

THE INFLUENCE OF THERMAL ACCLIMATION ON POWER PRODUCTION DURING SWIMMING

II. MECHANICS OF SCUP RED MUSCLE UNDER *IN VIVO* CONDITIONS

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Summary

We have previously shown that the power output of red muscle from warm-acclimated scup is greatly reduced when the fish swim at low temperatures. This reduction occurs primarily because, despite the slowing of muscle relaxation rate at cold temperatures, warm-acclimated scup swim with the same tail-beat frequency and the same stimulation durations, thereby not affording the slower-relaxing muscle any extra time to relax. We hypothesize that power output during swimming could be increased if the stimulus duration were reduced or if the relaxation rate of the red muscle were increased during cold acclimation.

Scup were acclimated to 10 °C (cold-acclimated) and 20 °C (warm-acclimated) for at least 6 weeks. Cold acclimation dramatically increased the ability of scup red muscle to produce power at 10 °C. Power output measured from cold-acclimated muscle bundles driven through *in vivo* conditions measured from cold-acclimated scup swimming at 10 °C (i.e. work loops) was generally much greater than that from warm-acclimated muscle driven through its respective *in vivo* conditions at 10 °C. The magnitude of the increase depended both on the anatomical location of the muscle and on swimming speed. Integrated over the length of the fish, the red musculature from cold-acclimated fish generated 2.7, 8.9 and 5.8 times more power than the red musculature from warm-acclimated fish while swimming at 30 cm s⁻¹, 40 cm s⁻¹ and 50 cm s⁻¹, respectively. Our analysis suggests that the cold-acclimated fish should be able to swim in excess of 40 cm s⁻¹ with just their red muscle whereas the warm-acclimated fish must recruit their pink muscle well below this speed. Because the red muscle is more aerobic than the pink muscle, cold acclimation may increase the sustained swimming speed at which scup perform their long seasonal migrations at cool temperatures.

We then explored the underlying mechanisms for the

increase in muscle power output in cold-acclimated fish. Contrary to our expectations, cold-acclimated muscle did not have a faster relaxation rate; instead, it had an approximately 50 % faster activation rate. Our work-loop studies showed that this faster activation rate, alone, can increase the mechanical power production during cyclical contractions to a surprising extent.

By driving cold-acclimated muscle through warm- and cold-acclimated *in vivo* conditions, we were able to partition the improvement in power production associated with increased activation rate and the approximately 20 % reduction in the duration of electromyographic activity found in the accompanying study. Depending on the position and swimming speed, approximately 60 % of the increase in power output was due to the change in the red muscle's contractile properties (i.e. faster activation); the remainder was due to the shorter stimulus duty cycle of cold-acclimated scup.

Thus, by both shortening the *in vivo* stimulation duration and speeding up the rate of muscle activation as part of cold-acclimation, scup achieve a very large increase in the power output of their red muscle during swimming at low temperature. This increase in power output probably results in an increase in muscle efficiency and, hence, a reduction in the energetic cost of swimming. This increase in power output also reduces reliance on the less aerobic and less fatigue-resistant pink muscle. Both these abilities may increase the swimming speed at which prolonged aerobic muscle activity can occur and thus reduce the travel time for the long seasonal migrations in which scup engage.

Key words: scup, *Stenotomus chrysops*, work loop technique, power, muscle, thermal acclimation.

Introduction

Fish are often required to locomote over a wide range of body temperatures either acutely as they swim through a thermocline

or gradually during seasonal changes or migrations (Rome, 1982; Rome, 1990). As reviewed in the accompanying study

(Rome and Swank, 2001), the red muscle of warm-acclimated scup generates a very low mechanical power output when the fish are subjected to an acute (i.e. over approximately 24 h) decrease in temperature and made to swim. This is due in part to the reduced steady-state power-generating capabilities of the muscle (i.e. the maximum shortening velocity, V_{\max}), but also to the slowing of non-steady-state properties of the muscle (i.e. rates of activation and relaxation). In addition, a major reduction in power is due to the interplay between the contractile properties of the muscles and the *in vivo* stimulation and length change conditions. The reduced relaxation rate at cold temperatures results in greatly reduced power output because fish swim with the same tail-beat frequencies (i.e. same muscle oscillation frequency) and with the same muscle stimulation durations at cold temperatures, thereby not affording the now more slowly relaxing muscle any extra time to relax (Rome et al., 2000; Swank and Rome, 2000). Hence, considerable improvement in the power output of the red muscle at low temperatures could be achieved if cold acclimation resulted in a faster relaxation rate or if the muscle were given more time to relax by reducing the stimulus duration.

As relaxation rate in scup has been shown to vary along the length of the fish (Swank et al., 1997) and cold acclimation has been shown to influence muscle mechanical properties in some species (Heap et al., 1985; Johnston et al., 1990; Fleming et al., 1990; Langfeld et al., 1991; Johnson and Bennett, 1995), it seemed possible that the relaxation rate (or some other contractile parameter) in scup might speed up after cold acclimation, resulting in higher *in vivo* power generation from the red muscle. This hypothesis was tested here by comparing the power generation of cold- and warm-acclimated muscle driven through identical warm-acclimated *in vivo* conditions.

