J. exp. Biol. 171, 261–281 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

THE INFLUENCE OF TEMPERATURE ON POWER OUTPUT OF SCUP RED MUSCLE DURING CYCLICAL LENGTH CHANGES

BY LAWRENCE C. ROME[1] AND DOUGLAS SWANK[2]

[1]*Department of Biology and* [2]*Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104, USA* and *Marine Biological Laboratories, Woods Hole, MA 02543, USA*

Accepted 16 June 1992

Summary

To gain insight into how temperature affects locomotory performance, we measured power output of scup red muscle during oscillatory length changes at 10°C and 20°C. When we optimized work loop parameters, we found that maximum power was 27.9 W kg^{-1} at 20°C and 12.8 W kg^{-1} at 10°C, giving a Q_{10} of 2.29. Maximum power was generated at a higher oscillation frequency at 20°C (5 Hz) than at 10°C (2.5 Hz), and the Q_{10} for power output at a given oscillation frequency ranged from about 1 at 1 Hz to about 5 at 7.5 Hz. An analysis of the results in terms of crossbridge kinetics suggests that the higher power output at 20°C is associated with both a higher V_{max} (i.e. more power per cycling crossbridge) and faster activation and relaxation (i.e. a greater number of cycling crossbridges).

To obtain a more realistic understanding of the functional importance of temperature effects on muscle, we imposed on isolated muscle *in vivo* length changes and oscillation frequencies (measured during previous experiments on swimming scup) and the *in vivo* stimulus duty cycle (measured from electromyograms in this study). At 20°C, muscle bundles tested under these *in vivo* conditions produced nearly maximal power, suggesting that the muscle works optimally during locomotion and, thus, important contractile parameters have been adjusted to produce maximum mechanical power at the oscillation frequencies and length changes needed during swimming. At 10°C, muscle bundles tested under *in vivo* conditions produced much less power than was obtained during the 'optimized' work loop experiments discussed above. Furthermore, although 'optimized' work loop experiments showed that maximum power output occurs at 2.5 Hz, scup do not appear to swim with such a low tailbeat frequency. Although the reason for these apparent discrepancies at 10°C are not known, it shows that simple extrapolation from isolated muscle to the whole animal, without knowledge of how the muscle is actually used *in vivo*, can be misleading.

Key words: muscle, power, temperature, scup, *Stenotomus chrysops*.

Introduction

Although animals can locomote over a wide range of temperatures, the fastest movements an animal can make with a given fibre type are slower at low temperatures, apparently because of low muscle power output (Rome *et al.* 1984, 1990, 1992; Rome, 1990). For example, using their red muscle, carp and scup are able to swim at a 1.5-fold faster swimming speed with a 1.6-fold faster muscle shortening velocity (V) at 20°C than at 10°C (Rome *et al.* 1990, 1992*a*,*b*; Rome, 1990). This is made possible by a 1.6-fold higher maximum velocity of shortening (V_{max}) of the red muscle at 20°C. Thus, at both 10°C and 20°C, fish can swim to V/V_{max} values of approximately 0.36, where analysis from steady-state force–velocity curves shows that maximum power is generated. Rome and colleagues concluded that V/V_{max} and power generation set the limits for use of a given muscle fibre type at a given temperature.

These authors (Rome *et al.* 1992*b*; Rome, 1992), as well as others before them (Josephson, 1985; Altringham and Johnston, 1990*a*), have suggested that studies relating swimming behaviour to *steady-state* properties of muscle may not be complete. Josephson (1985) pointed out that the rate of relaxation as well as activation may be important in determining how much power a muscle can generate during this type of cyclical locomotion and pioneered the use of work loops for such analyses. It seems that one might obtain a better insight into how temperature affects locomotory performance by comparing it to the power output during oscillatory contractions at different temperatures. Thus, the major goal of this paper is to determine the optimal power generated by scup red muscle as a function of oscillation frequency and temperature.

Although numerous papers have used the work loop technique, previous investigators have typically optimized as many parameters as possible to obtain maximum power output of the muscle *without regard to how the animal is actually using the muscle*. Drawing conclusions about the locomotory ability or muscle adaptation from these work loop measurements might be misleading. Lacking knowledge of how animals actually use their muscles, one might compare power outputs at oscillation frequencies over which the animals never use their muscles, and hence these power outputs would be irrelevant.

To understand fully how temperature affects locomotory performance, it is necessary to reproduce in isolated muscle the exact length changes and stimulation pattern that the muscle undergoes during locomotion. Although we do not have complete information, we have recently determined the sarcomere length (SL) changes and the oscillatory frequencies that the scup red muscle undergoes as a function of temperature and swimming speed (Rome *et al.* 1992*a*). In this paper, we have additionally determined the stimulation duration of the muscle during swimming (electromyogram duration). Thus, our final goal is to constrain the length changes and stimulation conditions of the isolated muscle to those actually occurring during locomotion and to measure performance.

Materials and methods

Preparation

Scup (*Stenotomus chrysops* L.), 19–23 cm long, were killed with a blow to the head and their scales were removed. Muscle bundles approximately 400 μm thick, 900 μm wide and 0.48 cm long were removed from above the midline of the fish, dissected at 4 °C under a stereomicroscope, and tied to the servomotor arm and force transducer as described by Rome and Sosnicki (1990). The volume of the bundle was estimated by photographing different aspects of the bundle through a dissecting microscope. Taking into account the large amount of connective tissue and fat in the bundle revealed by previous histological studies (see Rome *et al.* 1992*b*), we calculated that the cross section (and volume) occupied by muscle fibres was 43 % of the overall cross section. The dissection differed from that of Rome *et al.* (1992*b*) for scup, but was similar to Rome and Sosnicki's carp experiments (1990), in that the overlying skin was not removed from the muscle, it was only thinned. Leaving the skin on increased the probability of obtaining bundles giving consistent results. Sinusoidal length changes without stimulation showed that the work performed by passive components, including the skin, is relatively small (see Results). Thus, leaving the skin on had little effect on measurements of active contractions. Our ability to obtain acceptable bundles was further improved by testing the bundles for response to electrical stimulation prior to fine dissection and mounting.

Solutions

Dissection and experiments were carried out in a Ringer's solution containing (mmol l^{-1}) NaCl (132), KCl (2.6), $CaCl_2$ (2.7), imidazole (10), sodium pyruvate (10) and $MgCl_2$ (1), pH 7.7 at 15 °C, based on Altringham and Johnston (1990*a*).

Experimental apparatus

A Cambridge Technology 300S servosystem provided muscle length changes and a Cambridge Technology model 400 force transducer (natural frequency 2 kHz) was used to measure force. The experimental apparatus was controlled by an Analog Devices D/A, A/D board (RT-815) installed in an IBM-AT. The preparation was activated by monopolar stimulation through bright platinum plate electrodes running the full length of the bundle. Stimulus pulses were generated by a Grass S-48 stimulator and the current was amplified with an HP power amplifier. All force and length signals were recorded and stored using a Nicolet 4094 digital oscilloscope.

Software

A set of software programs was written to accomplish various experimental tasks. The computer generated a sinusoidal length change and triggered the stimulator to fire at the appropriate phase. In these experiments, unlike previous ones by other authors (Josephson, 1985; Altringham and Johnston, 1990*a*),

stimulus phase is defined with reference to the beginning of shortening from the maximum length during the sinusoid without regard for the twitch properties of the muscle. Hence at a phase of 0°, the stimulus would start at the moment the muscle started shortening, whereas at a phase of −60°, the stimulus would start one-sixth of a cycle prior to muscle shortening. Although all referencing systems are acceptable, we believed this was more appropriate for our purposes.

