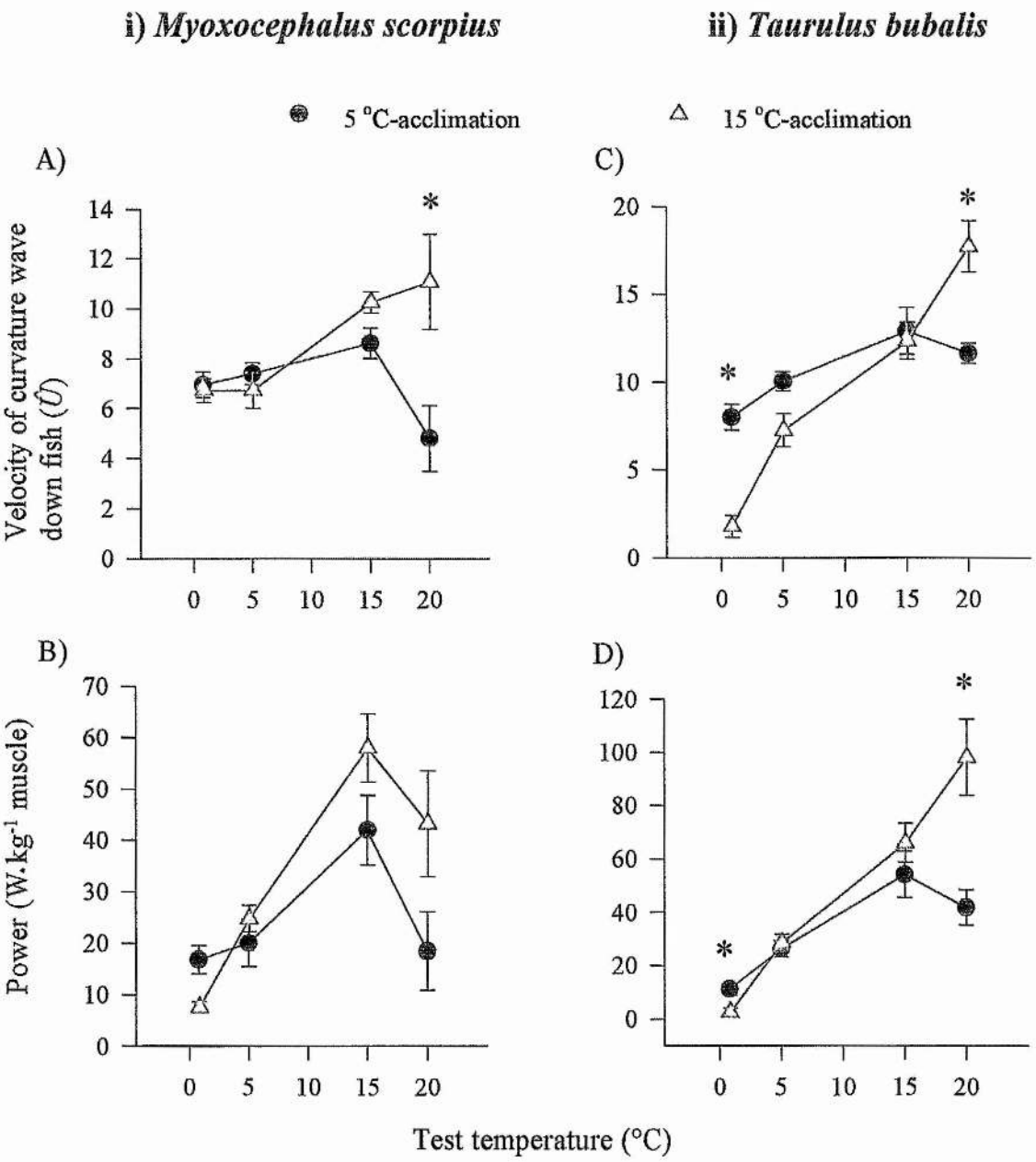

C) 5 @ 0.8 °C ($\hat{U}$ = 8.865)
D) 15 @ 0.8 °C ($\hat{U}$ = 2.423)
Time (ms)
Body position ($\hat{l}$ )
Non-dimensional curvature $\hat{c}$
3.00
2.25
1.50
0.75
0
-0.75
-1.50
-2.25
-3.00

Table 5.2. *$Q_{10}$ values for 5 and 15 °C acclimated fish over the temperature range 5.0 °C to 15.0 °C and 0.8 to 5.0 °C. $Q_{10}$s have also been included for long-spined sea scorpion over the temperature range 5.0-20.0 °C.*

| | ***Myoxocephalus scorpius*** | | ***Taurulus bubalis*** | |
|---|---|---|---|---|
| | Acclimation temperature | | | |
| | 5 °C | 15 °C | 5 °C | 15 °C |
| Variable | Temperature range, 5.0 - 15.0 °C | | | |
| $\hat{U}$, $s^{-1}$ | 1.16 | 1.52 | 1.29 | 1.70 |
| Power, $W.kg^{-1}$ | 2.09 | 2.34 | 2.06 | 2.30 |
| | Temperature range, 0.8 - 5.0 °C | | | |
| $\hat{U}$, $s^{-1}$ | 1.16 | 1.00 | 1.71 | 27.97 (5.0-20.0 °C = 1.81) |
| Power, $W.kg^{-1}$ | 1.53 | 16.76 | 7.58 | 280.11 (5.0-20.0 °C = 2.27) |

Figure 5.3. The velocity of the curvature wave, $\hat{U}$ (A, C) and power requirements (B, D) attained during the contralateral contraction of escape responses in i) short-horn sculpin and ii) long-spined sea scorpion. Asterisks indicate significant differences between acclimation groups at particular test temperatures (all Tukey multiple comparison tests, except for long-spined sea scorpion, 5 °C acclimated tested at 0.8 °C - Mann Whitney U tests. For short-horn sculpin groups, 5@0.8, 5@5.0, 5@15.0, 5@20.0, 15@20.0, 15@15.0, 15@5.0, 15@0.8 (acclimation temperature at test temperature, °C), $N$=9, 9, 9, 5, 5, 10, 11, 9, respectively. For long-spined sea scorpion, $N$=7, 12, 8, 7, 8, 13, 9, 2, respectively. Sculpin were 161.8 ± 3.0 mm $L$ and sea scorpion were 115.1 ± 1.3 mm $L$. Both groups, $N$=27.


i) Myoxocephalus scorpius
ii) Taurulus bubalis
5 °C-acclimation
15 °C-acclimation
A)
C)
B)
D)
Velocity of curvature wave down fish (Ú)
Power (W.kg-1 muscle)
Test temperature (°C)
*

## 5.4 Discussion

### *5.4.1 Validation of the methods*

The power requirements of short-horn sculpin escape responses as estimated from whole animal performance closely matched the power output calculated from work loop experiments (Chapter 3). In this study power ranged from 20 $W.kg^{-1}$ muscle in 5 °C-acclimated fish escaping at 5 °C, to 58 $W.kg^{-1}$ muscle in 15 °C-acclimated fish swimming at 15 °C. Comparative power outputs of the fast muscle fibres under conditions simulating the contralateral contraction were 33 and 66 $W.kg^{-1}$, respectively (Chapter 3). Moreover, the acclimation of power showed the same general pattern between the two methods. There was one inconsistency concerning 5 °C-acclimated short-horn sculpin swimming at 15.0 °C; power requirements estimated from whole animal performance showed an increase when 5 °C-acclimated fish were escaping at 15 °C, whereas work loop experiments revealed power output to decrease slightly with this acute increase in temperature. However, neither increase nor decrease was significant and the values for the two methods were not statistically different (Mann-Whitney, $P>0.1$).

A number of assumptions were made in this study including the added mass of water being 0.2 $m$ (Webb, 1982), the muscle mass in both species being a constant 0.298 $m$, and the hydrodynamic efficiency being 0.31 as estimated from pike fast-starts. It is believed that the muscle mass used did not differ greatly between fish acclimated to particular thermal conditions at different times of the year. In short-horn sculpin, the muscle of gravid fish delivers less force (R. James, unpublished results) and is likely to be reduced in mass, however gravid fish were not used in this study. It is likely that the mass of fast muscle does not differ dramatically between the two species, as both are benthic fish, have very similar body forms, inhabit similar niches and rely on fast-starts for prey capture and predator avoidance. On the other hand, it is likely that the efficiency of fast-starts in cottids is different from that of pike due to a number of variables including body size and the somewhat contrasting body forms influencing power requirements. Furthermore, it is possible that efficiency changes with temperature in a similar way to power and $\hat{U}$. Despite the inclusion of these various assumptions in this study, results match closely with more directly measurable muscle mechanics data

(Chapter 3). Thus the current procedure should reliably predict other acclimation patterns in the short-horn sculpin in addition to those in the closely related long-spined sea scorpion.