Another way that cold acclimation could increase *in vivo* muscle power would be by modifying the *in vivo* length change and stimulation conditions. In the accompanying study (Rome and Swank, 2001), we found that stimulus duty cycle was reduced by cold acclimation. In the present study, we also address whether these changes help to increase power production by comparing the power output of cold-acclimated red muscle bundles driven through two sets of conditions: the muscle length change and stimulation pattern of cold-acclimated scup swimming at 10 °C (i.e. cold-acclimated conditions), and the muscle length change and stimulation patterns of warm-acclimated scup swimming at 10 °C (i.e. warm-acclimated conditions).

Finally, the combined effects of acclimatory changes of the nervous system and muscle were assessed by comparing the power output at 10 °C of cold-acclimated muscle driven through cold-acclimated conditions with that of warm-acclimated muscle driven through warm-acclimated conditions.

Materials and methods

Fish

Scup (*Stenotomus chrysops* L.), 19–23 cm fork length, were caught at Woods Hole, MA, USA, and acclimated to either

10 °C (cold-acclimated) or 20 °C (warm-acclimated) in flowthrough tanks for at least 6 weeks (as in Rome and Swank, 2001). Scup were killed by a blow to the head followed by spinal transection and double pithing.

Solutions

Mechanics experiments and dissections were performed in a Ringer's solution containing (mmol l⁻¹): NaCl, 132; KCl, 2.6; CaCl₂, 2.7; imidazole, 10; sodium pyruvate, 10; and MgCl₂, 1, pH 7.7 at 15 °C (based on Altringham and Johnston, 1990).

Dissection

Muscle bundles, approximately 0.4 mm deep, 0.9 mm wide and 4.8 mm long, were removed from four locations above the midline of the fish: ANT-1, ANT-2, MID and POST (29, 40, 54 and 70 %, respectively, of fork length from the snout). Only one bundle was used per fish. The bundle was dissected to a single myotome and tied to a servomotor arm and force transducer as described previously (Rome and Swank, 1992).

Experimental protocol

A description of the experimental apparatus has been published previously (Rome and Swank, 1992). The optimal stimulus pulse duration and voltage for maximum isometric force at 20 °C were determined. Stimulus frequency for tetani was 200 Hz. The optimal length of the muscle was determined by generating a force/length curve (Rome and Swank, 1992). The initial muscle length for the work loop experiments was set on the plateau of this curve so that a 5 % length change would cause a similar decrement in active force at both the shortest and longest lengths. Generally, resting tension was less than 4 % of tetanic tension. Isometric twitches and tetani were recorded at 20 °C and 10 °C, and activation and relaxation times were measured. Three aspects of the relaxation time course were measured: from 90 % to 10 % of maximum tetanic force ($T_{r,90-10}$), from 95 % to 80 % of maximum tetanic force ($T_{r,95-80}$), and from the last stimulus pulse to 10 % of maximum force ($T_{r,last}$). Activation time was measured from 10 % to 90 % of maximum force ($T_{a,10-90}$).

Work loops

The work loop technique was used to measure oscillatory power production by muscle bundles for both *in vivo* and optimized stimulation and length change conditions (Rome and Swank, 1992). Note that a phase of 0 ° means that the stimulus starts at the moment the muscle starts shortening. When driven under *in vivo* conditions, a sinusoidal muscle length waveform was used for bundles from the ANT-1 and ANT-2 positions, while a triangular wave with the sharp corners digitally rounded was used for the MID and POST positions because these best fit the *in vivo* length changes (Rome et al., 1993; Rome et al., 2000; Swank and Rome, 2000). The maximum power output at 10 °C was found by optimizing phase, duty cycle and strain. An optimal frequency of 2.5 Hz was selected because it has been determined previously to be at the peak of the power-frequency curve for red muscle (Rome and Swank, 1992). The optimal conditions and maximum power output for

oscillation frequencies of 2.85, 3.55 and 4 Hz (corresponding to swimming speeds of 30, 40 and 50 cm s⁻¹) were then determined in the same manner.

In vivo power was measured when muscle bundles were driven through the stimulus and length change conditions determined from measurements made on scup swimming at 10 °C at 30 cm s⁻¹ and 50 cm s⁻¹ (Rome and Swank, 2001). *In vivo* conditions for 40 cm s⁻¹ at 10 °C were estimated by averaging the 30 and 50 cm s⁻¹ values. Typically, the first 10–20 oscillation cycles for a given work-loop condition increased in power, the next 10 produced similar amounts of power, and by 40 cycles the power started to decline. Work was measured from the cycle that provided the highest value after power production levelled off (Rome et al., 2000). The interval between work-loop sets was 10–30 min depending on how far from optimal the conditions were. As found previously (Rome et al., 2000), muscle bundles driven under non-optimal conditions required more time to recover. To control for possible muscle fatigue, after every four work loop sets, a set of work loops using optimal conditions was re-measured and the data were normalized (as detailed in Rome et al., 2000).

