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HOW FISH POWER PREDATION FAST-STARTS

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Summary

Short-horned sculpin (*Myoxocephalus scorpius* L.) were acclimated for 6–8 weeks to either 5 °C or 15 °C (12 h dark: 12 h light). Fast-starts elicited by prey capture were filmed from above in silhouette using a high-speed video camera (200 frames s^{-1}). Outlines of the body in successive frames were digitised and changes in strain for the dorsal fast muscle calculated from a knowledge of backbone curvature and the geometrical arrangement of fibres. For 15 °C-acclimated fish at 15 °C, muscle strain amplitude (peak-to-peak) during the first tail-beat was approximately 0.16 at 0.32*L*, 0.19 at 0.52*L* and 0.15 at 0.77*L*, where *L* is the total length of the fish. Fast muscle fibres were isolated and subjected to the strains calculated for the first tail-beat of the fast-start (abstracted cycle). Preparations were electrically stimulated at various times after the initiation of the fast-start using an *in vivo* value of duty cycle (27 %). Prior to shortening, muscle fibres at 0.52*L* and 0.77*L* were subjected to a pre-stretch of 0.055l_0 and 0.085l_0 respectively (where l_0 is resting muscle length). The net work per cycle was calculated from plots of fibre length and tensile stress. For realistic values of stimulus onset, the average power output per abstracted cycle was similar at different points along the body and was in the range 24–31 W kg^{-1} wet muscle mass. During shortening, the instantaneous power output reached 175–265 W kg^{-1} wet muscle mass in middle and caudal myotomes. At the most posterior position examined, the muscle fibres produced significant tensile stresses whilst being stretched, resulting in an initially negative power output. The fibres half-way down the trunk produced their maximum power at around the same time that caudal muscle fibres generated significant tensile stress. Fast muscle fibres at 0.37–0.66*L* produced 76 % of the total work done during the first tail-beat compared with only 14 % for fibres at 0.67–0.86*L*, largely reflecting differences in muscle mass. The effect of temperature acclimation on muscle power was determined using the strain fluctuations calculated for 0.52*L*. For 5 °C-acclimated fish, the average power per cycle (± S.E.M.; W kg^{-1} wet muscle mass) was 21.8±3.4 at 5 °C, falling to 6.3±1.8 at 15 °C. Following acclimation to 15 °C, average power per cycle increased to 23.8±2.8 W kg^{-1} wet muscle mass at 15 °C. The results indicate near-perfect compensation of muscle performance with temperature acclimation.

Key words: fish, short-horned sculpin, *Myoxocephalus scorpius*, skeletal muscle, muscle strain, muscle power output, temperature acclimation.

Introduction

During cyclic undulatory swimming, the length fluctuations of axial muscle fibres in several fish have been observed to be approximately sinusoidal (e.g. Hess and Videler, 1984; van Leeuwen *et al.* 1990). Several studies have applied sinusoidal length changes to isolated muscle fibres and optimised strain and stimulation parameters to determine maximum power output using the work loop technique as described by Josephson (1985). Optimal muscle power during cyclical contractions has been determined with respect to oscillation frequency (Altringham and Johnston, 1990*a*), temperature (Rome and Swank, 1992) and body length (Altringham and Johnston, 1990*b*; Anderson and Johnston, 1992).

However, muscle fibres at different positions along the trunk may not work optimally *in vivo* (van Leeuwen *et al.* 1990). Altringham *et al.* (1993) investigated this problem experimentally in the saithe (*Pollachius virens*). They used strains for slow fibres calculated from kinematic analyses (Hess and Videler, 1984) and set the timing and duration of stimulation within each cycle according to electrical recordings made during periodic swimming (Wardle and Videler, 1993). Muscle fibres towards the caudal fin were mainly active during lengthening (Wardle and Videler, 1993) and only produced positive work towards the end of the simulated cycle, resulting in net negative work (Altringham *et al.* 1993). The timing of maximum power output in rostral fibres coincided with the production of maximum force in caudal fibres. Altringham and co-workers suggested that caudal muscle fibres may have a role in transmitting force generated in the rostral and middle

myotomes towards the caudal fin, providing support for the earlier experimental and theoretical results on carp (*Cyprinus carpio* L.) by van Leeuwen *et al.* (1990).

Rome *et al.* (1993) experimentally determined fibre strain and, in conjunction with simultaneous electromyographical recordings (EMGs), measured the power output of slow fibres isolated from the scup (*Stenotomus chrysops* L.). They concluded that slow muscle fibres in rostral myotomes produced relatively little power because of the very low strain amplitude (3.2 % peak-to-peak), while fibres in caudal myotomes produced 81 % of the maximum possible power. It should be noted, however, that the most caudal position examined in scup was considerably more anterior than the position at which most of the negative work was found in the carp (van Leeuwen *et al.* 1990).

Much of fish swimming behaviour consists of unsteady manoeuvres involving varying degrees of acceleration and deceleration, while the distance travelled may be, respectively, increasing or decreasing between successive tail-beats. Fast-starts during predation or escape responses (powered by the fast muscle fibre type) are good examples of this type of behaviour. Muscle length fluctuations during fast-starts and turning manoeuvres are likely to be more complex than those occurring during steady swimming, varying in amplitude from tail-beat to tail-beat. So far, the only studies of muscle power output in unsteady swimming are by van Leeuwen *et al.* (1990) and van Leeuwen (1992), who investigated kick-and-glide swimming in carp. They calculated that the mean power output of the fast muscle fibres is positive over the whole trunk, with significant initial negative instantaneous power being produced in the most caudal myotomes. The present study on the short-horned sculpin is the first report of muscle power output during a fast-start. The changes in muscle fibre strain during the first tail-beat of fast-starts elicited by prey capture have been calculated and abstracted for use in work loop experiments. Since muscle contractile properties (Johnson and Johnston, 1991; Beddow and Johnston, 1995) and the kinematic parameters of fast-starts (Beddow *et al.* 1995) vary with temperature acclimation in this species, muscle power output was measured in 5 °C- and 15 °C-acclimated fish.

Materials and methods

Fish

Short-horned sculpin (*Myoxocephalus scorpius* L.), 230–350 g wet body mass and 22–25 cm total length (L), were caught locally and maintained in flow-through seawater aquaria. For the experiments on muscle power production at various points along the body, 14 summer-caught fish were used (nine for mechanical experiments and five for anatomical investigations). The fish were held for 1–2 months at 15±0.5 °C under a photoperiodic regime of 12 h:12 h light:dark. For experiments on the effects of thermal acclimation on muscle power output, sculpin caught between August and March (five fish at each temperature) were held for approximately 2 months at either 5 °C or 15 °C (±0.5 °C) (12 h:12 h light:dark). The relative proportion of fast muscle fibres at different positions along the trunk was determined in a further five fish caught in April. Fish were fed on a diet of shrimps (*Crangon crangon*), squid and fish flesh.

Swimming experiments

Fast-starts associated with prey capture were filmed in a static tank using a VHS high-speed video camera (Nac, Japan) at 200 frames s^{-1}. Sharp silhouettes of the fish, viewed from above, were obtained using a 45 ° mirror and a reflective background overlaid with a 10 cm calibration grid. The prey items used were 5–6 cm shrimps (*Crangon crangon*) or 2 cm crabs (*Carcinus carcinus*) acclimated to 14–15 °C. In preliminary experiments, EMGs were recorded from the fast muscle at points approximately 0.40L and 0.80L along the body in order to obtain information on the duty cycle (Johnston *et al.* 1993).

