

POWER OUTPUT OF FISH MUSCLE FIBRES PERFORMING OSCILLATORY WORK: EFFECTS OF ACUTE AND SEASONAL TEMPERATURE CHANGE

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Summary

Fast muscle fibres were isolated from the abdominal myotomes of the short-horned sculpin *Myoxocephalus scorpius* L. Sinusoidal length changes were imposed about resting muscle length and fibres were stimulated at a selected phase during the strain cycle. The work output per cycle was calculated from the area of the resulting force–position loops. The strain amplitude required for maximum work per cycle had a distinct optimum at $\pm 5\%$ of resting length, which was independent of temperature. Maximum positive work loops were obtained by retarding the stimulus relative to the start of the length-change cycle by 30° (full cycle = 360°). The maximum negative work output was obtained with a 210° stimulus phase shift. At intermediate stimulus phase shifts, work loops became complex with both positive (anticlockwise) and negative (clockwise) components. The number and timing of stimuli were adjusted, at constant strain amplitude ($\pm 5\%$ of resting muscle length), to optimize net positive work output over a range of cycle frequencies. The cycle frequency required for maximum power output (work per cycle times cycle frequency) increased from around 5–7 Hz at 4°C to 9–13 Hz at 15°C . The maximum tension generated per cycle at 15°C was around two times higher at all cycle frequencies in summer- relative to winter-acclimatized fish. Fast muscle fibres from summer fish produced consistently higher tensions at 4°C , but the differences were only significant at 15 Hz. Acclimatization also modified the relationship between peak length and peak force at 4°C and 15°C . The maximum power output of muscle fibres showed little seasonal variation at 4°C and was in the range $20\text{--}25\text{ W kg}^{-1}$. In contrast, at 15°C , maximum muscle power output increased from 9 W kg^{-1} in the winter- to 30 W kg^{-1} in the summer-acclimatized fish.

Introduction

The muscle fibres in fish myotomes undergo cyclical contractions of increasing frequency as swimming speed increases. Slow muscle fibres power low-speed swimming whereas fast muscle fibres are recruited for acceleration and at high cruising speeds (Johnston *et al.* 1977; Bone *et al.* 1978). During the initial few tail-

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beats of 'fast starts' sarcomere length changes are irregular and of large amplitude (van Leeuwen *et al.* 1990). However, kinematic analyses have shown that for steady swimming the strain fluctuations of muscle fibres are essentially sinusoidal (Hess and Videler, 1984; van Leeuwen *et al.* 1990). Altringham and Johnston (1990*a,b*) measured the power output of isolated fish muscle fibres subject to sinusoidal strain fluctuations designed to simulate their activity during swimming. The approach adopted was first used to measure the power output of asynchronous insect flight muscles by Machin and Pringle (1959) and later adapted for synchronous insect muscle by Josephson (1985). Briefly, isolated muscle fibres are subjected to sinusoidal length changes and phasically stimulated during the strain cycle whilst simultaneously recording fibre length and force. The area of the force–position loop produced during each cycle corresponds to the work done during shortening minus the work required to re-lengthen the muscle. At each cycle frequency the strain amplitude and the number and timing (phase) of electrical stimuli can be adjusted to maximize net work output. Altringham and Johnston (1990*a*) found that, in the marine teleost *Myoxocephalus scorpius* L., maximum mechanical power output (work per cycle times cycle frequency) was produced at a cycle frequency of 2 Hz for slow ($5\text{--}8\text{ W kg}^{-1}$) and 5–7 Hz for fast ($25\text{--}35\text{ W kg}^{-1}$) muscle fibres (at 5°C). Curtin and Woledge (1989) have also reported measurements of the power output of dogfish fast muscle fibres at 12°C using cyclical ramp length changes.

Temperature is a major factor determining the performance of fish muscle. In the common carp (*Cyprinus carpio* L.), as water temperature decreases fast muscle fibres are recruited at lower cruising speeds to compensate for the reduced mechanical power output of the slow muscle (Rome *et al.* 1985; Heap and Goldspink, 1986). After several weeks at low temperature, however, both the speed at which fast muscle fibres are first recruited and the swimming performance increase (Rome *et al.* 1985; Heap and Goldspink, 1986). These improvements in swimming performance are associated with a major remodelling of the skeletal muscles (reviewed in Johnston and Dunn, 1987). For example, in carp isometric twitch duration decreases whereas maximum tension and isotonic shortening velocity are increased following cold acclimation (Fleming *et al.* 1990; Johnston *et al.* 1990). Adaptations in contractile properties with temperature acclimation are more pronounced for fast than for slow muscle fibre types (Langfeld *et al.* 1991). The molecular mechanisms underlying these adjustments in muscle contractile properties include changes in sarcoplasmic reticulum ATPase activity (Fleming *et al.* 1990) and alterations in myosin light chain (Crockford and Johnston, 1990; Langfeld *et al.* 1991) and myosin heavy chain composition (Gerlach *et al.* 1990).

