

## MUSCLE POWER OUTPUT DURING ESCAPE RESPONSES IN AN ANTARCTIC FISH

CRAIG E. FRANKLIN\* AND IAN A. JOHNSTON†

*Gatty Marine Laboratory, School of Biological and Medical Sciences, University of St Andrews, St Andrews, Fife KY16 8LB, Scotland*

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### Summary

Escape responses (C-shaped fast-starts) were filmed at 500 frames  $s^{-1}$  in the Antarctic rock cod (*Notothenia coriiceps*) at 0 °C. The activation and strain patterns of the superficial fast myotomal muscle were measured simultaneously using electromyography and sonomicrometry respectively. In order to bend the body into the initial C-shape, the muscle fibres in the rostral myotomes (at 0.35L, where L is total length) shortened by up to 13% of their resting length at a maximum velocity of 1.68 fibre lengths  $s^{-1}$ . During the contralateral contraction, muscle fibres were stretched (by 5% and 7% at 0.35L and 0.65L, respectively) and were activated prior to the end of lengthening, before shortening by up to 12% of resting fibre length (peak-to-peak strain). Representative strain records were digitised to create cyclical events corresponding to the C-bend and contralateral contraction. Isolated fibres were subjected to the abstracted strain cycles and stimulated at the same point and for the same duration as occurs *in vivo*. During the early phase of shortening, muscle shortening velocity (V) increased dramatically whilst the load was relatively

constant and represented a substantial fraction of the maximum isometric stress. Pre-stretch of active muscle was associated with significant force enhancement. For the contralateral contraction, V exceeded that predicted by the steady-state force–velocity relationship for considerable periods during each tailbeat, contributing to relatively high maximum instantaneous power outputs of up to 290 W  $kg^{-1}$  wet muscle mass. *In vitro* experiments, involving adjusting strain, cycle duration and stimulation parameters, indicated that *in vivo* muscle fibres produce close to their maximum power. During escape responses, the maximum velocity and acceleration recorded from the centre of gravity of the fish were  $0.71 \pm 0.03 \text{ m s}^{-1}$  and  $17.1 \pm 1.4 \text{ m s}^{-2}$ , respectively (mean  $\pm$  S.E.M.,  $N=7$  fish). Muscle performance was sufficient to produce maximum velocities and accelerations that were within the lower end of the range reported for temperate-zone fish.

Key words: muscle, locomotion, kinematics, power output, temperature, swimming, work loops, Antarctic rock cod, *Notothenia coriiceps*.

### Introduction

Antarctic fish live in contact with ice and show a range of adaptations to avoid freezing (Clarke and Johnston, 1996). Muscle fibres in Antarctic fish remain excitable at an ambient temperature of  $-1.86^\circ\text{C}$ , but how does their performance compare with that of muscle from warm-water species? Some insight into this question can be obtained from measurements of steady-state isometric and isotonic contractions. Muscle fibres from Antarctic, temperate and tropical fish produce similar maximum isometric stresses ( $F_{\max}$ ) (180–250 kN  $m^{-2}$ ) at their respective normal body temperatures (Johnston and Brill, 1984). In contrast, unloaded shortening speed ( $V_{\max}$ ) is no faster at low temperatures in cold-water than in warm-water species. The maximum shortening velocity ( $V_{\max}$ ) for fast fibres is only 1.5–2.5 FL  $s^{-1}$ , where FL is fibre length, at 0 °C in Antarctic species compared with 15–20 FL  $s^{-1}$  at 25 °C in Indo-West Pacific species (Johnston and Brill, 1984; Johnson

and Johnston, 1991). Studies of the force–velocity relationship of fast muscle fibres indicate that, as a result, muscle power output during isotonic shortening is significantly lower in Antarctic than in tropical fish at their respective body temperatures (Johnston and Altringham, 1985; Johnson and Johnston, 1991). Isometric and isotonic contractions are, however, rarely used during swimming, and it is important to consider muscle properties measured under conditions that are more relevant to natural behaviours.

In order to escape predators or to capture prey, fish use fast-starts. Typically, in an escape response, the body is bent into a C-shape and the subsequent tailbeat(s) results in rapid acceleration from a resting position (Eaton *et al.* 1977; Webb, 1978). These escape manoeuvres involve large-amplitude body movements, rapid changes in direction and the recruitment of all the fast muscle fibres in the trunk. As a result, muscle strain

\*Present address: Department of Zoology, University of Queensland, Brisbane, Queensland 4072, Australia.

†Author for correspondence (e-mail: iaj@st-andrews.ac.uk).

and activation patterns are complex, varying along the body and from tailbeat to tailbeat (Johnston *et al.* 1995; van Leeuwen, 1995).

In order to investigate muscle performance during fast-starts in the Antarctic rock cod (*Notothenia coriiceps* Nybelin), a high-speed ciné camera (500 Hz) was used to film the initial C-bend and the subsequent contralateral contraction. The activation and strain patterns of the superficial layers of fast myotomal muscle fibres were measured simultaneously using sonomicrometry and electromyography, respectively. To investigate muscle power output during the fast-start, we adapted the work loop technique of Josephson (1985) using information from the *in vivo* recordings. Representative strain waveforms were digitised, and segments corresponding to the initial C-bend and the contralateral contraction were abstracted to create the cyclical events necessary for work loop experiments. This is the first of a series of such studies on polar, temperate and tropical fish designed to test the hypothesis that it is muscle power output, rather than hydromechanical constraints or other factors, that limits fast-start performance, as suggested by Frith and Blake (1995).

## Materials and methods

### Experimental animals

*Notothenia coriiceps* Nybelin (body mass  $154 \pm 24$  g, total length  $20.5 \pm 3.5$  cm; mean  $\pm$  S.D.,  $N=15$ ) were caught around Signy Island, South Orkneys, Antarctica ( $60^\circ 43'$  S,  $45^\circ 36'$  W), transported to St Andrews and maintained in a custom-built cold aquarium at  $0 \pm 0.5^\circ\text{C}$ . Fish were fed daily on krill and chopped squid.

