



## Seasonal changes in fast-starts in the short-horn sculpin: integration of swimming behaviour and muscle performance

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In short-horn sculpin *Myoxocephalus scorpius*, the power requirements for fast-start swimming and the length-specific velocity of the curvature wave travelling down the spine ( $\dot{U}$ ) were not influenced significantly by acclimation to summer and winter conditions at test temperatures of 5 and 15° C. However, *in-vivo* and *in-vitro* muscle performance exhibited acclimation responses at 15° C. Seasonal acclimation altered the escape performance curves for power and  $\dot{U}$  significantly over a wider temperature range of 0.8–20° C.  $\dot{U}$  was significantly higher at 20° C in the summer- than winter-acclimation group. The acclimation of lower levels of physiological organization at 15° C may thus serve to extend the thermal limits for escape performance in summer acclimated fish.

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Key words: short-horn sculpin; fast-start; escape response; fast muscle; temperature acclimation; muscle power output; hydrodynamic power.

### INTRODUCTION

At temperate latitudes, the muscle physiology of ectothermic organisms may change with the seasons. Studies on field-acclimatized animals have shown modifications at most levels of organization from the molecular to the whole animal. In rainbow trout *Oncorhynchus mykiss* (Walbaum), glycolytic and mitochondrial enzyme activities vary seasonally in both red and white muscle (Thibault *et al.*, 1997). The power output of isolated muscle from a marine fish, the short-horn sculpin *Myoxocephalus scorpius* L. was significantly higher at a summer temperature of 15° C in summer- than winter-caught fish (Johnson & Johnston, 1991). At the whole animal level, small mouth buffalo *Ictiobus bubalus* (Rafinesque) exhibited a seasonal modification in critical swimming speed (Adams & Parsons, 1998). In addition, seasonal changes in temperature altered the thermal optimum for growth in winter flounder *Pseudopleuronectes americanus* (Walbaum) (Buckley, 1982) and plaice *Pleuronectes platessa* L. (Hovenkamp & Witte, 1991).

Studies on thermal physiology have often investigated temperature acclimation under laboratory conditions. Johnston *et al.* (1975) found temperature acclimation increased significantly the white muscle myosin ATPase activity at 1° C in 1° C-acclimated goldfish *Carassius auratus* L. compared with 26° C-acclimated fish, in addition to altering the susceptibility of the ATPase to

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thermal denaturation. At the whole animal level, Fry & Hart (1948) were the first to demonstrate that temperature acclimation altered both the optimum cruising temperature and thermal range for swimming activity in goldfish.

By integrating the various levels of organization during behaviours which are plausible fitness correlates, it may be possible to elucidate the evolutionary significance of acclimation responses. Johnson & Bennett (1995) carried out an integrative study examining the effects of thermal acclimation on the molecular, cellular and whole animal levels of organization during fast-starts in goldfish and killifish *Fundulus heteroclitus* L. Interspecific differences in the acclimation of white muscle were evident at the various levels of organization and were reflected in the whole animal escape performance. These differences were thought to reflect local niche distributions.

However, recent studies in carp *Cyprinus carpio* L., have shown that the relationship between the whole animal and lower levels of organization is not a simple one. Wakeling *et al.* (2000) examined acclimation response in common carp using a temperature range (8–21° C) in which the fish forage actively in the wild. They found temperature acclimation altered the myosin heavy chain composition, ATPase activity, spine curvature, muscle strain and muscle strain rate without altering the fast-start swimming capability of the fish. Significantly altered swimming performance occurred only over a greater temperature range. Previous studies on the short-horn sculpin are consistent with this idea. Beddow & Johnston (1995) reported significant changes in fast muscle contractile properties at 5 and 15° C following acclimation to both temperatures. However, improved maximum fast-start velocity during escape responses occurred only at a test temperature of 20° C (Temple & Johnston, 1998).

The purpose of this study was to integrate the contractile properties of the muscle with the power output of fast muscle, organismal power requirements and swimming performance during fast-starts, whilst assessing the effect of seasonal temperature acclimation on these parameters. Summer- and winter-acclimation were carried out in the laboratory in the summer and winter, respectively, using constant but seasonal photoperiods. This was done in order to avoid disrupting the natural seasonal rhythms of the fish and thereby examine acclimation responses that may have more relevance to the field situation.

The muscle power output of isolated fast muscle from the short-horn sculpin acclimated to summer and winter water temperatures was measured under conditions simulating escape responses using work loop experiments. The power requirements for the fast-starts were calculated from whole animal performance using the methods of Wakeling & Johnston (1998). Since fast-start swimming, like steady swimming, involves a wave of bending travelling down the body of the fish, the speed of swimming is dependent on the speed of this curvature wave (Wakeling & Johnston, 1998). Thus, swimming performance during fast-starts was also assessed by calculating the velocity of the curvature wave.

## MATERIALS AND METHODS

### FISH

Short-horn sculpin were collected between March 1995 and March 1997 using trawls and creels off the Fife coast and Isle of Cumbrae, Scotland.