In the present study we have used the work loop approach to investigate the effects of acute and seasonal temperature change on muscle power output. All experiments were carried out on fast muscle fibres isolated from the short-horned sculpin *Myoxocephalus scorpius*. Locally, this species experiences sea temperatures ranging from 3–5°C in winter to 12–16°C during the summer.

Materials and methods

Fish

Short-horned sculpin (*Myoxocephalus scorpius* L.), were caught in St Andrews Bay during the winter (January–February) and summer (July–August) of 1990. Fish were maintained in seawater aquaria for 2–21 days prior to experiments at ambient temperatures and photoperiods; 4–5°C (8 h light:16 h dark) in the winter and 12–13°C (16 h light:8 h dark) in the summer. To reduce variation resulting from scaling (Altringham and Johnston, 1990b), fish of a similar size were used. The standard lengths and masses of the fish studied were 270.4±52.5 g, 22.4±2.0 cm ($N=7$) for winter specimens and 257.4±39.4 g, 22.3±1.5 cm ($N=7$) for summer specimens (mean±s.d.). A small number of winter-acclimatized fish were also used to determine the effects of varying stimulus phase and strain amplitude on muscle power output (272.8±65 g, 22.4±2.1 cm, $N=4$, mean±s.d.).

Isolation of fibre bundles

Sculpin were killed by a blow to the head, followed by pithing and decapitation. Bundles of superficial fast muscle fibres were isolated from anterior abdominal myotomes (numbers 4–6 counting from the head) and maintained in Ringer of the following composition (in mmol l⁻¹): 132.3, NaCl; 10, sodium pyruvate; 2.6, KCl; 1.0, MgCl₂; 18.5, NaHCO₃; 3.2, NaH₂PO₄; 2.7, CaCl₂; pH 7.2–7.4, at 4°C (Hudson, 1969; Altringham and Johnston, 1988). Preparations consisted of 20–30 fibres and had a mean length of 8.3±1.1 mm (mean±s.d., $N=31$). Reproducible results could be obtained from preparations over a 2-day period provided they were stored in fresh Ringer at 4°C.

Apparatus and experimental protocol

Fibre bundles were attached *via* foil clips and steel hooks between a servo-motor and a silicon blade force transducer (AME, Horten, Norway). Preparations were perfused with aerated Ringer from a reservoir at controlled temperature (±0.1°C). Muscle fibre length was adjusted to give an optimal twitch, which corresponded to a sarcomere length of 2.3–2.4 µm as measured by laser diffraction.

Muscle fibres were subjected to sinusoidal length changes about *in situ* resting length and the timing of stimulation in relation to the strain cycle (phase shift) selected. Stimulation was supramaximal, administered *via* two platinum electrodes lying on either side of the preparation (12 V, 2 ms duration, 50 Hz at 4°C and 75 Hz at 15°C). The stimulation frequencies used were those required to give a maximal fused tetanus. By plotting force against muscle length a series of force–position loops were obtained for each cycle, the area of which represented the net work done (Josephson, 1985). Anticlockwise components indicate positive work and clockwise components indicate negative work. Power output, which is net work multiplied by cycle frequency, is a complex function of the cycle frequency, strain amplitude, number of stimuli and stimulus phase shift. Strain

amplitude was expressed as a percentage of resting muscle fibre length. Stimulus phase was defined as the delay between the start of stimulation and the start of the length change cycle expressed in degrees (full cycle=360°). Eight cycles of work were performed during an experimental run. Maximum force and work output were relatively constant over eight cycles (see Figs 1A and 6). Power output was calculated using the mean of eight cycles. The apparatus was controlled and the data collected and analysed using a microcomputer (Amstrad PC1640) and in-house software.

Determination of muscle fibre cross-sectional area

At the end of each experiment fibre bundles were pinned to strips of a silicone elastomer base (Sylgard 184, Dow Corning, USA) and frozen at their resting length in isopentane cooled to near its freezing point with liquid nitrogen (-159°C). Frozen sections, 13 µm thick, were cut, stained for myosin ATPase activity (Johnston *et al.* 1974) and the cross-sectional area of muscle fibres was determined by digitising fibre outlines using a microscope drawing arm and digital planimeter interfaced to a Hewlett-Packard 86B computer.

Statistics

Results from winter- and summer-acclimatized fish were compared at a given cycle frequency and temperature using a two-sided *t*-test performed on the means of the eight cycles per experimental run.

Results

Effects of varying strain amplitude

The cycle frequency, optimal strain amplitude, number of stimuli and stimulus phase required to maximize power output at 4°C and 15°C were determined in preliminary experiments. Whilst maintaining the other parameters required for maximum power output constant, the effects of varying strain amplitude were determined. Typical results from a winter-acclimatized fish are shown in Fig. 2. The optimal strain amplitude occurred at $\pm 5\%$ of resting muscle length and was independent of temperature. Changes in work output with variations in strain amplitude were a consequence of changes in the shape and position of work loops (Fig. 1). The area of work loops is reduced at strain amplitudes of less than $\pm 5\%$ of muscle length. Under these conditions net work is further reduced because fibres fail to relax completely between successive cycles, thereby increasing the work required to return the muscle to its resting length (Fig. 1). Work output is reduced for strain amplitudes of more than $\pm 5\%$ resting length since force generation is limited by the faster imposed relaxation rate (shortening-induced inactivation) (Fig. 1).