To determine the approximate optimal phase for the stimulus at a given frequency, we used a program that would vary the phase during 10 consecutive loops. For instance, to initially screen the range of −90° to −30°, the computer would produce two consecutive loops each of −90°, −75°, −60°, −45° and −30° (for a total of 10 loops). Note that two consecutive loops of the same phase were necessary, because the force generated during lengthening in the first cycle of the pair is influenced by the phase of the stimulus in the preceding cycle. As the work generated varied with loop number, final values were obtained in series at a fixed phase. The first work loop in each series was a passive one (no stimulus given) so that the effects of passive force could be assessed.

Work output per cycle was calculated from length and force records by a program written in WAVEFORM Basic. The program obtained the data from the Nicolet oscilloscope and then calculated the values for negative work, positive work, net work per cycle and net power output.

Experimental protocol

Following attachment to the servo and force transducer, the muscle was given a series of twitches to determine the optimal stimulus pulse duration and voltage. Generally, a pulse duration of 2 ms was used at 10 °C and one of 1 ms was used at 20 °C. Stimulus rates were then adjusted to obtain maximum force generation (100–150 pulses s^{-1} at 10 °C and 200–300 pulses s^{-1} at 20 °C). A force–length curve was then obtained for the bundle. The initial length for the work loop experiments was set on the plateau of this curve where a 5 % length change would cause a similar decrement in active force at both the shortest and longest length. At this muscle length, resting tension was a small fraction of tetanic tension (i.e. 3±0.5 %, ±S.E., N=19).

The muscle bundle was studied at selected oscillation frequencies of 1, 2.5, 3.5, 5, 7.5, 10 and 12.5 Hz. Peak power output at each frequency was obtained by varying phase, stimulus duty cycle and amplitude of the length change. At the end of an experimental run at a given temperature, the optimal conditions at each oscillation frequency were run in sequence to allow comparison at equivalent levels of fatigue. After finding the optimal power output at a number of oscillation frequencies at one temperature, the temperature was changed.

Stimulation duration during swimming

To understand better how muscle is actually used during locomotion, specific oscillation frequencies and stimulation durations measured from swimming fish were also imposed on the muscle while power output was measured. The

stimulation durations in this experiment were determined by measuring the electromyogram (EMG) duration of the red muscle of scup swimming at various speeds at 10°C and 20°C using techniques detailed in Rome *et al.* (1992*a*). Briefly, scup were swum in a water treadmill at flow velocities which increased in steps of $10\,cm\,s^{-1}$. Bipolar EMG electrodes were placed in the red muscle below the dorsal fin. The EMG amplifiers were equipped with cutoff filters of 10 and 3000 Hz as well as a 60 Hz notch filter. The EMGs at each swimming speed were stored on the audio tracts (bandwidth of 20 Hz to 20 kHz) of the videotape while fish swimming was filmed. EMG duration for a red muscle burst was counted from the first spike to the last using the Nicolet oscilloscope. Only high-frequency peaks that exceeded 12 μV were considered to be spikes. Tailbeat frequency was taken to be the frequency at which red muscle bursts occurred.

Results

Isometric properties

Maximum isometric tension was $197\pm20\,kN\,m^{-2}$ (±S.E., $N=9$) at 20°C, at which the force–length curve was generally performed. Using a Q_{10} value of 1.09, taken from Rome *et al.* (1992*b*), gave a value of $181\,kN\,m^{-2}$ at 10°C.

Although tetanic tension was higher at 20°C than at 10°C, twitch force was higher at 10°C. On average, the twitch–tetanus ratio was 0.54±0.028 (±S.E., $N=8$) at 10°C and 0.37±0.037 (±S.E., $N=10$) at 20°C (Fig. 1).

Tension generation and particularly muscle relaxation were much faster at 20°C than at 10°C (Fig. 1). During twitches, the time in which the force level increased from 10 to 90 % maximum force ($T_{a,10-90}$) decreased 1.9-fold from 71 to 37 ms (Table 1) as temperature was raised from 10°C to 20°C. The time needed to relax from 90 to 10 % maximum tension ($T_{r,90-10}$), the time needed to relax from 95 % to 80 % maximum tension ($T_{r,95-80}$) and the time between the last stimulus and the point where tension fell to 10 % of the maximum level ($T_{r,last}$), were all more than twice as fast at 20°C as at 10°C. The largest difference was in $T_{r,95-80}$, which decreased 2.75-fold from 126 ms at 10°C to 46 ms at 20°C. $T_{r,last}$ also changed dramatically from 824 ms at 10°C to 350 ms at 20°C.

The temperature effects for short tetani (Table 1) were similar to those for twitches. $T_{a,10-90}$, however, was about 1.5-fold longer for tetani than for twitches at a given temperature. The relaxation parameters in tetani were similar to those in twitches, but tended to be somewhat faster (6–34 %). Values of $T_{r,95-80}$, which is thought to correlate with the period during which intracellular $[Ca^{2+}]$ declines exponentially (Cannell, 1986), were very similar and not statistically different between twitches and short tetani.

During isometric contractions, relaxation is exceedingly slow. If one assumes that the muscle cannot operate during locomotion at a frequency higher than the time between the last stimulus and the tension falling to 10 % of the maximum ($T_{r,last}$), then the muscle could not be used faster than 1.2 Hz at 10°C and 2.86 Hz at 20°C (a similar argument was first made by Marsh and Bennett, 1986). From our

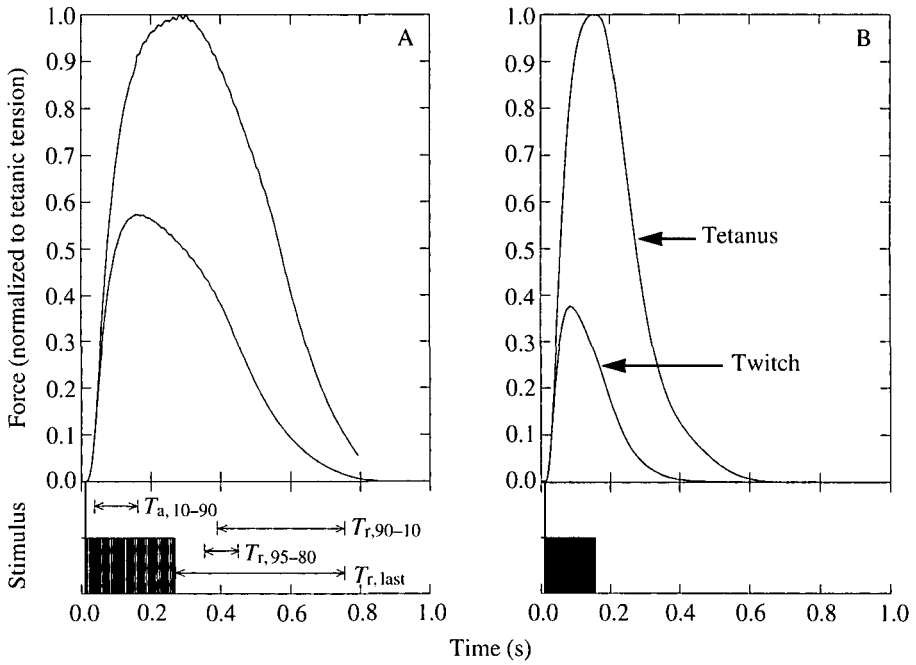

Fig. 1. Kinetics of twitch and tetanus at 10°C and 20°C. (A) A twitch and short tetanus at 10°C and (B) a twitch and short tetanus at 20°C for a different muscle bundle. In each bundle, the forces are normalized for peak tetanic tension. As can be seen, the twitch–tetanus ratio is much higher at 10°C than at 20°C. The rate of muscle activation and the rate of muscle relaxation, however, are much faster at 20°C than at 10°C. The various measures of force generation and relaxation are shown in A for the tetanus and are defined in the text. Timing of stimuli is shown by single vertical lines (twitches) or multiple vertical lines (tetanic).

