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RECRUITMENT PATTERNS AND CONTRACTILE PROPERTIES OF FAST MUSCLE FIBRES ISOLATED FROM ROSTRAL AND CAUDAL MYOTOMES OF THE SHORT-HORNED SCULPIN

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Summary

Muscle action during swimming and the contractile properties of isolated muscle fibres were studied in the short-horned sculpin *Myoxocephalus scorpius* at 5°C. Semi-steady swimming, startle responses and prey-capture events were filmed with a high-speed video at 200frames s^{-1}, using fish 22–26cm in total length (*L*). Electromyographical (EMG) recordings, synchronised with the video, were made from fast muscle in rostral and caudal myotomes at points 0.40*L* and 0.80*L* along the body. Fast muscle fibres were first recruited at tail-beat frequencies of 3.7–4.2Hz, corresponding to a swimming speed of 1.7 *L* s^{-1}. Electrical activity in the muscles occurred during 16–38% of each tail-beat cycle regardless of frequency. Muscle fibres were activated during the lengthening phase of the cycle. In caudal myotomes, the onset of the muscle activity occurred at a phase of 75–105° at 3.7Hz, decreasing to approximately 50° at frequencies greater than 4.5Hz (0° phase was defined as the point at which muscle fibres passed through their resting lengths in the stretch phase of the cycle; a full cycle is 360°). Prey capture was a stereotyped behaviour consisting of a preparatory movement, a powerstroke at 7–9Hz and a glide of variable duration. The delay between the activation of muscle fibres in rostral and caudal myotomes during prey capture and startle responses was approximately 10ms.

Fast muscle fibres isolated from rostral and caudal myotomes were found to have similar isometric contractile properties. Maximum tetanic stress was 220kN m^{-2}, and half-times for force development and relaxation were approximately 50ms and 135ms respectively. Power output was measured by the 'work loop' technique in muscle fibres subjected to sinusoidal length changes at the range of frequencies found during swimming. Under optimal conditions of strain and stimulation, muscle fibres from rostral and caudal myotomes produced similar levels of work (3.5 J kg^{-1}) and generated their maximum power output of 25–30 W kg^{-1} at the tail-beat frequencies used in swimming (4–8Hz). Progressively delaying the onset of stimulation relative to the start of the strain

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cycle resulted in an initial modest increase, followed by a decline, in the work per cycle. Maximum positive work and net negative work were done at stimulus phase values of 20–50° and 120–140° respectively. The EMG and swimming studies suggest that fast muscle fibres in both rostral and caudal myotomes do net positive work under most conditions.

Introduction

Subcarangiform swimming involves the alternate shortening and lengthening of myotomes on either side of the body. A wave of bending passes caudally down the fish and maximum thrust is developed towards the caudal fin (Lighthill, 1971). The cross-sectional area of muscle is greatest in the anterior half of the body, whereas the proportion of red to white muscle fibres increases towards the caudal penduncle (Greer-Walker and Pull, 1975). Electromyographical (EMG) studies have shown that there is a successive recruitment of slow red, fast red and fast white muscle fibres with increasing speed (Johnston *et al.* 1977). Kinematic analysis of subcarangiform swimming at uniform speed suggests that muscle strain approximates a sine wave and increases in magnitude from the head to the tail (Hess and Videler, 1984; van Leeuwen *et al.* 1990). Using the work loop technique pioneered by Josephson (1985), several studies have measured the power output of fast and slow muscle fibres subjected to sinusoidal length changes under conditions that maximise the work per cycle (Altringham and Johnston, 1990*a*; Johnson and Johnston, 1991; Anderson and Johnston, 1992; Rome and Swank, 1992). Maximum power output *in vitro* is developed by stimulating the muscle in the lengthening phase of the cycle such that peak force coincides with the onset of muscle shortening. However, the wave of electrical activity passes down the trunk faster than the wave of bending, resulting in systematic phase differences in muscle length and force cycles along the length of the body (Williams *et al.* 1989; van Leeuwen *et al.* 1990). van Leeuwen *et al.* (1990) modelled normalised force and power in red muscle fibres of the common carp (*Cyprinus carpio* L.) at different points along the body. During slow continuous swimming, red muscle fibres in rostral myotomes produced peak force at the onset of the shortening phase and, except for a short initial phase, performed net positive work over almost the entire tail-beat cycle (anticlockwise work loops). In contrast, stimulation was sufficiently delayed in caudal myotomes that these muscle fibres developed their maximum force whilst being lengthened by the antagonistic muscles. Under these conditions, work is done on the muscles (negative work) and clockwise loops are produced. Muscle fibres near the anus produced complex loops with both positive and negative work components (van Leeuwen *et al.* 1990). Similar changes in the size and shape of work loops for isolated muscles can be produced by progressively retarding the onset of muscle stimulation in relation to the length-change cycle (Johnson and Johnston, 1991).

The aim of the present study was to investigate the contractile properties and recruitment patterns of fast fibres isolated from rostral and caudal myotomes of the short-horned sculpin (*Myoxocephalus scorpius* L.). This fish is a typical sit-and-wait ambush predator, having a large head and reduced axial musculature and spending long periods

stationary on the sea bed. Because muscle contractile properties in this species can be modified by a period of temperature acclimation (Johnson and Johnston, 1991), all the experiments were performed on winter-acclimatised fish maintained at 5°C.