Short-horn sculpin of two size ranges ( $161.8 \pm 3.0$  mm total length ( $L$ ),  $59.4 \pm 3.2$  g wet mass,  $n=27$ ;  $215.71 \pm 3.37$  mm  $L$ ,  $161.74 \pm 9.14$  g wet mass,  $n=14$ ) were acclimated to one of two temperature regimes. During winter months (November–March) fish were caught and acclimated to  $5 \pm 0.5^\circ\text{C}$  for a minimum of 6 weeks in a flow-through seawater aquarium. Fish were kept under a 10 hL : 14 hD photoperiod regime. During the summer months (June to September) fish were caught and acclimated to  $15 \pm 0.5^\circ\text{C}$  and were kept under a 13 hL : 11 hD photoperiod regime. In both cases fish were fed to satiation twice weekly with chopped squid or live *Crangon*.

#### MEASUREMENT OF FAST-START PERFORMANCE

For this study, the smaller size-class of sculpin was used. Following acclimation to 5 or  $15 \pm 0.5^\circ\text{C}$ , fish were transferred to a  $2.0 \times 0.6 \times 0.25$  m (length  $\times$  width  $\times$  depth) static filming arena at their acclimation temperature. Escape responses were elicited by tactile stimulation by presenting a 4 mm diameter rod randomly from either side of the caudal fin of a stationary fish. Filming was carried out at a frame rate of  $125\text{ s}^{-1}$  using a high speed ciné camera (NAC-Japan), via a mirror set at  $45^\circ$  above the tank. Five 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, 15 and  $20^\circ\text{C}$ . To achieve this the tank temperature was changed by  $1^\circ\text{C}$  every 30 min and the fish left for 1 hour prior to filming at the new temperature.

Kinematic analysis was carried out using the methods of Wakeling & Johnston (1998) whereby velocity and acceleration were determined from the displacement of the centre of mass of the fish. Length-specific curvature,  $\hat{c} = Lr^{-1}$  (where  $r$  is the radius of curvature), was used as an index of the amount of bend in the spine, following the procedures described by Wakeling & Johnston (1998). 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 contour plots. A straight line was drawn through the second wave of curvature travelling down the fish, which is the second half tail-beat and equivalent to the contralateral contraction. The slope of the line was inversely proportional to the rate of the wave of curvature,  $\dot{U}$ .

Fast-starts involve rapid accelerations and, therefore, inertial power is a good estimate of the useful hydrodynamic power of fish fast-starts (Wakeling & Johnston, 1998). Fish motion also causes the acceleration of water adjacent to the body (Weihs, 1973). Inertial power is required to accelerate both the mass of the fish and the added mass of water that moves with the fish. The added mass of water for fast-starting fish was taken as 0.2 mass ( $m$ ), after Webb (1982).

Frith & Blake (1995) estimated the hydrodynamic efficiency of pike *Esox lucius* L. fast-starts, as the ratio of useful power to the total hydrodynamic power. The total hydrodynamic power is the resultant power expenditure of both forward and lateral components, whereas the useful hydrodynamic power is the component which propels the fish in the direction of travel. Wakeling & Johnston (1998) used Frith & Blake's data to estimate a value of hydrodynamic efficiency of 0.31 for pike fast-starts. In order to calculate the total hydrodynamic power of the fast-starts in the present study, the useful inertial power during the second half tail-beat was divided by this value of hydrodynamic efficiency. Values of power were converted to  $\text{W kg}^{-1}$  of muscle assuming the white muscle mass is 29.8% of the body mass (Wakeling & Johnston, 1998).

#### IN-VIVO MEASUREMENTS OF MUSCLE STRAIN AND ACTIVITY

In a further set of experiments muscle strain and activity were recorded in short-horn sculpin using sonomicrometry and electromyography (EMG), respectively. The larger size-class of fish was used for this study to improve accurate insertion of the sonomicrometry crystals. The methods were based on those of Franklin & Johnston (1997). A brief description is given here. Anaesthesia was induced with a 1 : 5000 (m/v) solution of tricaine methane sulphonate (MS222) and maintained during surgery at a concentration of 1 : 7000 (m/v) at the acclimation temperature. A pair of sonomicrometry (piezoelectric) crystals (Triton Technology Inc., San Diego, U.S.A.) were inserted to a depth of 1–1.5 mm into the superficial dorsal myotomal muscle where

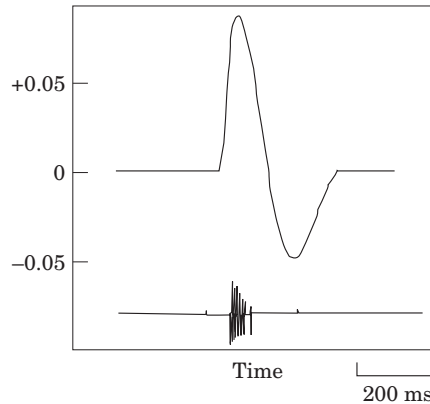


FIG. 1. A representative waveform of the *in-vivo* fast muscle strain and activation patterns obtained during the contralateral contraction of an escape response by a short-horn sculpin. This pattern has already been abstracted to create a cyclic event for use on bundles of fast muscle fibres *in vitro*. A total of four waveforms, similar to this were used in the *in-vitro* experiments.

the fast muscle fibres run parallel to the long axis of the fish. On each side of the fish the first crystal was inserted at *c.* 0.35 body lengths from the snout of the fish and the second 8–10 mm posterior to the first. An EMG electrode was inserted to a depth of 1–1.5 mm, *c.* 3 mm dorsally to the mid-point of the two sonomicrometry crystals on both sides of the fish. The procedure lasted *c.* 1 h. Fish were allowed to recover for 24 h before swimming experiments.