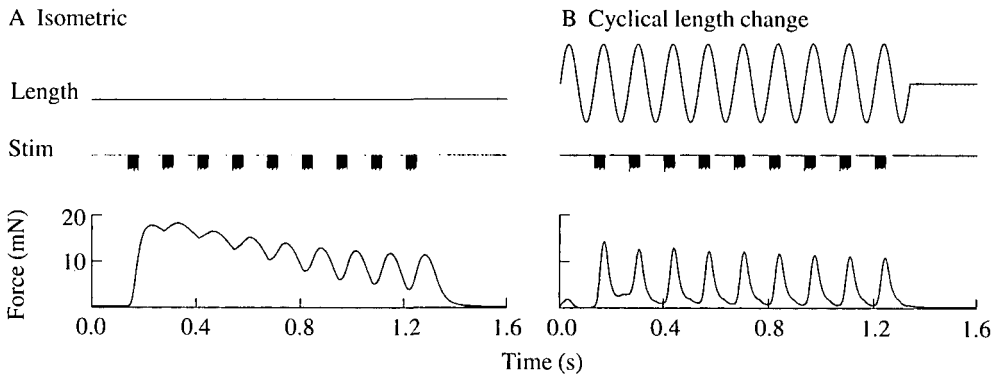

Fig. 2. Shortening deactivation during cyclical length changes allows for faster muscle relaxation. Whereas during isometric contractions (A), the scup red muscle does not completely relax between tetani, when given the same stimulus (40 ms, 300 pulses s^{-1}, 7.5 times s^{-1}) while undergoing 5.5% length changes (B), the muscle relaxes almost completely between tetani. Note that, at 20°C, scup can swim with a tailbeat frequency of 7.5 Hz. Muscle length was 0.5 cm.

Table 1. *Kinetics of force generation and muscle relaxation during twitches, tetani and oscillatory contractions*

	$T_{a,10-90}$	$T_{r,90-10}$	$T_{r,95-80}$	$T_{r,last}$
Twitch				
20°C (N=7)	37.4±1.68	230±17.5	46±6.15	350±22.3
10°C (N=7)	71±1.29	546±77.8	126±10.2	824±73.4
$1/Q_{10}$	1.90	2.37	2.75	2.36
Tetanus				
20°C (N=9)	57.6±4.3	193±19.5	40.5±2.8	261±23.3
10°C (N=9)	107.4±8.6	517±50.8	117±5.2	670±50.1
$1/Q_{10}$	1.87	2.67	2.89	2.56
Work loops				
20°C, 5 Hz (N=10)	27.6±0.8			73.8±4.3
20°C, 10 Hz (N=2)	25±3.53			42±1.4
10°C, 2.5 Hz (N=8)	47±1			152±20
10°C, 5 Hz (N=6)	36±3.3			86.3±5.2

For the twitches and tetani, the mean time (ms) for force generation ($T_{a,10-90}$) and for muscle relaxation ($T_{r,90-10}$, $T_{r,95-80}$, $T_{r,last}$) as well as the S.E. are given at 10°C and 20°C.

Force generation and muscle relaxation are faster at 20°C than at 10°C. This is signified by the Q_{10} for the rates (i.e. $1/Q_{10}$ for the time period) ranging from about 1.9 to 3.

For the oscillatory contraction, we show only $T_{a,10-90}$ and $T_{r,last}$ (see text for explanation). As can be seen, $T_{r,last}$ is much faster during shortening than during isometric twitches and tetani.

previous experiments, we know that scup use their red muscles to 4.5 Hz at 10°C and 7.5 Hz at 20°C (see Discussion). This is made possible because relaxation rate is greatly enhanced during shortening (as elegantly shown in crab muscle by Josephson and Stokes, 1989). This can be seen graphically in Fig. 2 (similar to Fig. 8 in Josephson and Stokes, 1989; see also Fig. 3 in Altringham and Johnston, 1990*b*) by comparing the force records of a muscle receiving an identical stimulation pattern (short tetani of 40 ms at 7.5 Hz at 20°C), but in one case being held isometric (Fig. 2A) and in the other case undergoing oscillatory length changes (Fig. 2B). In the isometric case, the force record resembles a partially fused tetanus, whereas when the muscle underwent oscillatory length changes it showed almost complete relaxation between tetani. Hence, oscillatory length changes seem to speed up the rate of force generation ($T_{a,10-90}$; Table 1) and the rate of muscle relaxation, making it possible for the muscle to operate effectively at higher oscillation frequencies.

Quantifying the faster relaxation rate is complicated by the fact that shortening causes a reduction in force associated with the force–velocity relationship, which is quite distinct from the reduction in force associated with muscle relaxation. This makes the 90 % tension start point during shortening difficult to interpret. The 10 % tension during shortening, however, occurs at a time when shortening has slowed to some extent and the muscle is closer to isometric. Therefore, the only relaxation parameter we report is $T_{r,last}$, because its start point is the last stimulus

rather than a force level. At the optimal oscillation frequencies for power output at both 10°C (2.5 Hz) and 20°C (5 Hz), the rate of relaxation is increased about fourfold compared to the respective isometric case (Table 1). As the muscle is driven through higher oscillation frequencies $T_{r,last}$ is further decreased; at 10 Hz at 20°C it was 42 ms, and at 5 Hz at 10°C it was 86 ms.

Work per cycle

During a series of oscillatory length changes, the work per cycle generally increased to a certain point, stayed constant, and then decreased. At slow oscillation frequencies (except 1 Hz), the maximum work was obtained after four or five cycles, but at higher frequencies, work per cycle increased up to about the tenth cycle. At 1 Hz, to avoid fatigue effects caused by long stimulation durations per cycle (approximately 500 ms), we reported the values for the second cycle at 20°C and the third cycle at 10°C.

At all frequencies, the negative work per cycle declined as the series proceeded (see Fig. 2), presumably because the muscle exhibited more complete relaxation. The positive work also dropped slightly. At high oscillation frequencies, this is probably due to more complete relaxation and thus lower force enhancement during subsequent stretching and shortening. At low frequencies, the positive work declined more dramatically, presumably as a result of fatigue. We measured work on the cycle that provided the highest value. At all frequencies, the net work generated by the passive forces under oscillating length changes (i.e. the first loop) was quite *small* (i.e. less than 5 % of the total net work).

Conditions for optimum work per cycle at a given oscillatory frequency

This study differed from many others in that stimulation durations were quite long. At 20°C, at oscillation frequencies of 5 Hz and below, the stimulation duty cycle for peak work was approximately 0.5 (Figs 3, 4A). Slightly more work could be achieved with duty cycles somewhat greater than 0.5 at very low oscillation frequencies (but this was not explored rigorously). However, at oscillation frequencies greater than 5 Hz, the optimal stimulus duty cycle was less than 0.5 and decreased with increasing oscillation frequency. At 10°C, the optimal duty cycle was approximately 0.5 only at 1 Hz oscillation frequency. As oscillation frequency increased, the optimal stimulation duty cycle decreased dramatically.

With increasing oscillation frequency, the stimulus phase for optimal work preceded the shortening portion of the length change by a greater percentage of the cycle (from −50° at 1 Hz to −100° at 12.5 Hz). This stimulus phase was similar at 10°C and 20°C (Figs 3, 4B).

We found that optimal strain decreased with increasing oscillation frequency, but over most of the range it was 5–6 % (Figs 3, 4C). In addition, optimal strain at a given oscillation frequency was higher at 20°C than at 10°C, but this difference was only statistically significant at 2.5 and 3.5 Hz.