Materials and methods

Short-horned sculpin (*Myoxocephalus scorpius* L.) were caught in St Andrews Bay, Scotland, between November and February, and maintained in tanks of filtered sea water in the Gatty Marine Laboratory, St Andrews, at 5°C for 1–2 months before use. All swimming and mechanical experiments were carried out at 5.0±0.2°C, on fish ranging from 20 to 26cm total length (*L*) (23.6±3.2cm, mean ± S.D., *N*=20). Fish were fed daily on shrimps and chopped squid.

Swimming experiments

Sculpins were transported from the Gatty to the Dunstaffnage Marine Laboratory and held under the same conditions for 1–3 weeks prior to experiments. Fish were anaesthetised with a 1:5000 (w/v) solution of tricaine methane sulphonate (MS 222) in sea water buffered to approximately pH7.0 with $NaHCO_3$ ($60 mg\,l^{-1}$). Bipolar hook electrodes, made from 200 μm diameter insulated copper wire or 150 μm diameter Teflon-coated silver wire, were inserted into the dorsal fast muscle region of left rostral and caudal myotomes at points 0.40*L* and 0.80*L* along the body (Fig. 1). The electrodes were securely sutured to the skin with surgical thread and the fish were allowed to recover overnight. The wires were loosely suspended above the fish using thin elastic cord. This enabled the fish to move unhindered around the tank. Electromyograms (EMGs) were recorded from these electrodes using a differential amplifier (A-M Systems, Everett, USA) with high- and low-pass filter settings of 100Hz and 1000Hz, respectively, and a gain of 1000. Records were displayed using a thermal array recorder (Summagraphics, Ltd) operated in either direct or memory mode.

Swimming sequences were filmed at $200 frames\,s^{-1}$ with a high-speed video (Nac, Japan) in a tank (200 cm×380cm×15 cm deep) held in a constant-temperature room at 5°C. Light from a 40W strobe lamp was reflected off a semi-silvered mirror set at an angle of 45° in front of the camera lens. Sharp silhouettes of the fish were obtained by using a Scotchlite reflex reflector background with a graduated scale. A timing signal consisting of a train of square pulses was displayed on both the chart recorder and the high-speed video (using a wave inserter) and was used to synchronise the EMG recordings with the video-taped swimming sequences. The pulse inserted onto the video and chart recorder could be correlated within 1/100th of the interframe interval (i.e. 0.05 ms).

Escape responses were elicited by tactile stimulation of the caudal fin using a 1cm diameter probe. Prey attack sequences were filmed following the introduction of shrimps (*Crangon crangon*) into the filming tank. Disturbance of the fish and changing light levels in the room were both found to be effective in inducing semi-steady swimming sequences over a range of speeds. Individual fish were filmed for up to 48h. At the end of experiments, fish were killed by a blow to the head and pithed. The locations of the tips of the recording electrodes were determined by dissection.