Muscle strain and activation were recorded simultaneously from both sides of the fish. In the present study, the C-bend refers to initial activation, shortening and then lengthening, thus returning the muscle to its resting length. On the other side of the fish the contralateral contraction consisted of initial passive lengthening (resulting from the C-bend), activation, shortening and then a return to resting length (Fig. 1). The cycle period refers to the duration of muscle length change. Escape responses were elicited in 5 and 15° C-acclimated fish at experimental temperatures of 5 and 15° C.

### CONTRACTILE PROPERTIES

Muscle fibre preparations consisting of 10–15 fast muscle fibres were isolated from the anterior abdominal myotomes as described by Franklin & Johnston (1997) but using Ringer solution described by James & Johnston (1998). The stimulus amplitude and width and the length of each muscle preparation were adjusted to give a maximum isometric twitch. The latter corresponded to a sarcomere length of 2.3–2.4  $\mu\text{m}$ , as measured by laser diffraction using a HeNe laser. The stimulus frequency was also optimized (77–132 Hz) to maximize isometric tetanic force.

For work loop experiments, cyclic events were created from representative strain waveforms recorded *in vivo* using sonomicrometry during the contralateral contraction of escape responses (Fig. 1). Strain patterns and thus length fluctuations started and finished at the resting length of the muscle. 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. Muscle preparations were allowed to recover for 6 min between each work loop cycle.

At the acclimation temperature of the fish, each muscle preparation did work loops using strain and activation patterns recorded at both test temperatures (5 and 15° C) in a fish acclimated to the same temperature as that of the preparation. Following a set of experiments at one temperature the temperature of the Ringer was changed over a period of 1–2 h to the next test temperature and the experiment repeated.

At the end of each experiment each muscle fibre preparation was frozen in isopentane cooled in liquid nitrogen. Transverse sections of 10  $\mu\text{m}$  thickness were cut at several

points along the preparation, transferred to microscope coverslips and stained for myosin ATPase activity (Johnston *et al.*, 1974). The cross-sectional area of each muscle preparation was determined by digitizing all live muscle fibres (VideoPlan, Kontron, Basel, Switzerland). Mass-specific power output was calculated using the measured cross-sectional area, the resting fibre length and by assuming a muscle density of  $1060 \text{ kg m}^{-3}$  (Méndez & Keyes, 1960).

## STATISTICS

For the experiments examining whole animal performance a two-way general linear model (GLM) ANOVA was used to investigate the effects of acclimation and acute temperature on power requirements and the velocity of the curvature wave travelling down the fish. A one-way ANOVA was employed to examine the effects of acclimation temperature at all four test temperatures. All statistical tests were computed using Minitab software (Minitab Inc., U.S.A.).

For the experiments involving sonomicrometry, EMG and work loops, 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 not normally distributed, the Kruskal-Wallis non-parametric test was used.

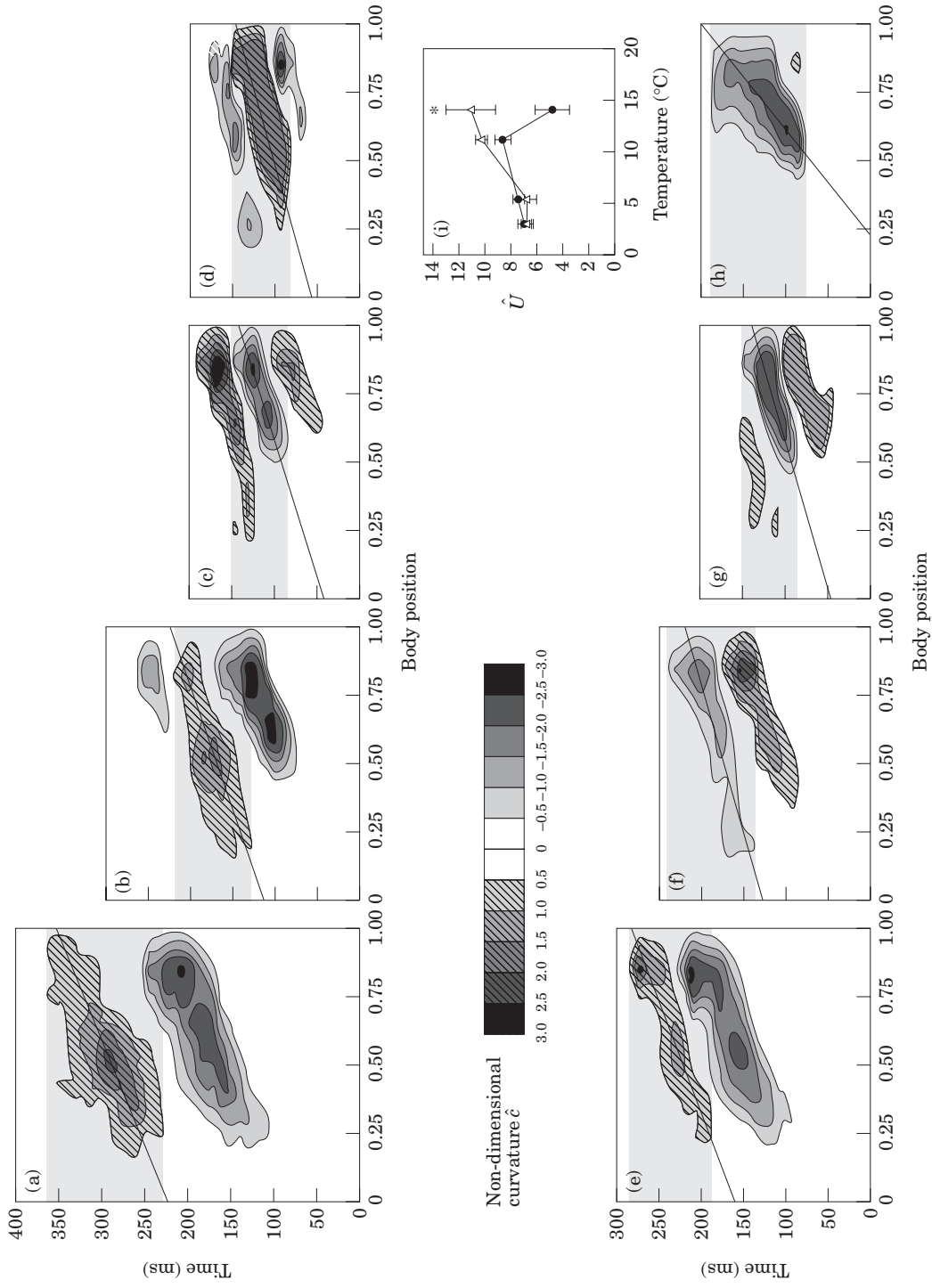
The effect of temperature on rate parameters was assessed using  $Q_{10}$ s. All values are presented as mean  $\pm$  s.e. unless otherwise stated.  $n$  represents the number of observations. Statistical results were judged significant at the 95% confidence level.