### Swimming kinematics

Escape responses to tactile stimuli were filmed in silhouette using a 16 mm NAC E10 high-speed ciné camera at  $500 \text{ frames s}^{-1}$  via a mirror suspended at  $45^\circ$  above a glass arena ( $850 \text{ mm} \times 850 \text{ mm} \times 100 \text{ mm}$ ; length  $\times$  width  $\times$  depth) illuminated from below. The arena was maintained at  $0^\circ\text{C}$  with a water-jacket containing antifreeze. The film (Ilford HP5) was over-exposed by one stop, developed 'open trap', and then viewed and analysed using a motion-analysis system (MOVIAS, NAC, Japan). The positions of the snout and centre of gravity of the fish were used to determine the kinematics of the startle response. Velocities and accelerations were calculated from the raw position data which had been smoothed using a moving average filter with 10 passes (MOVIAS, NAC, Japan).

### In vivo measurements

Anaesthesia was initiated with a 1:5000 (m/v) solution of bicarbonate-buffered MS222 and maintained by irrigation of the gills during surgery, which was performed in a constant-temperature room at  $0\text{--}1^\circ\text{C}$ . Piezoelectric crystals (1 mm in diameter) were attached to Perspex implants and calibrated in dissected blocks of muscle at  $0^\circ\text{C}$ . Each pair of crystals was inserted approximately 8 mm apart in the superficial layers of fast

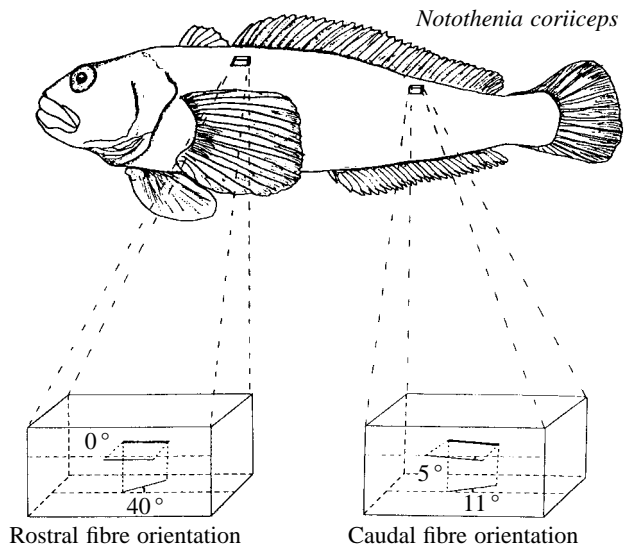


Fig. 1. Diagram to show the position of insertion of sonomicrometry crystals and electromyogram wires in the superficial layers of myotomal muscle in the Antarctic rock cod *Notothenia coriiceps*. The expanded boxes show the orientation of fast fibres with respect to the horizontal and median planes at the rostral and caudal recording sites. Recording sites were approximately 2 mm below the skin surface.

muscle (2–2.5 mm deep) and anchored by silk sutures to the skin. Sets of crystals were positioned at  $0.35L$  (rostral myotomes) and  $0.65L$  (caudal myotomes) as illustrated in Fig. 1. The orientation of the muscle fibres at these positions had been established previously by dissection of a dead fish (Fig. 1), enabling the crystals to be correctly aligned along the longitudinal axis of the muscle fibres. An oscilloscope connected to the sonomicrometer (Triton Technology Inc, model 120-1000) was used to check the alignment of the crystals. A correction of 5 ms was used for the lag in the sonomicrometry readings due to the data filter in the sonomicrometer circuit. The velocity of sound through fish muscle at  $0^\circ\text{C}$  ( $1507 \text{ m s}^{-1}$ ; C. E. Franklin, unpublished results) was used to calibrate the sonomicrometer, and errors in readings due to the change in the velocity of sound through the crystal lenses and to changes in muscle stiffness were calculated to be less than 4% of the measured strain. A pair of Teflon-coated, silver electromyography (EMG) wires ( $150 \mu\text{m}$  in diameter), with the insulation removed from the tips, was implanted near to each pair of sonomicrometry crystals. A differential amplifier (A-M Systems, Everett, USA) with low- and high-pass filter settings of 100 Hz and 1000 Hz, respectively, amplified the muscle potentials, which were recorded together with the sonomicrometry signals on a thermal array recorder (Summagraphics Ltd). At the end of the experiments, the location of the sonomicrometry crystals was determined by X-ray photography and dissection. Only those results from fish with crystals aligned along the longitudinal axis of the muscle fibres were used (see Fig. 1).

### Isolation of muscle fibres

Muscle preparations were obtained from the dorsal surface of the caudal myotomes. Bundles of 5–12 fast muscle fibres were isolated on an ice-cooled dissection stage with the aid of a

binocular microscope. The Ringer's solution used for dissection had the following composition (in mmol l<sup>-1</sup>): NaCl, 132.2; sodium pyruvate 10.0; KCl, 2.6; MgCl<sub>2</sub>, 1.0; CaCl<sub>2</sub>, 2.7; NaHCO<sub>3</sub>, 18.5; NaH<sub>2</sub>PO<sub>4</sub>, 3.2; pH 7.5 at 0°C). Aluminium T-clips were wrapped around remnants of myosepta as close to the insertion of the muscle fibres as possible (illustrated in Altringham and Johnston, 1994). Preparations were suspended *via* the T-clips between two hooks in a stainless-steel water-jacketed chamber with a glass bottom through which Ringer's solution was circulated at 0±0.2°C. One hook was attached to a strain gauge (AME 801, Senso-Nor, Horten, Norway) mounted on a micromanipulator and the other to a computer-controlled servomotor. The length of the preparation was adjusted to give a maximum isometric twitch, corresponding to a sarcomere length of 2.3–2.4 µm as measured by laser diffraction.

#### Work loop experiments

In work loop experiments, muscle fibres are subjected to cyclical length changes and are phasically stimulated during each cycle. The net work done by the muscle is obtained from plots of fibre length against the force generated (Josephson, 1985; Altringham and Johnston, 1990). In our experiments, information on the *in vivo* strain fluctuations during escape responses was used to define the cyclical length changes used for the work loop experiments. Muscle strain was recorded by sonomicrometry from the rostral and caudal positions for a minimum of six different escape responses from each of seven fish. Representative strain waveforms were selected and digitised. In order to create cyclical events, segments were abstracted corresponding to the initial C-bend and the subsequent contralateral contraction. Each 360° cycle started and ended at close to the resting sarcomere length. The resulting cycles were used as an input to the servomotor. In addition, the computer was programmed to stimulate the muscle at the same point in the strain cycle and for the same duration as was determined from the electromyogram (EMG) recordings in the representative *in vivo* experiments. Stimulus amplitude and pulse duration (1–2 ms) were optimised to give a maximal isometric twitch. The stimulation frequency was set within the range required to give a maximal fused isometric tetanus (50–70 Hz), and the number of pulses was adjusted to give the required stimulus duration. In each series of experiments, the servomotor was programmed with either the abstracted strain waveform for the C-bend or that for the subsequent contralateral contraction, and the muscle was stimulated. The effects of the abstraction procedure used were small owing to the negligible activation of fibres outside the period of the abstracted waveform. To allow the muscle to recover fully, 10 min was allowed between each contraction. The work done on unstimulated muscle was typically less than 2% of the work done by active muscle for the C-bend contraction and less than 4% of the work done for the contralateral contraction. Each experiment typically lasted 8 h, during which the maximum isometric tension usually declined by less than 5%. The instantaneous power output during work loop experiments was calculated over 1 ms intervals.