Positive work per cycle decreased monotonically with increasing oscillation frequency (Fig. 5B). At very low frequencies (1 Hz), some muscle bundles at 20°C

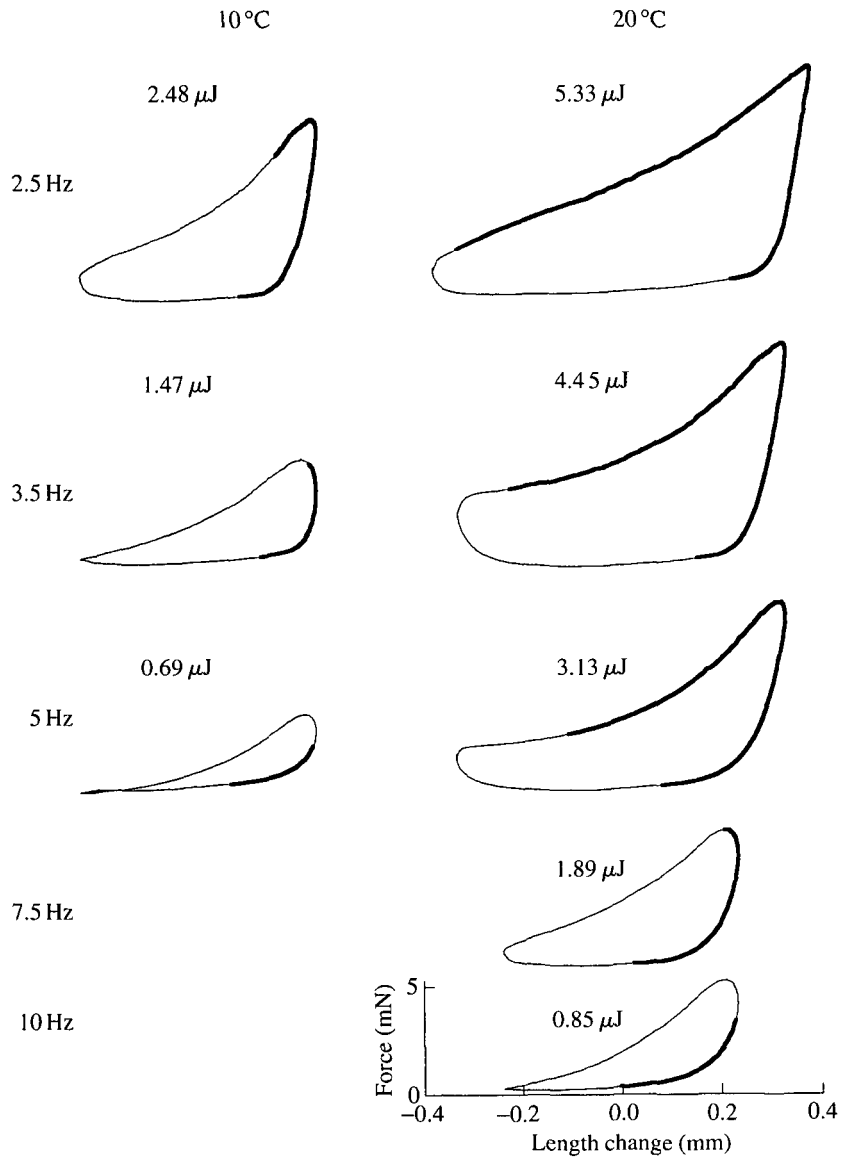

Fig. 3. Optimal work loops as a function of oscillation frequency at 10°C and 20°C. The timing of the stimulus (150 pulses s^{-1} at 10°C and 300 pulses s^{-1} at 20°C) for each work loop is denoted by the thicker section of the work loop trace. As can be seen at 20°C, as oscillation frequency increases, stimulation duty cycle decreases, strain decreases and the stimulus phase is shifted towards earlier times. The bundle length was 0.38 cm, muscle volume was 0.0023 cm^3 and tetanic force was 16 mN during the 10°C measurement and 15.1 mN during the 20°C experiments.

showed fatigue and the work per cycle was somewhat lower than at 2.5 Hz. The decrease in net work per cycle with increasing oscillation frequency is due almost exclusively to a decrease in positive work per cycle, as negative work per cycle is

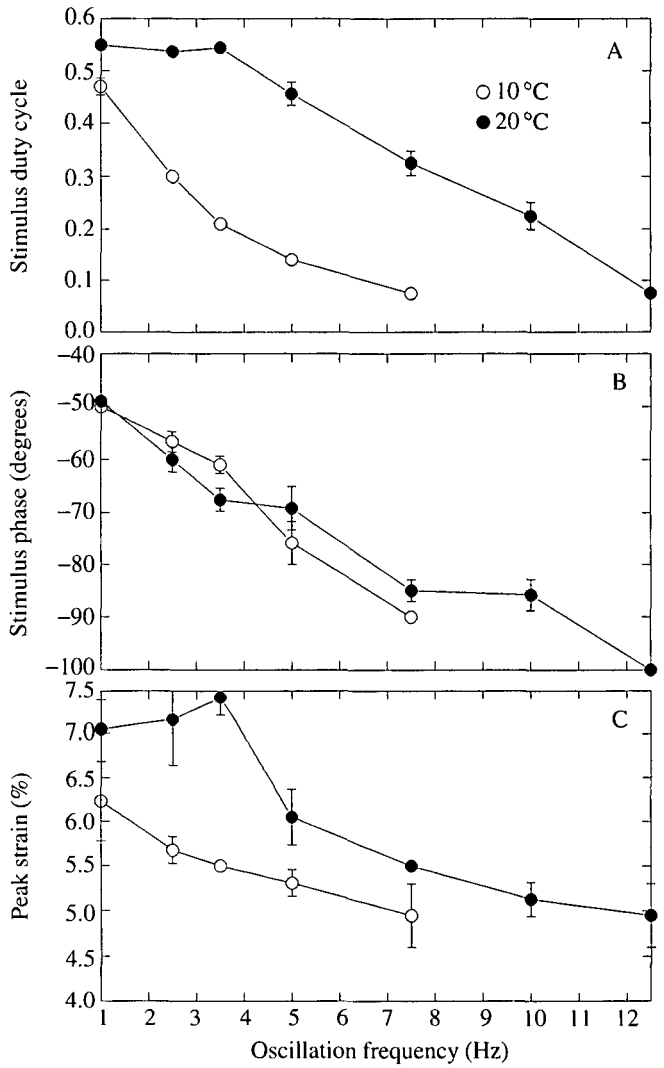

Fig. 4. Stimulus duty cycle, stimulus phase and peak strain for optimal power production as a function of oscillation frequency at 10°C and 20°C. Each point is the mean (N=6) with standard error bars. At the highest frequency used at both temperatures (7.5 Hz at 10°C and 12.5 Hz at 20°C), data points were obtained from only two bundles. Thus, to minimize effects of variation in absolute power between different bundles, values are normalized.

relatively independent of oscillation frequency (Fig. 5A). The net work per cycle was considerably higher at 20°C than at 10°C except at 1 Hz (Fig. 5B). The higher net work per cycle at 20°C is due almost exclusively to higher positive work per cycle, rather than to lower negative work per cycle. The positive work per cycle decreased far more rapidly with increasing oscillation frequency at 10°C than at 20°C (Fig. 5A).

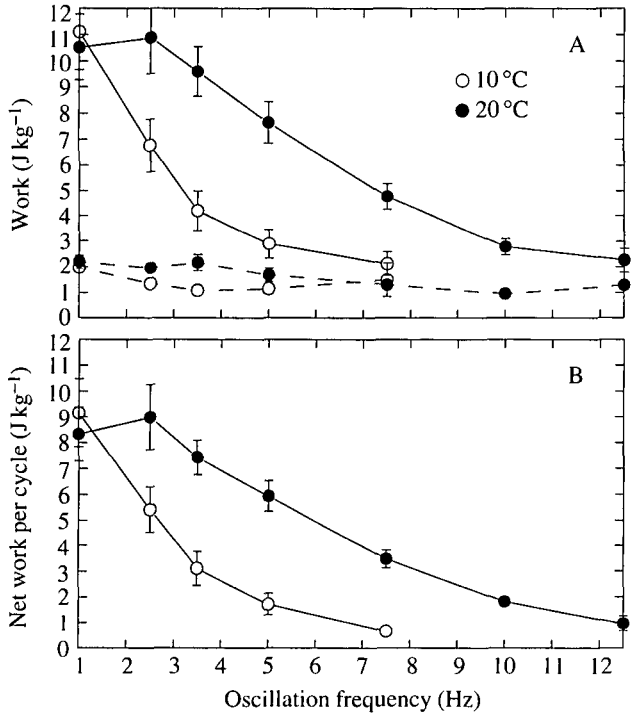

Fig. 5. Positive and negative work (A) and net work per cycle (B), as a function of oscillation frequency at 10°C and 20°C. Positive work and negative work are shown in A, with the negative work per cycle values being connected by dashed lines. The data come from the same bundles as in Fig. 4 and are presented in the same fashion.