Calculation of muscle strain

Outlines of the fish in successive frames were traced, enlarged on a photocopier, and digitised (Jandel Scientific, California, USA) relative to fixed reference points in order to provide a series of x,y-coordinates. About 100 points were used for each side of the body. A purpose-designed computer program was used to display the digitised outline data on screen and to transform the data into a suitable format for further calculations.

A second program calculated the shape of the axis of the fish during the swimming event from the digitised outlines. Each axis calculation was started at the most rostral point and finished at the most caudal point. Following van Leeuwen *et al.* (1990), it was assumed that the projected areas to the left and right of the axis were equal for each element of the axis. This has been supported by radiography of swimming carp (van Leeuwen *et al.* 1990). The calculated axis closely followed the central canal of the vertebral column as projected on the radiograph. In order to calculate the fish axis, it was divided into a number of straight-line segments. The (mathematical) segment length Δs was defined to vary linearly down the trunk of the fish:

$$\Delta s = [(L - s)s_1 + s \times s_2]/L , \quad (1)$$

where L is the total length of the fish, s is the distance of the most rostral point of the segment along the axis from the snout, and s_1 and s_2 are the most rostral and caudal segment lengths respectively. The variable segment length was chosen to improve the stability of the applied algorithm, while allowing enough flexibility to approach the actual curvatures as closely as possible. Therefore, the largest segment length chosen was in the head region (typically 0.1L), which is very stiff so that bending is negligible, whereas the smallest segment length was in the tail region (typically 0.03L) where bending is largest. These variable lengths correspond approximately to the anatomical segment lengths of the fish (skull, vertebrae and fin-ray segments respectively). We did not attempt to make the

mathematical segment length identical to the anatomical segment length since this would have slightly reduced the accuracy of the body curvature calculations.

A computer program was written to describe the axis data in parametric form, so that $x=F(s,t)$, and $y=G(s,t)$, where t is time. For each of the selected instants, the functions F and G were smoothed as a function of position down the trunk using cubic spline functions. For this purpose, the spline function package developed by Woltring (1986) was used. Smoothing was carried out by choosing an appropriate value of the smoothing parameter. The parameter was chosen such that (1) the axis in the head region was kept as straight as possible, (2) unrealistic small-scale fluctuations in the curvature were removed as far as possible, and (3) the fundamental characteristics of the wave of lateral curvature were preserved. With $F_{sp}(s,t)$ and $G_{sp}(s,t)$ as the smoothed functions, the absolute curvature at a particular position down the trunk was calculated as described by Lipschutz (1969):

$$k = [(d^2F_{sp}/ds^2)^2 + (d^2G_{sp}/ds^2)^2]^{0.5}/[(dF_{sp}/ds)^2 + (dG_{sp}/ds)^2] \quad (2)$$

For convenience, we defined the curvature $c(s,t)$ as positive ($c=k$) for bending to the right and negative for bending to the left. Using quintic spline functions and the generalised cross-validation criterion, as described by Woltring (1986), the curvature function $c(s,t)$ was smoothed as a function of time to obtain a function $c_{st}(s,t)$, which was used for the strain calculations. To calculate the strains of the selected muscle fibres, equations 4–7 from van Leeuwen *et al.* (1990) were applied. These formulae take into account the thickening and thinning of the body at the concave and the convex sides, respectively, as well as the position and orientation of the muscle fibres.

The computer programs were written for the Macintosh family of computers, using either Think Pascal 4.0.2 (Symantec Corp.) or Mac Fortran II 3.2 (Absoft Corp.) as programming languages. ANSI standards were used as far as possible (except for graphic routines).

Determination of muscle fibre geometry

Fish were placed flat on their lateral surface and the skin was carefully removed to reveal the musculature. An incision was made with a sharp razor blade at the mid-point of the dorsal fast muscle at points 0.32*L*, 0.52*L* and 0.77*L* along the body (Fig. 1A). The angles of the fibres were measured with respect to the median and frontal plane of the fish (Fig. 1B) as described by Alexander (1969). The ranges of angles measured for fast muscle fibres in the median plane were 14–24 ° for the

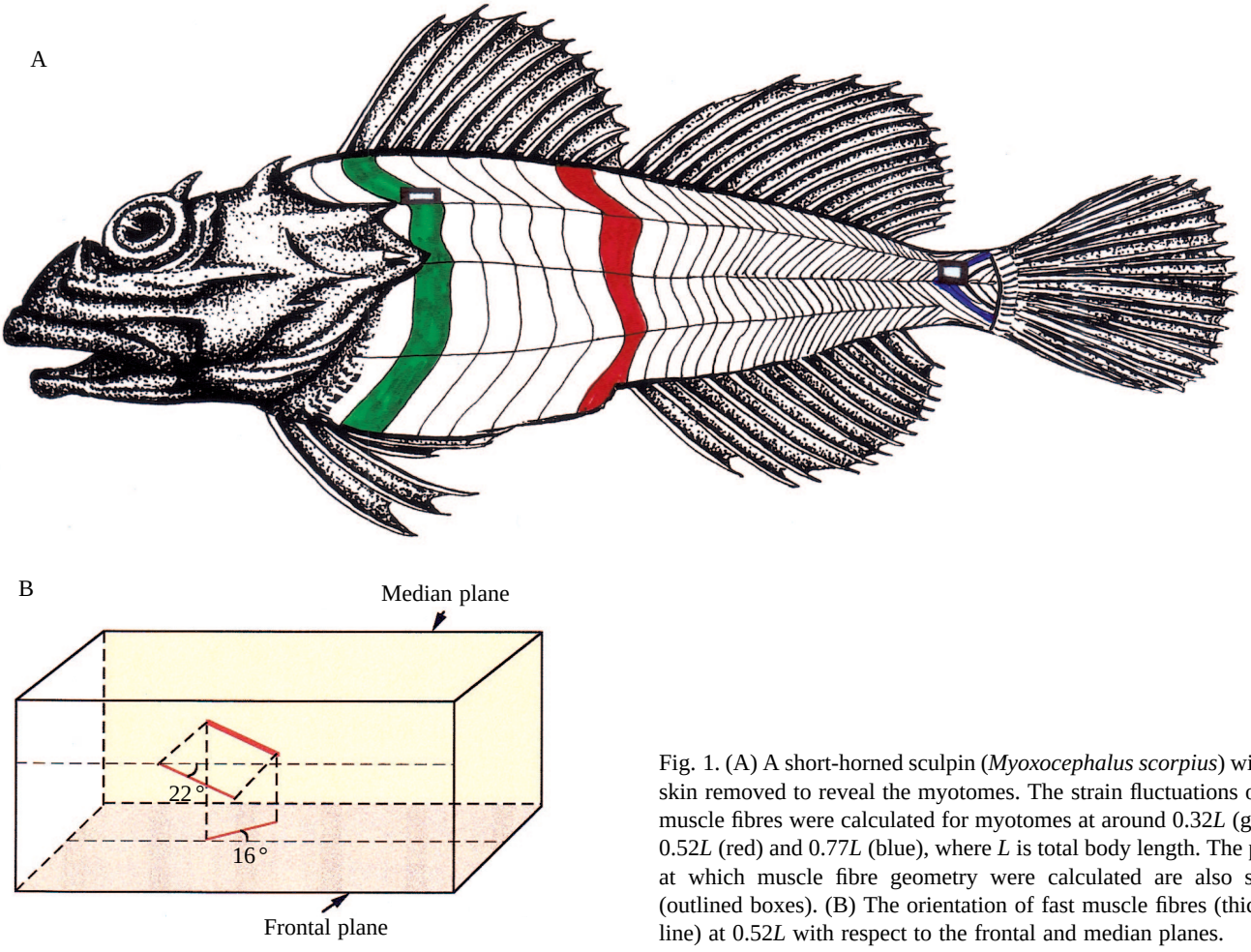

Fig. 1. (A) A short-horned sculpin (*Myoxocephalus scorpius*) with the skin removed to reveal the myotomes. The strain fluctuations of fast muscle fibres were calculated for myotomes at around 0.32*L* (green), 0.52*L* (red) and 0.77*L* (blue), where *L* is total body length. The points at which muscle fibre geometry were calculated are also shown (outlined boxes). (B) The orientation of fast muscle fibres (thick red line) at 0.52*L* with respect to the frontal and median planes.