### *5.4.2 Acute temperature effects*

In both species of Cottidae, the velocity of the curvature wave, $\hat{U}$, and power increased with acute temperature between 5 and 15 °C. $Q_{10}$s for $\hat{U}$ were between 1.2 and 1.7 with higher values in 15 °C-acclimated fish. The power requirements for escape responses had $Q_{10}$ values of between 2.1 and 2.3, and in 15 °C-acclimated fish these increases were significant; power increased in the short-horn sculpin from 24.8 to 58.1 W. $kg^{-1}$ and in the long-spined sea scorpion from 28.8 to 66.1 W.$kg^{-1}$. Similarly, in Chapter 3, the $Q_{10}$ for the power output of the fast muscle fibres under work loop conditions was 2.0 for 15 °C-acclimated fish, although as mentioned above the power output from 5 °C-acclimated fish did not increase with temperature. Increases in power output with acute temperature have been demonstrated in muscle from a variety of organisms including reptiles, e.g. the lizard (*Dipsosaurus dorsalis*) (Marsh and Bennett, 1985; Swoap *et al.* 1993), insects, e.g. the hawkmoth (*Manduca sexta*) (Stevenson and Josephson, 1990) and even humans (Ferretti *et al.* 1992). Most biological processes are highly sensitive to temperature and have $Q_{10}$ values between 2-3 (Precht *et al.* 1973; Prosser, 1973). Both power, the product of force and velocity, and $\hat{U}$ are governed by rate processes and are therefore temperature dependent.

### *5.4.3 Association between power, $\hat{U}$ and whole animal performance*

The overall configuration of power and $\hat{U}$ with temperature reflect patterns for maximum velocity and acceleration during escape responses and prey capture in these species (Beddow *et al.* 1995; Chapter 2). This is not a surprising result in view of the relationships between these parameters; the swimming velocity of the fish is positively correlated with $\hat{U}$ passing down the body and power was estimated from mass, velocity and acceleration components. Velocity and acceleration are proportional to the square root of the power requirements (Wakeling and Johnston, 1998). The $Q_{10}$ values for $\hat{U}$ are consequently lower than those of power. In Chapter 2, the $Q_{10}$s (5.0-15.0 °C) for

maximum length-specific velocity and acceleration of both acclimation groups were 1.2-1.3 in sculpin and 1.3-1.6 in sea scorpion. As can be seen from the preceding section these values are somewhat lower than power, although not its precise square root. The match is not exact as methods for calculating velocity and acceleration in the previous study used the centre of mass positioned on the spine.

### *5.4.4 Effect of acclimation temperature*

During periods of acclimation, compensations in physiological rate processes sometimes occur such that the rate resulting from acute exposure to the new thermal regime is altered. For example in perfect compensation the rate returns to that of the previous thermal environment (Precht, 1958). Acclimation effects on $\hat{U}$ and power were found in both species. In Chapter 2, it was hypothesized that improvements in escape performance in the short-horn sculpin after warm acclimation would be at the expense of performance at low temperature but this was found not to be the case. Therefore $\hat{U}$ and power were not compromised at low temperature by acclimation to 15 °C, although the $Q_{10}$ for power between 0.8 and 5.0 °C was higher in the 15 °C- than the 5 °C-acclimated sculpin, a result echoed by the maximum length-specific velocity (Chapter 2).

Chapter 2 revealed maximum velocity and acceleration were significantly influenced by acclimation only at low temperature during escape responses in the long-spined sea scorpion. In this study acclimation patterns were similar, although both $\hat{U}$ and power requirements in 15 °C-acclimated fish, continued to increase dramatically up to 20.0 °C. Therefore $\hat{U}$ and power showed significant acclimation effects after warm as well as cold acclimation and there was a trade-off in optimum temperature for performance with thermal acclimation. On Scottish coasts, long-spined sea scorpion are resident in the intertidal and shallow sublittoral zones where summer water temperatures can reach 22 °C (Morris and Taylor, 1983). Adult short-horn sculpin, on the other hand, live offshore at a depth of 30-50 m where water temperatures are cooler. Therefore the continued increase in $\hat{U}$ and power of sea scorpion acclimated to summer water temperatures may represent an adaptation to occupation of a warmer niche.

In both species of Cottidae acute exposure to 5.0 °C depressed values of $\hat{U}$ and power. Furthermore, following a period of acclimation to 5 °C there was no significant

evidence of any thermal compensation, with power requirements and $\hat{U}$ remaining relatively unchanged. The lack of thermal compensation in power output is not an uncommon result (see Bennett, 1991, for review), although as indicated above it obviously depends on the range of acclimation and test temperatures and the natural thermal habitat of the organism. The lower values of power for fast-starts in cold- compared to warm-acclimated fish, escaping at their acclimation temperatures, follow the same trend as found in stenothermal species living habitually at low temperature (Wakeling and Johnston, 1998).

Videler and Hess (1984) used the slippage ratio of the velocity of swimming to $\hat{U}$ during steady swimming as an indicator of hydrodynamic efficiency. Wakeling and Johnston (1998) used the ratio of maximum fast-start velocity to $\hat{U}$ to estimate efficiency during escape responses in a range of fish species. Using values of maximum swimming velocity taken from Chapter 2, slippage ratios for short-horn sculpin are 0.51 and 0.57 for 5 °C and 15 °C-acclimated fish swimming at their respective acclimation temperatures. In long-spined sea scorpion these values are 0.57 and 0.61, respectively. Wakeling and Johnston (1998) found slippage ratios to be greater at lower habitat temperatures indicating that Antarctic fish may compensate for a reduced muscle power output by increasing efficiency. In the present study, efficiency increased with acclimation temperature and therefore reduced muscle power output at low temperature does not appear to be compensated for by increased efficiency.

*Chapter 6*

# GENERAL DISCUSSION

Temperature change over both acute and seasonal time scales was found to alter fast-start escape performance at the whole animal level in both the short-horn sculpin and the long-spined sea scorpion and additionally at the cellular level in the sculpin. An acute increase in temperature increases the rate of many physiological processes (Schmidt-Nielson, 1990). During escape responses, an increase in temperature in many cases lead to an increase in maximum velocity, maximum acceleration (Chapter 2), power output of the fast muscle fibres (Chapter 3), velocity of the curvature wave travelling down the fish and power requirements (Chapter 5). However, alterations in these parameters with acute temperature were dependent on two factors: acclimation temperature and species. The short-horn sculpin exhibited higher speeds and accelerations at 15.0 and 20.0 °C following acclimation to 15 °C than 5 °C. Thus, in the 5 °C-acclimated group, speed and acceleration did not continue to increase with temperature up to 20 °C, but began to fall. In the goldfish (*Carassius auratus*) acclimation to high temperature has been found to be at the expense of escape performance at low temperature (Johnson and Bennett, 1995). However, the prediction that this would be the case in the short-horn sculpin was found to be false. At 0.8 °C and 5.0 °C, performance parameters were comparable between the 5 and 15 °C acclimation groups. The long-spined sea scorpion did not exhibit any acclimation response as measured by maximum speed, acceleration and the velocity of the curvature wave to average seasonal water temperatures of 5 and 15 °C. Over a greater test range, however, 5 °C-acclimation significantly improved these parameters at a test temperature of 0.8 °C. In addition, improvements in the velocity of the curvature wave following cold acclimation were at the expense of performance at high temperature. Thus the two species exhibited differences in the thermal dependence of their fast-start performance.

Short-horn sculpin and long-spined sea scorpion additionally exhibited differences in their acclimation ability during development (Chapter 2). None of the long-spined sea scorpion ranging in length from 45 - 160 mm *L* exhibited acclimation of maximum speed during the escape response between 5 and 15 °C. However, in the short-

horn sculpin the ability to thermally acclimate to these seasonal water temperatures was acquired during development. It was proposed that the differences between the two species may be partly due to local niche distributions. Adult short-horn sculpin live offshore at an average depth of 40 m (Unesco, 1986) around the British Isles, where water temperatures fluctuate seasonally. Juvenile short-horn sculpin and all stages of the long-spined sea scorpion, however, live intertidally and in the shallow subtidal zone (King and Fives, 1983; Unesco, 1986) where water temperatures are more variable, particularly in the summer (Morris and Taylor, 1983). For these fish the cost of acclimating fast-start performance may outweigh the benefits. Alternatively, the variability of the thermal regime may enable acclimation responses that are only manifest over broader temperature ranges as found in the long-spined sea scorpion. Cycling thermal regimes have been found to increase the thermal tolerance of pupfish (*Cyprinodon nevadensis amargosae*) (Feldmeth *et al.* 1974). Whether acclimation responses are manifest over broader temperature ranges in juveniles of both cottids remains to be seen.

In the short-horn sculpin, temperature was found to have profound effects on the parameters of the fast muscle strain cycle and the power output during escape responses (Chapter 3). Changes in the *in vivo* muscle shortening velocity with acclimation temperature were manifest at high temperature but not at low temperature due to increases in $V_{max}$, as reported by Beddow *et al.* (1995). Isometric twitch and tetanic activation rates were higher at 15 °C in 15 °C- than 5 °C-acclimated fish but no differences were manifest at low temperature between the two acclimation groups. The maximum instantaneous power output measured under conditions representing the C-bend and contralateral contraction using work loop experiments, and the mean power output of the contralateral contraction similarly showed acclimation responses only at high temperature. In addition, the power requirements as estimated using a hydrodynamic model only showed acclimation responses at high temperature (Chapter 5). Johnson and Johnston (1991) used the work loop technique under conditions simulating steady swimming in summer- and winter-acclimatised short-horn sculpin. These authors also found that alterations in the power output of the fast muscle fibres were principally manifest at high temperature.