Net power output (net work per cycle×oscillation frequency) increased to a maximum level and then decreased as oscillation frequency increased (Fig. 6A). Net power output was maximal at 2.5 Hz at 10°C and 5 Hz at 20°C. At 20°C, net power output as a function of oscillation frequency produced a broader curve, which extended to higher frequencies than at 10°C.

Maximum power output at 20°C was 27.9 W kg^{-1} and at 10°C was 12.8 W kg^{-1}. The Q_{10} for maximum power was 2.29. The data at 10°C were somewhat more variable than at 20°C. The Q_{10} for power output *at a given oscillation frequency* increased with increasing oscillation frequency from a value of about 1 at 1 Hz to about 5 at 7.5 Hz (Fig. 6B).

Stimulation duration during swimming

To understand fully how muscle is designed, it is necessary to reproduce *in vitro* the exact length changes and stimulation patterns that the muscle undergoes *in vivo*. We have previously determined the length changes and oscillation frequencies over which scup muscle operates as a function of swimming speed (Rome *et al.* 1992*a*). In this paper we additionally determined the duration for which the muscles were stimulated. The stimulation duty cycle (EMG duty cycle) was

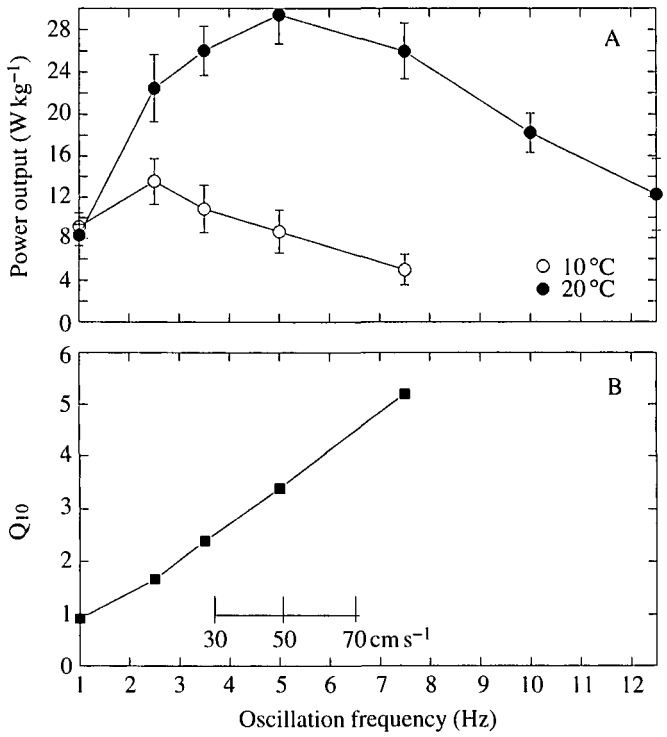

Fig. 6. Net power output (A) and Q_{10} (B) for power output as a function of oscillation frequency at 10°C and 20°C. The data come from the same bundles as in Figs 4 and 5 and are presented in the same manner. Note that the appropriate swimming speed (from Rome *et al.* 1992*a*) for each oscillation frequency over which the scup use their red muscle is shown.

measured in the anterior region of four scup. Data from the one that had the best records over the largest range of swimming speeds are plotted as a function of the measured tailbeat frequency (equivalent to oscillation frequency) in Fig. 7. Over most of the range of tailbeat frequencies (swimming speeds), the duty cycle is slightly less than 0.5 (i.e. 0.43–0.5), but there is a tendency for EMG duty cycle to decrease as swimming speed (or oscillation frequency) increases. There is, however, no difference in EMG duty cycle between 10°C and 20°C. Data from the other three scup are consistent with that shown in Fig. 7.

Reproducing in vivo *oscillation frequency, length changes and stimulation duration in isolated muscle*

Although we did not measure the phase of the stimulus with length change during swimming, it was of interest to measure power output when the muscle was forced to work under the values we had measured for the other *in vivo* parameters. Hence, we set the oscillation frequency, stimulation duty cycle and strain to the *in vivo* values and varied phase to achieve optimal power output. Fig. 7 shows the situation regarding stimulation duty cycle at the two temperatures. At the tailbeat

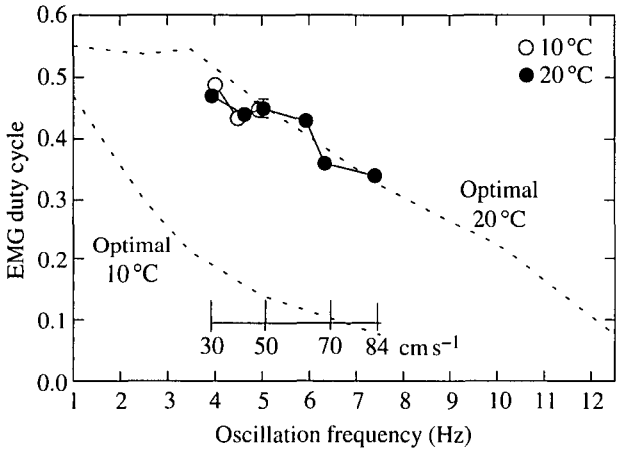

Fig. 7. EMG duty cycle as a function of tailbeat frequency (swimming speed) at 10°C and 20°C. Each data point represents the mean with standard error bars for 17–65 EMG bursts from one scup that had the best records. Note that there is no difference in duty cycle at 10°C and 20°C but that, as swimming speed increases EMG duty cycle declines. Note also that the swimming speed scale differs slightly from that in Fig. 6, as this scale was calculated for this particular fish. For comparitive purposes, the stimulation duty cycles that we found produced optimal power output in the isolated muscle experiments (from Fig. 4A) are shown with dashed lines.

frequencies used by scup, the EMG duty cycle at 20°C fell almost exactly on the stimulation duty cycle for optimal power output measured in the isolated muscle experiments. Thus, we anticipated that muscle run under *in vivo* conditions would produce nearly maximal power (Fig. 8). At 20°C we tested this in three muscle bundles at oscillation frequencies ranging from 3.93 Hz (equivalent to 30 cm s^{-1}) to 6.71 Hz (equivalent to 70 cm s^{-1}). At 3.93 Hz, the power produced with a 122 ms stimulus was equal to the maximum power generated by the muscle (i.e. the ratio was 1.00±0.01, ±S.E., N=3); however, the strain used (±8.4 %) was larger than that found *in vivo*. At a strain (±6.3 %) more like that used *in vivo*, the power was only slightly less than the maximum power we measured in the muscle (i.e. the ratio equalled 0.98±0.02; ±S.E., N=3). In the two bundles that we ran at 6.7 Hz, the average optimal strain was ±6.5 % (the same as that *in vivo*) and the power was about 0.87±0.05 (±S.E., N=2) of the maximum value, which is close to the expected value extrapolated from Fig. 6. Thus, when constrained to work under the oscillation frequency, strain and stimulation duration that occur *in vivo*, the muscle appears to be able to generate nearly maximal power, suggesting that the red muscle is designed to power swimming at these speeds and temperature.