fifth myotome at 0.32*L* (measured from the snout), 19–26 ° for the fifteenth myotome at 0.52*L* and 10–14 ° for the twenty-ninth myotome at 0.77*L* (angles inclined towards the tail). In the frontal plane, fast muscle fibres 2–3 mm deep made angles with respect to the longitudinal axis of 13 ° at 0.32*L*, 16 ° at 0.52*L* and 36 ° at 0.77*L*. These angles were used to correct strain calculations for the orientation of fast fibres (see above).

Measurement of contractile properties

Bundles of 3–10 fast muscle fibres were isolated on a cooled dissection stage under Ringer's solution with the following composition (in mmol l^{-1}): NaCl, 132.2; sodium pyruvate, 10.0; KCl, 2.6; $MgCl_2$, 1.0; $CaCl_2$, 2.7; $NaHCO_3$, 18.5; NaH_2PO_4, 3.2; pH 7.4 at 5 °C. Preparations were isolated from the anterior abdominal myotomes at 0.30–0.40*L* (rostral) and from the dorsal epaxial myotomes at 0.75–0.80*L* (caudal). Previous studies have found no differences in the contractile properties of the ventral and dorsal muscle fibres in the rostral myotomes (Altringham and Johnston, 1990*a*; Johnston *et al.* 1993). In the present study, fibres isolated from the ventral part of the myotome were used for the rostral position because of their ease of dissection. T-shaped clips, made from aluminium foil, were folded over and clamped around remnants of myosepta as close to the muscle fibres as possible. Preparations were attached *via* steel hooks to a computer-controlled servomotor and a force transducer (AME 801, SensorNor, Horten, Norway) mounted on a micromanipulator. The fibres were suspended in a stainless-steel water jacket with a glass bottom through which Ringer's solution was circulated from a thermostatically controlled reservoir (temperature was controlled to ±0.25 °C). The length of the preparation was set to give a maximal twitch, corresponding to a sarcomere length of 2.2–2.3 μm as measured by laser diffraction. The fibres were stimulated *via* two platinum wire electrodes lying either side of the preparation at intervals of 10 min to allow full recovery of the preparation between contractions. Using this stimulation protocol, reproducible results could usually be obtained over 36 h, although most experiments were completed within 8 h.

Muscle stimulation and length changes were fully programmable using hardware and software developed in-house. Muscle length changes calculated during the first tail-beat of the fast-start (see above) were abstracted for use in work loop experiments (shown as coloured lines in Fig. 2 and referred to as abstracted cycle throughout the text). The muscle length fluctuations for the abstracted cycle were entered into the computer *via* a graphics tablet (Jandel Scientific, California, USA). The abstraction was performed in order to create a cyclic event which could be given as an input to the servomotor system of the work loop apparatus. The effects of the abstraction procedure on the work and power output were probably small, owing to the low activation levels outside the period of the abstracted waveforms. For isometric contractions, preparations were stimulated at 50 Hz at 5 °C and 70 Hz at 15 °C in order to produce a fused tetanus (0.5–1 ms pulse width). For the derived cyclical contractions, the stimulation frequency was adjusted with four stimuli per cycle to give a constant duty cycle of 27 %, which was within the range of duty cycles found from EMG recordings (Johnston *et al.* 1993). Varying the number of stimuli and holding the stimulation interval constant to give a duty cycle of 27 % gave similar results. Stimulus time for each of the three positions along the body was defined with respect to the start of the cycle (Fig. 2). A range of stimulus times was investigated for each strain waveform, with the earliest times being close to those found *in vivo* (Johnston *et al.* 1993). The work done per tail-beat cycle was calculated from the area enclosed by plots of tensile stress against fibre length (work loops). Anticlockwise loops are indicative of positive work and clockwise loops imply negative work. Mean power output was obtained from the net work per abstracted cycle divided by the cycle duration. In some experiments, the velocity of fibre shortening was also calculated in order to determine the instantaneous power output. At the end of each experiment, fibre bundles were removed from the apparatus and pinned out at their resting length on a strip of silicone elastomer (Sylgard 184, Dow Corning, Seneffe, Belgium). Preparations were frozen in isopentane cooled to near its freezing point with liquid nitrogen (−159 °C). Frozen sections (10 μm thick) were cut at several

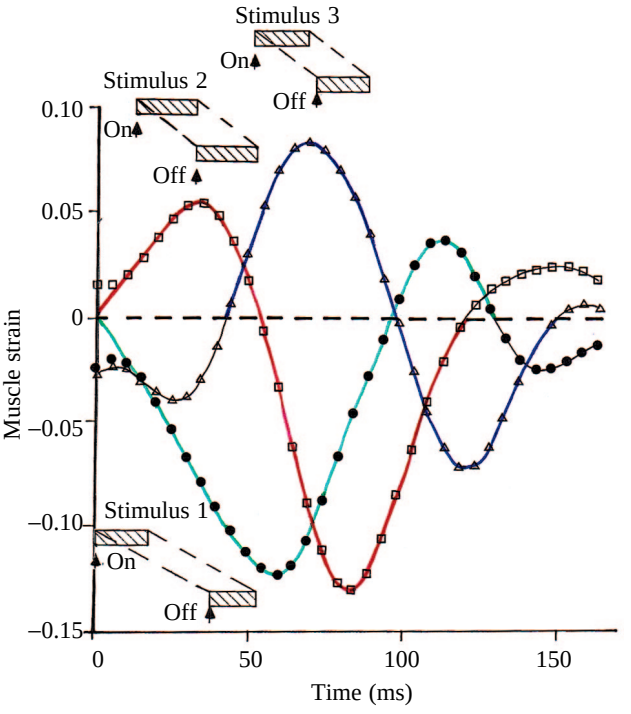

Fig. 2. Calculated values of muscle fibre strain during the first tail-beat of a predation fast-start at three points along the body (the coloured lines refer to the myotomes illustrated in Fig. 1A). Strain values were calculated as described in the text from high-speed video recordings of a 15 °C-acclimated fish attacking a shrimp at 15 °C. The strain waveforms input into the computer-controlled servomotor system for the work loop experiments are shown as coloured lines (the abstracted cycle). The actual strains deviate from zero at the start owing to a slight bend in the trunk. In our experiments, we investigated a range of stimulus phases, and the timings for the first and last stimuli at each position along the body are illustrated.

points along the preparation and stained for myosin ATPase activity (Johnston *et al.* 1974). The number and cross-sectional area of the fibres were determined using a microscope drawing arm and digital planimetry (VideoPlan, image analysis system, Kontron, Basel, Switzerland).