Muscle performance therefore mimics that of whole animal performance despite the fact that not all time-dependent properties of muscle function are altered to the same extent by temperature and that many time-independent properties are actually temperature dependent (Rall and Woledge, 1990). An example of the latter is peak tetanic force. In the present study, tetanic force did not alter with temperature. However this was only tested over a 10 °C temperature range. If tested over the broader temperature range used in the kinematic experiments in this study, force may increase with temperature due to increases in the force-generating capacity of the cross-bridges (Rall and Woledge, 1990; Beddow *et al.* 1995).

Beddow *et al.* (1995) found prey capture events in the short-horn sculpin to be affected by acclimation in a similar manner to that of escape responses. At 15 °C maximum velocity was 33 % higher in the 15 °C- than the 5 °C-acclimated fish, although acclimation responses at low temperature were not assessed. In Chapter 4 escape responses in the short-horn sculpin were found to be kinematically distinct from prey capture responses. Escape responses were always C-starts, whereas prey capture could be either C or S-starts. Escape involved varying degrees of turning, whereas prey capture involved maintaining a straight trajectory towards the prey and a feeding strategy which involves both suction and ram feeding. However, maximum speeds were not significantly different between the two behaviours. In addition, the power requirements for escape and prey capture were similar as were the energetic costs and efficiency. It is therefore probable that temperature acclimation responses for prey capture performance at low temperature and over broader temperature ranges would show the same trends as for escape performance. Morley and Batty (1996), however, found herring larvae exhibited different responses to temperature during S-strike feeding to that reported for C-start escape responses (Batty *et al.* 1993).

An important question arises from the results of this thesis: if fast-start performance is a direct correlate of fitness and thus influences differential survival and reproduction, how have cold-acclimated sculpin evolved? The short-horn sculpin possesses three opercular spines on each side of a large bony head. Perhaps under winter conditions predator avoidance is achieved using aggression in lieu of escape responses. In lizards, individuals with warm body temperatures flee from predators whereas those with

cold body temperatures stay their ground and fight (Hertz *et al.* 1982; Crowley and Pietruszka, 1983). Another potential strategy would be hiding. Prey capture may be possible in the winter as the velocities of prey may be similarly slowed. Alternatively feeding activities may be reduced in the winter. Such behavioural adaptations in the winter may conserve energy or channel reserves into cold tolerance such as antifreeze production (Fletcher *et al.* 1988). However, whether these behaviours actually occur in the field is uncertain. The assumption that fast-start performance is a good correlate of fitness must be questioned. It is only possible to test this assumption by direct measurements of fast-start behaviour under natural conditions preferably over several generations.

### *6.1 Future studies*

It would be interesting to see whether patterns of acclimation, if present over a broad temperature range, differed between juveniles of the two species of Cottidae. If juveniles of both species exhibited responses like those of the adult long-spined sea scorpion, then it may be possible to speculate that acclimation responses solely reflect local niche distributions in these species. On the other hand, if responses were dissimilar and each akin to those of the adult, i.e. acclimation responses in the short-horn sculpin manifest at high temperature (above 15 °C) and acclimation responses in the long-spined sea scorpion manifest at low or both high and low temperature, then patterns of acclimation would appear to be species-specific. However, the results are likely to be more complex than this. Walsh and colleagues (1997) did not find overwhelming evidence that the distribution patterns of several species of freshwater Cottidae were solely attributable to temperature.

Temperature acclimation has been found to alter the expression of muscle protein isoforms. Using capillary electrophoresis, Ball and Johnston (1996) found alterations in the myosin light chains with thermal acclimation in adult short-horn sculpin. During development different isoforms are expressed and temperature alters the timing of these developmental sequences (Crockford and Johnston, 1993). In the Clyde herring, the combination of isoforms present has been found to be dependent on rearing temperature (Johnston *et al.* 1997). Studies of the muscle proteins during development could indicate

when acclimation ability is acquired in the short-horn sculpin. Similar studies examining the muscle proteins, in addition to studies on enzyme kinetics, of the long-spined sea scorpion could explain the mechanisms underlying the acclimation responses manifest in this species. This could be carried out in conjunction with a study of the temperature effects on the muscle performance of the long-spined sea scorpion. Preliminary work indicated that mechanical studies on this species are possible despite the smaller size of the adult sea scorpion in comparison to the short-horn sculpin.

A brief encounter with "New Scientist" or "Nature" these days clearly demonstrates the common use of molecular techniques. The present study has examined fish locomotion at the whole animal and cellular levels. Perhaps the next natural progression would be the molecular level. Molecular techniques could potentially be a strong tool for elucidating the expression of acclimation dependent isoforms of muscle proteins. Imai *et al.* (1997) isolated cDNA clones which encoded three types of MHC isoforms from fast muscle of carp acclimated to 10, 20 and 30 °C. At the transcription level, northern hybridization revealed the level of mRNA of the 10 °C-type MHC isoform was six-fold higher in 10 °C-acclimated carp than in 30 °C-acclimated carp. Using northern blot analysis, Hirayama *et al.* (1997) found an increase in the ratio of LC3 to LC1 mRNAs, from 3.10 in 10 °C-acclimated carp, to 3.93 in 30 °C-acclimated fish.

It is important, however that molecular studies are not totally removed from studies of animal behaviour. Ideally experiments on whole animal performance should be conducted under ecologically relevant conditions as in the field, although this has often been a difficult and impractical task. However, just as much of the interest in fast-start behaviour has taken off in the last 40 years due mainly to the advancements in high speed ciné and video cameras, it should now be possible to conduct more ecologically relevant studies on fish locomotion with the advancement in techniques such as telemetry. This should furthermore enable studies to be conducted with a view to assessing the evolutionary significance of particular behaviours.

## REFERENCES


Al-Khayat, H.A., Yagi, N. and Squire, J.M. (1995). Structural changes in actin-tropomyosin during muscle regulation: computer modeling of low angle X-ray diffraction data. *J. Mol. Biol.* **252**, 611-632.

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

Altringham, J.D. and 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. and Johnston, I.A. (1989). The innervation pattern of fast myotomal muscle in the teleost *Myoxocephalus scorpius*: a reappraisal. *Fish Physiol. Biochem.* **6**, 309-313.

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

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

Altringham, J.D., Wardle, C.S. and Smith, C.I. (1993). Myotomal muscle functions at different locations in the body of a swimming fish. *J. exp. Biol.* **151**, 453-467.

Andraso, G.M. (1997). A comparison of startle response in two morphs of the brook stickleback (*Culaea inconstans*): Further evidence for a trade-off between defensive morphology and swimming ability. *Evol. Ecol.* **11**, 83-90.

Anson, M., Geeves, M.A. Kurzawa, S.E. and Manstein, D. (1996). Myosin motors with artificial lever arms. *EMBO J.* **15**, 6069-6074.

Archer, S.D., Altringham, J.D. and Johnston, I.A. (1990). Scaling effects on the neuromuscular system, twitch kinetics and morphometrics of the cod, *Gadus morhua*. *Mar. Behav. Physiol.* **17**, 137-146.

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

Arloing, S. and Lavocat, A. (1875). Recherches sur l'anatomie et la physiologie des muscles striés pâles et foncés. *Mem. Acad. Sci. Belles Lett. Toulouse* 7, 177-194.

Arnold, S. J. (1983). Morphology, performance and fitness. *Am. Zool.* **23**, 347-361.

Bagshaw, C.R. (1993). *Muscle Contraction*. pp. 155. London: Chapman and Hall.

Bailey, K. (1948). Tropomyosin: a new asymmetrical protein component of the muscle myofibril. *Biochem. J.* **43**, 271-279.

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

Ball, D. and Johnston, I.A. (1996). Molecular mechanisms underlying the plasticity of muscle contractile properties with temperature acclimation in the marine fish, *Myoxocephalus scorpius*. *J. exp. Biol.* **199**, 1363-1373.

Barets, A. (1961). Contribution à l'étude des systèmes moteurs lent et rapide du muscle latéral des téléostéens. *Arch. Anat. Morphol. Exp.* **50**, Suppl., 91-187.

Baron, G., Demaille, J. and Dutruge, E. (1975). The distribution of parvalbumins in muscle and other tissues. *FEBS Lett.* **56**, 155-160.

Batty, R.S. (1984). Development of swimming movements and musculature of larval herring (*Clupea harengus*). *J. exp. Biol.* **110**, 217-229.

Batty, R.S. and Blaxter, J.H.S. (1992). The effect of temperature on the burst swimming performance of fish larvae. *J. exp. Biol*. **170**, 187-201.

Batty, R.S., Blaxter, J.H.S. and Bone, Q. (1991). The effect of temperature on the swimming of a teleost (*Clupea harengus*) and an ascidian larva (*Dendrodoa glossularia*). *Comp. Biochem. Physiol.* **100A**, 297-300.

Batty, R.S., Blaxter, J.H.S. and Fretwell, K. (1993). Effect of temperature on the escape responses of herring larvae, *Clupea harengus*. *Mar. Biol.* **115**, 523-528.