## RESULTS

### TEMPERATURE AND WHOLE ANIMAL PERFORMANCE

Acclimation temperature affected significantly the length-specific velocity of the curvature wave,  $\dot{U}$ , travelling down the body of the fish ( $F_{(1,57)}=11.12$ ,  $P=0.002$ ) (Fig. 2). The shapes of the two acclimation curves were different [Fig. 2(i)]. The mean  $\dot{U}$  was  $8.7 \text{ s}^{-1}$  in summer- and  $7.0 \text{ s}^{-1}$  in winter-acclimated fish. At low temperature, the two acclimation groups produced similar values of  $\dot{U}$ , however at  $20^\circ \text{C}$  acclimation to  $15^\circ \text{C}$  improved  $\dot{U}$  ( $11.1 \text{ s}^{-1}$ ) significantly whereas acclimation to  $5^\circ \text{C}$  caused a very much reduced performance ( $4.8 \text{ s}^{-1}$ ). Therefore, it was only at a test temperature of  $20^\circ \text{C}$  that there were significant differences between acclimation groups ( $P<0.001$ , Tukey multiple-comparison tests). Test temperature also had a significant effect on  $\dot{U}$  ( $F_{(3,57)}=5.93$ ,  $P=0.001$ ) (Fig. 2). Between test temperatures of  $0.8$  and  $15^\circ \text{C}$ , the adjusted mean  $\dot{U}$  for both acclimation groups increased by 38%.

Power requirements were estimated from whole body performance and were affected significantly by acclimation ( $F_{(1,57)}=5.72$ ,  $P=0.02$ ). In addition, there was significant interaction between acclimation and test temperature ( $F_{(3,57)}=3.59$ ,  $P=0.019$ ) (Fig. 3). Mean power was again higher for the  $15^\circ \text{C}$ - than the  $5^\circ \text{C}$ -acclimated group ( $33.4$  compared with  $24.3 \text{ W kg}^{-1}$ ). Tukey tests did not reveal any significant differences between the two acclimation groups at specific test temperatures. However, the  $Q_{10}$ s were dramatically different for the two acclimation groups between  $0.8$  and  $5^\circ \text{C}$ ; the  $Q_{10}$  for power in  $5^\circ \text{C}$ -acclimated fish was  $1.17$  compared with  $19.45$  for the  $15^\circ \text{C}$ -acclimated fish. Consequently at low temperature the  $5^\circ \text{C}$ -acclimation curve extends to the left of the  $15^\circ \text{C}$ -acclimation curve. Likewise, at high temperature the  $15^\circ \text{C}$ -acclimation curve extends beyond that of the  $5^\circ \text{C}$ -acclimation curve. Thus, acclimation has shifted the performance curves. Test temperature also affected



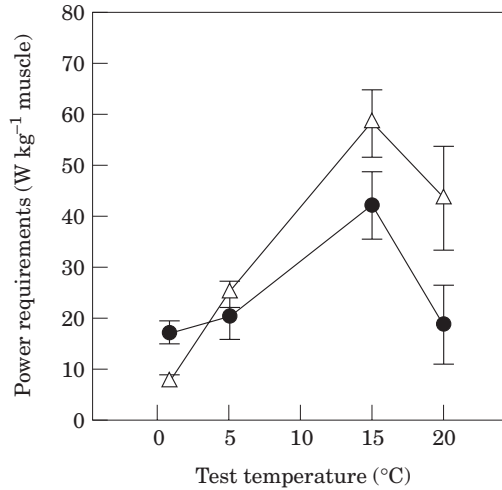


FIG. 3. The power requirements of the contralateral contraction of escape responses. ●, 5° C-acclimated fish; △, 15° C-acclimated fish. For sculpin groups 5 at 0.8, 5 at 5, 5 at 15, 5 at 20, 15 at 20, 15 at 15, 15 at 5, 15 at 0.8 (acclimation temperature at test temperature, °C),  $n=9, 9, 9, 5, 5, 10, 11, 9$ , respectively. Error bars represent S.E.

the power requirements for fast-start swimming ( $F_{(3,57)}=21.03$ ,  $P<0.0005$ ) (Fig. 3). Tukey tests revealed that in 15° C-acclimated fish, power increased significantly between 5 and 15° C ( $P<0.05$ ). Between test temperatures of 0.8 and 15° C mean power increased from 12.2 to 50 W kg<sup>-1</sup>.