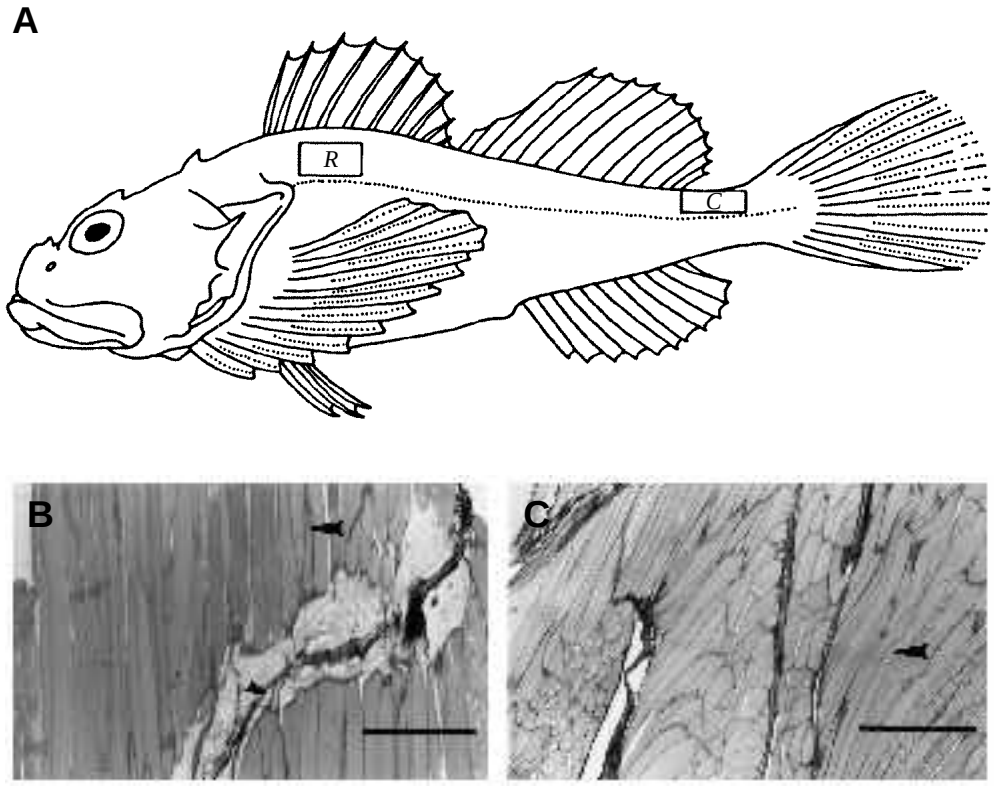

Fig. 1. (A) Diagram of a short-horned sculpin showing the sites in rostral (*R*) and caudal (*C*) myotomes at which EMG electrodes were inserted and from which muscle fibres were isolated. The light micrographs (B,C) are saggital sections stained with haemotoxylin and eosin to show the sites and orientation (illustrated by arrowheads) of the fast muscle fibres used for mechanical experiments. The rostral (B) and caudal (C) myotomes sampled were approximately 0.40 and 0.80 of the distance from the tip of the snout to the end of the caudal fin. Scale bars, 750 μm.

Measurement of contractile properties

Bundles of 3–10 fast muscle fibres were isolated from the superficial layers of the same rostral and caudal myotomes studied in the EMG experiments (Fig. 1). Fibre dissection was carried out on a cooled microscope stage in chilled Ringer (composition in mmol l^{-1}: NaCl, 132.2; sodium pyruvate, 10; KCl, 2.6; $MgCl_2$, 1; $CaCl_2$, 2.7; NaH_2CO_3, 18.5; NaH_2PO_4, 3.2; pH7.4 at 5°C). T-shaped clips of aluminium foil were attached to the ends of the preparation by short lengths of myosepta as close to the tendinous insertions as possible. Preparations were transferred to a chamber through which aerated Ringer's solution was circulated at constant temperature. The ends of the fibre bundle were attached by steel hooks to a servo-motor and force transducer (AME801, SensorNor, Horten, Norway). Stimuli were applied *via* two platinum electrodes lying on either side of the preparation. The length of the fibre bundle was adjusted so as to obtain the maximum twitch (2ms pulses, 12V). This was found to correspond to a sarcomere length of approximately 2.2 μm measured by laser diffraction (resting fibre length, l_0). Muscle fibre

length was measured with a binocular microscope (magnification ×20). Preparations were left to recover for 10min between cycles of stimulation.

During continuous swimming, the muscle fibres in the myotomes undergo approximately sinusoidal length changes (Hess and Videler, 1984). In order to simulate their activity *in vivo*, fibres were subject to sinusoidal length changes around l_0 and stimulated during each cycle. The frequency of applied length changes and the duty cycle of stimulation chosen for our experiments included the range of values obtained during the EMG and high-speed video recordings. The number and frequency of stimuli were adjusted at each frequency of movement to produce the maximum work per cycle. Muscle stimulation and length changes were controlled by a microcomputer and the data were collected and analysed on-line using in-house software. Stimulus phase was defined from the point when the fibres passed through their resting length (0˚ phase) whilst lengthening; a full cycle is 360˚. In order to investigate the influence of stimulus phase on the maximum work output, the number and frequency of stimuli were adjusted at a fixed cycle frequency of 5Hz and a strain of ±5% l_0. The fibres were typically subjected to eight cycles of work, with a 10–15min rest between trains of cycles. The work performed in each cycle was calculated by plotting force against fibre length to obtain work loops (anticlockwise loops indicate positive work and clockwise loops indicate negative work). Power output is obtained from the net work per cycle multiplied by cycle frequency. At the end of experiments, preparations were pinned out at their resting length on a strip of silicone elastomer (Sylgard 184, Dow Corning, Seneffe, Belgium) and frozen in isopentane cooled to near its freezing point with liquid nitrogen (−159˚C). The frozen preparation was mounted on a cryostat chuck, equilibrated to −20˚C, and sectioned transversely at 10 μm at 3–5 points along its length. Sections were stained for myosin ATPase activity (Johnston *et al.* 1974) and the cross-sectional area of undamaged fibres was determined using a digital planimeter (VideoPlan, Image Analysis System, Kontron, Basel). Data on the contractile properties of muscle fibres isolated from rostral and caudal myotomes were compared using either a paired or a two-tailed Student's *t*-test.