Beamish, F.W.H. (1978). Swimming capacity. In *Fish Physiology* Vol. VII (ed. W.S. Hoar and D.J. Randall), pp. 101-187. London: Academic Press.

Beddow, T.A. and Johnston, I.A. (1995). Plasticity of muscle contractile properties following temperature acclimation in the marine fish *Myoxocephalus scorpius*. *J. exp. Biol.* **198**, 193-201.

Beddow, T.A., van Leeuwen, J.L. and Johnston, I.A. (1995). Swimming kinematics of fast starts are altered by temperature acclimation in the marine fish *Myoxocephalus scorpius*. *J. exp. Biol.* **198**, 203-208.

Bennett, A.F. (1978). Activity metabolism of the lower vertebrates. *Ann. Rev. Physiol.* **40**, 447-469.

Bennett, A.F. (1984). The thermal dependence of muscle function. *Am J. Physiol.* **247** (*Regulatory Interactive Comp. Physiol.* **16**): R217-R229.

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

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

Bennett, A.F. (1991). The evolution of activity capacity. *J. exp. Biol.* **160**, 1-23.

Bennett, A.F. and Lenski, R.E. (1997). Evolutionary adaptations to temperature. VI. Phenotypic acclimation and its evolution in *Escherichia coli*. *Evolution* **51**, 36-44.

Best, A.C.G. and Bone, Q. (1973). The terminal neuromuscular junctions of lower chordates. *Z. Zellforsch. Mikrosk. Anat.* **143**, 495-504.

Blaxter, J.H.S. and Batty, R.S. (1985). The development of startle responses in herring larvae. *J. mar. biol. Ass. U.K.* **65**, 737-750.

Blum, H., Balschi, J.A. and Johnson, R.G. (1991). Coupled *in vivo* activity of the membrane band $Na^+K^+$ ATPase in resting and stimulated electric organ of the electric fish *Narcine brasiliensis*. *J. Biol. Chem.* **266**, 10254-10259.

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

Bone, Q. (1966). On the function of the two types of myotomal muscle fibre in elasmobranch fish. *J. Mar. Biol. Ass. U.K.* **46**, 321-349.

Bone, Q. (1970). Muscular innervation and fish classification. *Simp. Int. Zoofil., 1st Univ. Salamanca* pp. 369-377.

Bone, Q. (1978). Locomotor muscle. In *Fish Physiology* Vol. VII (ed. W.S. Hoar and D.J. Randall), pp. 361-424. London: Academic Press.

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

Bone, Q., Marshall, N.B. and Blaxter, J.H.S. (1995). *Biology of Fishes*. pp. 332. London: Chapman and Hall.

Breder, C.M. (1926). The locomotion of fishes. *Zoologica (N.Y.)* **50**, 159-297.

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

Brooks, S. and Johnston, I.A. (1993). Influence of development and rearing temperature on the distribution, ultrastructure and myosin sub-unit composition of myotomal muscle-fibre types in the plaice, *Pleuronectes platessa*. *Mar. Biol.* **117**, 501-513.

Buckley, L.J. (1982). Effects of temperature on growth and biochemical composition of larval winter flounder *Pseudo-pleuronectes americanus*. *Mar. Ecol. Prog. Ser.* **8**, 181-186.

Bullock, T.H. (1955). Compensation for temperature in the metabolism and activity of poikilotherms. *Biol. Rev.* **30**, 311-342.

Canfield, J.G. and Rose, G.J. (1993). Activation of Mauthner neurons during prey capture. *J. comp. Physiol.* **172 A**, 611-618.

Carlson, F. D. and Siger, A. (1959). The creatine phosphoryl-transfer reaction in iodoacetate poisoned muscle. *J. gen. Physiol.* **43**, 301-313.

Chanoine, C., Lefant-Guyot, M., El Attari, A., Saadi, A. and Gallien, C.-L. (1992). White muscle differentiation in the eel (*Anguilla anguilla* L.): changes in the myosin isoform pattern and ATPase profiles during post-metamorphic development. *Differentiation* **39**, 69-75.

Clarke, A. (1991). What is cold adaptation and how do we measure it? *Am. Zool.* **31**, 81-92.

Cohen, C. (1975). The protein switch of muscle contraction. *Sci. Am.* **233**, 36-45.

Cossins, A.R. and Bowler, K. (1987). *Temperature Biology of Animals*. Cambridge: Cambridge University Press.

Coughlin, D.J., Valdes, L. and Rome, L.C. (1996). Muscle length changes during swimming in scup: sonomicrometry verifies the anatomical high-speed cine technique. *J. exp. Biol.* **199**, 459-463.

Coughlin, D.J., Zhang, G. and Rome, L.C. (1996). Contraction dynamics and power production of pink muscle of the scup (*Stenotomus chrysops*). *J. exp. Biol.* **199**, 2703-2712.

Coutant, C.C. (1970). Biological aspects of thermal pollution. I. Entraiment and discharge canal effects. *Crit. Rev. Env. Cont.* **1**, 341-381.

Covell, J.W., Smith, M., Harper, D.G. and Blake, R.W. (1991). Skeletal muscle deformation in the lateral muscle of intact rainbow trout *Oncorhynchus mykiss* during fast start maneuvers. *J. exp. Biol.* **156**, 453-466.

Crockford, T, and 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.* **160B**, 23-30.

Crockford, T. and Johnston, I.A. (1993). Developmental changes in the composition of myofibrillar proteins in the swimming muscles of Atlantic herring, *Clupea harengus*. *Mar. Biol.* **115**, 15-22.

Crowley, S.R. and Pietruszka, R.D. (1983). Aggressiveness and vocalization in the leopard lizard (*Gambelia wislizennii*): the influence of temperature.

Curtin, N.A., Kushmerick, M.J., Wiseman, R.W. and Woledge, R.C. (1997). Recovery after contraction of white muscle fibres from the dogfish *Scyliorhinus canicula*. *J. exp. Biol.* **200**, 1061-1071.

Curtin, N. A. and Woledge, R. C. (1991). Efficiency of energy conversion during shortening of muscle fibres from the dogfish *Scyliorhinus canicula*. *J. exp. Biol.* **158**, 343-353.

Curtin, N.A. and Woledge, R.C. (1993). Efficiency of energy conversion during sinusoidal movement of white muscle fibres from the dogfish *Scyliorhinus canicula*. *J. exp. Bio.* **183**, 137-147.

Curtin, N.A. and Woledge, R.C. (1994). Effect of distance shortened on the efficiency of white muscle isolated from dogfish. *J. Physiol., Lond.* **479**, 158P.

Curtin, N.A. and Woledge, R.C. (1996). Power at the expense of efficiency in contraction of white muscle fibres from dogfish *Scyliorhinus canicula*. *J. exp. Biol.* **199**, 593-601.

Dalla Libera, L., Carpene, E., Theibert, J. and Collins, J.H. (1991). Fish myosin alkali light chains originate from two different genes. *J. Muscle Res. Cell Motil.* **12**, 366-371.

Darwin, C. (1859). *The origin of species*. pp. 477. Great Britain: Richard Clay Ltd.

Domenici, P. and Blake, R.W. (1991). The kinematics and performance of the escape response in the angelfish (*Pterophyllum eimekei*). *J. exp. Biol.* **156**, 187-205.

Domenici, P. and Blake, R.W. (1993*a*). Escape trajectories in angelfish (*Pterophyllum eimekei*). *J. exp. Biol.* **177**, 253-272.

Domenici, P. and Blake, R.W. (1993*b*). The effect of size on the kinematics and performance of angelfish (*Pterophyllum eimekei*) escape responses. *Can. J. Zool.* **71**, 2319-2326.

Domenici, P. and Blake, R.W. (1997). The kinematics and performance of fish fast-start swimming. *J. exp. Biol.* **200**, 1165-1178.

Duthie, G. G. (1982). The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. *J. exp. Biol.* **97**, 359-373.

Eaton, R.C., Bombardieri, R.A. and Meyer, O.H. (1977). The Mauthner-initiated startle response in teleost fish. *J. exp. Biol.* **66**, 65-81.

Eaton, R.C. and Emberley, D.S. (1991). How stimulus direction determines the trajectory of the Mauthner-initiated escape response in a teleost fish. *J. exp. Biol.* **161**, 469-487.

Eaton, R.C. and DiDomenico, R. (1986). Role of the teleost escape response during development. *Trans. Am. Fish. Soc.* **115**, 128-142.

Eaton, R.C. and DiDomenico, R. and Nissanov, J. (1988). Flexible body dynamics of the goldfish C-start: implication for reticulospinal command mechanism. *J. Neurosci.* **8**, 2758-2768.

Eaton, R.C., Lavender, W.A. and Wieland, C.M. (1981). Identification of Mauthner-initiated response patterns in goldfish: evidence from simultaneous cinématography and electrophysiology. *J. comp. Physiol.* **144**, 521-531.

Ebashi, S. and Kodama, A. (1966*a*). A new factor promoting aggregation of tropomyosin. *J. Biochem.* **58**, 107-108.