Effects of varying stimulus phase

The effects of varying stimulus phase shift were investigated for winter-

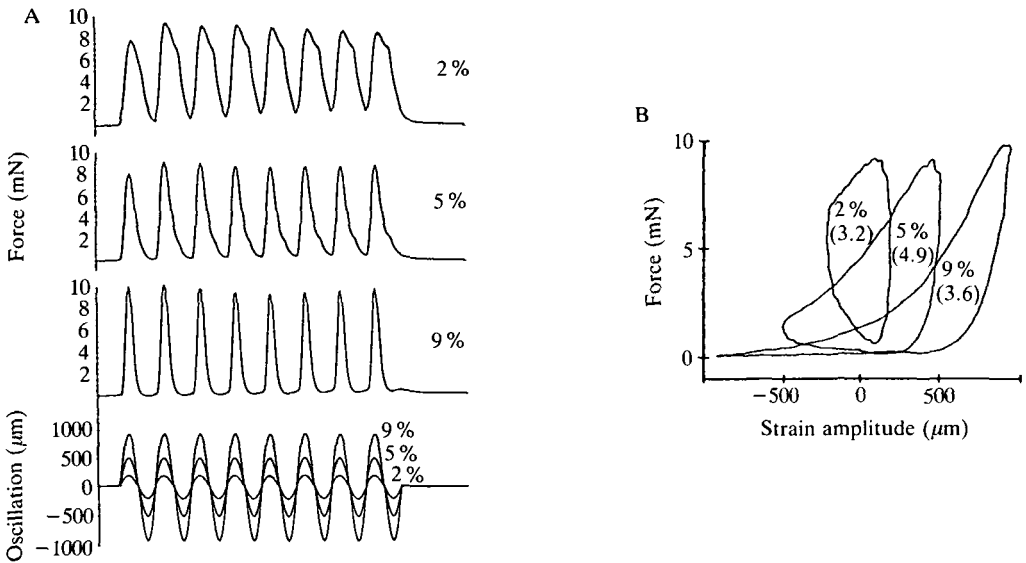


Fig. 1. (A) Force and length records of fibres from a winter-acclimatized fish performing oscillatory work at 4°C. Fibres were stimulated two times every cycle, with a stimulus phase shift of 25°, at a cycle frequency of 5 Hz. Muscle strain amplitude was ±2%, ±5% and ±9% of resting length. (B) Force has been plotted against fibre length for the fourth cycle of oscillatory work to produce the corresponding work loops. The value in brackets represents work done (J kg^{-1}).

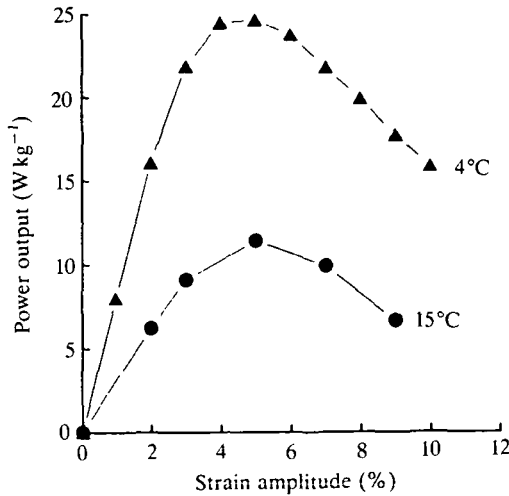


Fig. 2. The relationship between strain amplitude (as a percentage of fibre length) and power output at 4°C and 15°C for a single representative preparation from a winter-acclimatized fish. Stimulation parameters and cycle frequency were adjusted to maximize power output at each temperature: 4°C, 3 stimuli per cycle, 30° stimulus phase shift, 5 Hz cycle frequency; 15°C, 2 stimuli per cycle, 25° phase shift, 13 Hz cycle frequency.