We measured power generation under two sets of *in vivo* conditions for each muscle preparation. Cold-acclimated muscle was first driven through the *in vivo* conditions measured from cold-acclimated scup swimming at 10 °C. Second, the muscle was driven through the *in vivo* conditions previously measured in warm-acclimated scup swimming at 10 °C (Swank and Rome, 2000). This procedure allowed us to determine how much the difference in *in vivo* conditions contributed to changes in power output. Further, by comparing the results with previous measurements of warm-acclimated muscle driven through warm-acclimated conditions for swimming at 10 °C (Rome et al., 2000), we could examine how much the changes in muscle contractile properties contributed to increased muscle power.

All mechanics experiments were conducted at 10 °C unless specified otherwise. Maximum velocity of shortening was determined experimentally, as reported previously (Rome et al., 1992).

Analysis

Total power generated by the entire red musculature was calculated (assuming that all fibres are recruited) using the volume of red muscle from successive longitudinal positions (Zhang et al., 1996). The *in vivo* power per kilogram muscle for each position multiplied by the mass of muscle at that position was summed to determine the total power the red muscle is capable of producing. Note that if the *in vivo* power per kilogram was negative for a given position, we assumed that recruitment would be minimal and hence assigned a value of zero for that position. This calculation was performed for swimming speeds of 30 cm s⁻¹, 40 cm s⁻¹ and 50 cm s⁻¹. Warm-acclimated muscle power values, activation times and relaxation times are taken from Rome et al. (Rome et al., 2000).

Statistical analyses

Statistical analyses were performed using SigmaStat

software (Jandel) and are described in the accompanying paper (Rome and Swank, 2001). Values are means \pm 1 S.E.M.

Results

In vivo power production of red muscle in cold-acclimated fish

Except for the ANT-2 position at 50 cm s⁻¹, red muscle bundles from all four positions of cold-acclimated scup produced positive power for swimming at all speeds (Fig. 1) when driven through their *in vivo* stimulus and length change conditions measured at 10 °C. Isolated muscle bundles from the ANT-1 and ANT-2 positions generated between 0 W kg⁻¹ and 2.5 W kg⁻¹ at *in vivo* conditions measured from scup swimming at speeds of 30, 40 and 50 cm s⁻¹, while muscle bundles from the MID and POST regions produced up to 14.4 W kg⁻¹ (Fig. 1). These values are in sharp contrast to those from warm-acclimated muscle bundles. When driven through their 10 °C *in vivo* conditions at 40 and 50 cm s⁻¹, they produced no net power at any position except for the POST position, where power output was quite low (Fig. 1B,C).

The increase in power at 10 °C found in cold-acclimated compared with warm-acclimated fish was greatest at the lower swimming speeds especially at the MID and POST positions (Fig. 1A,B). At 40 cm s⁻¹, cold-acclimated muscle at the MID and POST positions produced 8 and 14 W kg⁻¹ compared with -2 and 3.4 W kg⁻¹ from warm-acclimated muscle. At 30 cm s⁻¹, cold-acclimated muscles at the MID and POST positions generated between 13.4 and 14.4 W kg⁻¹ compared with 2 and 8 W kg⁻¹ from warm-acclimated muscle. At 30 cm s⁻¹, power production from cold-acclimated muscle bundles from the ANT-1 and ANT-2 positions under their *in vivo* conditions was not significantly greater than those of their warm-acclimated counterparts (Fig. 1A). However, at 40 cm s⁻¹ and 50 cm s⁻¹, cold-acclimated ANT-1 muscle (and ANT-2 muscle at 40 cm s⁻¹) produced positive power instead of substantial 'negative' power values measured from warm-acclimated muscle (Fig. 1B,C). Therefore, in addition to the MID and POST positions producing higher power at 10 °C, some of the anterior musculature could contribute power for swimming at 40 and 50 cm s⁻¹ in cold-acclimated scup, whereas no power is generated at these positions in the warm-acclimated fish.

Power production at cold temperatures is increased by acclimation of muscle properties

There was a substantial increase in power output of the muscle from cold-acclimated fish when driven under their *in vivo* (i.e. cold-acclimated) conditions. We found that there were two acclimatory changes responsible for this increase in power: changes in the contractile properties of the muscles, and changes in the output of the nervous system.

Overall, cold-acclimated muscle generated more power than warm-acclimated muscle when both were driven through identical *in vivo* conditions (Fig. 2). This signifies that there was some change in the properties of the red muscle during thermal acclimation. The increase in power depended on the *in vivo* conditions used, which varied with swimming speed

and muscle position. When run under the warm-acclimated conditions, cold-acclimated muscle bundles from the MID and POST positions generated substantially more power than warm-acclimated muscle. Power output from cold-acclimated ANT-1 was significantly higher than that from the warm-acclimated muscle at 40 and 50 cm s⁻¹; however, at 30 cm s⁻¹, it was not significantly different. Unlike muscle from the other regions, there was no statistically significant increase in power generation in the cold-acclimated ANT-2 muscle compared with the warm-acclimated muscle (Fig. 2).