In order to determine whether the simulated *in vivo*

parameters used in work loop experiments gave the maximum possible mean power output, we systematically varied the strain cycle duration, strain amplitude, stimulus phase and stimulus duration. Only one parameter was varied at a time, the remaining three parameters were kept constant at the values recorded *in vivo*.

#### Force–velocity relationship

The isometric properties and force–velocity characteristics of *N. coriiceps* white (fast) myotomal muscle fibres were also determined. Maximum contraction velocities ( $V_{\max}$ ) were determined using the slack-test method (Edman, 1979) and from force–velocity curves using isovelocity releases (Altringham and Johnston, 1988). In the slack-test method, fibres were given a step release sufficient to abolish force during the plateau phase of an isometric tetanus. The time taken to re-develop force was measured for 8–10 different step length changes. A linear regression of step length *versus* slack time was used to calculate  $V_{\text{slack}}$  for each preparation. In constructing force–velocity curves, an initial step release (1–2 ms) was given during the plateau phase of an isometric tetanus to reduce the stress to a new level. A second slower release was adjusted to hold stress constant after the step. 15–22 isovelocity releases were used to construct each curve. The hyperbolic–linear equation described by Marsh and Bennett (1986) was used to fit the steady-state data:

$$V = [B(1 - F)/(A + F)] + C(1 - F),$$

where  $V$  is velocity in  $FLs^{-1}$ ,  $F$  is stress and  $A$ ,  $B$  and  $C$  are constants. Data for individual preparations were fitted using a non-linear curve-fitting programme (Regression, Blackwell Scientific Software, Oxford, England).

#### Determination of muscle fibre cross-sectional area

At the end of the experiments, muscle fibres were pinned out at their resting lengths and immersed in liquid isopentane cooled to –159°C using liquid nitrogen. Frozen transverse sections (10 µm) were cut at several points along the length of the preparation, transferred to coverslips and stained for myosin ATPase activity (Johnston *et al.* 1974). Cross-sectional areas of intact fibres were determined using digital planimetry and image-analysis software (VideoPlan, Kontron, Basel, Switzerland). The total cross-sectional area of the fibres together with measurements of fibre length were used to calculate mass-specific power outputs.

#### Statistics

Results are presented as means ± S.E.M. Seven fish were used for the swimming experiments, nine for steady-state isometric contractile properties and five for work loop experiments. Significant differences were determined using Wilcoxon signed-rank tests.

## Results

#### Kinematics of escape responses

Escape responses consisted of an initial C-bend in which the

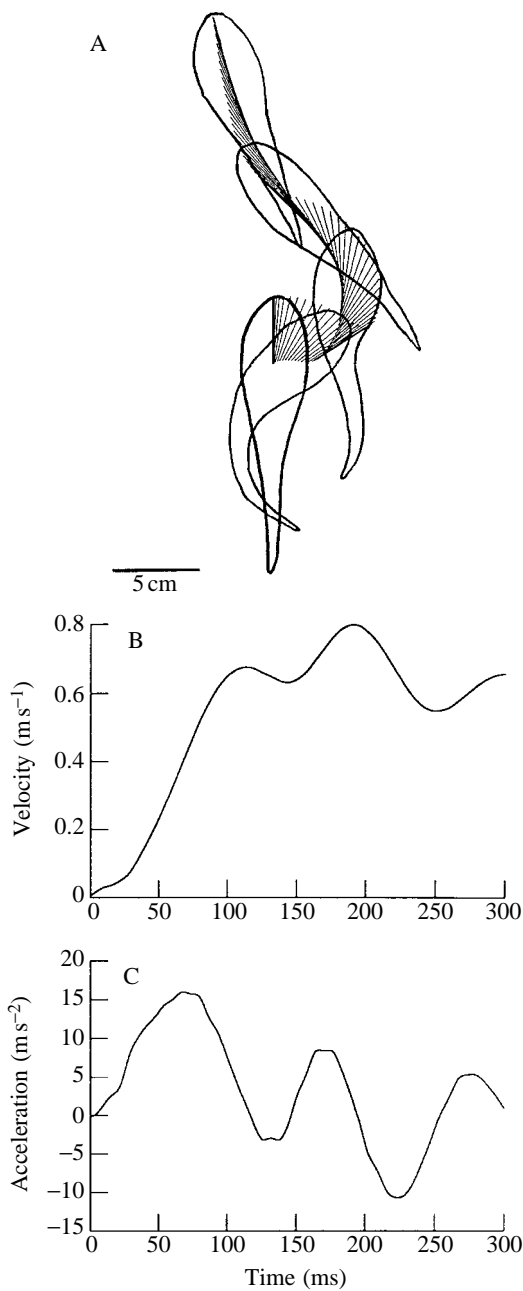


Fig. 2. Swimming kinematics of *Notothenia coriiceps* during a representative escape response at 0 °C. (A) Body outlines with the path of the line connecting the centre of mass and the snout also shown. (B) Velocity and (C) acceleration of the centre of gravity of the fish in the direction of travel.

head and tail of the fish bend in the same direction away from the centre of mass (half tailbeat), followed by one or more complete tailbeats and an unpowered glide of variable duration (Fig. 2). The escape responses were highly variable, especially with respect to the final direction of travel. Only in a few cases did *N. coriiceps* swim in a straight line (relative to the starting position of the fish): there was usually an angular component dictated by the initial C-bend and subsequent contralateral contraction. The presence of the sonomicrometry and

Table 1. Kinematics of escape responses in the Antarctic teleost *Notothenia coriiceps* at 0 °C over 300 ms before (control fish) and after insertion of sonomicrometry crystals and electromyography electrodes (instrumented fish)