Results

Muscle fibre recruitment

In the laboratory, *Myoxocephalus scorpius* spends long periods stationary on the bottom of the aquarium interspersed with bouts of subcarangiform swimming at relatively low speed (less than $1\,L\,s^{-1}$). Semi-steady swimming sequences at intermediate speeds were elicited by tactile stimulation and typically consisted of 3–5 relatively uniform strides. In contrast, the fastest swimming speeds were always unsteady, involving periods of acceleration and deceleration. Most of these events were either associated with prey capture or were startle responses. Prey-capture behaviour was highly stereotyped. Following visual contact with the shrimp, the sculpins moved forward slowly to within 15–25cm of their prey. The prey-capture sequence consisted of a preparatory stroke (C-start), a powerstroke with the pectoral fins abducted, and a glide during which the mouth was extended to suck in the prey and the pectoral fins were

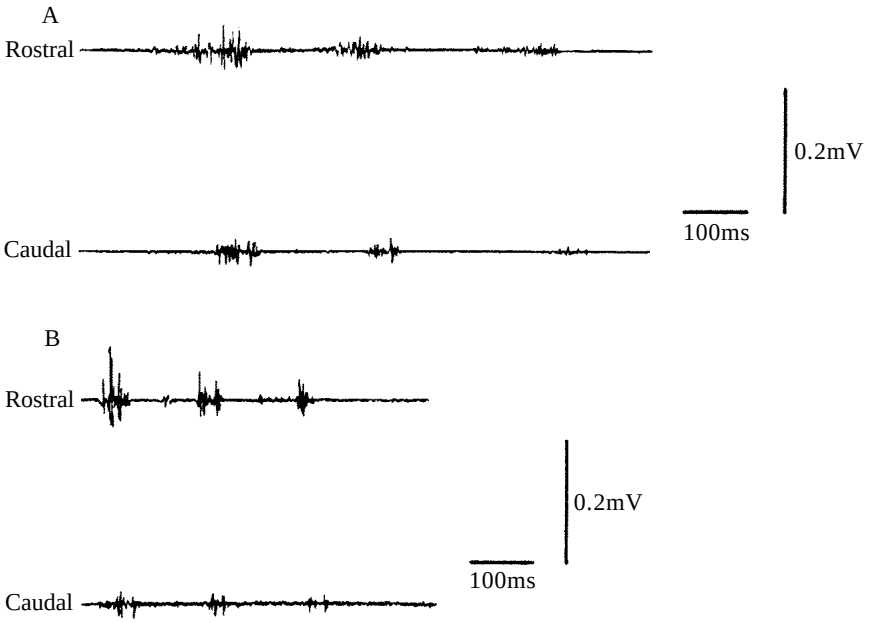

Fig. 2. Muscle recruitment patterns in the short-horned sculpin (*Myoxocephalus scorpius*). Electromyograms (EMGs) were recorded from fast muscle fibres in rostral and caudal myotomes at points 0.40*L* and 0.80*L*, respectively, from the tip of the snout to the end of the caudal fin. (A) Semi-steady swimming at the tail-beat frequency (4.2Hz) at which EMGs were first recorded from the fast muscle fibres (*L*=24cm). (B) Semi-steady swimming at a tail-beat frequency of 6.5Hz.

adducted to serve as brakes. The frequency of the power-stroke ranged from 7 to 9Hz, at a tail-beat amplitude of approximately $0.26\,L$. The average speed during the power-stroke ranged from 2 to $4\,L\,s^{-1}$, whereas maximum speeds measured over any 20ms interval reached $5\,L\,s^{-1}$.

EMGs from the fast muscle fibres were first recorded at tail-beat frequencies of 3.7–4.2Hz, corresponding to a swimming speed of $1.7\,L\,s^{-1}$ (Fig. 2A). During semi-steady swimming, significant variation in tail-beat amplitude was observed at a given frequency, even for individual fish. The duration of electrical activity in the caudal myotomes was also variable, ranging from 16% to 38% of the tail-beat cycle, and was independent of frequency (Fig. 3). The length of time between the onset of electrical activity in rostral (0.40*L*) and caudal (0.80*L*) myotomes decreased with increasing tail-beat frequency (Fig. 2A,B). During startle responses and prey capture, the delay between the activation of anterior and posterior myotomes was typically 10ms at a tail-beat frequency of 7–9Hz.

In order to obtain information about the timing of electrical activity in the muscles in relation to changes in muscle length, videotapes were examined frame-by-frame (5ms intervals). Only sequences that consisted of a minimum of three complete strides were analysed. The frame in which the caudal myotomes had no curvature was identified for each cycle in order to establish the point at which muscle fibres passed through their

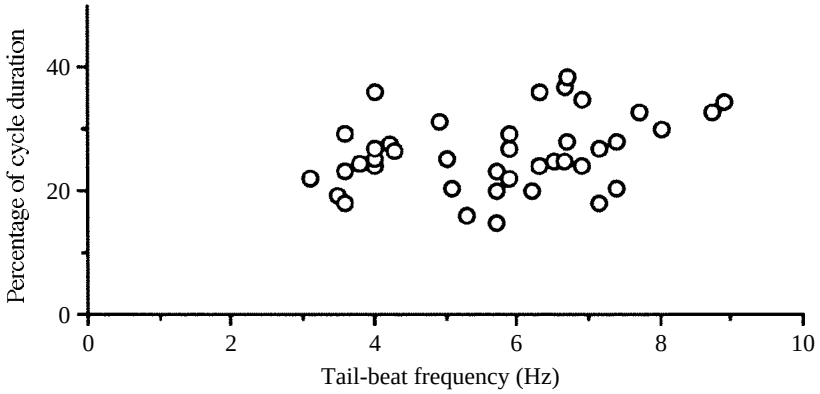

Fig. 3. The relationship between the duration of the muscle activity expressed as a percentage of the tail-beat cycle time (percentage of cycle duration) and tail-beat frequency. Data are from the caudal myotomes of five fish.