Measurements of muscle mass

The entire fast muscle was dissected from 0.1*L* segments and weighed, taking care to remove red muscle fibres and small bones.

Statistics

All the results are expressed as mean ± standard error (S.E.M.). Six three-way repeated-measures analyses of variance (ANOVAs) were carried out on the data on isometric contractile properties using the SPSS-PC statistical analyses package (SPSS Inc, Chicago, USA). In each case, there was one between-subjects factor, acclimation temperature (5 °C or 15 °C), and two within-subjects factors, muscle position (rostral *versus* caudal myotomes) and experimental temperature (5 °C or 15 °C). In cases where significant interactions were found, Newman–Kuels tests were carried out to locate the source of the interaction. Data on the maximum average power output per cycle at 0.52*L* during fast-starts at 5 °C in 5 °C-acclimated fish were compared with similar data for 15 °C-acclimated fish at 5 °C and 15 °C using a *t*-test.

Results

Muscle strain waveforms during fast-starts

There was significant variation in the kinematics of fast-start performance between fish. For this study, it was decided to carry out a detailed analysis on two selected individuals. The path of the chosen fish deviated only minimally from a straight-line trajectory in the direction of travel and had representative values of velocity and acceleration similar to those determined in a more extensive kinematic study (Beddow *et al.* 1995).

The maximum instantaneous fast-start velocity of fish 1 (15 °C-acclimated at 15 °C) was 1.51 m s^{-1} and its maximum acceleration was 27.4 m s^{-2}. Calculations were made as described in Beddow *et al.* (1995), using 5 ms intervals between subsequent data points. The calculated strains of fast muscle fibres for the side of this fish where fibres at the most rostral position (0.32*L*) shortened at the initiation of the fast start are shown in Fig. 2 (green line). In the calculation, it was assumed that the fibres were at their resting length (l_0) when the trunk was straight. On the same body side, fibres at 0.52*L* and 0.77*L* were initially stretched by the bend to the contralateral side, receiving a pre-stretch prior to shortening of 0.055 and 0.085l_0 respectively (Fig. 2). The maximum strain ranges (peak-to-peak) were approximately 0.160 at 0.32*L*, 0.186 at 0.52*L* and 0.154 at 0.77*L* (Fig. 2). Fibres shortened in

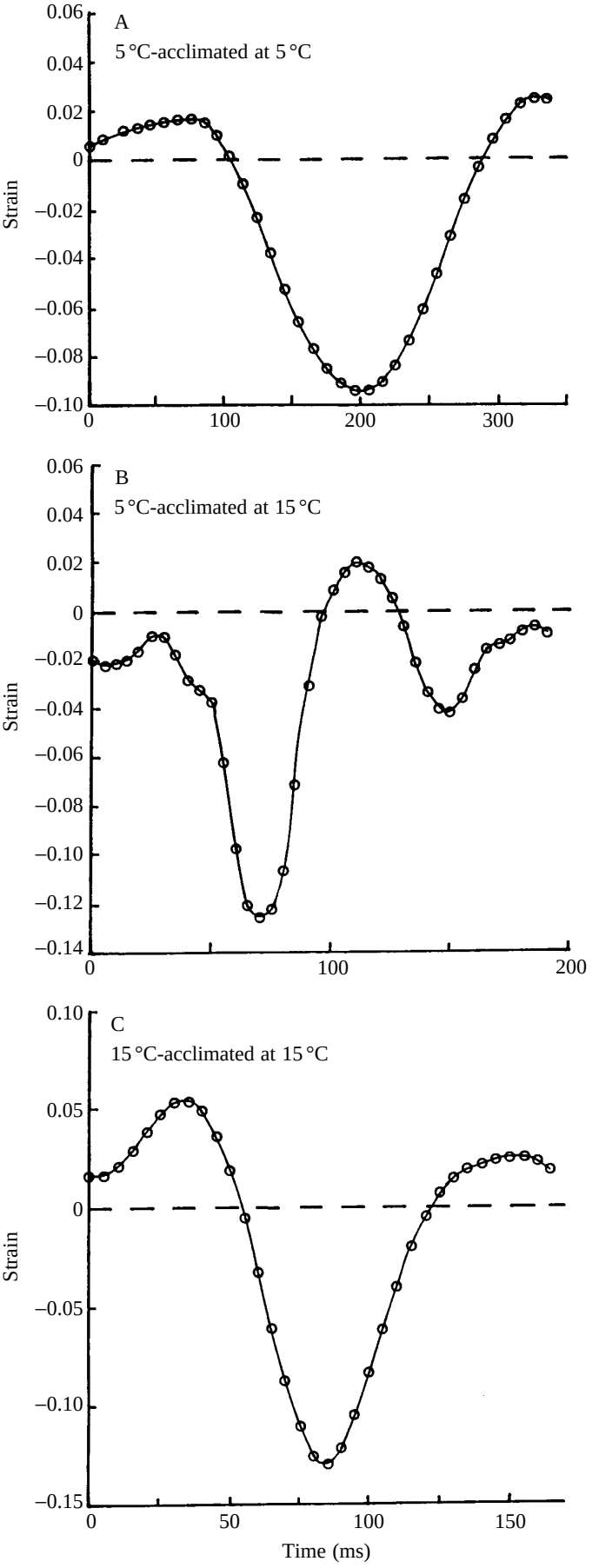

Fig. 3. Calculated values of muscle fibre strain fluctuations at 0.52*L* during fast-starts in short-horned sculpin acclimated to either 5 °C or 15 °C.

Table 1. *Isometric contractile properties of fast muscle fibres isolated from the rostral myotomes (0.3–0.4*L*) of short-horned sculpin (*Myoxocephalus scorpius *L.) acclimated to either 5 or 15 °C*

	Experimental temperature			
	5 °C		15 °C	
Variable	5 °C-acclimated	15 °C-acclimated	5 °C-acclimated	15 °C-acclimated
Twitches				
Peak tensile stress, F_{max} (kPa)	103.0±10.9	123.2±19.3	32.8±9.8	129.2±22.9
Time to F_{max}/2 (ms)	22.2±1.7	21.4±1.7	11.8±1.1	13.0±0.4
Half-relaxation time (ms)	39.4±4.6	36.3±9.7	23.2±4.0	18.1±1.1
Tetanic contractions				
Maximum tensile stress, F_{max} (kPa)	149.4±12.4	219.0±17.8	60.4±22.9	201.6±21.7
Time to F_{max}/2 (ms)	27.3±1.6	26.0±2.9	24.0±2.4	17.8±1.6
Half-relaxation time (ms)	109.9±9.9	120.8±14.4	62.5±9.7	45.1±2.9

Values represent mean ± S.E.M. of data from five fish per acclimation temperature.

the first tail-beat (from the first maximum in the strain wave) by $0.105l_0$, $0.18l_0$ and $0.15l_0$ at 0.32*L*, 0.52*L* and 0.72*L* respectively (Fig. 2). This shortening lasted about 55 ms at 0.32*L*, 50 ms at 0.52*L* and 53 ms at 0.77*L* (Fig. 2), during which maximum specific shortening speeds ($l_0\,s^{-1}$) of 2.44 at 0.32*L*, 4.88 at 0.52*L* and 3.78 at 0.77*L* were reached.