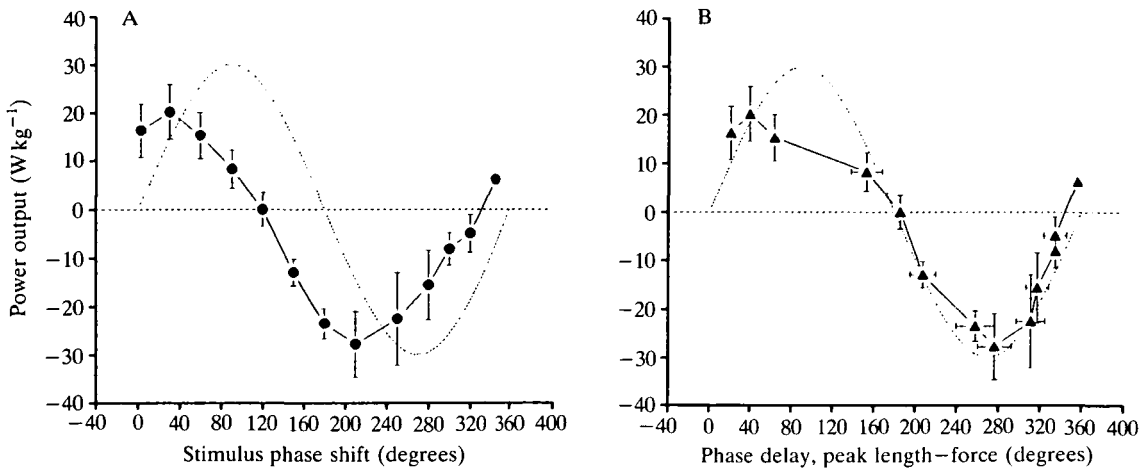


Fig. 3. The relationship between power output and stimulus phase shift (A), and the phase delay between peak length and peak force (B), for fibre bundles performing oscillatory work at 4°C, 5 Hz cycle frequency, $\pm 5\%$ strain amplitude and stimulated 3 times per cycle (dashed line, resting length; dotted line, muscle length). Data represent mean \pm s.e., obtained from four winter-acclimatized fish of mean mass, 273 ± 90.7 g and standard length 22.4 ± 2.1 cm (mean \pm s.d.).

acclimatized fish whilst maintaining the other parameters required to produce maximum power output constant. Maximum positive work loops were obtained over a narrow range of stimulus phase shift angles for which the fibre was given a small stretch prior to stimulation. At a phase shift of 30° net positive work was greater than at 5° or 60° , owing to optimum force enhancement by stretch (Figs 3 and 4). Under these conditions the additional work produced during the shortening part of the cycle, by virtue of the higher force, was greater than the extra negative work performed during stretch. At 210° phase shift, negative work output was maximal (clockwise work loop) and exceeded the maximum positive work output at 30° phase shift by 20–30% (Fig. 3A). Between 30° phase shift (maximum positive work) and 210° phase shift (maximum negative work), power output systematically varied with stimulus phase shift (Fig. 3A) and work loops became complex with both positive and negative components (Fig. 4). As with stimulus phase shift, the phase delay between peak length and peak force is also expressed in degrees, with a full cycle equal to 360° . When fibres were stimulated during shortening (90 – 180° phase), the phase delay between peak length and peak force increased dramatically (Fig. 3B). This resulted in a non-linear relationship between stimulus phase shift and the phase delay between peak length and peak force (Fig. 5).

Effects of temperature

The effects of temperature on the work output produced during cyclical

contractions varied between summer- and winter-acclimatized fish (Figs 6 and 7A). Work output was maximized at a range of cycle frequencies by adjusting the number and phase shift of stimuli per cycle at a strain amplitude of $\pm 5\%$ of resting muscle length. For both acclimatization groups, work per cycle decreased with increasing cycle frequency and at very low cycle frequencies ($< 3\text{ Hz}$) (Fig. 7A). The decline in work output with increasing cycle frequency was less pronounced at 15°C than at 4°C ; thus, the cycle frequency required to produce maximum power

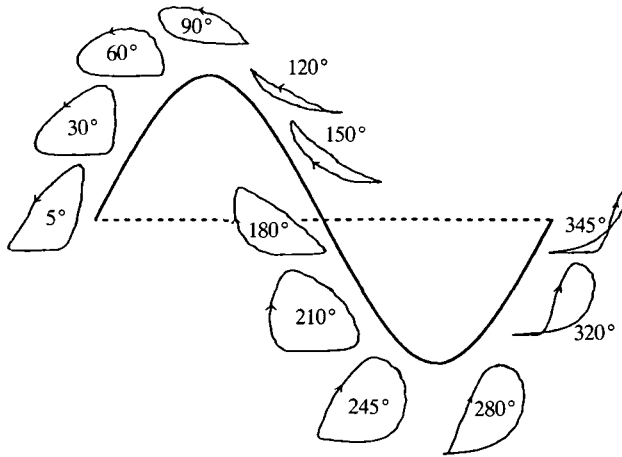


Fig. 4. The effect of stimulus phase shift on the work loops of a representative preparation from the data presented in Fig. 3. The numerical values represent the stimulus phase shift in degrees. The dashed line represents resting length and the solid line corresponds to the muscle length change ($\pm 5\%$ of resting length). Work loops are positioned according to the timing of stimulation in relation to the length change.