Ebashi, S. and Kodama, A. (1966*b*). Native tropomyosin-like action of troponin on trypsin-treated myosin B. *J. Biochem.* **60**, 733-734.

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

Edman, K.A.P. (1980). Depression of mechanical performance by active shortening during twitch and tetanus of vertebrate muscle fibres. *Acta Physiol. Scand.* **109**, 15-26.

Edman, K.A.P., Elzinga, G. and Noble, M.I.M. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *J. Physiol.* **281**, 139-155.

Edsall, T.A. and Yocom, T.G. (1972). *Review of recent technical information concerning the adverse effects of once-through cooling on Lake Michigan.* U.S. Fish Wildl. Serv. Gt. Lakes Fish. Lab., Ann Arbor, Michigan pp. 86.

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

Ellington, C.P. (1984). The aerodynamics of hovering insect flight. VI. Lift and power requirements. *Phil. Trans. R. Soc. Lond. B.* **305**, 145-181.

Ellington, C.P. (1985). Power and efficiency in insect flight muscle. *J. exp. Biol.* **115**, 293-304.

Engelhardt, W.A. and Ljubimowa, M.N. (1939). Myosin and adenosinetriphosphatase. *Nature* **144**, 668-669.

Ennion, S., Gauvry, L., Butterworth, P. and Goldspink, G. (1995). Small-diameter white myotomal muscle fibres associated with growth hyperplasia in the carp (*Cyprinus carpio*) express a distinct myosin heavy chain gene. *J. exp. Biol.* **198**, 1603-1611.

Feldmeth, C.R. Stone, E.A. and Brown, J.H. (1974). An increased scope for thermal tolerance upon acclimating Pupfish (*Cyprinodon*) to cycling temperatures. *J. comp. Physiol.* **89**, 39-44.

Ferretti, G., Ishii, M., Moia, C. and Cerretelli, P. (1992). Effects of temperature on the maximal instantaneous muscle power of humans. *Eur. J. app. Physiol. occ. Physiol.* **64**(2), 112-116.

Fisher, K.C. (1958). An approach to the organ and cellular physiology of adaptation to temperature in fish and small mammals. In *Physiological Adaptation* (ed. C.L. Prosser), pp. 3-49. Washington, DC: American Physiological Society.

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

Fletcher, G.L., King, M.J., Kao, M.H. and Shears, M.A. (1989). Antifreeze proteins in the urine of marine fish. *Fish Physiol. Biochem.* **6**, 121-127.

Focant, B. and Huriax, F. (1976). Light chains of carp and pike skeletal muscle myosins. Isolation and characterisation of the most anodic light chains on alkaline pH electrophoresis. *FEBS Lett.* **65**, 16-19.

Foreman, M.B. and Eaton, R.C. (1993). The direction change concept for reticulospinal control of goldfish escape. *J. Neurosci.* **13**, 4101-4133.

Foster, M.A. (1969). Ionic and osmotic regulation in three species of Cottus (Cottidae, Teleost). *Comp. Biochem. Physiol.* **30**, 751-759.

Franklin, C.E. and Johnston, I.A. (1997). Muscle power output during escape responses in an Antarctic fish. *J. exp. Biol.* **200**, 703-712.

Franklin, C. E., Johnston, I. A., Batty, R. S. and Yin, M. C. (1996). Metabolic recovery in herring larvae following strenuous activity. *Journal of Fish Biology.* **48**, 207-216.

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

Frith, H.R. and Blake, R.W. (1995). The mechanical power output and hydromechanical efficiency of northern pike (*Esox lucius*) fast-starts. *J. exp. Biol.* **198**, 1863-1873.

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

Fuiman, L.A. (1986). Burst-swimming performance of larval zebra danios and the effects of diel temperature fluctuations. *Trans. Am. Fish. Soc.* **115**, 143-148.

Fuiman, L.A. (1991). Influence of temperature on evasive responses of Atlantic herring larvae attacked by yearling herring, *Clupea harengus* L. *J. Fish Biol.* **39**, 93-102.

Fuiman, L.A. and Batty, R.S. (1997). What a drag it is getting cold: partitioning the physical and physiological effects of temperature on fish swimming. *J. exp. Biol.* **200**, 1745-1755.

Gaffney, B. and Cunningham, E.P. (1988). Estimation of genetic trend in racing performance of thoroughbred horses. *Nature* **332**, 722-724.

Gazith, J., Himmelfarb, S. and Harrington, W.F. (1970). Studies on the subunit structure of myosin. *J. Biol. Chem.* **245**, 15-22.

Geeves, M.A. and Conibear, P.B. (1995). The role of three-state docking of myosin S1 with actin in force generation. *Biophys. J. Suppl.* **68**, 194-201.

Gergely, J. (1994). Biochemical aspects of muscular structure and function. In *Disorders of Voluntary Muscle* (ed. J. Walton, G. Kaparti and D. Hilton-Jones), pp. 179-230. Edinburgh: Churchill-Livingstone.

Gerlach, G.-F, Turay, L., Malik, K.T.A., Lida, J., Scutt, A. and Goldspink, G. (1990). Mechanisms of temperature acclimation in the carp: a molecular biology approach. *Am. J. Physiol.* **259**, R237-R244.

Gibson, R.N. (1969). The biology and behaviour of littoral fish. *Oceanogr. Mar. Biol. Ann. Rev.* 7, 367-410.

Gibson, S. and Johnston, I.A. (1995). Scaling relationships, individual variation and the influence of temperature on maximum swimming speed in early settled stages of the turbot *Scophthalmus maximus*. *Mar. Biol.* **121**, 401-408.

Gillis, J.M., Thomason, D., Lefevre, J. and Kretsinger, R.H. (1982). Parvalbumins and muscle relaxation: a computer simulation study. *J. Muscle Res. Cell Motil.* **3**, 377-398.

Goldspink, G., Turay, L., Hansen, E., Ennion, S. and Gerlach, G. (1992). Switches in fish myosin genes induced by environmental temperature in muscle of the carp. *Symp. Soc. exp. Biol.* **46**, 139-149.

Gordon, A.M., Huxley, A.F. and Julian, F.J. (1966*a*). Tension development in highly stretched vertebrate muscle fibres. *J. Physiol.* **184**, 143-169.

Gordon, A.M., Huxley, A.F. and Julian, F.J. (1966*b*). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol.* **184**, 170-192.

Gray, J. (1933). Studies in animal locomotion. I. The movement of fish with special reference to the eel. *J. exp. Biol.* **10**, 586-588.

Greaser, M.L. and Gergely, J. (1973). Purification and properties of the components from troponin. *J. Biol. Chem.* **248**, 2125-2133.

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

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

Hanson, J. and Huxley, H.E. (1953). The structural basis of the cross-striations in muscle. *Nature* **172**, 530-532.

Harper, D.G. and Blake, R.W. (1988). Energetics of piscivorous predator-prey interactions. *J. theor. Biol.* **134**, 59-76.

Harper, D.G. and Blake, R.W. (1989). On the error involved in high-speed film used to evaluate maximum acceleration of fish. *Can J. Zool.* **67**, 1929-1936.

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

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

Harrington, W.F. (1979). On the origin of the contractile force in skeletal muscle. *Proc. Natl. Acad. Sci. USA* **76**, 5066-5070

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

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

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

Heglund, N.C. and Cavagna, G.A. (1985). Efficiency of vertebrate locomotory muscles. *J. exp. Biol.* **115**, 283-292.

Heglund, N.C. and Cavagna, G.A. (1987). Mechanical work, oxygen consumption, and efficiency in isolated frog and rat muscle. *Am. J. Physiol.* **253**, C22-C29.

Heilbrunn, L.V. and Wiercinski, F.J. (1947). The action of various cations on muscle protoplasm. *J. cell. comp. Physiol.* **29**, 15-32.

Hertz, P.E., Huey, R.B. and Nevo, E. (1982). Fight versus flight: body temperature influences defensive responses in lizards. *Anim. Behav.* **30**, 676-679.

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

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

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

Hirayama, Y., Kanoh, S., Nakaya, M. and Watabe, S. (1997). The two essential light chains of carp fast skeletal myosin, LC1 and LC3, are encoded by distinct genes and change their molar ratio following temperature acclimation. *J. exp. Biol.* **200**, 693-701.

Hochachka, P.W. (1983). Protons and glucose metabolism in shock. *Adv. Shock Res.* **9**, 49-65.

Hochachka, P.W. (1994). *Muscles as Molecular and Metabolic Machines*. pp. 158. USA: CRC Press, Inc.

Hoogland, R., Morris, D. and Tinbergen, N. (1956). The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as a means of defense against predators (*Perca* and *Esox*). *Behav.* **10**, 205-236.

Horowits, R., Kempner, E.S., Bisher, M.E. and Podolski, R.J. (1986). A physiological role of titin and nebulin in skeletal muscle. *Nature* **323**, 160-164.

Hovenkamp, F. and Witte, J.IJ. (1991). Growth, otolith growth and RNA/DNA ratios of larval plaice *Pleuronectes platessa* in the North Sea 1987 to 1989. *Mar. Ecol. Prog. Ser.* **70**, 105-116.