The maximum instantaneous fast-start velocity of fish 2 (5 °C-acclimated at 5 °C) was $0.40\,m\,s^{-1}$ and its maximum acceleration was $5.20\,m\,s^{-2}$. At 15 °C, the maximum velocity and acceleration of this 5 °C-acclimated fish were $1.04\,m\,s^{-1}$ and $15.9\,m\,s^{-2}$ respectively. To investigate the effect of acclimation on muscle performance, only the strain sequence from 0.52*L* was used (Fig. 3). In the 5 °C-acclimated fish examined, the first tail-beat lasted 294 ms at 5 °C and 128 ms at 15 °C. The maximum strain (peak-to-peak) was 0.11 at 5 °C and 0.145 at 15 °C (Fig. 3A,B), whereas the maximum specific shortening speeds were 1.28 and $6.40\,l_0\,s^{-1}$, respectively.

Isometric contractions

A three-way ANOVA revealed a main effect of muscle position on peak twitch tensile stress [$F(1,8)=45.3$, $P<0.001$] and maximum tetanic tensile stress [$(F(1,8)=7.9$, $P<0.025$]. Although tensile stress was significantly higher for fast muscle fibres from rostral than from caudal myotomes, the differences were relatively minor (Tables 1, 2). The half-time for relaxation of twitch tension was slightly prolonged for muscle fibres in caudal compared with rostral myotomes [$F(1,8)=6.5$, $P<0.04$]. However, half-activation times for twitches and tetani and half-relaxation times for tetani were not significantly different between rostral and caudal myotomes (Tables 1, 2).

There were main effects of acclimation and experimental temperature on tensile stress, which were modified by an interaction between these factors [$F(1,8)=10.7$, $P<0.02$ for twitches and $F(1,8)=7.9$, $P<0.025$ for tetanic contractions]. Peak twitch and tetanic tension were lower at 15 °C than at 5 °C in the 5 °C-acclimated fish ($P<0.01$; Tables 1, 2). The decrease in force production of muscle fibres from cold-acclimated fish at high temperatures was fully reversible. For tetanic contractions, the maximum tensile stress F_{max} was 34 % higher at 5 °C and 68 % higher at 15 °C in 15 °C-acclimated than in 5 °C-acclimated fish ($P<0.001$; Tables 1, 2). In 15 °C-

Table 2. *Isometric contractile properties of fast muscle fibres isolated from the caudal myotomes (0.75–0.8*L*) of short-horned sculpin (*Myoxocephalus scorpius *L.) acclimated to either 5 or 15 °C*

	Experimental temperature			
	5 °C		15 °C	
Variable	5 °C-acclimated	15 °C-acclimated	5 °C-acclimated	15 °C-acclimated
Twitches				
Peak tensile stress, F_{max} (kPa)	68.8±10.8	117.3±16.2	21.9±9.9	94.7±19.5
Time to F_{max}/2 (ms)	23.5±1.4	21.8±1.4	13.9±0.9	13.4±0.6
Half-relaxation time (ms)	51.7±7.0	51.7±9.8	20.8±1.1	33.3±3.4
Tetanic contractions				
Maximum tensile stress, F_{max} (kPa)	117.2±16.2	183.3±14.2	59.8±6.8	152.0±23.8
Time to F_{max}/2 (ms)	32.3±4.2	27.5±1.4	31.8±7.1	17.5±1.1
Half-relaxation time (ms)	124.8±13.0	125.4±11.5	67.2±8.4	69.6±5.8

Values represent mean ± S.E.M. of data from five fish per acclimation temperature.

acclimated fish, F_{max} values during twitches and tetanic contractions were not significantly different (Newman–Keuls tests) between 5 °C and 15 °C (Tables 1, 2). F_{max} for tetanic contractions was around three times higher in warm- than in cold-acclimated fish at 15 °C (Tables 1, 2).

The time from the first stimulus to half-activation (F_{max}/2)

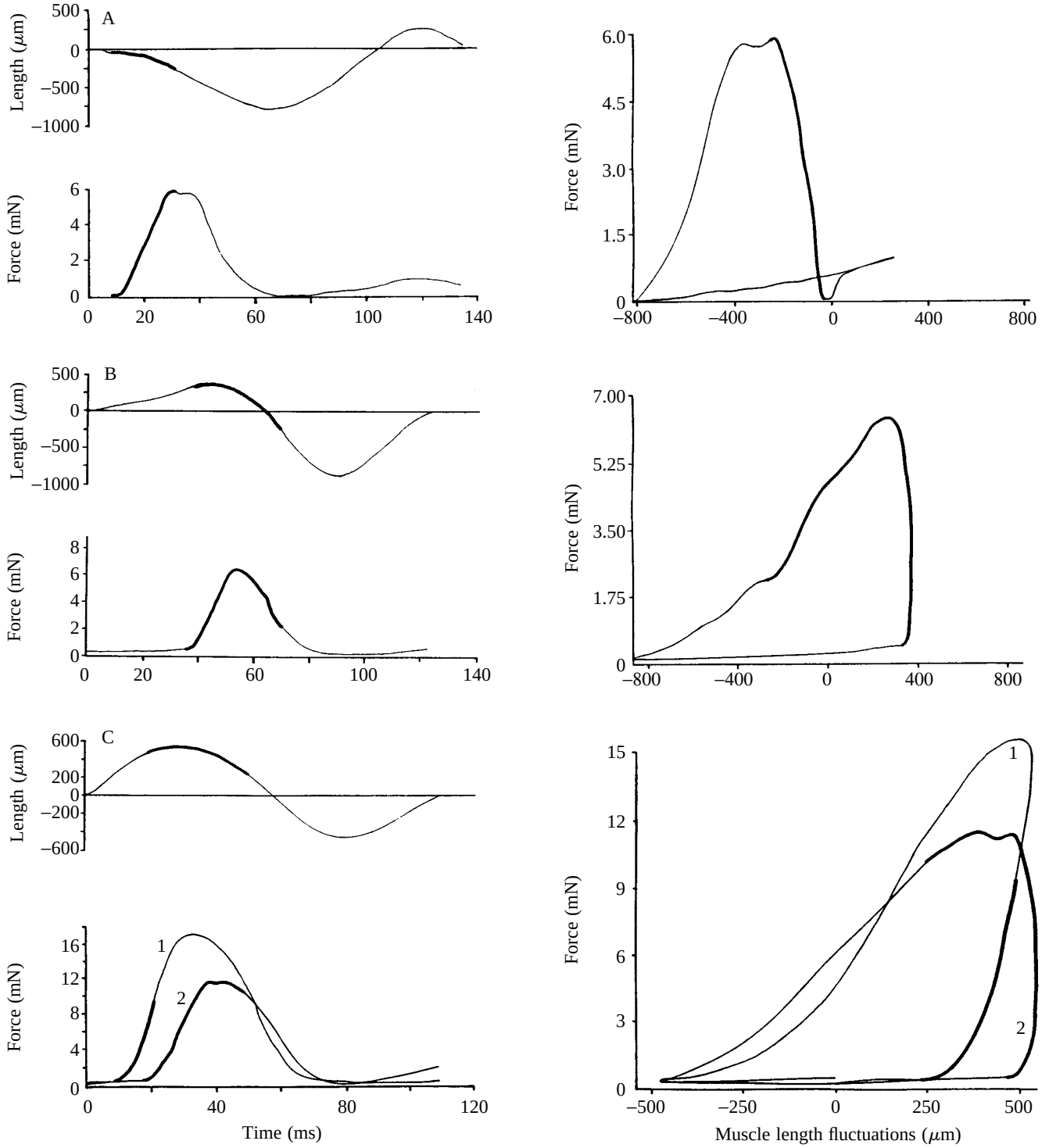

Fig. 4. Examples of work loops for a fast muscle preparation isolated from a rostral myotome (0.30–0.40*L*). Left-hand side: changes in muscle length (upper traces) and tensile stress (force; lower traces) giving rise to the tensile stress *versus* fibre length work loops illustrated on the right. Preparations were driven using strain values calculated for a fast-start at 15 °C for three positions along the body and stimulated with a duty cycle of 27 %. The bold parts of the curves show the time when stimuli were applied. The work loops in A and B are for values of stimulus onset giving close to the maximum work per cycle (7.4 ms at 0.32*L* and 24 ms at 0.52*L*, respectively). Two work loops are shown in C; loop 1 had a stimulus onset of around 52 ms (net work 5.3 μJ) and loop 2 a stimulus onset of around 64 ms (net work 5.8 μJ).