Acclimation of the muscle stimulation pattern increases power production at low temperatures

In most of the muscle, there was a large increase in power output associated with thermal acclimation of the muscle alone (Fig. 2). However, in general, this did not account for the total increase in power associated with thermal acclimation of the whole locomotory system of the fish (broken blue lines in Fig. 2). The remaining increase in power output associated with cold-acclimation came from changes in the output of the nervous system that favoured power production at cold temperatures. In the accompanying study (Rome and Swank, 2001), we found that, although *in vivo* length change and oscillation frequency (tail-beat frequency) did not change during cold acclimation, there was a significant reduction in the stimulus duty cycle that we predicted should increase the power output of the muscle. Indeed, we found that changes in the *in vivo* conditions contributed significantly to the higher power production of cold-acclimated red muscle. As shown in Fig. 3, at 40 and 50 cm s⁻¹, cold-acclimated muscle produced significantly higher power when activated under cold-acclimated *in vivo* swimming conditions at 10 °C compared with warm-acclimated conditions. When driven though cold-acclimated 40 cm s⁻¹ conditions, MID and POST muscle bundles produced 2.5 and 1.5 times more power, respectively, compared with warm-acclimated conditions (Fig. 3B). Under 50 cm s⁻¹ cold-acclimated conditions, there was a 1.6-fold increase in power output for the POST position. Cold-acclimated conditions also significantly raised power output from ANT-1 and ANT-2 muscle at 50 cm s⁻¹ (Fig. 3C). In contrast, at 30 cm s⁻¹, there was no statistically significant difference in power generation between *in vivo* conditions from the two acclimation groups (Fig. 3A).

The mechanism for improved contractile performance with cold acclimation: rates of activation and relaxation

In general, changes in both the muscle properties and the output of the nervous system contributed to the increase in power output. We tried to determine which contractile property was responsible for power increases due to cold acclimation of the muscle. The increase in power-generating ability was not due to a speeding up of the relaxation rate, as hypothesized; instead, it appears to be due to an increased activation rate (Figs 4, 5). Activation rates of cold-acclimated red muscle were far faster than those of warm-acclimated muscle for both tetani and twitches at 10 °C (Figs 4, 5A,B). Compared with warm-acclimated muscle, cold-acclimated isometric activation

rates for tetani were 1.43, 1.65 and 1.58 times faster (i.e. the activation times were 30 %, 39 % and 36 % shorter) for ANT-2, MID and POST bundles, respectively (Fig. 5A). Similarly, the twitch activation rates were approximately 1.25 times faster (i.e. activation time was approximately 20 % shorter) than the warm-acclimated muscle for all four positions (Fig. 5B). In contrast, we found no difference in tetanic relaxation rate ($T_{r,90-10}$) or in twitch relaxation rate (except for ANT-1; Fig. 5C,D) between cold-acclimated and warm-acclimated red muscle. Note that a similar lack of acclimation effect was observed for $T_{r,95-80}$ and $T_{r,last}$ (not shown).

Steady-state properties: isometric force and V_{max}

No difference in maximum isometric force was found between cold- and warm-acclimated groups: 136±7.4 kN m⁻² ($N=23$) and 118±6.8 kN m⁻² ($N=34$), respectively, at 10 °C (t -test, $P=0.10$), and 151±10.0 kN m⁻² and 144±9.3 kN m⁻², respectively, at 20 °C (t -test, $P=0.63$). At 10 °C, the twitch-to-tetanus ratio was approximately 35 % and 18 % higher for cold-acclimated muscle from the ANT-1 and ANT-2 positions, respectively. The ratios were 0.81±0.03 ($N=4$), 0.79±0.03 ($N=5$), 0.69±0.03 ($N=10$) and 0.56±0.04 ($N=4$) for cold-acclimated muscle bundles and 0.60±0.03 ($N=5$), 0.67±0.02 ($N=5$), 0.66±0.04 ($N=17$) and 0.62±0.03 ($N=7$) for warm-acclimated muscle for the ANT-1, ANT-2, MID and POST positions, respectively. There was no statistical difference between the acclimation groups in the twitch-to-tetanus ratio of the MID and POST positions.

The increase in power output of cold-acclimated muscle was also not due to an increase in the maximum velocity of shortening (V_{max}) as no difference in V_{max} was found between acclimation groups. We found in this study that warm-acclimated red muscle had a V_{max} of 5.63±0.3 ML s⁻¹ ($N=3$), which agrees quite well with the V_{max} of 5.42±0.2 ML s⁻¹ at 20 °C ($N=12$; t -test, $P=0.6$) obtained previously from cold-acclimated muscle (Swank et al., 1997). Both sets of values are similar to V_{max} values obtained previously (5.55±0.25 ML s⁻¹, $N=5$; Rome et al., 1992) for fish maintained at seawater temperature, which changed with the environment (average temperature approximately 15 °C).

Thermal acclimation of muscle leads to greater maximum power output

The observed changes in contractile properties not only permit cold-acclimated muscles to generate higher power under their *in vivo* length change and stimulation conditions but also increase the maximum power these muscles can generate during oscillatory contractions. When stimulus and length change conditions were optimized for power generation, cold-acclimated muscle bundles produced up to 2.5-fold the power produced by warm-acclimated muscle bundles (Table 1). This finding again demonstrates the higher power-producing ability of cold-acclimated muscles compared with warm-acclimated red muscle and also demonstrates that the increase in power associated with cold acclimation is general and is not restricted to a narrow set of *in vivo* conditions.