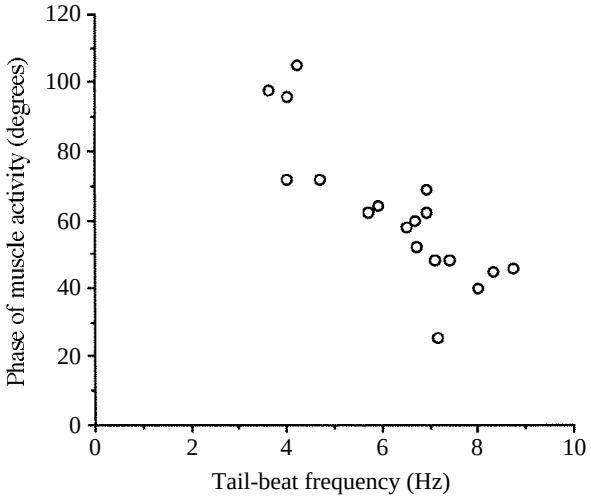

Fig. 4. The relationship between the phase of muscle activity and tail-beat frequency for muscle fibres in caudal myotomes of the short-horned sculpin. Phase was defined from the point at which the muscle fibres passed through their resting lengths (trunk straight) on the stretch phase of the cycle. A full cycle is 360˚.

resting length (l_0). Values of the phase of muscle activation (ϕ) calculated using this reference point are independent of any assumptions about the waveform of muscle length changes. There was a trend for values of ϕ to decrease with increasing tail-beat frequency (Fig. 4). At tail-beat frequencies just above the threshold for the recruitment of fast muscle fibres, ϕ ranged from 75 to 105˚, whereas at frequencies greater than 4.5Hz, ϕ decreased from 70˚ to a minimum of 26˚ (Fig. 4). Because of the small amplitude of body movements at the front of the fish, it was not possible to carry out a similar analysis for the rostral myotomes using this methodology.

Table 1. *Mechanical properties of fast muscle fibres isolated from the rostral and caudal myotomes of the short-horned sculpin*

Variable	Rostral	Caudal
Isometric contractions	(*N*=6)	(*N*=6)
Maximum tetanic stress (kNm^{-2})	222±67	218±66
$T_{1/2P}$ (ms)	48.7±7.8	51.4±10.0
$T_{1/2R}$ (ms)	136.4±41.5	133.6±50.7
Twitch tetanus ratio	0.44±0.04	0.47±0.07
Oscillatory work at 5Hz	(*N*=5)	(*N*=5)
Maximum work per cycle (Jkg^{-1})	5.7±1.9	5.8±3.1
Maximum power output (Wkg^{-1})	28.4±9.7	29.1±11.9
Optimal number of stimuli per cycle	3	3
Optimal stimulus phase (degrees)	19±4	20±4
Phase delay between peak length and peak force (degrees)	33±11	29±6

All experiments were conducted at 4°C.
Values represent mean ± S.D.
$T_{1/2P}$, time to 50% peak tetanic force; $T_{1/2R}$, time from last stimulus to 50% of peak tetanic force.
Maximum tetanic stress was produced at a stimulation frequency of 50–60Hz for preparations from both rostral and caudal myotomes.

Isometric contractile properties

Muscle fibre preparations gave reproducible contractions for periods of up to 48 h (maximum stress falling by less than 5%), although experiments were usually completed within 10h. Multiple stimulation at 50–60Hz caused fused tetani in all cases. Fast muscle fibres isolated from rostral and caudal myotomes also produced similar maximum isometric stresses (P_0) and twitch tetanus ratios (Table 1). The half-times for force development (from first stimulus to $0.5P_0$) and relaxation (from last stimulus to $0.5P_0$) were approximately 50 and 135ms, respectively, for fibres isolated both from rostral and from caudal myotomes.

Work loop experiments

As reported previously for fast fibres from sculpin abdominal myotomes (Altringham and Johnston, 1990*a*), force declined by 10–20% over the first 3–4 contraction cycles before reaching a steady state. Results calculated for the second and fourth cycles of oscillatory work were found to be similar: data illustrated are for the fourth cycle. The relationship between work per cycle and strain at optimum values of stimulus phase and duty cycle are shown in Fig. 5. Maximum work per cycle was 3.5–4.0 $J\,kg^{-1}$ wetmusclemass for strains of ±3 % l_0 to ±9 % l_0, but declined to approximately 1 $J\,kg^{-1}$ at ±1 % l_0 (Fig. 5). There were no significant differences in the maximum work performed by muscle fibres from rostral and caudal myotomes at any of the strain values. For fibres oscillating at 5Hz at a strain of ±5 % l_0, net positive work increased with increasing values of stimulus phase until a maximum was reached at 20–50˚ (Fig. 6). At even higher values of stimulus phase, the net work per cycle declined,