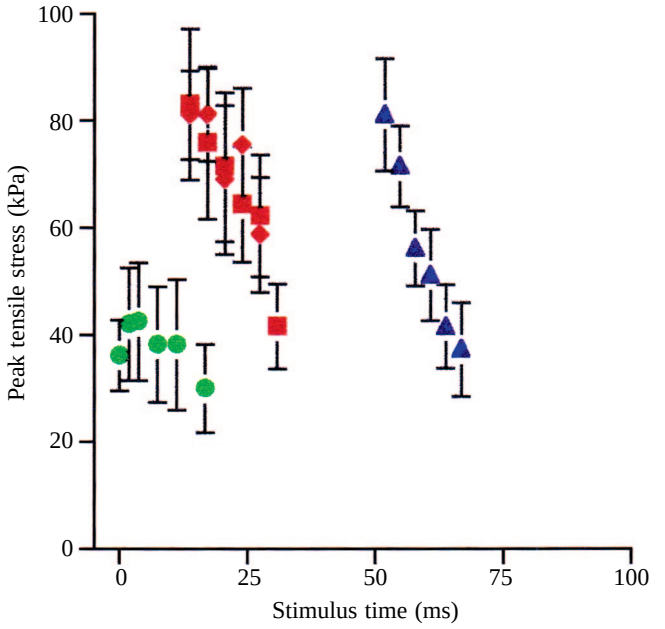

Fig. 5. The peak tensile stress (kPa) generated during the abstracted cycle at various values of stimulus onset using muscle strains calculated for a fast-start at 15 ˚C in a 15 ˚C-acclimated short-horned sculpin. The timing of stimuli were with respect to the initiation of the fast-start with a duty cycle of 27 %. Fast muscle fibres were isolated from rostral (green circles, red squares) and caudal (blue triangles, red diamonds) myotomes. The colours also refer to the positions illustrated in Fig. 1A. Values represent mean ± S.E.M. for 8–9 preparations for each experimental condition.

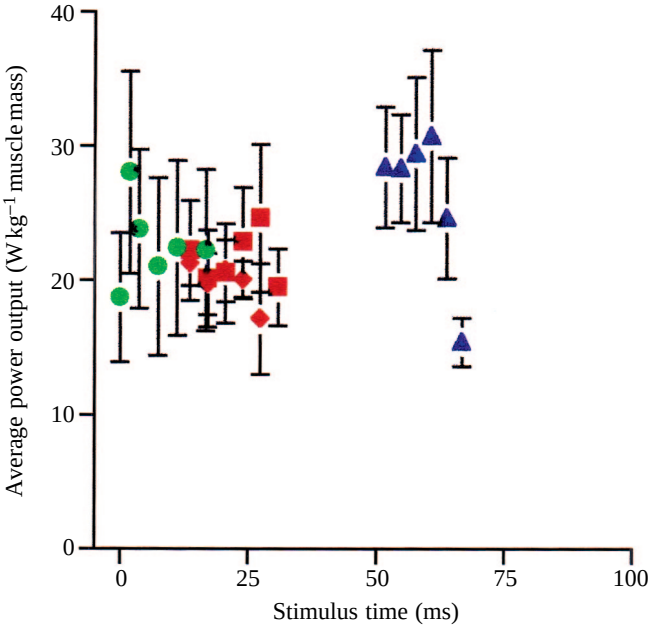

Fig. 6. Average values of power output (W kg^{-1} wet muscle mass) per abstracted cycle at various values of stimulus onset using muscle strains calculated for a fast-start at 15 ˚C in a 15 ˚C-acclimated short-horned sculpin. The timing of stimuli were with respect to the initiation of the fast-start with a duty cycle of 27 %. Fast muscle fibres were isolated from rostral (green circles, red squares) and caudal (blue triangles, red diamonds) myotomes. The colours also refer to the positions illustrated in Fig. 1A. Values represent mean ± S.E.M. for 8–9 preparations for each experimental condition.

showed a main effect of experimental temperature for both twitches [$F(1,8)=70.1$, $P<0.01$] and tetanic contractions [$F(1,8)=26.1$, $P<0.001$]. Half-activation times were significantly shorter at 15 ˚C than at 5 ˚C for twitches in both acclimation groups ($P<0.05$; Newman–Kuels tests). However, there was a significant interaction between experimental and acclimation temperature for tetanic contractions [$F(1,8)=11.2$, $P<0.01$)]. Newman–Kuels tests showed that although half-activation times were significantly shorter at 15 ˚C than at 5 ˚C in 15 ˚C-acclimated fish ($P<0.05$), there was no significant difference for the 5 ˚C-acclimated fish (Tables 1, 2).

Half-relaxation times (time from last stimulus to $F_{max}/2$) showed a main effect of experimental temperature for both twitches [$F(1,8)=35.2$, $P<0.001$] and tetani [$F(1,8)=72.2$, $P<0.001$], with values being significantly shorter ($P<0.05$) at 15 ˚C than at 5 ˚C. There were no significant effects of acclimation temperature on relaxation times (Tables 1, 2).

Work loop experiments

The tensile stress *versus* fibre length curves (work loops) produced with the abstracted strain cycles and *in vivo* stimulation duration and realistic stimulus phase at 0.32*L*, 0.52*L* and 0.77*L* are shown in Fig. 4. The strain fluctuations were calculated for a 15 ˚C-acclimated fish swimming at 15 ˚C. Work loops at the three positions examined along the body had characteristic shapes (Fig. 4). At 0.32*L*, the tensile stress increased rapidly to a peak of about 40 kPa and the fibres shortened from their resting length associated with the initiation of the fast-start (Figs 4, 5). Peak stress and the average power output per cycle (19–28 W kg^{-1} wet muscle mass) were maintained until a stimulus onset of around 17 ms (Figs 5, 6) before declining significantly (not illustrated).

Muscle fibres at 0.52*L* were actively stretched prior to shortening, resulting in peak tensile stresses (83 kPa) significantly higher than at 0.32*L* (Fig. 5). Peak tensile stress (Fig. 5) and the average power output per cycle (Fig. 6) were similar for fibres isolated from rostral and caudal myotomes using the strain waveform calculated for 0.52*L*. At 0.52*L*, optimal values of stimulus onset were in the range 13.5–31 ms (Figs 5, 6). The peak stress declined by around 50 % from the earliest to the latest stimulus onset time (Fig. 5), whereas the mean power output remained relatively constant (Fig. 6). Delaying the stimulus resulted in a decrease in force enhancement due to active stretch of the fibres, but resulted in a less rapid decline in force during the shortening phase of the cycle. The time of stimulus onset for maximum power was around 60 ms for muscle fibres at 0.77*L* (Figs 4, 5). Muscle fibres at 0.77*L* were subjected to lower strains but were given a larger pre-stretch prior to shortening than fibres at 0.52*L* (Fig. 2). The peak stress per cycle at 0.77*L* was around 82 kPa for stimuli starting at 51 ms, decreasing markedly as the stimulus onset was delayed (Fig. 5). On average, for a stimulus onset of

67 ms, peak tensile stress was only 50 % of that for 51 ms (Fig. 5).