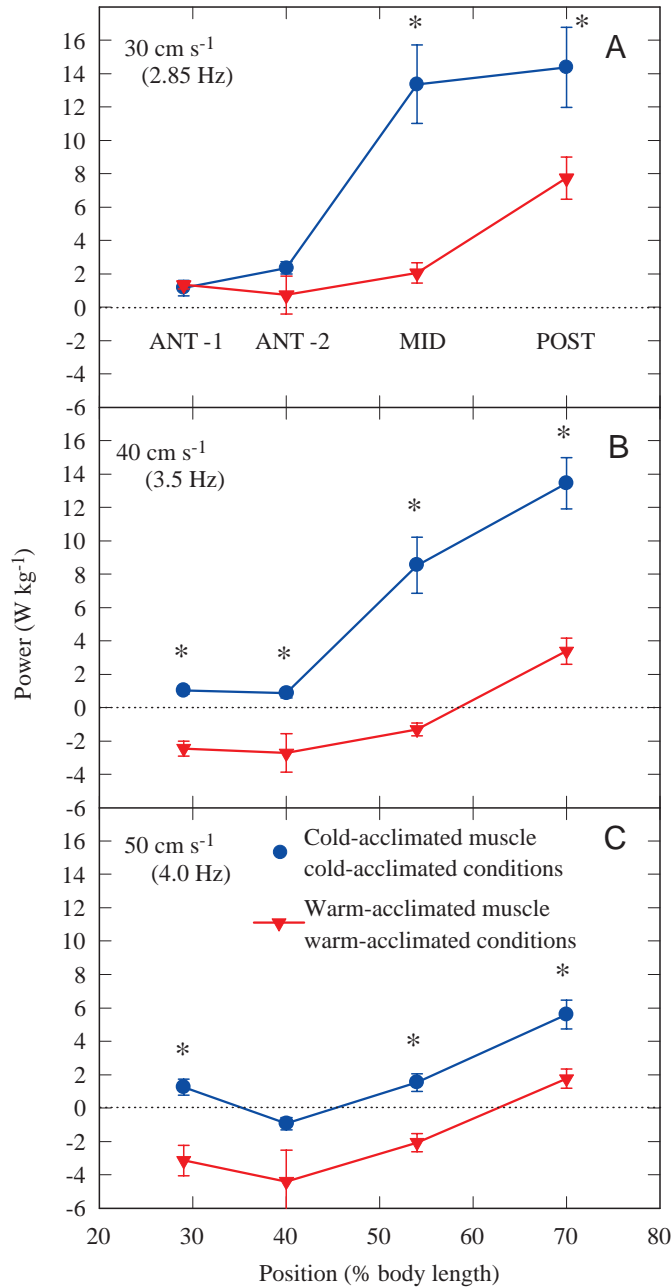


Fig. 1. *In vivo* power output from scup red muscle. Muscle bundles from cold-acclimated scup were driven through cold-acclimated *in vivo* conditions at 10°C (blue) and muscle bundles from warm-acclimated scup were driven through warm-acclimated *in vivo* conditions at 10°C (red). Power differences due to acclimation at all swimming speeds were statistically significant (two-way ANOVA; $P < 0.001$ for all swimming speeds). An asterisk indicates a significant difference due to acclimation at individual positions (t -test, $P < 0.05$). Values are means ± 1 S.E.M. For cold-acclimated muscle working under cold-acclimated conditions, $N=3, 4, 4$ and 5 (30 cm s^{-1}), $N=3, 4, 7$ and 7 (40 cm s^{-1}) and $N=3, 5, 7$ and 7 (50 cm s^{-1}), respectively, for the ANT-1, ANT-2, MID and POST positions. For warm-acclimated muscle working under warm-acclimated conditions, $N=6, 6, 6$ and 7 (30 cm s^{-1}), $N=5, 5, 8$ and 7 (40 cm s^{-1}) and $N=4, 3, 5$ and 6 (50 cm s^{-1}), respectively, for the ANT-1, ANT-2, MID and POST positions.