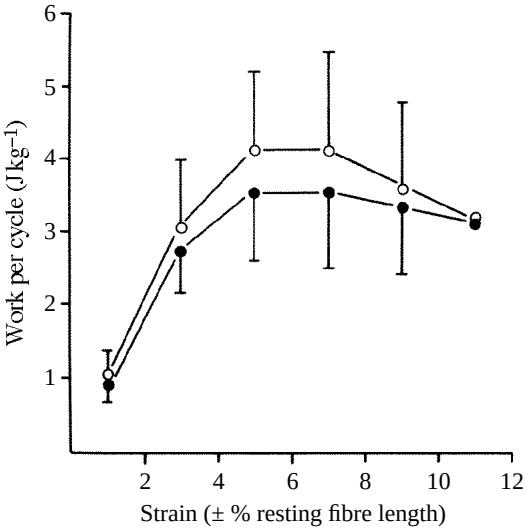

Fig. 5. Work per cycle at various values of strain for fast muscle fibres isolated from rostral (filled symbols) and caudal myotomes (open symbols) of the short-horned sculpin. Muscle fibres were subjected to sinusoidal length changes about their resting length at 7Hz and given a single supramaximal stimulus in each cycle. The stimulus phase was varied from 10° to 60° to determine the optimal value for each preparation. This optimal value was then used for all subsequent experiments with that preparation. Values are mean ± S.D., N=5.

reaching zero at 120–140°, and the work loops became complex with both negative and positive work components (not illustrated, but see Fig. 4 in Johnson and Johnston, 1991). There were no significant differences in the work per cycle at equivalent values of stimulus phase for fast muscle fibres isolated from rostral and caudal myotomes (Fig. 6). The stimulus phase and duty cycle were adjusted to give the maximum work per cycle at a constant strain (±5% l_0) over a range of frequencies. Fast muscle fibres were capable of large amounts of work at movement frequencies below their recruitment threshold, but only if large numbers of stimuli were given. The net work per cycle declined from approximately $7\,J\,kg^{-1}$ wetmass at 4Hz to about $1.5\,J\,kg^{-1}$ at 13Hz (Fig. 7). The optimal power output was within its maximum range (25–30 $W\,kg^{-1}$) for cycle frequencies of 4–8Hz, dropping by 40–60% at 13Hz (Fig. 8). Under the conditions for optimal work, there were no significant differences in the performance of fast muscle fibres isolated from rostral and caudal myotomes (Table 1).

Discussion

Fast muscle fibres isolated from the dorsal region of rostral and caudal myotomes of the short-horn sculpin have similar *in vitro* contractile properties. Muscle fibres from different points along the body have similar twitch contraction times and produce approximately the same maximum tensions (Table 1, Figs 6 and 8). In contrast, Wardle (1985) has shown that the twitch contraction times of large blocks of myotomal muscle in

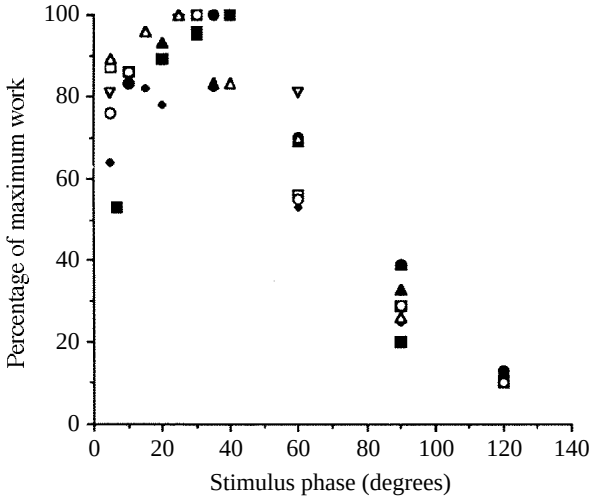

Fig. 6. The relationship between stimulus phase and work per cycle at a strain of ±5 % resting muscle fibre length and an optimal stimulation duty cycle. Fast muscle fibres were isolated from rostral (filled symbols) and caudal myotomes (open symbols) of the short-horned sculpin.

some other teleosts increase from the head towards the caudal penduncle. Studies with small bundles of isolated fast muscle fibres have recently confirmed Wardle's observations for the Atlantic cod (*Gadus morhua*) (Davies and Johnston, 1993) and the saithe (*Pollachius virens*) (Altringham *et al.* 1993). For example, for fast muscle fibres isolated from the cod, the duration (stimulus to 95% relaxation) of isometric twitches at 5 °C increased from 85±9ms in rostral myotomes (N=13) to 137±28ms (N=7) in caudal myotomes (mean ± S.D., P<0.01) (Davies and Johnston, 1993). These results cannot be extrapolated to intact fish because strain fluctuations differ along the length of the fish (Hess and Videler, 1984; Rome *et al.* 1992) and have profound effects on twitch duration (Altringham and Johnston, 1990*b*). In cod muscle fibres, force is higher and relaxation more rapid during oscillatory contractions than during isometric twitches (Altringham and Johnston, 1990*b*). Other factors which influence twitch duration *in vivo* include the length and properties of tendons and other elastic structures, which in turn may also differ between rostral and caudal myotomes.