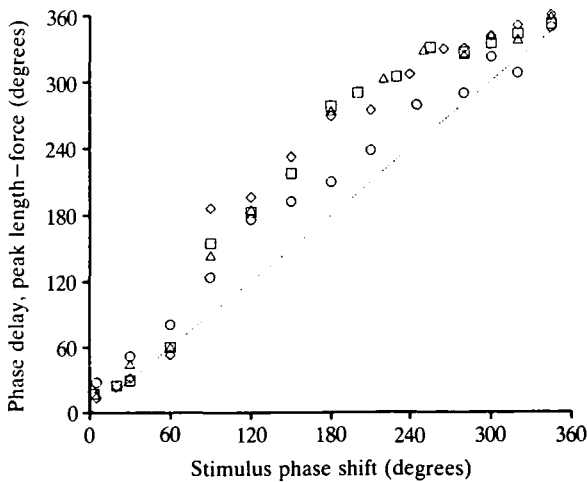


Fig. 5. The relationship between stimulus phase shift and the phase delay between peak length and peak force for four preparations isolated from winter-acclimatized fish. The dotted line represents linearity. Other details are given in the legend to Fig. 3.

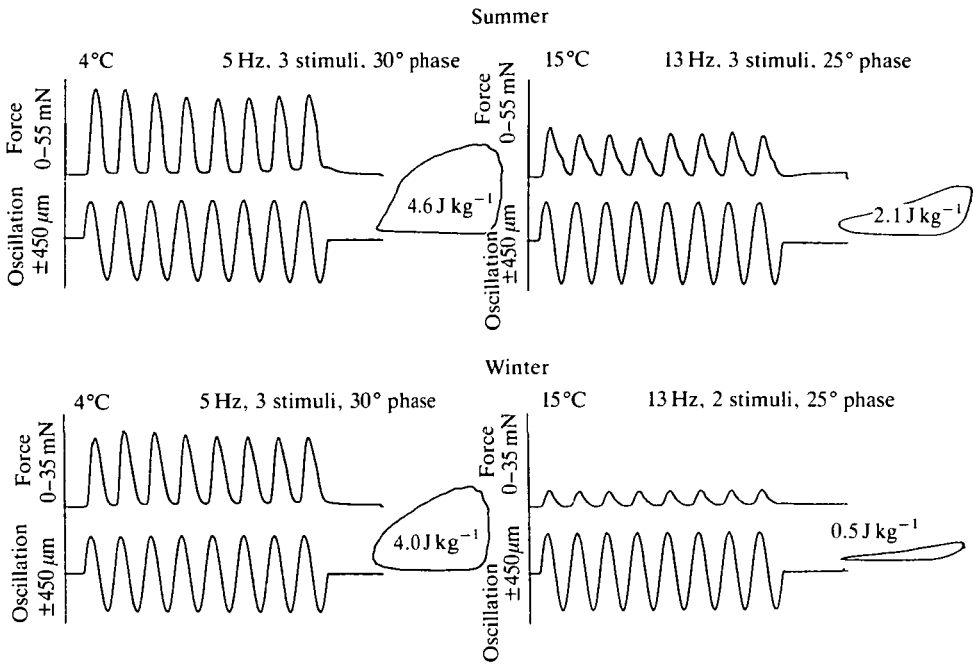


Fig. 6. Representative tension and length records with corresponding work loops of fibre bundles isolated from a winter- and a summer-acclimatized fish. Each preparation was stimulated at 4°C and 15°C under conditions required to yield optimum power output.

output increased from around 5–7 Hz at 4°C to 9–13 Hz at 15°C (Fig. 7B). At 4°C, the maximum mechanical power output, 19–26 W kg⁻¹, was not significantly different in summer- and winter-acclimatized fish ($P > 0.05$). However, at an experimental temperature of 15°C, work per cycle and power output were significantly higher in summer- than in winter-adapted fish ($P < 0.05$), particularly above a cycle frequency of 5 Hz (Figs 6, 7A,B). For example, at 13 Hz, maximum power output was 9.3 ± 1.6 W kg⁻¹ for winter fish and 29.2 ± 4.9 W kg⁻¹ for summer fish (mean \pm s.d.) ($P < 0.01$) (Fig. 7B).

The maximum tension generated per cycle decreased by around half between cycle frequencies of 3 and 8 Hz and thereafter remained relatively constant (Fig. 8A). At 15°C, maximum tension per cycle was higher at all cycle frequencies in summer- compared to winter-acclimatized fish ($P < 0.05$ at 3 Hz, $P < 0.01$ at 5–20 Hz) (Fig. 8A). Although maximum tension was consistently slightly lower at 4°C in winter than in summer fish, this difference was only statistically significant at 15 Hz ($P < 0.01$) (Fig. 8A).

The number of stimuli per cycle required to maximize work output decreased with increasing cycle frequency and increasing temperature (Fig. 8B). Subtle changes in the number of stimuli required to maximize work per cycle were