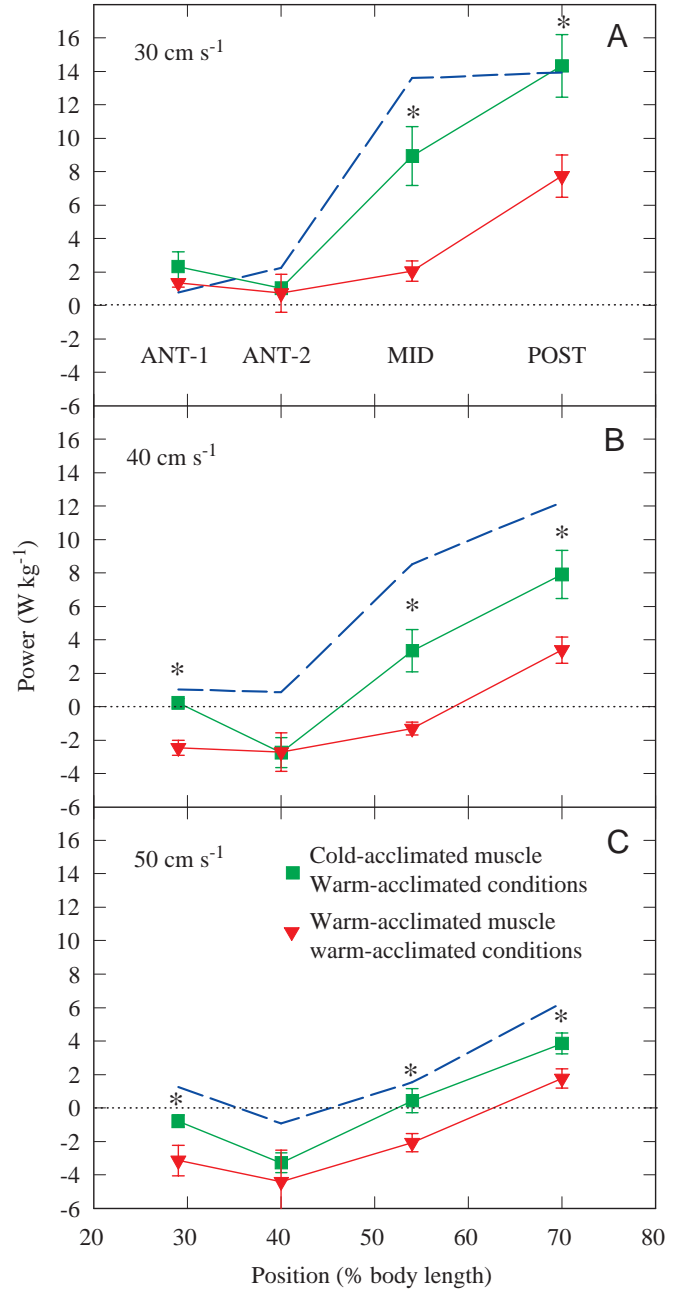


Fig. 2. Power from cold-acclimated (green) and warm-acclimated (red) muscle bundles driven through warm-acclimated *in vivo* conditions, showing the contribution of acclimation-induced changes in red muscle contractile properties to power production ability. Power differences due to acclimation at all swimming speeds were statistically significant (two-way ANOVA; $P < 0.001$ for 30 and 40 cm s^{-1} , $P = 0.004$ for 50 cm s^{-1}). Values are means ± 1 S.E.M. An asterisk indicates a significant difference due to acclimation at individual positions (t -test, $P < 0.05$). For comparison with the total change in power output associated with cold acclimation, the power output of the cold-acclimated muscle running under cold-acclimated conditions is shown as a dashed blue line. For cold-acclimated muscle working under warm-acclimated conditions, $N=3, 4, 4$ and 5 (30 cm s^{-1}), $N=3, 4, 7$ and 6 (40 cm s^{-1}) and $N=3, 5, 6$ and 7 (50 cm s^{-1}), respectively, for the ANT-1, ANT-2, MID and POST positions. Other N values are given in the legend to Fig. 1.