	Control fish	Instrumented fish	Combined data
Distance travelled (cm)	16.5±0.9	15.8±0.3	16.3±0.3
Maximum velocity (m s <sup>-1</sup> )	0.69±0.03	0.72±0.04	0.71±0.03
Maximum acceleration (m s <sup>-2</sup> )	16.3±1.2	17.6±2.1	17.1±1.4
Tailbeat amplitude (L)	0.23±0.10	0.24±0.13	0.24±0.11

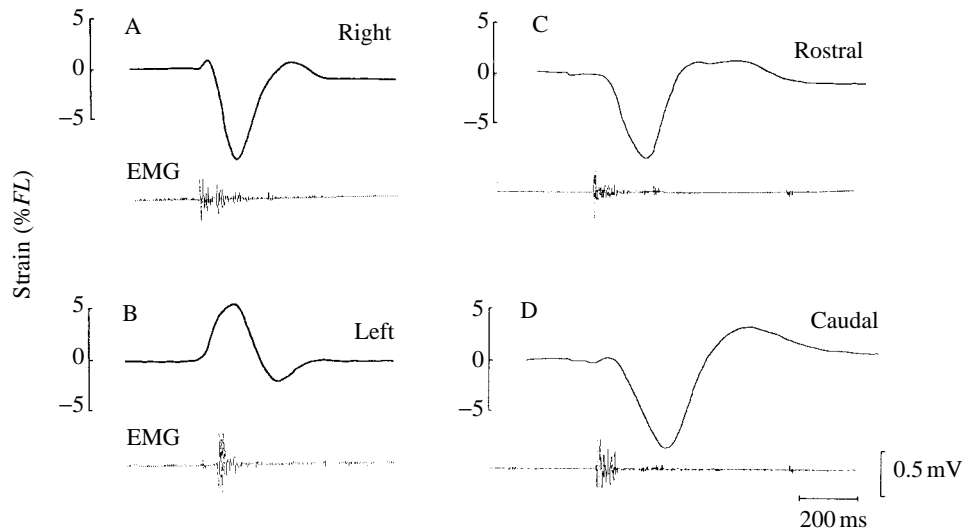
Values represent mean ± S.E.M., N=7 fish.  
L is total length.

electromyography (EMG) wires had no significant effect on swimming velocity and acceleration relative to unwired fish (signed-rank test) (Table 1). In 300 ms, *N. coriiceps* travelled 16.3±0.3 cm, reaching a maximum velocity and acceleration (as recorded from the centre of gravity of the fish) of 0.71±0.03 m s<sup>-1</sup> and 17.1±1.4 m s<sup>-2</sup>, respectively (Table 1).

In vivo measurements of muscle action

Sonomicrometry and EMG recordings were made from the superficial white muscle of *N. coriiceps* during escape responses (Figs 3, 4). There was considerable variation in muscle strain (Fig. 4) and activation patterns, reflecting the variation seen in the kinematics of the escape response. Fig. 3 presents results for a typical escape response showing a C-start initiated by muscles on the right-hand side resulting in fibres in the rostral myotomes (0.35L) shortening by 9 % of their resting length (FL) (Fig. 3A). The strain ranged between 5 % and 13 %FL (Fig. 4), with the magnitude of the strain being dependent upon the final direction of travel. For example, sonomicrometry recordings of strain taken from the right myotomes tended to be larger if the fish was moving to the right during the escape response than if it was going straight ahead or veering off to the left. The modal strain recorded from the rostral myotomes during the C-bend was 10 %, with a mean strain of 9.5±1.5 % (mean ± S.D., seven fish). The mean fibre-length-specific speed of muscle shortening for the largest strain recorded for each fish was 1.68±0.21 FL s<sup>-1</sup> (N=7). In some cases, there was a small pre-stretch (never more than 0.01FL), presumably due to the shortening of more anterior muscles and/or changes in muscle stiffness (Figs 3A, 5A). Electrical activity (EMGs) was recorded in rostral myotomes 10–20 ms before shortening and continued for 100–150 ms (Fig. 3A,C). The muscle fibre strain waveforms recorded from the caudal myotomes (0.65L) on the same side of the fish were of similar amplitude (5–12 %FL; modal value 9.5 %) and resembled those of the rostral myotomes in shape. At 0.65L, electrical activity was recorded 60–90 ms after the initiation of the fast-start whilst the muscle fibres were still lengthening (Fig. 3D). The pre-stretch recorded from the caudal myotomes was always greater than that recorded from the rostral myotomes. The C-bend caused muscle fibres on the contralateral side of the body (Fig. 3B) to be stretched by 5.0 %FL and

Fig. 3. Muscle fibre strain and activation patterns (EMG) in rostral (0.35L) and caudal (0.65L) myotomes for a typical escape response in *Notothenia coriiceps*. The records show the C-bend (A) and contralateral contraction (B) for the rostral position in a fish initiating the escape response on its right-hand side. C and D illustrate recordings on the same side of the fish at 0.35L (C) and 0.65L (D) for the C-bend in a fish initiating the escape response on its right-hand side. FL, muscle fibre length.



7.0%FL at 0.35L and 0.65L, respectively, producing nearly sinusoidal strain waveforms (Fig. 4B). During the contralateral contraction, muscle fibres in both rostral and caudal positions shortened by up to 12%FL (peak-to-peak strain) (Fig. 4B) at a maximum contraction speed of  $1.62 \pm 0.22 \text{ FL s}^{-1}$  ( $N=7$ ). In the rostral position, muscle strain during the contralateral contraction varied from 5 to 12%FL, with a mean of  $10.0 \pm 1.0 \text{ %FL}$ , whereas in the caudal location muscle strain was 6–12%FL, with a mean of  $9.5 \pm 1.0 \text{ %FL}$  (Figs 3, 4). Again, this variation reflected the final direction of travel of the fish. EMGs recorded from rostral and caudal positions started 40–65 ms after the beginning of fibre lengthening and finished 75–100 ms later, just after the onset of shortening (Fig. 3B).

#### In vitro muscle experiments

All experiments were performed using muscle fibres isolated from the caudal myotomes as it proved impossible to dissect preparations from the rostral myotomes. In a previous study with this species, using skinned muscle fibres isolated from the rostral myotomes, we obtained similar values for maximum isometric stress ( $180 \text{ kN m}^{-2}$ ) and unloaded contraction velocity ( $2.1 \text{ fibre lengths s}^{-1}$ ) as reported for caudal muscle fibres in the present study (Mutungi and Johnston 1988; G. Mutungi and I. A. Johnston, unpublished results). However, it should be noted that the properties of live muscle fibres may differ along the trunk.