Hubley, M.J., Locke, B.R. and Moerland, T.S. (1997). Reaction-diffusion analysis of the effects of temperature on high-energy phosphate dynamics in goldfish skeletal muscle. *J. exp. Biol.* **200**, 975-988.

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

Huey, R.B. and Berrigan, D.A. (1996). Testing evolutionary hypotheses of acclimation. In *Animals and Temperature Phenotypic and Evolutionary Adaptation* (ed. I.A. Johnston and A.F. Bennett.), pp. 205-237. Cambridge: Cambridge University Press.

Huey, R.B. and Stevenson, R.D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *Am. Zool.* **19**, 357-366.

Huxley, A.F. and Niedergerke, R. (1954). Structural changes in muscle during contraction. Interference microscopy of living muscle fibres. *Nature* **173**, 971-973.

Huxley, H.E. (1953). Electron microscope studies of the organisation of the filaments in striated muscle. *Biochem. biophys. Acta* **12**, 387-394.

Huxley, H.E. (1969). The mechanism of muscular contraction. *Science* **164**, 1356-1366.

Huxley, H.E. and Hanson, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature*, **173**, 973-976.

Huxley, H.E. and Niedergerke, R. (1954). Structural changes in muscle during contraction. Interference microscopy of living muscle fibres. *Nature* **173**, 971-973.

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

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

Imai, J,-I, Hirayama, Y., Kikuchi, K., Katinuma, M. and Watabe, S. (1997). cDNA cloning of myosin heavy chain isoforms from carp fast skeletal muscle and their gene expression associated with temperature acclimation. *J. exp. Biol.* **200**, 27-34.

James, R.S., Cole, N.J., Davies, M.L.F. and Johnston, I.A. (1998). Scaling of intrinsic properties and myofibrillar composition of fast-muscle fibres in the short-horn sculpin (*Myoxocephalus scorpius*). *J. exp. Biol.* In press.

James. R.S. and Johnston. (1998). Scaling of muscle performance during escape responses in the fish, Myoxocephalus scorpius. *J. exp. Biol.* In press.

Jayne, B.C. and Bennett, A.F. (1990*a*). Scaling of speed and endurance in garter snakes: a comparison of cross-sectional and longitudinal allometries. *J. Zool., Lond.* **220**, 257-277.

Jayne, B.C. and Bennett, A.F. (1990*b*). Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**, 1204-1229.

Jayne, B.C. and Lauder, G.V. (1993). Red and white muscle activity and kinematics of the escape response of the bluegill sunfish during swimming. *J. comp. Physiol.* **173A**, 495-508.

Jayne, B.C. and Lauder, G.V. (1994). How swimming fish use low and fast muscle fibres: implications for models of vertebrate muscle recruitment. *J. comp. Physiol.* **175A**, 123-131.

Jayne, B.C. and Lauder, G.V. (1995). Red muscle motor patters during steady swimming in largemouth bass - effects of speed and correlations with axial kinematics. *J. exp. Biol.* **198**, 1575-1587.

Johnson, T.P. and Bennett, A.F. (1995). The thermal acclimation of burst swimming escape performance in fish: an integrated study of molecular and cellular physiology and organismal performance. *J. exp. Biol.* **198**, 2165-2175.

Johnson, T.P., Cullum, A.J. and Bennett, A.F. (1993). The thermal dependence of C-start performance in fish: physiological versus biophysical effects. *Am. Zool.* **33**, 65A.

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

Johnson, T.P., Johnston, I.A. and Moon, T.W. (1991). Temperature and the energy cost of oscillatory work in teleost fast muscle fibres. *Pflügers Arch* **419**, 177-183.

Johnston, I.A. (1983). On the design of fish myotomal muscles. *Mar. Behav. Physiol.* **9**, 83-98.

Johnston, I.A. and Battram, J. (1993). Feeding energetics and metabolism in demersal fish species from Antarctic, temperate and tropical environments. *Mar. Biol.* **115**, 7-14.

Johnston, I.A., Cole, N.J., Vieira, V.L.A. and Davidson, I. (1997). Temperature and developmental plasticity of muscle phenotype in herring larvae. *J. exp. Biol.* **200**, 849-868.

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

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

Johnston, I.A., Franklin, C.E. and Johnson, T.P. (1993). Recruitment patterns and contractile properties of fast muscle fibres isolated from rostral and caudal myotomes of the short-horned sculpin. *J. exp. Biol.* **185**, 251-265.

Johnston, I.A. and Horne, Z. (1994). Immunocytochemical investigations of muscle differentiation in the Atlantic herring (*Clupea harengus*: Teleostei). *J. mar. biol. Ass. U.K.* **74**, 79-91.

Johnston, I.A., Johnson, T.P. and Battram, J.C. (1991). Low temperature limits burst swimming performance in Antarctic fish. In *Biology of Antarctic fishes* (ed. B. Guido de Prisco, B. Maresca, B. Tota), pp. 179-190. Heidelburg: Springer Verlag.

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

Johnston, I.A. and Maitland, B. (1980). Temperature acclimation in crucian carp: a morphometric study of muscle fibre ultrastructure. *J. Fish Biol.* **17**, 113-125.

Johnston, I.A. and Moon, T.W. (1981). Fine structure and metabolism of multiply innervated muscle fibres in teleost fish. *Cell Tissue Res.* **219**, 93-109.

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

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

Johnston, I.A., van Leeuwen, J.L., Davies, M.L.F. and Beddow, T. (1995). How fish power predation fast-starts. *J. exp. Biol.* **198**, 1851-1861.

Jontes, J.D., Wilson-Kubalek, E.M. and Milligan, R.A. (1995). The brush border myosin-1 tail swings through a 32 ° arc upon ADP release. *Nature* **378**, 751-753.

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

Josephson, R.K. (1989). Power output from skeletal muscle during linear and sinusoidal shortening. *J. exp. Biol.* **147**, 533-537.

Josephson, R.K. (1993). Contraction dynamics and power output of skeletal muscle. *Annu. Rev. Physiol.* **55**, 527-546.

Josephson, R.K. and Stevenson, R.D. (1991). The efficiency of flight muscle from the locust *Schistocera americana*. *J. Physiol.* **442**, 413-420.

Josephson, R.K. and Stokes, D.R. (1989). Strain, muscle length and work output in a crab muscle. *J. exp. Biol.* **145**, 45-61.

Kennett, J.P. (1982). *Marine Geology*. New Jersey: Prentice-Hall.

King, P.A. and Fives, J.M. (1983). Littoral and benthic investigations on the west coast of Ireland - XVI. The biology of the long-spined sea scorpion *Taurulus bubalis* (Euphrasen, 1786) in the Galway Bay area. *Proc. R. Ir. Acad.* **83B**, 215-239.

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

Kirkpatrick, S.W., Covell, J.W. and Friedman, W.F. (1972). A new technique for the continuous assessment of fetal and neonatal cardiac performance. *Am. J. Obstet. Gynec.* **116**, 963-972.

Kleckner, N.W. and Sidell, B.D. (1985). Comparisons of maximal activities of enzymes from tissues of thermally-acclimated and naturally-acclimatized chain pickerel (*Esox niger*). *Physiol. Zool.* **58**, 18-28.

Kock, K-H. (1992). *Antarctic Fish and Fisheries*. Cambridge: Cambridge University Press.

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

Korneliussen, H., Dahl, H.A. and Paulsen, J.E. (1978). Histochemical definition of muscle fibre types in the trunk musculature of a teleost fish (cod, *Gadus morhua*, L.). *Histochem.* **55**, 1-16.

Kuffler, S.W. (1946). The relation of electrical potential changes to contracture in skeletal muscle. *J. Neurophysiol.* **9**, 367-377.

Labeit, S. and Kolmerer, B. (1995). Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* **270**, 293-296.

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

Lauder, G.V. (1983). Prey capture hydrodynamics in fishes: experimental test of two models. *J. exp. Biol.* **104**, 1-13.

Law, T.C. and Blake, R.W. (1996). Comparison of the fast-start performance of closely related, morphologically distinct threespine sticklebacks (*Gasterosteus* spp.). *J. exp. Biol.* **199**, 2595-2604.

Lehrer, S.S. (1994). The regulatory switch of the muscle thin filament: $Ca^{2+}$ or myosin heads? *J. Mus. Res. Cell Motil.* **15**, 232-236.

Leroi, A.M., Bennett, A.F. and Lenski, R.E. (1994). Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1917-1921.

Liem, K.F. (1980). Acquisition of energy by teleosts: adaptive mechanisms and evolutionary patterns. In *Environmental Physiology of Fishes* (ed. M.A. Ali), pp. 299-334. New York: Plenum Publishing Corp.

Lighthill, M.J. (1969). Hydromechanics of aquatic animal propulsion. *Annu. Rev. Fluid Mech.* **1**, 413-446.

Lindsey, C.C. (1978). Form, function, and locomotory habits. In *Fish Physiology* Vol. VII (ed. W.S. Hoar and D.J. Randall), pp. 1-100. London: Academic Press.

Lou, F., Curtin, N.A. and Woledge, R.C. (1997). The energetic cost of activation of white muscle fibres from the dogfish *Scyliorhinus canicula*. *J. exp. Biol.* **200**, 495-501.