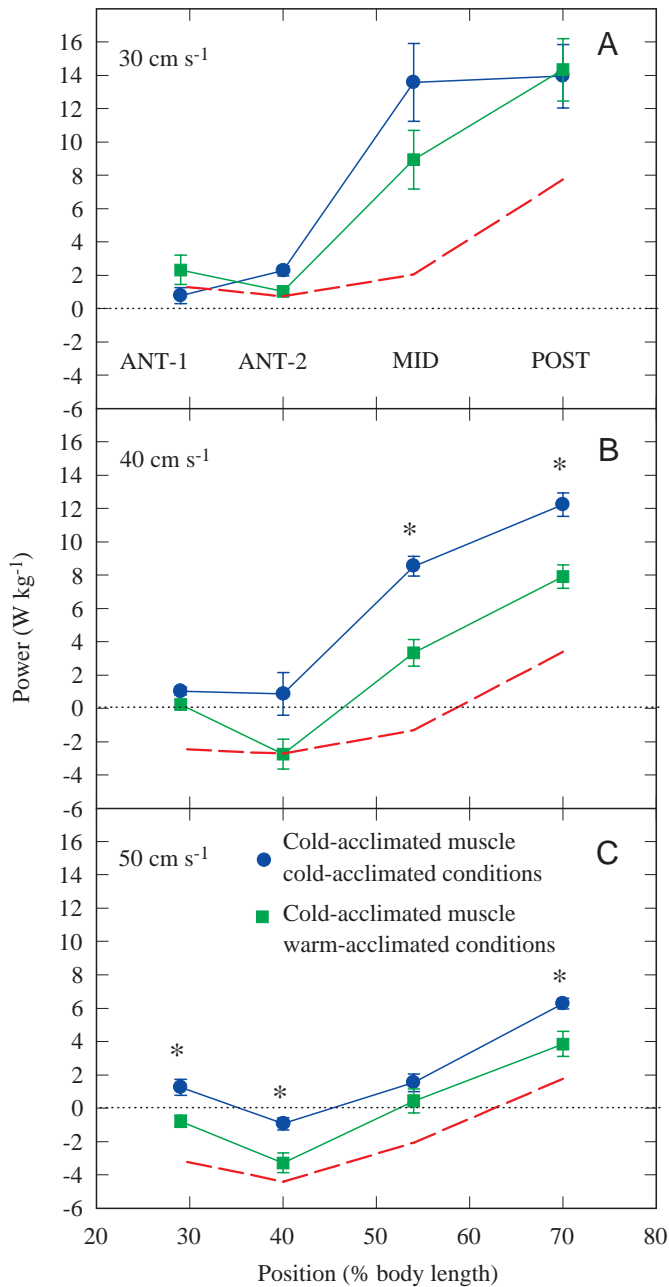


Fig. 3. Power from cold-acclimated red muscle when run under cold-acclimated (blue) *in vivo* conditions compared with warm-acclimated (green) conditions, showing the contribution of acclimation-induced changes in *in vivo* conditions (primarily EMG duty cycle) to power production ability. At 40 and 50 cm s⁻¹, power differences due to acclimation were statistically significant (two-way ANOVA; $P < 0.001$, 40 cm s⁻¹, $P = 0.003$, 50 cm s⁻¹). In contrast, at 30 cm s⁻¹, power differences due to acclimation were not statistically significant (two-way ANOVA; $P = 0.441$). An asterisk indicates a significant difference due to different *in vivo* acclimation conditions at individual positions (*t*-test, $P < 0.05$). Values are means ± 1 S.E.M. *N* values given in the legends to Figs 1 and 2. For comparison with the total change in power output associated with cold acclimation, the power output of warm-acclimated muscle running under warm-acclimated conditions is shown as a dashed red line.

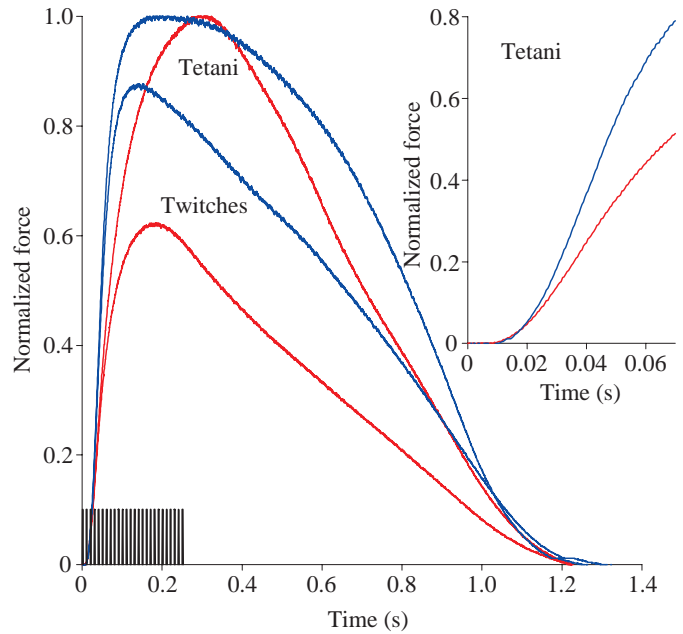


Fig. 4. Isometric twitches and tetani from warm- (red) and cold-acclimated (blue) scup red muscle under identical conditions. Bundles are from the MID position. The stimulus duration for the tetanus is 250 ms. Force levels were normalized to the maximum isometric force of each muscle. Note the longer time to reach maximum force in the warm-acclimated muscle. The inset shows force recordings for tetani on a faster time base.

Discussion

Increase in power output with thermal acclimation

Cold acclimation resulted in changes to the muscle and to the stimulation pattern of the muscle that dramatically increased the amount of power that scup were able to produce with their red muscle at 10 °C. The work loops in Fig. 6 illustrate how these changes combine to lead to greater power production at the POST position. The warm-acclimated bundle (WA), when driven through its *in vivo* conditions for 10 °C (WC; red), generated a very flat loop with nearly equal positive and negative work. This resulted in little net power output: only 1.4 W kg⁻¹. The cold-acclimated muscle (CA), with its faster activation rate, when run under the same conditions (WC; green) generated a much fuller loop with much more positive and net work (9.1 W kg⁻¹). However, under these warm-acclimated conditions, the cold-acclimated muscle generated considerable negative work during lengthening. The negative work was reduced when the bundle was run under the cold-acclimated conditions (CC; blue) because the shorter duty cycle gave the muscle more time to relax. Because positive work was maintained, there was a large increase in the net work generated (12.6 W kg⁻¹).