Work loops were produced by 'playing back' *in vivo* strains and stimulation patterns to stimulated isolated muscle fibres (Fig. 5). Four abstracted (cyclical) events corresponding to the C-bend at 0.35L and 0.65L (Fig. 5A,C,E,G), and the subsequent contralateral contraction at rostral and caudal positions (Fig. 5B,D,F,H), were studied. In the rostral position, the C-bend produced a maximum stress of  $115.0 \pm 7.3 \text{ kN m}^{-2}$  ( $N=5$ ) and the maximum power reached  $144.1 \pm 16.8 \text{ W kg}^{-1}$  wet muscle mass (Fig. 5C,E; Table 2). In the caudal position, during the C-bend, the increased pre-stretch prior to shortening (Fig. 5A) contributed to a 24% increase in the maximum stress and a more than twofold increase in the peak instantaneous power output relative to rostral

myotomes (Table 2;  $P < 0.05$ , signed-rank test). Although the maximum power outputs differed along the length of the trunk, the average power outputs over the abstracted cycles were not significantly different (Fig. 5G; Table 2).

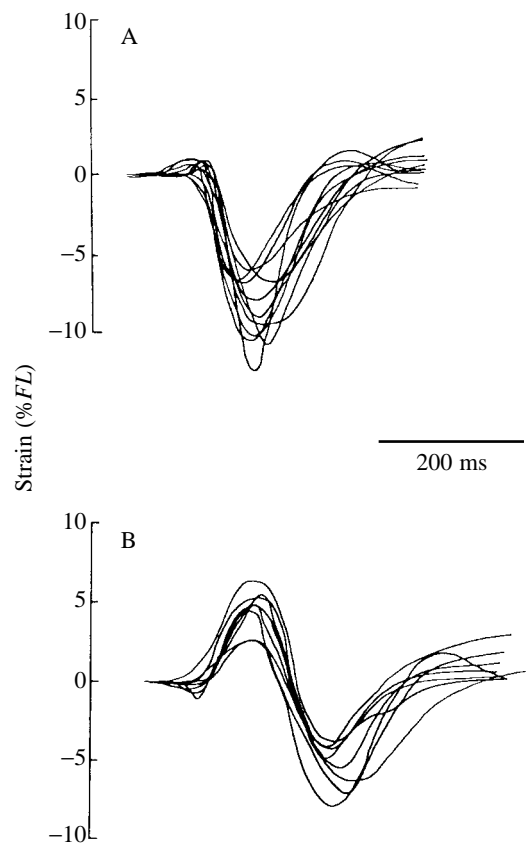


Fig. 4. The range of muscle strain measurements in rostral myotomes recorded using sonomicrometry for escape responses in *Notothenia coriiceps* for (A) the C-bend and (B) the contralateral contraction. Recordings were made from seven fish. The variation in muscle strain between different fast-starts was greater than the variation between individuals (see text for details). FL, muscle fibre length.

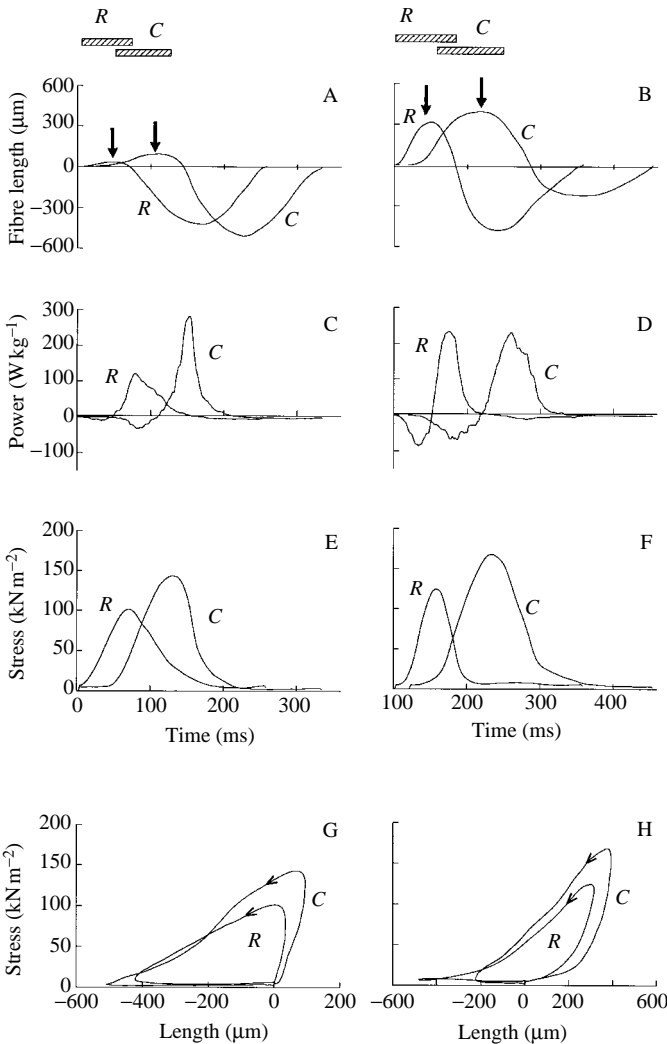


Fig. 5. Work loop experiments on isolated fast muscle fibres using the representative *in vivo* recordings of strain and activation. Changes in muscle fibre length with respect to the starting length (A,B), instantaneous power (C,D), stress (E,F) and the corresponding work loops (G,H) are illustrated for the C-bend (left-hand panels) and contralateral (right-hand panels) contraction. The shaded boxes show when the stimuli were applied. R, rostral myotome (0.35L); C, caudal myotome (0.35L). The arrows in A and B indicate the pre-stretch associated with the fast-start.

During the subsequent contralateral contraction, the muscle fibres were initially active whilst lengthening, producing maximum stresses of  $124.2 \pm 12.7 \text{ kN m}^{-2}$  and  $168.0 \pm 10.0 \text{ kN m}^{-2}$  at 0.35L and 0.65L, respectively (Fig. 5F; Table 2). This resulted in maximum power outputs of  $269.6 \pm 10.9 \text{ W kg}^{-1}$  wet muscle mass and  $247.5 \pm 30.8 \text{ W kg}^{-1}$  wet muscle mass being generated in the rostral and caudal locations respectively (Fig. 5D; Table 2). The average power outputs over the abstracted cycles for the contralateral contractions were not significantly different at different points along the body and reached 15.8 and  $19.6 \text{ W kg}^{-1}$  wet muscle mass for rostral and caudal positions respectively (Table 2).