#### IN-VIVO MEASUREMENTS OF FAST MUSCLE STRAIN AND ACTIVATION

The peak to peak strain amplitude ranged from 0.03 to 0.16 and was independent of acute or acclimation temperature. Acclimation to 15° C significantly reduced the cycle period of both the C-bend and contralateral contraction at both test temperatures (Tukey multiple comparison tests,  $P<0.05$ ) (Table I). The shortening durations were also significantly less, consequently increasing the shortening velocities. At 15° C, shortening velocity was 60 and 154% higher, for the C-bend and contralateral contraction respectively, in 15° C- compared with 5° C-acclimated fish (Tukey multiple comparison tests,  $P<0.05$ ).

Only the *in-vivo* cycle and shortening durations of 5° C-acclimated fish were influenced significantly by acute temperature. However, the trends differed for

FIG. 2. (a)–(h) Representative contour plots showing how the non-dimensional body curvature,  $\hat{c}$ , changes with time and body position (0=snout, 1.0=tail) during fast-starts. The slope of the solid line is inversely proportional to the rate the wave of curvature ( $\dot{U}$ ) travels down the fish. (a) 15° C-acclimated fish tested at 0.8° C,  $\dot{U}=6.9$ ; (b) 15° C-acclimated fish tested at 5° C,  $\dot{U}=8.9$ ; (c) 15° C-acclimated fish tested at 15° C,  $\dot{U}=9.5$ ; (d) 15° C-acclimated fish tested at 20° C,  $\dot{U}=14$ ; (e) 5° C-acclimated fish tested at 0.8° C,  $\dot{U}=7.8$ ; (f) 5° C-acclimated fish tested at 5° C,  $\dot{U}=9.3$ ; (g) 5° C-acclimated fish tested at 15° C,  $\dot{U}=8.9$ ; (h) 5° C-acclimated fish tested at 20° C,  $\dot{U}=3.1$ . The grey area indicates the period over which the mean inertial power requirements were calculated. (i) The velocity of the wave of curvature ( $\dot{U}$ ) travelling down the body of the fish. ●, 5° C-acclimated fish; △, 15° C-acclimated fish; \* significant difference between acclimation groups at 20° C. For sculpin groups 5 at 0.8, 5 at 5, 5 at 15, 5 at 20, 15 at 20, 15 at 15, 15 at 5, 15 at 0.8 (acclimation temperature at test temperature, °C),  $n=9, 9, 9, 5, 5, 10, 11, 9$ , respectively. Error bars represent S.E.

TABLE I. *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	
	5	15	5	15
C-bend	<i>n</i> =5	<i>n</i> =5	<i>n</i> =5	<i>n</i> =5
Cycle period (ms)	247.64 ± 12.25**	233.38 ± 8.76**	107.77 ± 14.07	69.48 ± 6.10
Strain	0.111 ± 0.014	0.076 ± 0.006	0.084 ± 0.010	0.085 ± 0.016
Shortening velocity (s <sup>-1</sup> )	1.50 ± 0.205	1.56 ± 0.11**	2.34 ± 0.19	2.49 ± 0.28
Shortening duration (ms)	115.58 ± 7.80***	81.09 ± 2.52**	56.01 ± 9.14	46.80 ± 8.34
Contralateral contraction	<i>n</i> =5	<i>n</i> =5	<i>n</i> =7	<i>n</i> =6
Cycle period (ms)	223.35 ± 9.27***	279.96 ± 11.11**	144.84 ± 14.56	126.44 ± 9.59
Strain	0.098 ± 0.013	0.100 ± 0.011	0.103 ± 0.015	0.103 ± 0.008
Shortening velocity (s <sup>-1</sup> )	1.57 ± 0.15	1.27 ± 0.11**	2.24 ± 0.29	3.23 ± 0.35
Shortening duration (ms)	76.79 ± 9.59*	118.90 ± 4.04**	60.47 ± 6.37	45.14 ± 3.60

\*Denotes a significant difference ( $P<0.05$ ) between test temperatures in one acclimation group; \*\*denotes a significant difference ( $P<0.05$ ) between acclimation groups at the same test temperature.

the two stages of the escape response, with the shortening duration decreasing significantly between 5 and 15° C in the C-bend (Tukey multiple comparison tests,  $P<0.05$ ), but the cycle and shortening durations increasing significantly between these two temperatures during the contralateral contraction (Tukey multiple comparison tests,  $P<0.05$ ).