The average work per cycle (J kg^{-1} wet muscle mass) was 2.75±0.35 (stimulus onset 1.9 ms) for fibres at 0.32*L*, 2.71±0.28 (stimulus onset 24 ms) for fibres at 0.52*L* and 3.38±0.46 (stimulus onset 64 ms) for fibres at 0.77*L* (eight or nine preparations per position). Within individual preparations, both the average (Fig. 6) and instantaneous power output per cycle (Fig. 7) usually increased in a rostral-to-caudal direction; however, the mean values were not significantly different owing to variability in the data. At the onset of shortening, the instantaneous muscle power output reached 175–265 W kg^{-1} wet muscle mass in the middle and caudal myotomes (Fig. 7B). For the earliest stimulus time studied (and probably the most realistic one, 51.8 ms), muscle fibres at 0.77*L* initially produced negative power output (Fig. 7B). The timing of maximum power production for muscle fibres towards the middle of the fish coincides with significant tensile stress in fibres at 0.77*L* (Fig. 7A).

The effects of temperature acclimation on the average work and power output per abstracted cycle were determined for the strain fluctuations at 0.52*L* (Fig. 8). In 5 °C-acclimated fish, under optimal stimulation conditions, the average work per cycle (J kg^{-1} wet muscle mass) was 6.41±1.06 at 5 °C, falling to 0.81±0.23 at 15 °C. The corresponding values for average power output (W kg^{-1} wet muscle mass) were 21.8 at 5 °C and 6.3 at 15 °C (P<0.001; Fig. 8). Following approximately 2 months of acclimation to 15 °C, average work was 2.87±0.28 J kg^{-1} wet muscle mass and average power was 23.8±2.8 W kg^{-1} wet muscle mass. The average power delivered per abstracted cycle in 5 °C-acclimated fish at 5 °C was not significantly different from that in 15 °C-acclimated fish at 15 °C (Fig. 8).

Discussion

There was significant variation in the amplitude and the pattern of strain fluctuations during fast-start behaviour for fast muscle fibres at different points along the trunk (Fig. 2), resulting in characteristic differences in the shape of work loops (Fig. 4). In the rostral myotomes (0.32*L*), maximum tensile stress was produced soon after the onset of shortening and only declined to 50 % of its peak value after 68–80 % of the total shortening (Fig. 4A). At 0.52*L*, the strain amplitude (0.19l_0, peak-to-peak) was slightly greater than that at 0.32*L* (0.16l_0), but the muscle fibres were stretched by 0.055l_0 prior to shortening. Using strain patterns at 0.52*L*, and a stimulus onset of 24 ms, the maximum force was produced at a fibre length greater than l_0, but fell to 50 % of its peak value shortly after shortening through l_0 (Fig. 4B). The pre-stretch was even greater at 0.72*L* (0.085l_0); however, fibres only shortened to −0.07l_0 before being lengthened again (Fig. 2). If the fibres were stimulated shortly after they passed through l_0 (stimulus onset 51 ms), then high forces were produced due to stretch activation, but submaximal amounts of work were done because of the rapid decrease in force during the shortening phase of the cycle (Fig. 4C, loop 1). For later stimulus onsets (60–70 ms), the force–length integral was maximised and on average the total work done per cycle was similar (Fig. 4C, loop 2) to that for fibres towards the front and middle of the fish (Fig. 6). The earlier stimulus times probably correspond more closely to the *in vivo* situation (Johnston *et al.* 1993).

During fast-starts, fast muscle fibres from different points along the body produce rather similar average power outputs per abstracted cycle of around 24–31 W kg^{-1} wet muscle mass (Fig. 6). These values are close to the average power output (30 W kg^{-1} wet muscle mass) determined at 15 °C using sine-

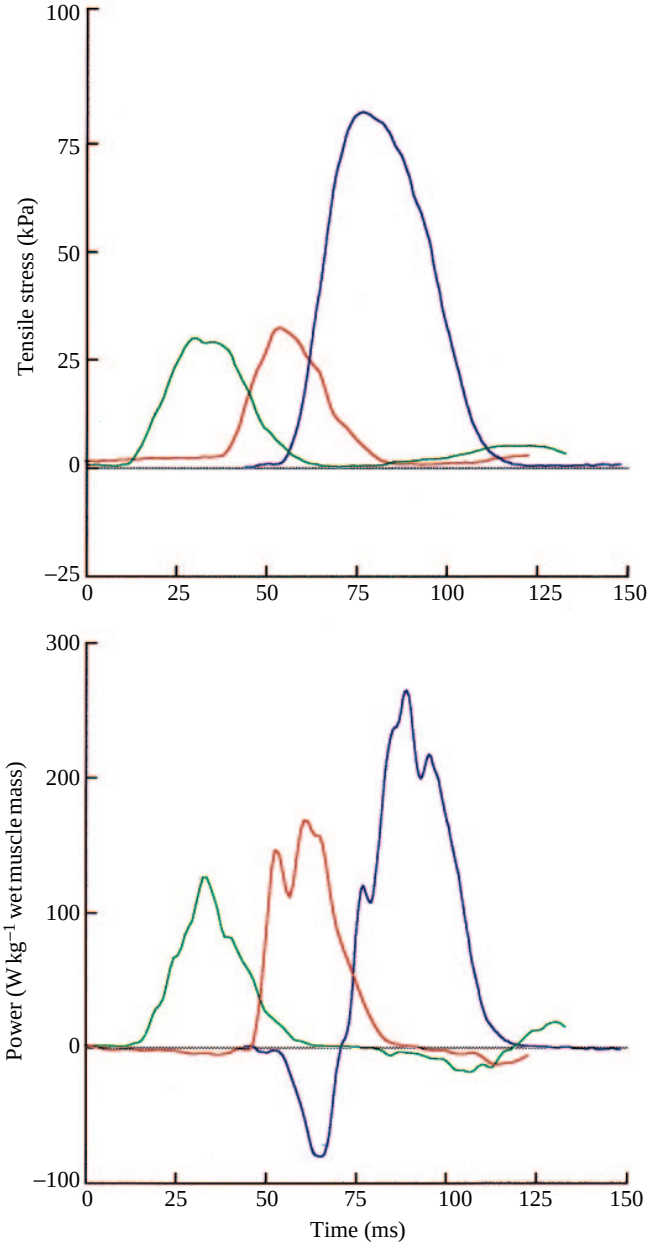

Fig. 7. (A) Tensile stress and (B) instantaneous power output during the abstracted cycle of the fast-start for the data illustrated in Fig. 4: green line, 0.32*L*; red line, 0.52*L*, and blue line, 0.77*L*.