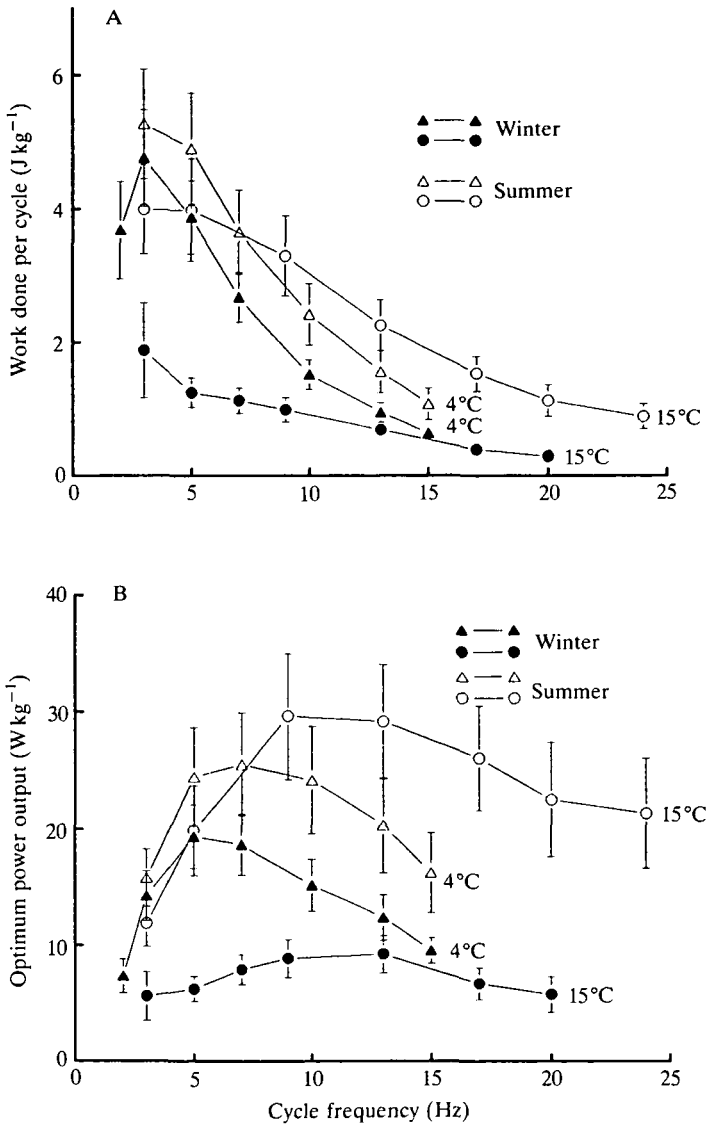


Fig. 7. The relationship between cycle frequency and (A) the work done per cycle (J kg^{-1}) and (B) power output (W kg^{-1}) for muscle fibre bundles from summer- ($N=11$) and winter- ($N=10$) acclimatized fish. Strain amplitude was $\pm 5\%$ of resting fibre length; cycle frequency and stimulation parameters were adjusted to maximize work output at a range of cycle frequencies. See the legend to Fig. 2 for further details. Data represent mean \pm s.e.

evident between summer- and winter-acclimatized fish (Fig. 8B). In general, summer-adapted fish required more stimuli at 15°C and fewer stimuli at 4°C (Fig. 8B). The stimulus phase shift required to maximize work decreased with