#### IN-VITRO ISOMETRIC STUDIES

Acclimation temperature had no significant effect on the maximum tetanic stress but did affect maximum twitch stress significantly (Table II). At a test temperature of 15° C twitch stress was 96% higher in fibres from 15° C- than 5° C-acclimated fish (Tukey multiple comparison tests,  $P<0.05$ ). The rates of force development were affected by acclimation temperature at 15° C. At this temperature, the rates of twitch and tetanic activation were 123 and 83% higher, respectively, in 15° C- than 5° C-acclimated fish (Tukey multiple comparison tests,  $P<0.005$ ). Twitch relaxation rates were also significantly greater at 15° C in 15° C- than 5° C-acclimated fast muscle (Tukey multiple comparison tests,  $P<0.001$ ). Tetanic relaxation rates, on the other hand, showed no acclimation effects, but were significantly higher at 15° C in both acclimation groups.

In 5° C-acclimated fish, twitch stress was significantly less at 15° C and the twitch : tetanus ratio declined by 33% between 5 and 15° C. There were no acute temperature effects on the rates of force development in 5° C-acclimated fish (Tables II and III). In 15° C-acclimated fish, tetanic activation increased significantly between 5 and 15° C, with a  $Q_{10}$  of 1.7 (Tables II and III).

#### TEMPERATURE EFFECTS ON THE POWER OUTPUT OF FAST MUSCLE FIBRES

Bundles of fast muscle fibres were subjected to the muscle strain and activation patterns for the contralateral contraction. There was no significant variation in

TABLE II. Influence of acute temperature and temperature acclimation on the isometric contractile properties of the fast myotomal muscle fibres of short-horn sculpin ( $n=7$ )

	5° C-acclimated		15° C-acclimated	
	5	15	5	15
<b>Twitch</b>				
Stress ( $\text{kN m}^{-2}$ )	92.6 ± 7.5*	48.4 ± 6.6**	106.6 ± 10.7	94.8 ± 12.1
Rate of force activation ( $\text{kN m}^{-2} \text{ms}^{-1}$ )	1.8 ± 0.4	1.7 ± 0.5**	2.5 ± 0.9	3.8 ± 1.5
Relaxation rate ( $\text{kN m}^{-2} \text{ms}^{-1}$ )	1.2 ± 0.2	1.3 ± 0.6**	1.6 ± 0.5*	2.5 ± 0.5
<b>Tetanus</b>				
Stress ( $\text{kN m}^{-2}$ )	194.7 ± 9.0	150.0 ± 12.8	181.4 ± 17.7	176.7 ± 10.1
Rate of force activation ( $\text{kN m}^{-2} \text{ms}^{-1}$ )	1.6 ± 0.5	1.8 ± 0.5**	2.0 ± 0.6*	3.3 ± 1.3
Relaxation rate ( $\text{kN m}^{-2} \text{ms}^{-1}$ )	0.8 ± 0.2*	1.2 ± 0.2	0.8 ± 0.3*	1.4 ± 0.2
Twitch : tetanus ratio	0.48	0.32	0.59	0.54

\*Denotes significant difference ( $P<0.05$ ) between test temperatures within one acclimation group; \*\*denotes significant difference ( $P<0.05$ ) between acclimation groups at the same test temperature.

TABLE III.  $Q_{10}$  values for the rates of force development and relaxation in fast muscle from 5° C- and 15° C-acclimated short-horn sculpin

Rate ( $\text{kN m}^{-2} \text{ms}^{-1}$ )	Acclimation temperature (° C)	
	5	15
Twitch activation	0.94	1.52
Tetanic activation	1.13	1.65
Twitch relaxation	1.08	1.56
Tetanic relaxation	1.5	1.75

maximum stress during work loop experiments with acute or acclimation temperature (one-way ANOVA,  $P>0.05$ ). The mean stress value for the contralateral contraction was  $122.8 \text{ kN m}^{-2}$ .

Acclimation temperature affected the power output of fast muscle fibres significantly (Fig. 4). At 15° C, maximum instantaneous power output was  $275.6 \text{ W kg}^{-1}$  in fibres from 15° C-acclimated fish compared with  $178.5 \text{ W kg}^{-1}$  in 5° C-acclimated fish (Tukey multiple comparison tests,  $P<0.05$ ) [Fig. 4(a)]. Similarly, mean muscle mass-specific power output at 15° C was  $69.3 \text{ W kg}^{-1}$  in 15° C-acclimated fish compared with  $28.6 \text{ W kg}^{-1}$  in 5° C-acclimated fish (Tukey multiple comparison tests,  $P<0.001$ ) [Fig. 4(b)]. There were no significant differences in power output between acclimation groups at a test temperature of 5° C.