Lowey, S., Slayter, H.S., Weeds, A.G. and Baker, H. (1969). Substructure of the myosin molecule I. Subfragments of the myosin by enzymic degradation. *J. Mol. Biol.* **42**, 1-29.

Lowey, S., Waller, G. and Trybus, K.M. (1993). Skeletal muscle myosin light chains are essential for physiological speeds of shortening. *Nature* **365**, 454-456.

Luiker, E.A. and Stevens, E.D. (1992). Effect of stimulus frequency and duty cycle on force and work in fish muscle. *Can J. Zool.* **70**, 1135-1139.

Luther, P.K. (1991). Three-dimensional reconstruction of a simple Z-band in fish muscle. *J. Cell Biol.* **113**, 1043-1055.

Luther, P.K., Munro, P.M.G. and Squire, J.M. (1995). Muscle ultrastructure in the teleost fish. *Micron* **26** (5), 431-459.

Luther, P.K., Squire, J.M. and Forey, P.L. (1996). Evolution of myosin filament arrangements in vertebrate skeletal muscle. *J. Morph.* **229** (3), 325-335.

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

Marden, J.H. (1995). Large-scale changes in thermal sensitivity of flight performance during adult maturation in a dragonfly. *J. exp. Biol.* **198**, 2095-2102.

Marden, J.H., Kramer, M.G. and Frisch, J. (1996). Age-related variation in body temperature, thermoregulation and activity in a thermally polymorphic dragonfly. *J. exp. Biol.* **199**, 529-535.

Marey, E.J. (1895). *Movement*. London: Heinemann.

Marsh, R.L. and Bennett, A.F. (1985). Thermal dependence of isotonic contractile properties of skeletal muscle and sprint performance in the lizard *Dipsosaurus dorsalis*. *J. Comp. Physiol. B.* **155**, 541-555.

Marsh, R.L. and 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.

Martinez, I. and Christiansen, J.S. (1994). Myofibrillar proteins in developing white muscle of the Arctic charr, *Salvelinus alpinus* (L.). *Comp. Biochem. Physiol.* **107B**, 11-20.

McConnaughey, B.H. (1978). *Introduction to Marine Biology*. St. Louis: The C.V. Mosby Company.

Méndez, J. and Keyes, A. (1960). Density and composition of mammalian muscle. *Metabolism* **9**, 184-188.

Miller, T.J., Crowder, L.B., Rice, J.A. and Marschall, E.A. (1988). Larval size and recruitment mechanisms in fishes: towards a conceptual framework. *Can. J. Fish. Aquat. Sci.* **45**, 1657-1670.

Moon, T. W., Altringham, J. D. and Johnston, I. A. (1991). Energetics and power output of isolated fish fast muscle fibres performing oscillatory work. *J. exp. Biol.* **158**, 261-273.

Morgan, D.L. and Proske, U. (1984). Vertebrate slow muscle - its structure, pattern of innervation and mechanical properties. *Physiol. Rev.* **64**, 103-169.

Morley, S.A. and Batty, R.S. (1996). The effects of temperature on "S-strike" feeding of larval herring, *Clupea harengus* L. *Mar. Fresh. Behav. Physiol.* **28**, 123-136.

Morris, S. and Taylor, A.C. (1983). Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Est., Coast. Shelf Sci.* **17**, 339-355.

Nemeth, D.H. (1997*a*). Modulation of buccal pressure during prey capture in *Hexagrammos decagrammus* (Teleostei: Hexagrammidae). *J. exp. Biol.* **200**, 2145-2154.

Nemeth, D.H. (1997*b*). Modulation of attack behaviour and its effect on feeding performance in a trophic generalist fish, *Hexagrammos decagrammus*. *J. exp. Biol.* **200**, 2155-2164.

Norton, S.F. (1991). Capture success and diet of cottid fishes: the role of predator morphology and attack kinematics. *Ecology* **72**(5), 1807-1819.

Norton, S.F. and Brainerd, E.L. (1993). Convergence in the feeding mechanics of ecomorphologically similar species in the Centrarchidae and Cichlidae. *J. exp. Biol.* **176**, 11-29.

Osse, J.W.M. and Muller, M. (1980). A model of suction feeding in teleostean fishes with some implications for ventilation. In *Environmental Physiology of Fishes* (ed. M.A. Ali), pp. 334-352. New York: Plenum Press.

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

Peterson, B.W. (1984). The reticulospinal system and its role in the control of movement. In: *Brain Stem Control of Spinal Cord Function* (ed. C.D. Barnes), 27-86, New York: Academic Press.

Precht, H. (1958). Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In *Physiological Adaptation* (ed. C.L. Prosser), pp. 50-78. Washington, DC: American Physiological Society.

Precht, H., Christophersen, J., Hensel, H. and Larcher, W. (1973). *Temperature and Life.* Berlin: Springer Verlag.

Prosser, C.L. (1973). Temperature. In *Comparative Animal Physiology* (ed. C.L. Prosser), pp. 362-428. London: W.B. Saunders Company.

Rall, J.A. and Woledge, R.C. (1990). Influence of temperature on mechanics and energetics of muscle contraction. *Am. J. Physiol.* **259**, R197-R203.

Rayment, I., Holden, H.M., Whittaker, M. Yohn, C.B., Lorenz, M., Holmes, K.C. and Milligan, R.A. (1993*b*). Structure of the actin-myosin complex and its implications for muscle contraction. *Science* **261**, 58-65.

Rayment, I., Rypniewski, W.R., Schmidtbase, K., Smith, R., Tomchick, D.R., Benning, M.M., Winkelmann, D.A., Wesenberg, G. and Holden, H.M. (1993*a*). 3-dimensional structure of myosin subfragment-1 - a molecular motor. *Science* **261**, 50-58.

Reebs, S.G., Whoriskey, F.G., Jr. and FitzGerald, G.J. (1984). Diel patterns of fanning activity, egg respiration, and the nocturnal behaviour of male three-spined sticklebacks, *Gasterosteus aculeatus* L. (f. *trachurus*). *Can. J. Zool.* **62**, 329-334.

Ritzmann, R.E. and Eaton, R.C. (1997). Neural substrates for initiation of startle responses. In *Neurons, Networks, and Motor Behavior* (ed. P.S.G. Stein, S. Grillner, A.I. Selverston and D.G. Stuart), pp. 33-44. London: The MIT Press.

Rome, L.C., Choi, I-H, Lutz, G. and Sosnicki, A. (1992). The influence of temperature on muscle function in the fast swimming scup. I. Shortening velocity and muscle recruitment during swimming. *J. exp. Biol.* **163**, 259-279.

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

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

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

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

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

Rome, L.C. and Swank, D. (1992). The influence of temperature on power output of scup red muscle during cyclical length changes. *J. exp. Biol.* **171**, 261-281.

Rossi, A.M., Eppenberger, H.M., Volpe, P., Cotrufo, R. and Wallimann, T. (1990). Muscle-type MM creatine-kinase is specifically bound to sarcoplasmic reticulum and can support $Ca^{2+}$ uptake and regulate ATP/ADP ratios. *J. Biol. Chem.* **265**, 5258-5266.

Rushmer, R.F., Franklin, D.L. and Ellis, R.M. (1956). Left ventricular dimensions recorded by sonocardiometry. *Circ. Res.* **14**, 684-688.

Scapolo, P.A., Veggetti, A., Mascarello, F., Romanello, M.G. (1988). Developmental transitions of myosin isoforms and organisation of the lateral muscle in the teleost *Dicentrarchus labrax* (L.). *Anat. Embryol.* **178**, 287-296.

Scheiner, S.M. (1993). Genetics and Evolution of Phenotypic Plasticity. *Annu. Rev. Ecol. Syst.* **24**, 35-68.

Schmidt-Nielsen, K. (1990). *Animal Physiology Adaptation and Environment.* Cambridge: Cambridge University Press.

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

Sidell, B.D., Johnston, I.A., Moerland, T.S. and Goldspink, G. (1983). The eurythermal myofibrillar protein complex of the mummichog (*Fundulus heteroclitus*): adaptation to a fluctuating thermal environment. *J. comp. Physiol.* **153**, 167-173.

Spedding, G.R. (1992). The aerodynamics of flight. In: *Advances in Comparative and Environmental Physiology 11. Mechanics and Animal Locomotion.* (ed. R. McN. Alexander), pp. 51-111. Berlin: Springer Verlag.

Squire, J.M. (1981). *The Structural Basis of Muscular Contraction.* New York: Plenum Press.

Stevens, E.D. (1993). Relation between work and power calculated from force-velocity curves to that done during oscillatory work. *J. Muscle Res. Cell Motil.* **14**, 518-526.

Stevenson, R.D. and Josephson, R.K. (1990). Effects of operating frequency and temperature on mechanical power output from moth flight muscle. *J. exp. Biol.* **149**, 61-78.

Swain, D.P. (1992). The functional basis of natural selection for vertebral traits of larvae in the stickleback *Gasterosteus aculeatus*. *Evolution* **46**, 987-997.

Swoap, S.J., Johnson, T.P., Josephson, R.K. and Bennett, A.F. (1993). Temperature, muscle power output and limitations on burst locomotor performance of lizard *Dipsosaurus dorsalis*. *J. exp. Biol.* **174**, 185-197.