At 10°C, however, the stimulus duty cycle for maximal power generation falls well below the EMG duty cycle during swimming. Thus, we anticipated that, at this temperature, the power output of muscle run under *in vivo* conditions might be low, because it would not have sufficient time to relax. We examined oscillation frequencies of 4 Hz and 4.93 Hz, corresponding to swimming speeds of 30 and

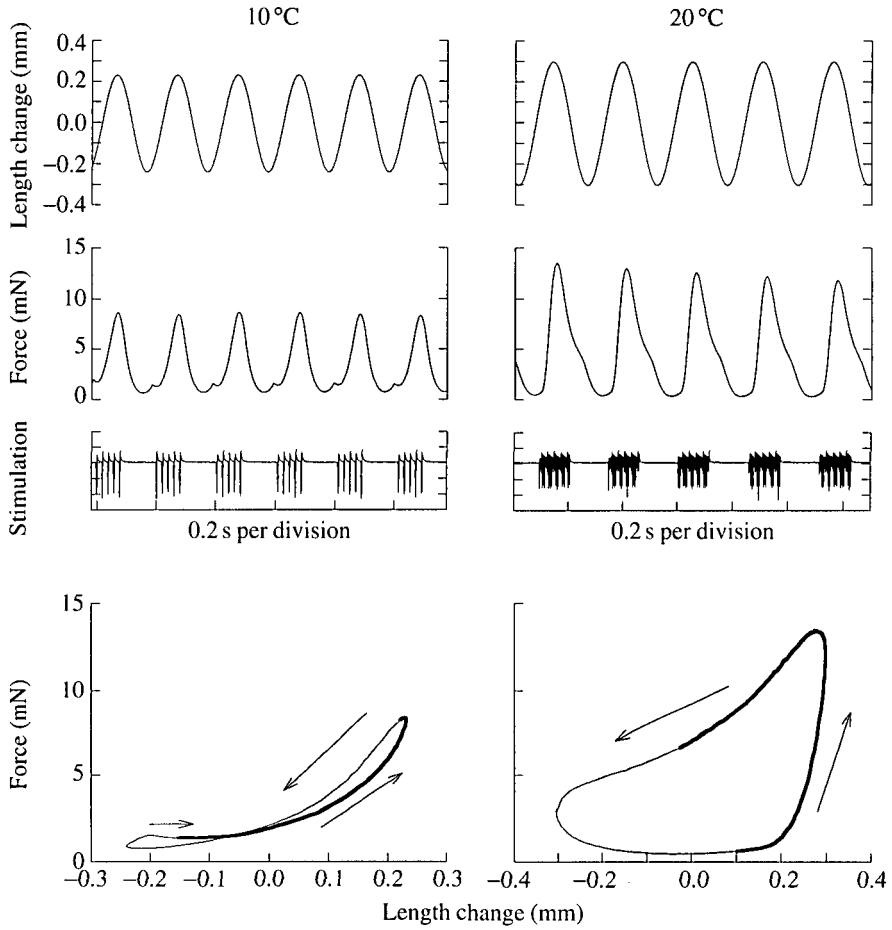

Fig. 8. Muscle bundles stimulated under *in vivo* conditions at 10°C and 20°C. The length, force and stimulation records, as well as the corresponding work loops, are shown for one bundle run under *in vivo* conditions at 10°C and another bundle run under *in vivo* conditions at 20°C. The length change, oscillation frequency and stimulation duration were set at each temperature, and phase was varied to obtain optimal power output. At 10°C, a 4.93 Hz oscillation frequency and 50 pulses s^{-1} stimulus were used. At 20°C, a 3.93 Hz oscillation frequency and 250 pulses s^{-1} were used. At 20°C, there is a nicely formed work loop, whose net work is 3.93 μJ, whereas at 10°C, the work loop curves back across itself and the net work is only 0.17 μJ. Note that to try to reduce negative work due to slow relaxation, the stimulus at 10°C must be shifted back to the beginning of the lengthening phase and it ends just as shortening starts. The length, volume and isometric force of the bundle used at 10°C are 0.38 cm, 4.3×10^{-4} cm^3 and 15.4 mN, respectively. The corresponding values for the bundle used at 20°C are 0.44 cm, 6.6×10^{-4} cm^3 and 21.7 mN.

50 cm s^{-1} in four bundles. Although the response of the bundles was much more varied than at 20°C, in general the bundles generated less than 20% maximal power (see Fig. 8). Bundles that showed rapid relaxation during isometric

contractions, tended to generate higher powers than those exhibiting slower relaxation rates.

Discussion

Power production during oscillatory contractions

In this study, we found that scup red muscle could generate 27.9 W kg^{-1} at 20 °C and 12.8 W kg^{-1} at 10 °C. Comparing these values to other published values is difficult because of the different temperature ranges used. Our values are higher than those for sculpin (5–8 W kg^{-1}; Altringham and Johnston, 1990*a*), the only other fish for which red muscle values have been reported. The sculpin experiments, however, were performed at a lower temperature (3 °C) and were not corrected for the non-muscular component of the bundles (if this component is similar to that in scup, this correction would approximately double the value). Our 20 °C value is only slightly lower than fast white muscle values of 30 W kg^{-1} at 15 °C in summer-acclimated sculpin (Johnson and Johnston, 1991), and is similar to that for moth flight muscle at 20 °C (Stevenson and Josephson, 1990).

The values for maximum power output during oscillatory length change are much less than those of 134 W kg^{-1} and 71 W kg^{-1} at 20 °C and 10 °C, respectively, found by multiplying force and velocity during force-clamp experiments (near steady-state contractions) on the same preparation (Rome *et al.* 1992*b*). There are several factors that contribute to this difference, some of which have been described elsewhere (Josephson and Stokes, 1989; Woledge, 1992). (1) During oscillatory contractions, the muscle shortens for only half of the cycle. (2) Sinusoidal length changes do not permit the muscle to shorten at the appropriate V/V_{max} for maximum power during the entire length change. (3) In force-clamp experiments, velocity was measured only over about a 20 ms period, which is too brief for shortening deactivation to be significant and thus does not lower power output as it does in work loops. (4) Negative work is performed, which represents 20–25 % of the positive work per cycle at the optimal oscillation frequencies. (5) To avoid even larger negative work, stimulation of the muscle is ended well before the end of shortening (see Fig. 3), resulting in incomplete activation during the later part of shortening. As the decrease in power during oscillatory contractions is greater than can be accounted for by items 1 and 2 (see Josephson, 1989), reduced muscle activation and slow muscle relaxation must limit, to some extent, net power production at both temperatures.

The influence of temperature on power production

Fig. 6B shows the Q_{10} for net power production. The Q_{10} of 2.29 for maximum power generation was much greater than the 1.2-fold increase in power output of sculpin white muscle reported by Johnson and Johnston (1991), as temperature was raised from 4 °C to 15 °C, but agrees well with the Q_{10} found for moth flight muscle between 20 °C and 30 °C (Stevenson and Josephson, 1990).

It is of considerable interest to determine the factors that limit power production

at a given temperature and the factors that influence the Q_{10} of power production. During near steady-state shortening contractions in scup red bundles, the Q_{10} for power production (1.88) is approximately the product of the Q_{10} for V_{max} (1.69) and the Q_{10} for isometric force (1.08; Rome *et al.* 1992*b*). Although the Q_{10} for power production in steady-state contractions is easy to explain, it is more complex to understand it for oscillatory contractions. Not only do the temperature dependences of V_{max} and isometric force enter in, but the temperature dependences of the kinetics of force generation, the kinetics of muscle relaxation and shortening deactivation may also be important.

Insight into how these components affect the Q_{10} of power output can be attained by making a simplifying assumption. If we assume that the processes of activation, relaxation and shortening deactivation act only by altering the number of cycling crossbridges per half-sarcomere, then the power output of the muscle, is described by:

$$\text{Power} = \text{power per cycling crossbridge} \times \text{percentage of crossbridges which are cycling}. \quad (1)$$

The first term on the right-hand side, power per cycling crossbridge, is a function of V/V_{max} and V_{max}, whereas the second term, the proportion of the crossbridges involved in cycling (as in Sweeney and Stull, 1990), is a function of both myofilament overlap (i.e. where on the SL–tension curve the muscle is operating) and of activation and relaxation processes.