Acute temperature effects were also evident in 15° C-acclimated fish. The maximum instantaneous power output increased significantly from 160 to

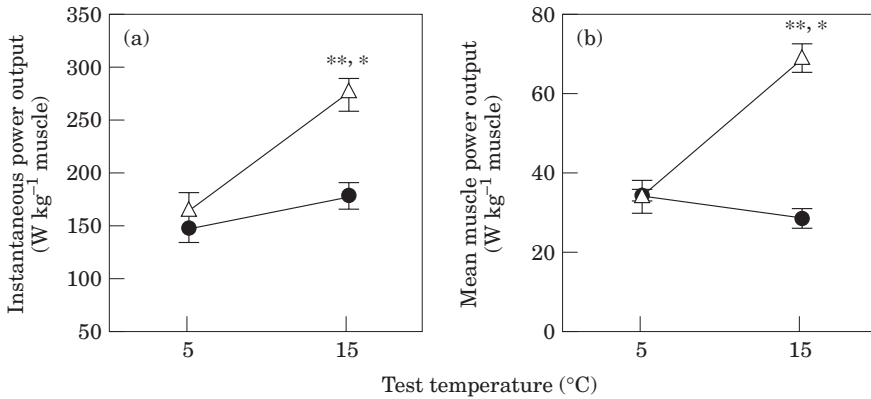


FIG. 4. (a) Maximum instantaneous power output and (b) mean power output of sculpin fast muscle fibres during the contralateral contraction. ●, 5° C-acclimated fish; △, 15° C-acclimated fish; \* significant difference ( $P < 0.05$ ) between test temperatures within the 15° C-acclimation group; \*\* significant difference ( $P < 0.05$ ) between acclimation groups at a test temperature of 15° C;  $n = 6$  for 5° C-acclimated sculpin and  $n = 7$  for 15° C-acclimated fish. Error bars represent s.e.

275.6 W kg<sup>-1</sup> and the mean muscle power output increased from 33.8 to 69.3 W kg<sup>-1</sup> between 5 and 15° C (Tukey multiple comparison tests,  $P < 0.001$ ).

## DISCUSSION

### ACCLIMATION AND FAST-START PERFORMANCE

Fast-starts are initiated by simultaneous EMG activity down one side of the musculature (Kashin *et al.*, 1979; Jayne & Lauder, 1993; Wakeling & Johnston, 1999a) which, due to the hydrodynamic resistance of the body, causes a wave of bending to travel down the fish (Wakeling & Johnston, 1999a). In the present study, there was no acclimation of the speed of the wave of curvature ( $\dot{U}$ ) at cold test temperatures of 5 and 0.8° C.  $\dot{U}$  was different between acclimation groups at a warm test temperature of 15° C (19% greater in 15° C-compared with 5° C-acclimated fish), but only significantly so at a test temperature of 20° C (131% greater in 15° C- than in 5° C-acclimated sculpin).

In a previous study, Temple & Johnston (1998) examined the acclimation of maximum velocity during fast-start escape responses in this species. It was predicted that, in accordance with the beneficial acclimation hypothesis (Leroi *et al.*, 1994), improved fast-start performance at high temperature following summer acclimation conditions (15° C) would be at the expense of performance at low temperature compared with winter-acclimated fish (5° C). As in the present study, this was not the case; at cold temperatures fish acclimated to summer water temperatures had values of maximum velocity similar to those of winter-acclimated fish. Again at 15° C there were more obvious differences between acclimation groups but significant differences occurred only at the highest test temperature of 20° C.

In contrast, Beddow *et al.* (1995) reported a significant acclimation of maximum velocity during prey capture in the short-horn sculpin at a test temperature of 15° C between fish acclimated to 5 and 15° C. However, although

prey capture and escape responses are both fast-starts, they involve significantly different kinematics in this species (R. S. James, G. K. Temple & I. A. Johnston, unpubl. results). During prey capture, sculpin had greater tail-beat amplitudes, implying greater body bending, at 15° C in 15° C- than 5° C-acclimated fish (Beddow *et al.*, 1995). In carp, body bending and muscle strain were significantly different between acclimation groups over the range 8–21° C, but maximum velocity of the true centre of mass exhibited no temperature acclimation (Wakeling *et al.*, 2000). Wakeling & Johnston (1998) demonstrated a 15% difference in the values for maximum velocity depending on the method of calculation used. When velocity is calculated from positions on the spine, body bending and thus additional lateral components, lead to higher estimations of velocity compared with estimations derived from the true centre of mass, which maintains a straighter trajectory. Beddow *et al.* (1995) used snout positions to calculate velocity during prey capture. Therefore, the significant acclimation of maximum velocity would have been influenced by the acclimation of body bending. However, warm-acclimation is clearly beneficial in terms of an improved prey capture performance at 15° C as Beddow *et al.* (1995) also reported a 73% prey capture success at this temperature in 15° C-acclimated fish compared with a 23% capture success in 5° C-acclimated fish.

#### EFFECTS OF ACUTE TEMPERATURE ON MUSCLE PROPERTIES

The peak tetanic force of sculpin fast muscle fibres did not change with temperature as has been found in muscle from other phyla (Bennett, 1985). However, in 15° C-acclimated fish the muscle had significantly faster tetanic activation rates at 15° C than at 5° C (Tables II and III). In the time course of muscle contractions during fast-starts, the muscle fibres will not reach a full activation state and so the forces produced are influenced by the activation level (Wakeling & Johnston, 1999b) and, therefore, at any given time during the contraction, muscles activating faster at 15° C will produce greater force.

In addition to the above, the ratio of the *in-vivo* shortening velocity to the maximum unloaded shortening velocity,  $v : V_O$ , has important implications for the pattern of force generation (Rome *et al.*, 1988; Rome, 1990). In this study there were no significant changes in muscle strain and shortening velocity with acute swimming temperature in 15° C-acclimated fish (Table I). This is in contrast to decreases in muscle strain and shortening velocity with increasing temperature that have been observed in carp (Wakeling *et al.*, 2000). However, in both studies increases in acute temperature resulted in a decrease in  $v : V_O$  ( $V_O$  for sculpin in the present study taken from Beddow & Johnston, 1995). Wakeling & Johnston (1999b) have shown that a decrease in  $v : V_O$  leads to an increase in the maximum force produced, as well as an increase in the rate of force production and a decrease in the ratio of force activation to deactivation.