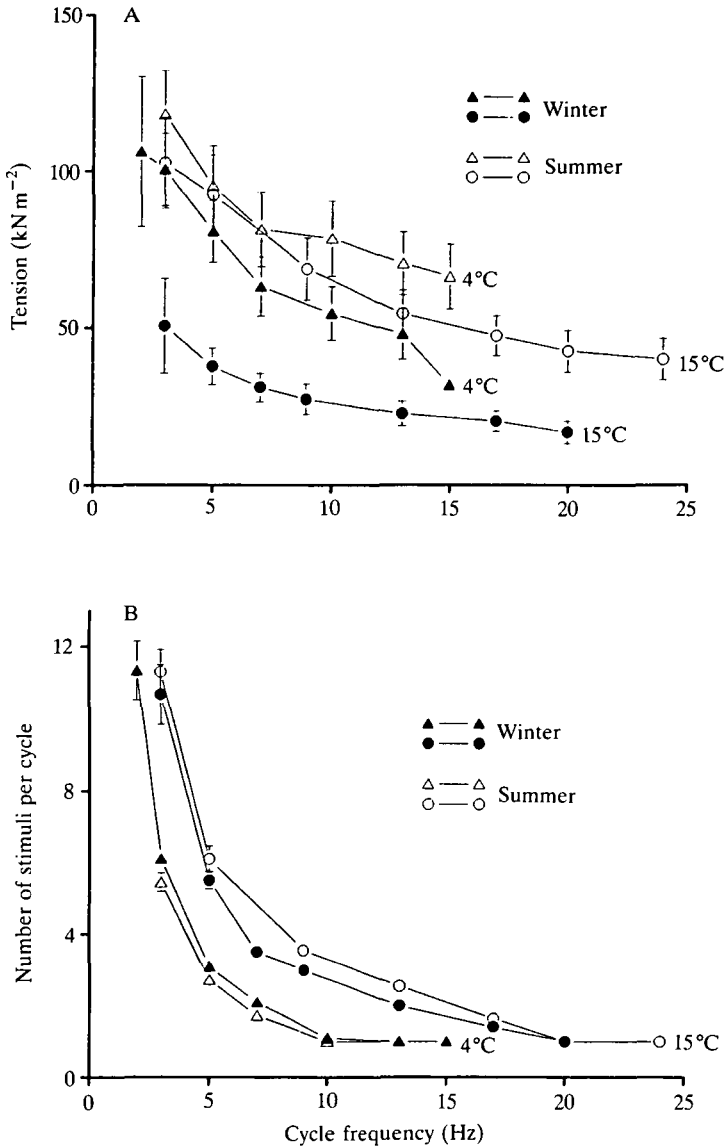


Fig. 8. The relationship between cycle frequency and (A) tension (kN m^{-2}) and (B) the number of stimuli per cycle for muscle fibres from summer- ($N=11$) and winter- ($N=10$) acclimatized fish. Strain amplitude was $\pm 5\%$ of resting length; cycle frequency and stimulation parameters were adjusted to maximize power output at a range of cycle frequencies. See the legend to Fig. 2 for details. Data represent mean \pm s.e.

increasing cycle frequency, particularly at 4°C (Fig. 9A). The optimal stimulus phase shift was independent of acclimatization temperature at all cycle frequencies (Fig. 9A). However, the phase delay between peak length and peak force was

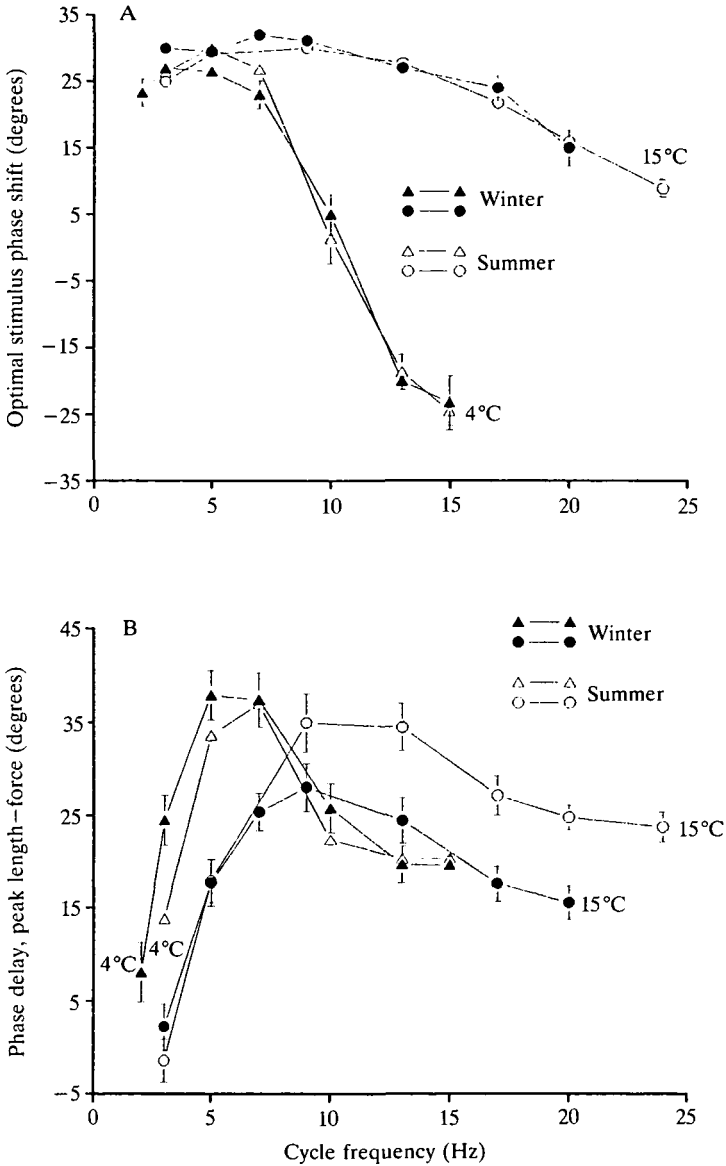


Fig. 9. The relationship between cycle frequency and (A) stimulus phase shift and (B) the phase delay between peak length and peak force for muscle fibres from summer- ($N=11$) and winter- ($N=10$) acclimatized fish. Strain amplitude was $\pm 5\%$ of resting length; cycle frequency and stimulation parameters were adjusted to maximize work output at a range of cycle frequencies. See the legend to Fig. 2 for details. Data represent mean \pm s.e.

prolonged at 15°C and high cycle frequencies (9–20 Hz) and reduced at 4°C and low cycle frequencies (≤ 3 Hz) in summer- relative to winter-acclimatized fish ($P < 0.01$) (Fig. 9B). The maintenance of higher force levels during the shortening

part of the cycle contributes to the greater work output per cycle of muscle fibres in summer-acclimatized fish at 15°C (Figs 6 and 7A).