Table 2. Mean values of maximum stress and power output generated during work loop experiments using *in vivo* strain and stimulation patterns

Maximum stress ( $\text{kN m}^{-2}$ )	
Rostral, C-bend	$115.0 \pm 7.3$
Caudal, C-bend	$143.3 \pm 7.0$
Rostral, contralateral contraction	$124.2 \pm 12.7$
Caudal, contralateral contraction	$168.0 \pm 10.0$
Maximum instantaneous power output ( $\text{W kg}^{-1}$ wet muscle mass)	
Rostral, C-bend	$144.1 \pm 16.8$
Caudal, C-bend	$289.9 \pm 18.6$
Rostral, contralateral contraction	$269.6 \pm 10.9$
Caudal, contralateral contraction	$247.5 \pm 30.8$
Mean power output for the abstracted cycle ( $\text{W kg}^{-1}$ wet muscle mass)	
Rostral, C-bend	$16.4 \pm 2.8$
Caudal, C-bend	$18.1 \pm 3.1$
Rostral, contralateral contraction	$15.8 \pm 2.3$
Caudal, contralateral contraction	$19.6 \pm 2.1$
Values represent mean $\pm$ S.E.M., $N=5$ .	
All experiments were performed at $0^\circ\text{C}$ .	

Table 3. Steady-state isometric contractile properties of fast myotomal muscle fibres from *Notothenia coriiceps* at  $0^\circ\text{C}$

Maximum twitch stress ( $\text{kN m}^{-2}$ )	$90.2 \pm 16.8$
Time to maximum twitch stress (ms)	$28.6 \pm 2.3$
Half-relaxation time (ms)*	$53.4 \pm 3.8$
Tetani contractions at 70 Hz	
Maximum isometric stress, $F_{\text{max}}$ ( $\text{kN m}^{-2}$ )	$185 \pm 15.3$
Time to half-maximum isometric stress (ms)	$52.4 \pm 8.1$
Half-relaxation time (ms)†	$122.9 \pm 10.7$

Values represent mean  $\pm$  S.E.M.,  $N=9$  fish.  
The number of preparations used is indicated in brackets.  
\*Time from peak to half-maximum stress; †time from the last stimulus to  $F_{\text{max}}/2$ .

Effect of varying *in vivo* parameters

The effect of varying, in turn, strain amplitude, cycle duration, stimulus duration and stimulus timing on muscle power output are shown in Figs 6 and 7. In all cases, the *in vivo* recorded ranges (indicated by the shaded boxes) produced between 90 and 100 % of the maximum *in vitro* power outputs.

Isometric and isotonic contractile properties

The steady-state isometric contractile properties of the muscle fibres are shown in Table 3 and Fig. 8.  $V_{\text{max}}$  was calculated from the Marsh-Bennett (1985) equation and was  $2.34 \pm 0.11 \text{ FL s}^{-1}$  ( $N=3$  preparations). Values for the constants A, B and C in the Marsh-Bennett equation were  $0.22 \pm 0.018$ ,

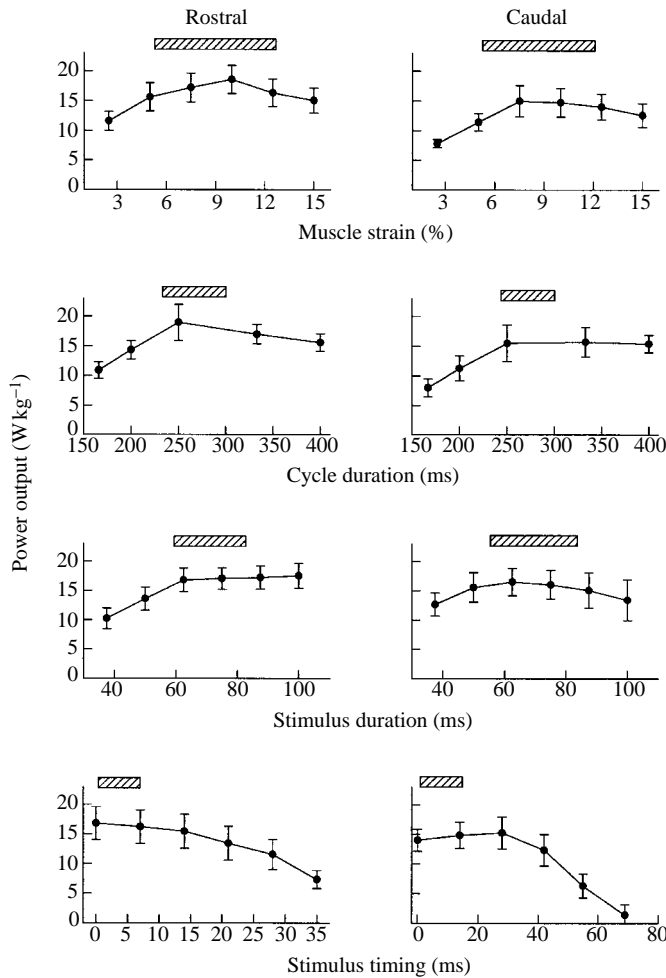


Fig. 6. The effect of varying strain, cycle duration, stimulus duration and stimulus timing on the average power output of fast muscle fibres undergoing the *in vivo* muscle length changes measured for the C-bend at rostral and caudal locations (see Fig. 3). The shaded blocks represent the range of values recorded *in vivo*. Time 0 corresponds to the initiation of the fast-start.

$0.027 \pm 0.0014$  and  $1.14 \pm 0.22$  respectively.  $V_{\text{slack}}$  determined for the same three preparations was  $2.39 \text{ FL s}^{-1}$ .