There is considerable variation in body shape and swimming style and in the relative proportions of red and white muscles between species (Greer-Walker and Pull, 1975; Lindsey, 1978). A corresponding diversity in muscle recruitment patterns has also been observed, particularly in relation to the range of swimming speeds at which the fast motor system first comes into operation (Bone *et al.* 1978; Johnston and Moon, 1980*a*,*b*; Rome *et al.* 1985). Most studies of muscle fibre recruitment have involved athletic pelagic species and the analysis of continuous swimming at a steady speed. For example, saithe (L=17cm) were found to recruit their fast muscle fibres at swimming speeds of 1.8–2.0 $L\,s^{-1}$. Fish of this length that swam at 2.1 $L\,s^{-1}$ for 3 weeks in an exercise chamber showed a marked hypertrophy of the fast muscle fibres (Johnston and Moon, 1980*a*). In other species, such as the Pacific herring (Bone *et al.* 1978) and striped bass

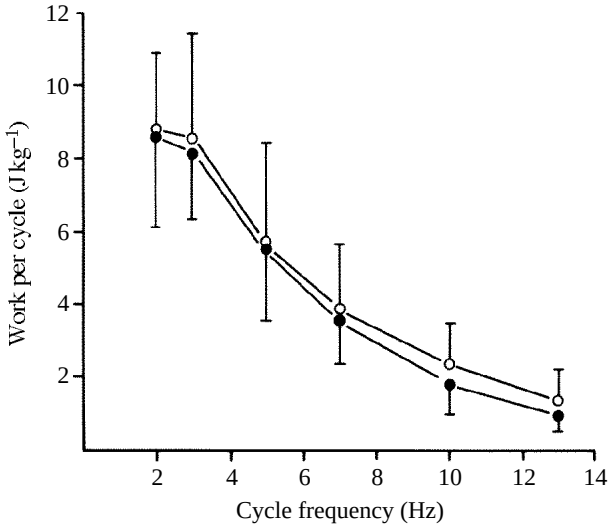

Fig. 7. The relationship between work per cycle and cycle frequency for fast muscle fibres isolated from rostral (filled symbols) and caudal myotomes (open symbols) of the short-horned sculpin. Muscle fibres were subject to sinusoidal length changes about their resting length at a strain of ±5% l_0. Stimulus phase and duty cycle were adjusted in order to maximise the work per cycle. Values are mean ± S.D., N=5.

(Sissons and Sidell, 1987), the recruitment of fast muscle fibres leads to fatigue within a few minutes. The short-horned sculpin only swims continuously at tail-beat frequencies of 1.0–3.5Hz, speeds less than $1\,L\,s^{-1}$, during which no electrical activity is recorded from the fast muscle fibres. In fish 22–26cm long, fast muscle fibres are first recruited at tail-beat frequencies of 3.7–4.2Hz, corresponding to an average speed of $1.7\,L\,s^{-1}$. The fast muscle fibres are rarely active for more than 10 consecutive tail beats before the fish settles on the substratum or slows to speeds that are powered by the red muscle alone. Relatively few of the recorded sequences contained more than 3–5 similar tail beats (referred to as semi-steady swimming in the present study).

Values for the phase of muscle activation calculated for fast muscle fibres in caudal myotomes of the short-horned sculpin during semi-steady swimming varied from approximately 100° at 3.7–4.2Hz to 45° at 7–8Hz (Fig. 4). The point at which caudal muscle fibres passed through their resting lengths (l_0) was determined by identifying the frame in which the trunk was straight with respect to the swimming track. This enabled muscle l_0 to be estimated with a theoretical accuracy of 7° at 4Hz and 16° at 9Hz. The effects of varying stimulus phase on work output were investigated in experiments with isolated fast muscle fibres at a constant strain of ±5% l_0. Stimulus variables were adjusted to obtain the maximum work at each phase: this required duty cycles of 33% at 4Hz and 18% at 9Hz. At the threshold speed for the recruitment of fast muscle (3.7–4.2Hz), the stimulus phase for fibres in caudal myotomes was 75–105°, resulting in 30–40% of the maximum work per cycle (Fig. 6). For tail-beat frequencies in the range 5–7Hz, the stimulus phase at caudal myotomes decreased to 70–45° which, in the *in vitro* experiments, resulted in 65–100% of the maximum work. During prey capture, caudal

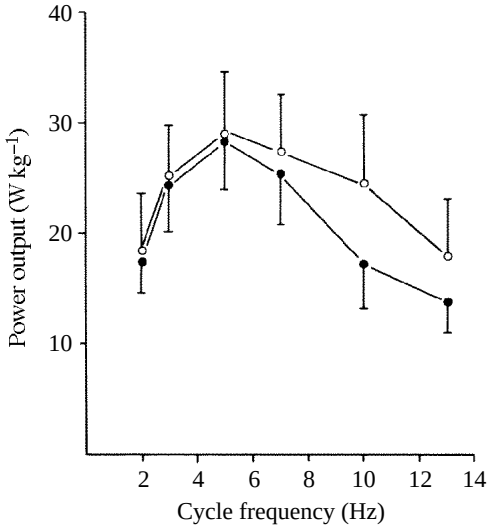

Fig. 8. The relationship between maximum power output and cycle frequency for fast muscle fibres isolated from rostral (filled symbols) and caudal myotomes (open symbols) of the short-horned sculpin. Details of the experimental conditions are given in the legend to Fig. 7. Values are mean ± S.D., N=5.