Tamura, Y. T., Hatta, I., Matsuda, T., Sugi, H. and Tsuchiya, T. (1982). Changes in muscle stiffness during contraction recorded using ultrasonic waves. *Nature* **299**, 631-633.

Taylor, E.B. and McPhail, J.D. (1985). Variation in burst and prolonged swimming performance among British Columbia populations of Coho Salmon, *Oncorhynchus kisutch*. *Fish. Aquat. Sci.* **42**, 2029-2033.

Toyoshima, Y.Y., Kron, S.J., McNally, E.M., Niebling, K.R., Toyoshima, C. and Spudich, J.A. (1987). Myosin subfragment-I us sufficient to move actin filaments *in vitro*. *Nature* **328**, 536-539.

Trinick, J. (1996). Cytoskeleton - titin as a scaffold and a spring. *Curr. Biol.* **6**, 258.

Unesco (1986). *Fishes of the north-eastern Atlantic and the Mediterranean.* (ed. P.J.P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen and E. Tortonese), Vol. 3. Paris: Unesco.

Uyeda, T.Q.P., Abramson, P.D. and Spudich, J.A. (1996). The neck region of the myosin motor domain acts as a lever arm to generate movement. *Proc. Natl. Acad. Sci USA* **93**, 4459-4464.

van Leeuwen, J.L. (1983). Optimum prey capture techniques in fish. Ph.D. Thesis. Agricultural University, Wageningen, The Netherlands.

van Leeuwen, J.L., Lankheet, M.J.M., Akster, H.A. and Osse, J.W.M. (1990). Function of red axial muscles of carp (*Cyprinus carpio*): recruitment and normalized power output during swimming in different modes. *J. Zool., Lond.* **220**, 123-140.

Videler, J.J. (1985). Fish swimming movements: a study of one element of behaviour. *Neth. J. Zool.* **35**, 170-185.

Videler, J.J. and Hess, F. (1984). Fast continuous swimming of two pelagic predators, saithe (*Pollachius virens*) and mackerel (*Scomber scombrus*): a kinematic analysis. *J. exp. Biol.* **109**, 209-228.

Vigoreaux, J.O. (1994). The muscle Z-band: lessons in stress management. *J. Mus. Res. Cell Motil.* **15**, 237-255.

Wakeling, J.M. and Johnston, I.A. (1998). Muscle power output limits fast-start performance in fish. Submitted to *J. exp. Biol*.

Walker, J.B. (1979). Creatine: biosynthesis, regulation, and function. *Adv. Enzymol.* **50**, 177-242.

Walsh, S.J., Haney, D.C. and Timmerman, C.M. (1997). Variation in thermal tolerance and routine metabolism among spring- and stream-dwelling freshwater sculpins (Teleostei: Cottidae) of the southeastern United States. *Ecol. Fresh. Fish* **6**, 84-94.

Ward, G. and FitzGerald, G.J. (1983). Macrobenthic abundance and distribution in tidal pools of a Quebec salt marsh. *Can J. Zool.* **61**, 1071-1085.

Wardle, C.S. (1975). Limit of fish swimming speed. *Nature* **255**, 725-727.

Wardle, C.S. (1980). Effects of temperature on the maximum swimming speed of fishes. In *Environmental Physiology of Fishes* (ed. M.A. Ali), pp. 519-531. New York: Plenum Press.

Wardle, C.S. and He, P. (1988). Burst swimming speeds of mackerel, *Scomber scombrus* L. *J. Fish Biol.* **32**, 471-478.

Watabe, S., Imai, J., Nakaya, M., Hirayama, Y., Okamoto, Y., Masaki, H., Uozomi, T., Hirono, I. and Aoki, T. (1995). Temperature acclimation induces light meromyosin isoforms with different primary structures in carp fast skeletal muscle. *Biochem. biophys. Res. Commun.* **208**, 118-125.

Watkins, T.B. (1996). Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla. Physiol. Zool.* **69**, 154-167.

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

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

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

Webb, P.W. (1978*a*). Temperature effects on acceleration of rainbow trout. *J. Fish. Res. Bd. Can.* **35**, 1417-1422.

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

Webb, P.W. (1982). Fast-start resistance in trout. *J. exp. Biol.* **96**, 93-106.

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

Webb, P.W. (1990). How does benthic living affect body volume, tissue composition and density of fishes. *Can. J. Zool.* **68**, 1250-1255.

Webb, P.W. (1993). Swimming. In *The Physiology of Fishes* (ed. D.H. Evans), pp. 47-73. USA: CRC Press Inc.

Webb, P.W. and Johnstrude, C.L. (1988). The effect of size on the mechanical properties of the myotomal-skeletal system of rainbow trout (*Salmo gairdneri*). *Fish Physiol. Biochem.* **5**, 163-171.

Webb, P.W. and Weihs, D. (1986). Functional locomotor morphology of early life history stages of fishes. *Trans. Am. Fish. Soc.* **115**, 115-127.

Webb, P.W. and Zhang, H. (1994). The relationship between responsiveness and elusiveness of heat-shocked goldfish (*Carassius auratus*) to attacks by rainbow trout (*Oncorhynchus mykiss*). *Can. J. Zool.* **72**, 423-426.

Weeds, A.G. and Lowey, S. (1971). Substructure of the myosin molecule II. The light chains of myosin. *J. Mol. Biol.* **61**, 701-725.

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

Weis-Fogh, T. (1973). Quick estimates of flight fitness in hovering animals, including novel mechanisms for lift production. *J. exp. Biol.* **59**, 169-230.

Whittaker, M., Wilson-Kubalek, E.M., Smith, J.E., Faust, L., Milligan, R.A., Sweeney, H.L. (1995). Smooth muscle myosin moves 35Å upon ADP release. *Nature* **378**, 748-751.

Wilkie, D.R. (1960). Thermodynamics and interpretation of biological heat measurements. *Prog. Biophys. Chem.* **10**, 259-298.

Witthames, P.R. and Greer-Walker, M. (1982). The activity of myofibrillar and actomyosin ATPase in the skeletal muscle of some marine teleosts in relation to their length and age. *J. Fish Biol.* **20**, 471-478.

Woledge, R.C. (1989). Energy transformation in living muscle. In *Energy Transformations in Cells and Organisms* (ed. W. Weiser and E. Gnaiger), pp. 36-45. New York: Georg Thieme Verlag.

Woledge, R.C. and Reilly, P.J. (1988). Molar enthalpy change for the hydrolysis of phosphocreatine under conditions in muscle cells. *Biophys. J.* **54**, 97-104.

Yocom, T.G. and Edsall, T.A. (1974). Effect of acclimation temperature and heat shock on vulnerability of fry of lake whitefish (*Coregonus clupeaformis*) to predation. *J. Fish. Res. Bd. Can.* **31**, 1503-1506.

Zamudio, K.R., Huey, R.B. and Crill, W.D. (1995). Bigger isn't always better: body size, developmental and parental temperature, and territorial success in *Drosophila melanogaster*. *Anim. Behav.* **49**, 671-677.

Zar, J.H. (1996). *Biostatistical Analysis,* pp. 662. London: Prentice-Hall International.

## ACKNOWLEDGEMENTS

The completion of this thesis would certainly not have been possible without the guidance, encouragement and friendship of some wonderful people. Firstly, I would like to thank my supervisor, Professor Ian Johnston, for always finding time to inspire and help in his hectic schedule and for having the faith to now be my employer. What excitement is in store for us!

I owe everything to Dr. Rob. James and Dr. James Wakeling. Dr. Rob. James, without whom fish would still be swimming but the world of muscle mechanics would be a dreaded mystery, gave me continual support and put up with a sometimes frantic student. I am eternally grateful. Gratitude also goes to him for the HPLC analysis of Chapter 4, the running practice and relay races, the fishing expeditions and the great cooking. I thank Dr. James Wakeling for the use of his programmes in Chapter 5, his enthusiasm, his patience, his instruction on proper soldering, for always brimming with ideas and for being a splendid teacher. I now know what a genius is.

The nature of this thesis also required the expertise of a number of other people. Thanks to Iain Tech for always being so helpful and cheerful and in short for all things technical; Jimmy Murdoch for the temperature data, a fantastic job in the aquarium and for being totally incomprehensible to my english ears; Bob Wilson for keeping me well supplied with sculpin; Marguerite for her Kontron guidance and store of rulers; Micha for his willing to sacrifice his time in order to give statistics lessons; Murray Couttes for fixing the muscle rig and attempting to explain electronics; Christina and Jane for their assistance and laughter; Pete for the orders needed yesterday; Derek Ball for the pipetting tips; Craig Franklin for his guidance and help during my first year; and Robbie Wilson for the helpful comments on my manuscript and for breaking everything in sight.

I also thank my family, particularly Mum, Frank, Granjie and Poppa, for all their love and support, and for having faith in me.

Finally, thanks go to my two dear house-mates, Lesley and Nick, who have made the unimaginable of our Liverpool days become real. Lesley - for that laugh, for her contagious enthusiasm for life and for being my talk specialist. I thank Nick with all my heart for his patience, friendship and love and making me so happy.