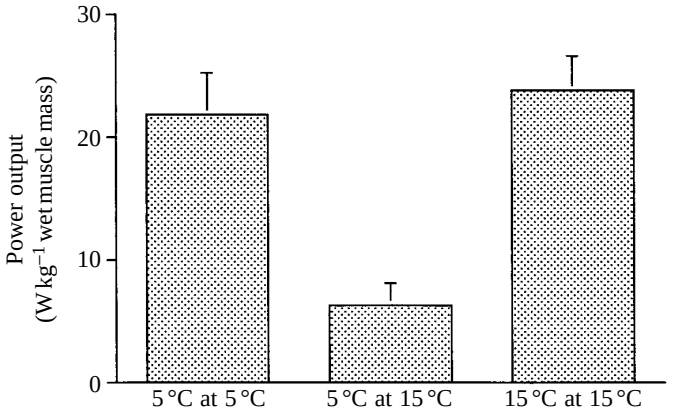

Fig. 8. The effects of temperature and thermal acclimation on the average power output of fast muscle fibres from short-horned sculpin measured under conditions simulating the first tail-beat of a fast-start. Muscle strain values were calculated for a point mid-way along the body (0.52*L*). Values represent means + S.E.M. for five fish; the data for rostral and caudal preparations were averaged.

waves of stimuli after optimising strain amplitude, the number and timing of stimuli, and cycle frequency (Johnson and Johnston, 1991). Values for instantaneous (maximum) power output at the onset of shortening were in the range 175–265 W kg^{-1} wet muscle mass for myotomes in the middle and caudal regions of the fish, which is similar or somewhat greater than that obtained from the force–velocity relationship (206 W kg^{-1}; Beddow and Johnston, 1995). The enhancement of power at 0.52*L* and 0.77*L* is probably related to the effects of pre-stretch, as was reported by Stevens (1993) for frog muscle. Rome *et al.* (1988) suggested from studies of muscle recruitment and the force–velocity relationship that *in vivo* muscle fibres are used over a narrow range of V/V_{max} values at which power and efficiency are near optimal. The maximum contraction velocity (V_{max}) of fast muscle fibres at 15 °C is around 8.2 muscle lengths s^{-1} at 15 °C (Beddow and Johnston, 1995). Therefore, in fast-starts the fast muscle fibres contract with V/V_{max} values of 0.29 at 0.32*L*, 0.59 at 0.52*L* and 0.46 at 0.72*L* during the main shortening phase of the first tail-beat. Thus (as expected for optimisation of mean power output), under dynamic conditions the *in vivo* peak velocity is greater than that predicted from the steady-state force–velocity relationship, again probably a function of pre-stretch (van Leeuwen, 1995).

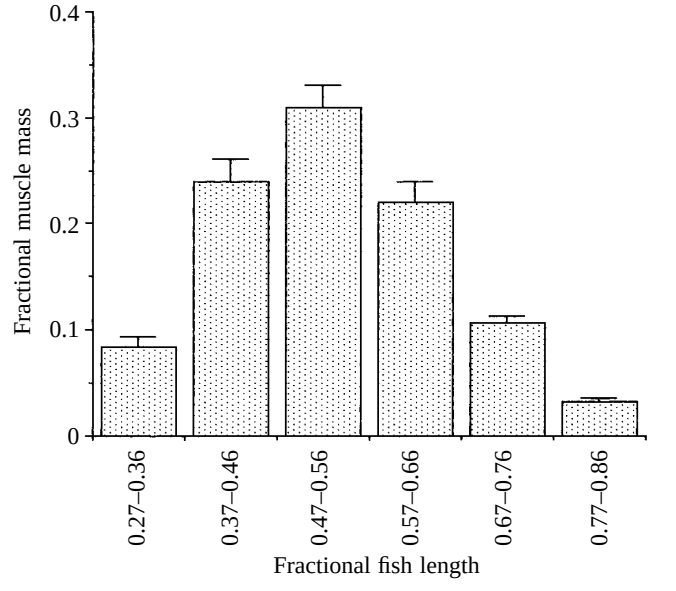

Fig. 9. The fractional mass of fast muscle fibres at different points along the trunk of the short-horned sculpin. The values represent means + S.E.M. for five fish.

The distribution of fast muscle at different points along the trunk is shown in Fig. 9. Middle myotomes, 0.37–0.66*L*, contain 77 % of the fast muscle mass and produce a similar proportion of the total power during the first tail-beat. At the time that fibres in the middle of the fish are producing their maximum instantaneous power, fibres towards the tail are generating significant tensile stress whilst producing negative power (Fig. 7). However, since the fibres at 0.77–0.86*L* only constitute 3.3 % of the fast muscle mass, this initial phase of negative power production is relatively minor and fibres at this position actually perform appreciable amounts of net positive work. The results suggest that muscle fibres towards the tail have a role in the transmission of power from the middle myotomes to the tail blade, in agreement with previous studies of kick-and-glide swimming in carp (van Leeuwen *et al.* 1990) and cyclical swimming in the saithe (Altringham *et al.* 1993).

The contractile properties of muscle fibres in the short-horned sculpin show marked plasticity to temperature change over seasonal time scales (Beddow and Johnston, 1995), as has been reported for some freshwater fish including goldfish (*Carassius auratus*) (Johnston *et al.* 1975; Heap *et al.* 1987) and the common carp (*Cyprinus carpio*) (Johnston *et al.* 1990; Langfeld *et al.* 1991). Average sea temperatures in St Andrews Bay, Scotland, vary from a minimum of 4–5 °C in February to a maximum of 15 °C during late August (Beddow and Johnston, 1995). Muscle contractile properties (Table 1 in Beddow and Johnston, 1995) and fast-start performance (Beddow *et al.* 1995; I. A. Johnston and C. E. Franklin, unpublished results) are altered at both high and low temperatures following a period of acclimation. V_{max} of fast muscle fibres was two times higher in 15 °C-acclimated than in 5 °C-acclimated fish at 5 °C (Beddow and Johnston, 1995), whereas F_{max} for tetanic contractions was around three times higher (Tables 1, 2). The contractile properties of fast muscle fibres in the short-horned sculpin are highly dependent on the acclimation state and condition of the fish, and some differences were found (Tables 1, 2) relative to previous studies (Johnston *et al.* 1993; Beddow and Johnston, 1995). In particular, compared with previous studies, F_{max} was higher and half-activation times were shorter for twitches and tetani at all temperatures in 15 °C- than in 5 °C-acclimated fish. Beddow and Johnston (1995) found that at 5 °C the force–velocity relationship was less curved in fast muscle fibres from 5 °C- than from 15 °C-acclimated fish. Normalizing the curves for maximum isometric stress (P_0) and V_{max} revealed that the change in curvature with

temperature acclimation was sufficient to produce a 40 % increase in relative power output at 5 ˚C in the cold-acclimated fish (Beddow and Johnston, 1995). Using strain waveforms derived from fast-starts, the average power output ($W\,kg^{-1}$ wet muscle mass) per abstracted cycle was found to decline from 21.8 at 5 ˚C to 6.3 at 15 ˚C in 5 ˚C-acclimated fish (Fig. 8). However, after 2 months of acclimation to 15 ˚C, average power output was similar at 5 ˚C and 15 ˚C ($23.8\,W\,kg^{-1}$ wet muscle mass), indicating near-perfect temperature compensation. At the whole-animal level, maximum velocity, tail-beat frequency and amplitude were all significantly higher in 15 ˚C- than in 5 ˚C-acclimated fish at 15 ˚C, which was sufficient to increase the percentage of successful attacks during prey capture from 23.2 to 73.4 % (Beddow *et al.* 1995).

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