muscle fibres were always activated at phase values (25–45˚) that produced the maximum net work and power. It was not possible to estimate the phase of muscle activation for the rostral myotomes because of the small amplitude of body movements at the front of the fish. However, Wardle and Videler (1993) have made such measurements during steady straight swimming in the saithe and mackerel (*Scomber scrombrus*). The phase delay between the onset of muscle activity and the strain cycle in rostral (0.4L) myotomes was 30–40˚ at all tail-beat frequencies. *In vitro* work loop experiments show that maximum work is produced at these values of stimulus phase (Fig. 6; Altringham and Johnston, 1990*a*; Johnson and Johnston 1991; Altringham *et al.* 1993). The extra work done in stretching the active muscle is more than compensated for by the increase in force caused by stretch activation, which peaks at the onset of the shortening phase of the cycle, thereby maximising positive work (Johnston, 1991). Wardle and Videler (1993) obtained different results from ours for the timing of muscle activation in the caudal myotomes of mackerel and saithe swimming at uniform speed. The onset of electrical activity in myotomes at 0.85L occurred at a phase of 330˚. Thus, fibres towards the tail were activated when shorter than l_0 and the period of rising force production occurred whilst the muscle was being stretched (Altringham *et al.* 1993). Under these conditions, muscle fibres resist stretch, and stiffness is maximised, leading Altringham *et al.* (1993) to suggest that the caudal fibres transmit power generated by rostral myotomes to the tail blade. According to this idea, a transition zone, in which the muscle's role changes from a power generator to a power transmitter, travels caudally down the body. The net result is that rostral power output, caudal force, bending moment and force at the tail tip are maximal as the tail blade crosses the swimming track.

There are important kinematic differences between steady and unsteady swimming.

For steady swimming at uniform velocity, such as is found in pelagic schooling species, stride length is a constant fraction of body length over a wide range of speeds. In contrast, in many other fish, such as the short-horned sculpin, fast muscle fibres are recruited during unsteady movements which always contain periods of acceleration or deceleration resulting in shorter or longer strides respectively (Videler and Wardle, 1991). The timing of muscle activation in relation to muscle strain is likely to vary between steady and unsteady motion (van Leeuwen *et al.* 1990) and with different swimming styles (Williams *et al.* 1989). For example, in the lamprey (which uses an anguilliform mode of swimming), the mid-point of electrical activity in the muscle is approximately in phase with the peak velocity of the segment of the body that is doing work against the water (Williams *et al.* 1989). In contrast, during steady swimming in rainbow trout (which use a carangiform mode of swimming), the myotomal muscles are activated in phase with the maximum velocity of the tail tip (Williams *et al.* 1989), as has been reported for saithe and mackerel (Wardle and Videler, 1993).

Using the values for stimulus phase obtained in the swimming experiments, and optimal values of strain and stimulation, it was found that fast muscle fibres in the sculpin produced their maximum power output over the range of tail-beat frequencies found in swimming (4–9 Hz). It is not clear whether muscle fibres work optimally *in vivo*. In isolated muscle fibres, the duty cycle required to optimise the amount of work per cycle decreased from 33 % at 5Hz to only 18% at 9Hz. In contrast, electrical activity in the muscles occupies a relatively constant proportion of the cycle over a wide range of tail-beat frequencies during swimming. If more than the optimal number of stimuli are delivered *in vitro* then fibres fail to relax completely between contractions, resulting in a component of negative work (Altringham and Johnston, 1990*a*). During swimming, the amplitude of body movements, and hence the strain of superficial muscle fibres, increases from the head to the tail. For example, Hess and Videler (1984) estimated from an analysis of bending forces and bending moments that, during continuous swimming in the saithe, the strain of superficial muscle fibres increased from $\pm 3\% \, l_0$ in rostral myotomes to $\pm 6\% \, l_0$ in myotomes near the tail. Superficial muscle fibres run parallel to the longitudinal axis, whereas deeper muscle fibres follow complex helical trajectories, making angles of up to 40° with the body axis (Alexander, 1969). Rome and Sosnicki (1991) provided evidence that the strain of the deep fibres was approximately 25% of that for the superficial muscle fibres. Thus, strain is likely to vary along the length of the body and between superficial and deep muscle fibres. In the present study, involving relatively superficial layers of fast muscle, the power output of fibres for optimal conditions of stimulation was relatively constant for strain values of $\pm 3\% \, l_0$ to $\pm 8\% \, l_0$. *In vivo* muscle length changes during unsteady swimming may not be sinusoidal and are likely to change in waveform and amplitude from tail beat to tail beat, particularly during ballistic movements such as prey capture. The simulation of muscle power output under these conditions *in vivo* will require realistic estimates of strain in addition to information about the timing and duration of muscle activation.

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