### Discussion

During escape responses at  $0^\circ\text{C}$ , the Antarctic rock cod (*N. coriiceps*) reached swimming velocities and accelerations comparable to escape responses recorded for some other fish species of similar size but at water temperatures  $5\text{--}15^\circ\text{C}$  warmer (Beamish, 1978). Archer and Johnston (1989) recorded even faster velocities in *N. coriiceps* during burst swimming (up to  $1.28 \text{ m s}^{-1}$ ); however, their measurements were made using trained fish and during the transition from labriform to subcarangiform swimming, and not from a standing start as in the present study. During prey capture, Beddow *et al.* (1995) recorded a maximum swimming velocity and acceleration of  $0.85 \text{ m s}^{-1}$  and  $17 \text{ m s}^{-2}$ , respectively, for the short horn sculpin *Myoxocephalus scorpius* at  $10^\circ\text{C}$ ; the

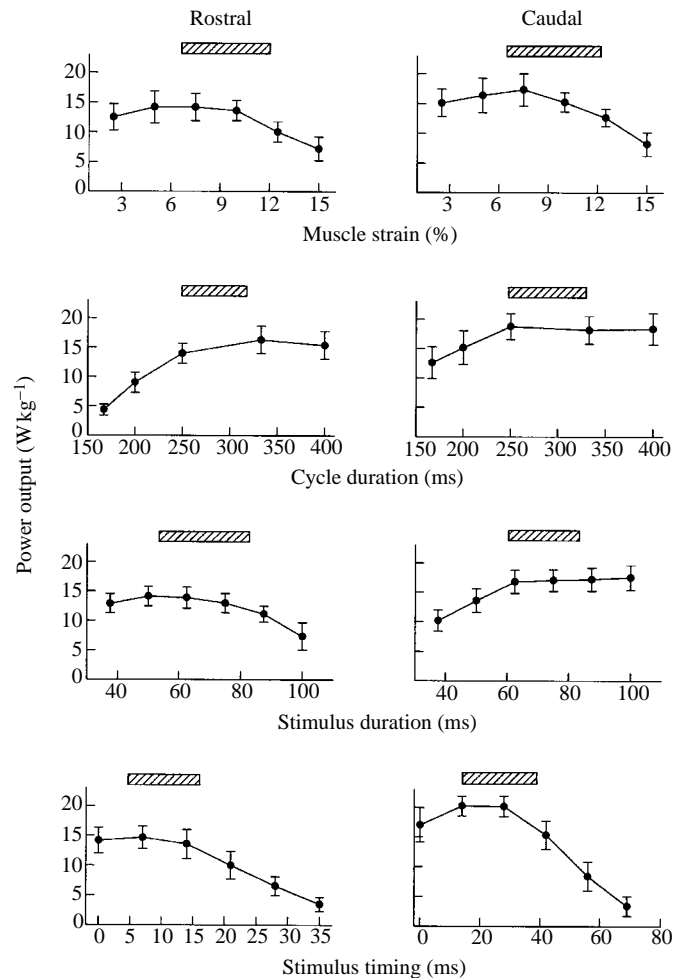


Fig. 7. The effect of varying strain, cycle duration, stimulus duration and stimulus timing on the average power output of fast muscle fibres undergoing the *in vivo* muscle length changes measured for the contralateral contraction at rostral and caudal locations (see Fig. 3). The shaded blocks represent the range of values recorded *in vivo*.

short horn sculpin is a temperate species which has a similar body form and locomotory behaviour to *N. coriiceps*. The maximum swimming performance in more-streamlined acceleration specialists such as northern pike *Esox lucius* is, however, considerably higher than for these other fish (Webb, 1978; Harper and Blake, 1990; Domenici and Blake, 1991).

The magnitudes of the muscle strains developed during the escape response in *N. coriiceps* were comparable to those calculated for the short horn sculpin during prey capture (S-shaped fast-starts) (Johnston *et al.* 1995), although the maximum muscle contraction velocities were considerably slower in *N. coriiceps*. The maximum shortening velocities of fast muscle fibres in caudal myotomes was  $3.8 \text{ FL s}^{-1}$  for *M. scorpius* at  $15^\circ\text{C}$  (Johnston *et al.* 1995), compared with only  $1.6 \text{ FL s}^{-1}$  for *N. coriiceps* of similar length at  $0^\circ\text{C}$ . This is not surprising as the maximum unloaded isotonic shortening velocity ( $V_{\text{max}}$ ) of fast muscle fibres in fish shows no evidence for evolutionary temperature compensation, ranging from approximately  $2 \text{ FL s}^{-1}$  in Antarctic species at  $-1^\circ\text{C}$  to