Discussion

During swimming the sequential activation of myotomes on alternate sides of the body produces a wave of lateral bending from the head to the tail. The electromyographical wave proceeds faster than the mechanical wave of bending, resulting in systematic phase differences in muscle force and length along the body (Hess and Videler, 1984; Williams *et al.* 1989). Several studies have modelled the strain fluctuations of muscle fibres in successive segments during steady swimming from a detailed study of body movements and information on fibre recruitment patterns (Hess and Videler, 1984; van Leeuwen *et al.* 1990). These studies show that, in anterior myotomes, force is maximal as the muscle fibres shorten through their resting lengths, such that power output is positive throughout most of the tail-beat cycle (Hess and Videler, 1984; van Leeuwen *et al.* 1990). Using the approach outlined in the present study, the stimulus phase shift can be adjusted to approximate these conditions (see also Altringham and Johnston, 1990*a,b*). Maximum positive work is obtained by giving muscle fibres a small initial stretch prior to shortening, corresponding to a stimulus phase shift of 30° (Fig. 3). In contrast, modelling studies show that more posterior myotomes are stretched whilst active, producing significant amounts of negative work (Hess and Videler, 1984; van Leeuwen *et al.* 1990). Towards the end of the abdomen the amounts of positive and negative work almost balance each other, whereas in the caudal region net negative work is done (Hess and Videler, 1984; van Leeuwen *et al.* 1990). Increasing the stimulus phase shift in the present study produced increasing amounts of negative work (Fig. 4). Thus, by adjusting the timing of stimuli in relation to the length change cycle, it was possible at least partially to mimic the predicted work output of posterior myotomes (Hess and Videler, 1984; van Leeuwen *et al.* 1990). Maximum negative work output also exceeded maximum positive work output in line with the predictions of swimming models (see Fig. 7 in Hess and Videler, 1984). The stimulation of muscle fibres with a 90–150° phase shift markedly increases the time to peak force (Fig. 5), owing to shortening inactivation of the contractile apparatus (Josephson and Stokes, 1989).

Several features of the behaviour of isolated muscle fibres performing oscillatory work have a correspondence with muscle action during locomotion. For example, in steady swimming, tail-beat frequency increases with temperature (Sisson and Sidell, 1987) and this is paralleled by an increase in the cycle frequency required for maximum power output in isolated muscle fibres (Fig. 7B). The cycle frequencies required for maximum power output *in vitro* (Fig. 7) are within the range reported for maximum tail-beat frequencies in teleosts of similar size (see Table 3 in Archer and Johnston, 1989). Interestingly, the relationship between cycle frequency and power output has a relatively broad optimum (Fig. 7), and this may reflect the use of fast muscle fibres for both 'fast starts' and high cruising

speeds. *In vitro* maximum muscle power output is produced at a strain amplitude of $\pm 5\%$ of resting length, over a range of temperatures (Fig. 2) and cycle frequencies (Altringham and Johnston, 1990a). Similarly, tail-beat amplitude during fast cruising is relatively constant over a wide range of tail-beat frequencies (Webb *et al.* 1984) and temperatures (I. A. Johnston, unpublished observations). In contrast, the optimal strain amplitude for isolated flight muscle of the tobacco hawkmoth (*Manduca sexta*) increases with temperature (Stevenson and Josephson, 1990). However, this again parallels the *in vivo* situation, since low-amplitude wing movements are used to raise the thoracic temperature prior to flight (Stevenson and Josephson, 1990). Electromyographical (EMG) studies have shown that the EMG occupies an almost constant proportion of each tail-beat cycle, decreasing in duration with increasing tail-beat frequency (Grillner and Kashin, 1976). The functional significance of this observation may be that at higher cycle frequencies fewer stimuli per cycle are required to produce maximal power output (Fig. 8B). Since twitch duration increases at low temperature, small adjustments in the number of stimuli per cycle and in the stimulus phase shift are required to allow complete relaxation between successive cycles and thereby maximize power output (Figs 8B and 9A).

The short-horned sculpin is a cold-water species distributed in the seas around northern Europe and into the Arctic circle. The liver secretes a polypeptide antifreeze to enable it to survive freezing during the winter (Fletcher *et al.* 1989). A major finding of the present study is that the mechanical properties of muscle in this species are not fixed but can be modulated according to seasonal temperature changes. However, in contrast to the more widely studied carp *Cyprinus carpio*, the major adjustments in mechanical properties occur at high temperatures (Fig. 6). At 15°C maximum muscle power output increased from 9 W kg^{-1} in winter to 30 W kg^{-1} in summer. The higher power output of muscles in summer-acclimatized fish is largely due to an increase in average force during the shortening part of the cycle, particularly at high cycle frequencies (Figs 6 and 8A). This is reflected by an increase in the phase delay between peak length and peak force (Fig. 9B).

Adaptations in power output can be explained by an increase in maximum tension generation (Fig. 8A) and/or a change in the shape of the force-velocity relationship. The twitch duration of carp fast muscle fibres is altered by temperature acclimation (Fleming *et al.* 1990). In the present study a small increase in the average number of stimuli was required to maximize power output at 15°C in summer-adapted fish, consistent with a decrease in twitch duration (Fig. 8B). Whatever the underlying mechanisms, the observed increase in muscle power output in summer-acclimatized fish will contribute to an improvement in swimming performance at high temperature. To our knowledge this is the first report of seasonal changes in the contractility of muscle fibres in a marine fish.

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