Owing to the complexity of activation–relaxation processes, it is not possible to model mechanistically or to measure the percentage of crossbridges which are cycling. However, it is possible to assess how power per cycling crossbridge might be affected by temperature. Thus, we should be able to determine whether this term limits performance at a given temperature and how it affects Q_{10} of power production.

Using the approach of Josephson (1989), we iteratively determined the work performed in the shortening portion of a work loop by assuming that the muscle was completely active (i.e. all crossbridges are actively cycling) during the entire shortening period and that the force the muscle generated is described by the force–velocity curves determined previously (Rome *et al.* 1992*b*). This approach shows that, as oscillation frequency is increased above the optimal value at each temperature, the observed decrease in power is *not* caused by V/V_{max} becoming too high for maximum power generation. For instance, the calculated positive power from work loops (using the strains as in Fig. 4A), does not begin to fall until oscillation frequencies exceed 7.5 Hz at 10 °C and between 10 and 12.5 Hz at 20 °C, even though the measured net power and measured positive power reach a maximum at much lower frequencies (2.5 Hz at 10 °C, 5 Hz at 20 °C). Thus, this decrease in power must involve activation, relaxation and shortening deactivation processes.

It would be incorrect, however, to conclude that the effect of temperature on

the power per cycling crossbridge does not contribute to the effect of temperature on power output during oscillatory contractions. If equation 1 is correct, the Q_{10} for positive power during oscillatory contractions would be the product of the Q_{10} of the power per cycling crossbridge and the Q_{10} of the number of active cycling crossbridges.

Thus, the iterative analysis shows that, at 5 Hz, active cycling crossbridges at 20°C generate about 1.8-fold greater power than at 10°C. The observation that during oscillatory contractions the muscle at 20°C generates about 2.67-fold greater positive power (see Fig. 5A) than at 10°C suggests that the Q_{10} for the number of actively cycling crossbridges would be 1.45 (from equation 1). Unfortunately, with our present knowledge, it is not possible to model or measure the number of actively cycling crossbridges as a function of time during the work loop cycle (Marsh, 1990; Rome, 1992) in order to test this calculation.

A higher number of cycling crossbridges during the shortening portion of the oscillatory length change would require that, at high temperatures, the muscle should relax more rapidly, so that most of the crossbridges have time to detach prior to the lengthening portion of the oscillatory length change. The Q_{10} of 2.75 for $T_{r,95-80}$ (shown to represent the period in which myoplasmic $[Ca^{2+}]$ falls exponentially; Cannell, 1986) suggests that one of the important processes of relaxation (the rate of Ca^{2+} pumping) is faster in warmer muscle.

It should be finally emphasized that the simplification on which equation 1 is based (i.e. that the processes of activation, relaxation and shortening deactivation affect only the number of actively cycling crossbridges) may not be completely true. To understand more fully the design of the muscle, further work in integrating various muscle properties (as in Malamud and Josephson, 1991) must be performed.

Influence of muscle power on swimming performance

The increased Q_{10} for power at higher oscillation frequencies clarifies a discrepancy we have previously observed. We found that, at 20°C, scup could swim 1.5-fold faster with their red muscle than at 10°C ($80\,cm\,s^{-1}$ *vs* $54\,cm\,s^{-1}$; Rome *et al.* 1992*a*). This should require about 2.7-fold greater mechanical power at 20°C than at 10°C (i.e. power for swimming increases with the 2.5 exponent of swimming speed and this is nearly independent of temperature; Webb, 1975, 1978), whereas maximum mechanical power output during steady-state muscle shortening increases only 1.88-fold (Rome *et al.* 1992*b*). Fig. 6B shows, however, that the Q_{10} for power production during oscillatory contractions is a function of frequency. Comparing the power outputs of muscles at 4.5 Hz at 10°C and 7.5 Hz at 20°C (i.e. the highest used during swimming) gives approximate values of $9\,W\,kg^{-1}$ at 10°C and $27\,W\,kg^{-1}$ at 20°C, representing a Q_{10} of 3. This Q_{10} is quite close to what is thought to be needed to power swimming at the two temperatures.

Comparing power output during oscillatory length changes at a given frequency gives further insight into muscle recruitment at the two temperatures than can be obtained from steady-state mechanics. Fish must generate nearly the same power

while swimming at a given speed at 10°C and 20°C, because drag is largely independent of water temperature (Webb, 1975). Rome *et al.* (1984, 1985) and Rome (1990) have suggested that, at low temperatures, fish recruit more muscle fibres by compressing their recruitment order to make up for the reduction in power at low temperatures. Rome *et al.* (1992*a*) have found some electromyographic evidence in scup that supports this hypothesis. Based on the V measured during swimming and the steady-state force–velocity characteristics of muscle, it was predicted that, at 10°C, scup must recruit about 1.8-fold more muscle fibres than at 20°C (Rome *et al.* 1992*a*). However, because scup use the same tailbeat frequency at a given swimming speed at 10°C and 20°C, at the fastest frequency that scup use at 10°C (4.5 Hz), the Q_{10} for power production during oscillatory length changes is about three times higher at 20°C than at 10°C. This suggests that, at 10°C, the fish must recruit three times as many fibres to produce the same swimming movements.

Power output at in vivo *strains, oscillation frequencies and stimulation durations*

Further insight into the design of muscle can be gained by comparing how the muscle is used during locomotion with its isolated properties and by reproducing the *in vivo* length changes and stimulation pattern on the isolated muscle. In scup swimming at 20°C, the red muscle was used at between about 3.7 and 7.5 Hz, which encompasses the peak of the power output graph but represents only a limited range of the oscillation frequencies over which the muscle was made to work (Fig. 6). In addition, the strain used during locomotion (±6 %) was optimal for isolated muscle (although, in the anterior part of the fish, the length change is likely to be smaller; Rome *et al.* 1992*a*). Finally, the EMG duty cycle overlapped the optimal stimulation duty cycle for power output in the isolated muscle (Fig. 7). Thus, it is no surprise to find that, at 20°C, when the muscle is driven under *in vivo* conditions of stimulation duty cycle, strain and oscillation frequency, it generates nearly maximal mechanical power. This suggests that the muscle is working optimally during locomotion. Conversely, this also suggests that the muscle is designed, both in terms of crossbridge kinetics and in terms of the kinetics of activation and relaxation, to produce the maximum mechanical power at the frequencies and strains that the animals need to use during swimming.

At 10°C, however, the apparent agreement between how the muscle is used during swimming and its isolated properties was not found. Maximum power was generated at 2.5 Hz, yet we never observed fish swimming with such a slow tailbeat frequency. This points to a potentially serious problem in extrapolating from isolated muscle work loops to the whole animal without knowledge of the animal's locomotory performance. Without this knowledge, one might conclude that a change in maximum power at a particular oscillation frequency represents an important adaptation, yet this may be of no functional importance if the animals never use their muscle under those conditions. Further, the Q_{10} of 2.29 for power output obtained by comparing the maximum power at 10°C (found at 2.5 Hz) to

the maximum power at 20°C (found at 5 Hz) may have little bearing on locomotory performance, because the muscle is never used at 2.5 Hz at 10°C.

The observation that at 10°C the muscle does not function optimally might be because the muscle we studied is designed to work optimally during swimming at 20°C (normal environmental temperature in summer) and that 10°C is an unusual temperature for summer fish. Scup do appear to be sensitive to low temperatures because in the autumn, as water temperature drops, they migrate off-shore to avoid low in-shore water temperatures. In the winter they are commonly found in water at 7°C or above (i.e. warmed by the Gulf Stream; Neville and Talbot, 1964), but this exposure to cold water comes only after they have had a considerable time to acclimate. We are still left, however, with the puzzle that the isolated muscle does not appear to generate very much power at 10°C under conditions where red muscle is *actually* powering swimming movements (Rome *et al.* 1992*a*).