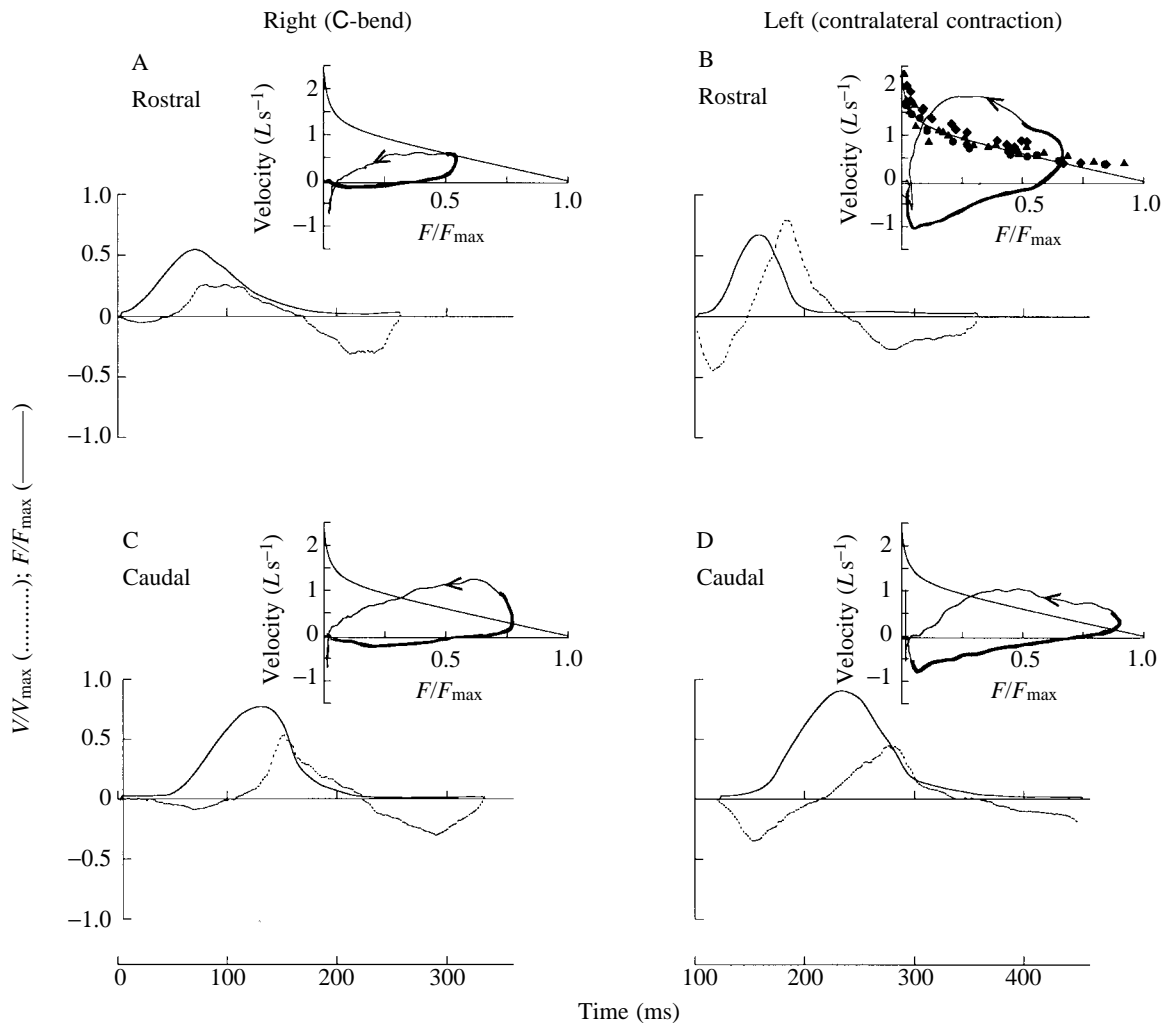


Fig. 8. Plots of  $V/V_{\max}$  (dotted lines) and  $F/F_{\max}$  (solid lines) versus time for the work loop experiments illustrated in Fig. 5A,B for C-start and contralateral contraction, respectively. Inset: steady-state and work loop force-velocity ( $F$ - $V$ ) relationships for muscle fibres from *Notothenia coriiceps* at  $0^{\circ}\text{C}$ . The bold line on the power loops indicates the period of stimulation. The full data sets for the steady-state  $F$ - $V$  curves are shown in B.

$15\text{--}20 FLs^{-1}$  in various tropical fish at  $25^{\circ}\text{C}$  (Johnston and Brill, 1984; Johnson and Johnston, 1991). This is also reflected in the kinematics of fast-starts, where length-specific tailbeat frequencies of polar fish are lower than those for temperate and tropical species (Archer and Johnston, 1989; Harper and Blake, 1990; Domenici and Blake, 1991).

The maximum isometric stress and the force-velocity relationship are intrinsic properties of muscle fibres, and we have used them as reference points for performance under non-steady-state conditions. During escape responses, power is initially absorbed by the muscle because the fibres are active whilst lengthening. This results in significant force enhancement due to stretch (Fig. 8), as has been reported for cyclical contractions in frog muscle (Stevens, 1993). The mechanism of force enhancement with active stretch is unknown, but it may involve altered cross-bridge interaction with the thin filament (Edman *et al.* 1978). It is unlikely to involve an increase in the number of cross bridges recruited,

since force enhancement after stretch increases with decreasing overlap of thick and thin filaments (Edman *et al.* 1978). Whatever the mechanism, active pre-stretch enhances performance, resulting in a significant deviation in behaviour from the steady-state force-velocity relationship (Fig. 8). For the contralateral contraction, differences in the rate and duration of stretch produced marked differences in the shape of the work (Fig. 5H) and power loops at  $0.35L$  and  $0.65L$  (Fig. 8). At both positions,  $V$  exceeded that predicted from the force-velocity curve for considerable periods. The velocity increased dramatically during the early part of shortening, whilst the load was relatively constant and still at an appreciable fraction of  $F_{\max}$ , 0.65 at  $0.35L$  and 0.85 at  $0.65L$  (Fig. 8). The particularly high levels of force generation at  $0^{\circ}\text{C}$  during isometric (Johnston and Brill, 1984; Johnston and Altringham, 1985) and cyclical (Fig. 7) contractions may reflect the unusual structure of the myosin molecule in Antarctic fish. Skeletal muscle myosin from Antarctic fish is readily denatured and loses its

ATPase activity on isolation (Johnston *et al.* 1975). The myofibrillar ATPase activity of fast muscle from several species of Antarctic fish at 0°C was higher, and the free activation energies ( $\Delta G^\ddagger$ ) were lower, than for the homologous enzyme in warm-water fish (Johnston and Goldspink, 1975). Values of  $F/F_{\max}$  during the early part of shortening in *N. coriiceps* are much higher than those in muscle fibres from temperate fish measured under comparable conditions at 12°C (R. S. James and I. A. Johnston, in preparation). Our results also contrast with those for continuous swimming, where steady-state muscle properties are better predictors of muscle performance. Red muscle fibres in carp (*Cyprinus carpio*) and scup (*Stenotomus chrysops*) have been shown to shorten at values of  $V/V_{\max}$  of 0.18–0.36, precisely the values predicted to generate maximum power from the  $P$ – $V$  relationship (Rome *et al.* 1988, 1992).

During the escape response, *N. coriiceps* generated maximum and mean power outputs comparable to those for predation fast-starts in a temperate fish, *Myoxocephalus scorpius*, at 15°C. *M. scorpius* fast muscle fibres produced maximum instantaneous and mean power outputs of 175–265 and 21–24 W kg<sup>-1</sup> wet muscle mass, respectively (Johnston *et al.* 1995). No other similar data are available for fish; however, maximum instantaneous power output,  $W_{\max}$ , for jet propulsion swimming in scallop is approximately 200 W kg<sup>-1</sup> wet muscle mass at 10°C (Marsh *et al.* 1992), whereas mouse fast muscle delivers 300 W kg<sup>-1</sup> wet muscle mass at 35°C during sinusoidal contractions (James, 1994). The similarity of these values during locomotion in Antarctic fish, scallop and mouse muscles is striking given the large differences in operating temperature, morphology and phylogeny of the animals being compared. In contrast, maximum muscle power output in *N. coriiceps* calculated from the  $P$ – $V$  relationship, is only 60–70 W kg<sup>-1</sup> wet muscle mass, significantly lower than for fast muscles in most temperate fish and in homeotherms measured at their normal body temperature (Josephson, 1993).

When muscle fibre strain, the duration of the abstracted cycle, stimulation duty cycle and stimulus timing were each systematically optimised to give maximum power whilst holding all other parameters constant, the average power per cycle was between 90 and 100% of that recorded using the *in vivo* parameters. Thus, during fast-starts, the muscle fibres in Antarctic fish are operating at close to their maximal performance.

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