The rate of muscle activation and hence force production affects  $\dot{U}$ . The tail of the fish has a large hydrodynamic resistance and requires a large force to swing it around but the caudal region has little muscle to generate this required force and therefore there is a delay before the tail moves (Wakeling & Johnston, 1999a). At 15° C, the fibres generate force faster and, therefore, the tail appears to move sooner with a resulting increase in  $\dot{U}$  and power requirements. However, whilst this is true for the initial C-bend of a fast-start when muscle

activation is simultaneous, there is a wave of muscle activation down the body of the fish during the contralateral contraction (Johnston *et al.*, 1993). Thus, acute temperature effects may be influenced additionally by the co-ordination of muscle contraction along the fish.

#### ACCLIMATION AND THE INTEGRATION OF WHOLE ANIMAL AND MUSCLE PERFORMANCE

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 & Blake, 1991; Bone *et al.*, 1995). Several studies in our laboratory have measured power output from isolated muscle bundles during fast-start escape responses using work loop experiments (Johnston *et al.*, 1995; Franklin & Johnston, 1997; James & Johnston, 1998; Wakeling & Johnston, 1998). Wakeling & Johnston (1998) employed a method for estimating power requirements from whole body performance that correlated well with values of muscle power output. Wakeling & Johnston's methods were similar in principle to those of an earlier study by Frith & Blake (1995) and involved estimating the inertial power required for the fast-start acceleration.

The sculpin used to estimate power requirements were shorter (16 cm) than those used for work loop experiments (21 cm). However, the mean mass-specific muscle power output during the contralateral contraction was independent of body size over the size range 17–30 cm in this species (James & Johnston, 1998), and so scaling effects in the present study are likely to be negligible. A GLM ANOVA revealed no significant differences in the values of power requirements and muscle power output ( $P=0.14$ ). Power requirements ranged from 20 W kg<sup>-1</sup> muscle in 5° C-acclimated fish escaping at 5° C, to 58 W kg<sup>-1</sup> muscle in 15° C-acclimated fish escaping at 15° C. Comparative power outputs of the fast muscle fibres under conditions simulating the contralateral contraction were 34 and 69 W kg<sup>-1</sup>, respectively.

The power output of the fast muscle fibres, however, showed a significant acclimation response at 15° C, as found before in this species (Johnston *et al.*, 1995). The present study also revealed acclimation of the *in-vitro* rate of fast muscle force development, *in-vivo* cycle periods, shortening velocity and shortening duration at a test temperature of 15° C. At the whole animal level, power requirements,  $\dot{U}$ , maximum velocity and maximum acceleration all showed no significant acclimation responses at 15° C (Temple & Johnston, 1998). However, all the measured parameters exhibited shifts in the positions of the summer and winter acclimation curves. In addition,  $\dot{U}$  and maximum velocity showed acclimation responses at a higher temperature of 20° C (Temple & Johnston, 1998). Therefore, whole animal performance shows the same trends with temperature acclimation as those at the cellular level, but the effects appear to be less marked.

The present results are similar to those of Wakeling *et al.* (2000). When acclimated to 9 and 30° C, carp had significantly improved fast-start performance at their acclimation temperatures, compared with those exposed acutely to the temperatures (Wakeling *et al.*, 2000). However, there was no beneficial improvement in fast-start swimming performance in carp across a narrower range of acclimation and acute temperatures (8–21° C), despite acclimation

occurring at lower levels of physiological organization. The benefit of the lower level acclimation responses may be to extend the thermal range of the fish rather than improve swimming performance significantly at typically experienced temperatures (Wakeling *et al.*, 2000).

Wakeling *et al.* (2000) have outlined recently potential problems when testing for acclimation responses at the whole animal level. Fish which are swum at acute temperatures which differ from their acclimation state by *c.* 15° C or more may show a pathological decrease in performance which is not related to the functioning of the neuromuscular system. Using the same experimental set-up as the present study, Temple & Johnston (1998) examined the seasonal acclimation of maximum velocity during fast-starts in the long-spined sea scorpion *Taurulus bubalis* (Euphrasen). In this species, significant acclimation effects were found only at 0.8° C. At this temperature, only 40% of 15° C-acclimated sea scorpion could swim compared with 100% of 5° C-acclimated fish. Johnston & Bennett (1995) reported a similar phenomenon with gold fish acclimated to 35° C and swum at 10° C. Therefore, it is intrinsically difficult to quantify the magnitude of acclimation effects at the whole animal level due to the limitations in the test for acclimation.

## CONCLUSIONS

Short-horn sculpin acclimated to summer and winter water temperatures, exhibited acclimation of fast muscle cycle periods, shortening velocity, shortening duration, twitch stress, rate of force development and muscle power output at a test temperature at 15° C but not at 5° C. However, the acclimation of these lower levels of organization cannot be used as a proxy for whole animal performance, as this showed no acclimation at 5° C or 15° C, in the parameters measured. Since there are many factors comprising the various levels of organization, it is not surprising that the acclimation of whole animal performance does not exactly mimic that of individual components.

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