There may be several factors that explain this apparent discrepancy. We can obtain some insights into this problem from our measurements. At 10°C, the response of the muscle seemed to be quite dependent on the stimulation frequency and small changes in temperature, although neither was systematically studied. In the bundles undergoing the *in vivo* stimulation duty cycle, slowing the stimulation rate to 25 or 50 pulses s^{-1} increased the power output. This leaves open the possibility that the muscle properties that we measured at 10°C might be very dependent on the stimulus parameters (e.g. stimulus rate) and that some of these stimulus parameters might be different during swimming than in the isolated muscle experiments.

In addition, in the one case tested, increasing the temperature of the bundle by 1°C (i.e. from 10 to 11°C) substantially increased power output during the *in vivo* stimulation regime. This demonstrates that the muscle is very sensitive to temperature around 10°C and that small acclimatory differences might cause large changes in muscle performance. Dramatic changes in muscle power output associated with thermal acclimation are known to occur (Johnson and Johnston, 1991). Although the fish for the swimming measurements and for the work loop experiments were obtained from the same water temperature, we cannot exclude the possibility of thermal acclimation differences because the work loop measurements were made 2 years after the swimming ones (Rome *et al.* 1992*a*).

Finally, we measured EMGs in only one place in the fish (beneath the dorsal fin), and EMG duration may vary along the length of the fish (Van Leeuwen *et al.* 1990). It is also possible that the contractile properties of red muscle (e.g. relaxation rate and power output) may vary along the length of the fish. To understand muscle function during locomotion more fully, we must measure the EMG duration and phase at several places along the fish and then reproduce on muscle from the *same* fish the *in vivo* length changes and stimulation pattern.

Design constraints of muscle

Rome *et al.* (1988, 1990), Rome and Sosnicki (1991), Rome (1992) and Sosnicki *et al.* (1991) have argued that myofilament overlap and V/V_{max} are important

design constraints of muscle. This was based on comparison of SL changes during swimming with steady-state measurements of muscle contraction (i.e. force–velocity curve). The data presented here show that the processes of activation, relaxation and shortening deactivation are also important design considerations and probably affect power production during locomotion. It would be incorrect, however, to say that activation and relaxation supplant the first two design constraints. As equation 1 argues, all three terms simultaneously influence the power produced by the muscle. Unlike the first two design considerations, however, we do not know what the design constraint is for activation and relaxation processes, nor can we provide a convincing argument of what it should be. The only way to determine this is to reproduce *in vitro* the muscle length changes and stimulation pattern that occur *in vivo*, and to observe how evolution has designed the muscular system.

We thank Dr Tim Johnson and Dr Bob Josephson for providing some useful advice about interpreting work loops from fish muscle and Dr Choi for helping analyze the EMGs. We also thank Cathy Kelly for help preparing the manuscript, and Gordon Lutz for providing useful comments on the manuscript. This work was supported by NIH AR38404.

References

ALTRINGHAM, J. D. AND JOHNSTON, I. A. (1990*a*). Modelling muscle power output in a swimming fish. *J. exp. Biol.* **148**, 395–402.

ALTRINGHAM, J. D. AND JOHNSTON, I. A. (1990*b*). Scaling effects on muscle function: power output of isolated fish muscle fibres performing oscillatory work. *J. exp. Biol.* **151**, 453–467.

CANNELL, M. B. (1986). Effect of tetanus duration on the free calcium during the relaxation of frog skeletal muscle fibres. *J. Physiol., Lond.* **376**, 203–218.

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

JOSEPHSON, R. K. (1985). Mechanical power output from striated muscle during cyclic contraction. *J. exp. Biol.* **114**, 493–512.

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

JOSEPHSON, R. K. AND STOKES, D. R. (1989). Strain, muscle length and work output in a crab muscle. *J. exp. Biol.* **145**, 45–61.

MALAMUD, J. G. AND JOSEPHSON, R. K. (1991). Force–velocity relationships of a locust flight muscle at different times during a twitch contraction. *J. exp. Biol.* **159**, 65–87.

MARSH, R. L. (1990). Deactivation rate and shortening velocity as determinants of contractile frequency. *Am. J. Physiol.* **259**, R223–R230.

MARSH, R. L. AND BENNETT, A. F. (1986). Thermal dependence of sprint performance of the lizard *Sceloporus occidentalis*. *J. exp. Biol.* **126**, 79–87.

NEVILLE, W. C. AND TALBOT, G. B. (1964). The fishery for scup with special reference to fluctuations in yield and their causes. *U.S. Dept Int. U.S. Fish Wildl. Serv., Spec. Sci. Rep. Fish.* **459**, 61pp.

ROME, L. C. (1990). The influence of temperature on muscle recruitment and function *in vivo*. *Am. J. Physiol.* **259**, R210–R222.

ROME, L. C. (1992). The mechanical design of the muscular system. In *Comparative Vertebrate Exercise Physiology* (ed. J. H. Jones), Orlando: Academic Press (in press).

ROME, L. C., CHOI, I., LUTZ, G. AND SOSNICKI, A. A. (1992*a*). The influence of temperature on

muscle function in fast swimming scup. I. Shortening velocity and muscle recruitment during swimming. *J. exp. Biol.* **163**, 259–274.

ROME, L. C., FUNKE, R. P. AND ALEXANDER, R. M. (1990). The influence of temperature on muscle velocity and sustained performance in swimming carp. *J. exp. Biol.* **154**, 163–178.

ROME, L. C., FUNKE, R. P., ALEXANDER, R. M., LUTZ, G., ALDRIDGE, H. D. J. N., SCOTT, F. AND FREADMAN, M. (1988). Why animals have different muscle fibre types. *Nature* **355**, 824–827.

ROME, L. C., LOUGHNA, P. T. AND GOLDSPINK, G. (1984). Muscle fiber recruitment as a function of swim speed and muscle temperature in carp. *Am. J. Physiol.* **247**, r272–r279.

ROME, L. C., LOUGHNA, P. T. AND GOLDSPINK, G. (1985). Temperature acclimation improves sustained swimming performance at low temperatures in carp. *Science* **228**, 194–196.

ROME, L. C. AND SOSNICKI, A. A. (1990). The influence of temperature on mechanics of red muscle in carp. *J. Physiol., Lond.* **427**, 151–169.

ROME, L.C. AND SOSNICKI, A.A. (1991).Myofilament overlap in swimming carp. II. Sarcomere length changes during swimming. *Am. J. Physiol.* **260**, C289–C296.

ROME, L. C., SOSNICKI, A. A. AND CHOI, I. (1992*b*). The influence of temperature on muscle function in the fast swimming scup. II. The mechanics of red muscle. *J. exp. Biol.* **163**, 281–295.

SOSNICKI, A. A., LOESSER, K. AND ROME, L. C. (1991). Myofilament overlap in swimming carp. I. Myofilament lengths of red and white muscle. *Am. J. Physiol.* **260**, C283–C288.

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

SWEENEY, H. L. AND STULL, J. T. (1990). Alteration of cross-bridge kinetics by myosin light chain phosphorylation in rabbit skeletal muscle: implications for regulation of actin–myosin interaction. *Proc. natn. Acad. Sci. U.S.A.* **87**, 414–418.

VAN LEEUWEN, J. L., LANKHEET, M. J. M., AKSTER, H. A. AND OSSE, J. W. M. (1990). Function of red axial muscle of carp (*Cyprinus carpio* L.): recruitment and normalised power output during swimming in different modes. *J. Zool., Lond.* **220**, 123–145.

WEBB, P. W. (1975). Hydrodynamics and energetics of fish propulsion. *Bull. Fish. Res. Bd. Can.* **190**, 1–159.

WEBB, P. W. (1978). Hydrodynamics: non-scombroid fish. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar and D. J. Randall), pp. 189–237. New York: Academic Press.

WOLEDGE, R. C. (1992). Relaxation as a determinant of the locomotory role of muscle. Abstract at the Annual Meeting of S.E.B. (in press).