The level of power increase and the extent to which the muscle and nervous stimulation changes contributed varied considerably with muscle position and swimming speed. The largest increases in power were at the POST and MID positions, which provide the majority of power for swimming in scup (Rome et al., 1993). The increase in power in these regions, particularly at 30 and 40 cm s⁻¹, is extremely large. In

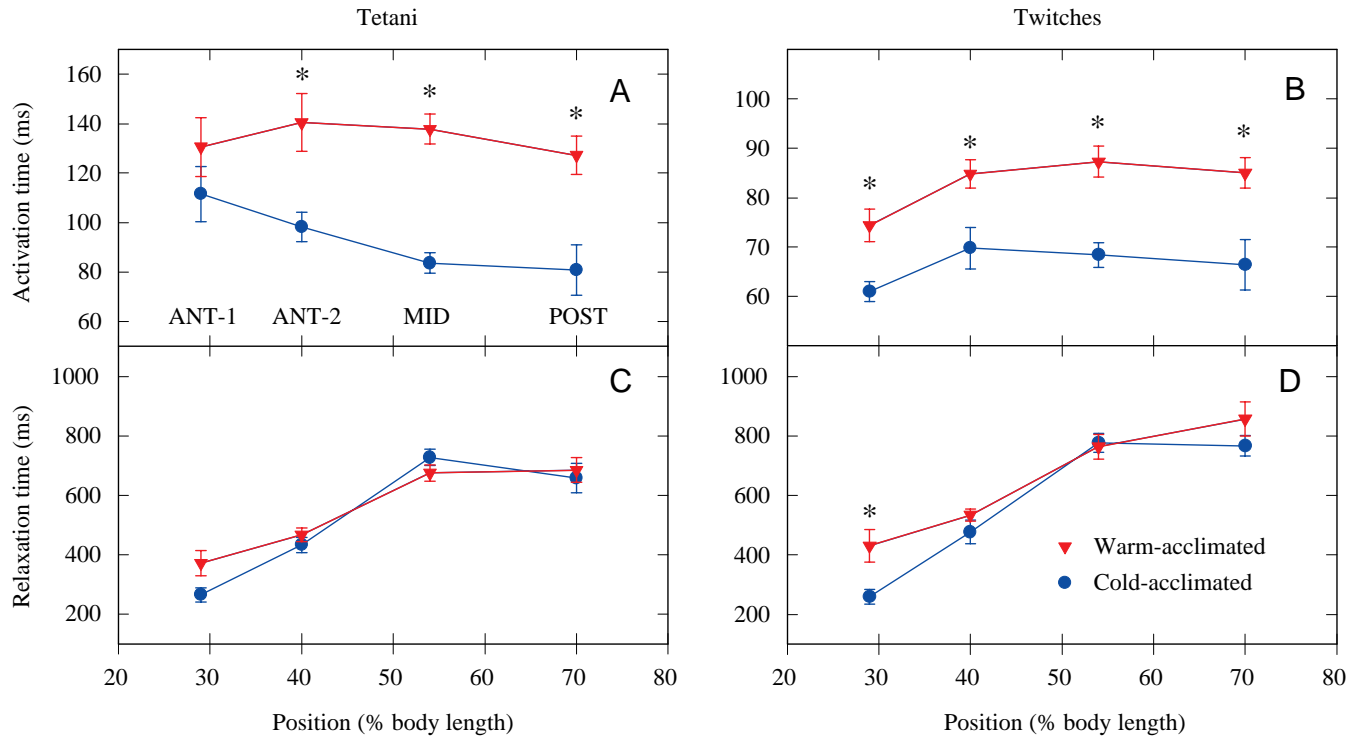


Fig. 5. Tetanic activation time (A) and relaxation time ($T_{r,90-10}$) (C) of cold- and warm-acclimated scup red muscle. Activation time was statistically significant due to acclimation (two-way ANOVA; $P < 0.001$). Relaxation differences due to acclimation were not statistically significant by two-way ANOVA ($P = 0.3$ for $T_{r,90-10}$, $P = 0.6$ for $T_{r,95-80}$ and $P = 0.4$ for $T_{r,last}$; note that $T_{r,95-80}$ and $T_{r,last}$ are not shown). See Materials and methods for an explanation of the different relaxation measurements. Twitch activation time (B) and relaxation time ($T_{r,90-10}$) (D) of cold- and warm-acclimated scup red muscle. Activation differences due to acclimation were statistically significant (two-way ANOVA; $P < 0.001$). Relaxation differences as measured by $T_{r,90-10}$ and $T_{r,last}$ were statistically significant by two-way ANOVA ($P = 0.045$ for $T_{r,90-10}$ and $P = 0.004$ for $T_{r,last}$; this is due to the difference at ANT-1), but not as measured by $T_{r,95-80}$ ($P = 0.6$). An asterisk indicates a significant difference due to acclimation at individual positions (t -test, $P < 0.05$). Values are means \pm 1 s.e.m. For cold-acclimated muscle, $N = 4, 5, 10$ and 4 and for warm-acclimated muscle, $N = 5, 5, 17$ and 7, respectively, for the ANT-1, ANT-2, MID and POST positions.

Table 1. Optimized power output of cold- and warm-acclimated scup red muscle bundles at 10 °C

Frequency (Hz)	Acclimation	Optimized power output (W kg^{-1})			
		ANT-1	ANT-2	MID	POST
2.5	Cold	16.7 \pm 3.2 (4)	16.9 \pm 1.4 (5)	16.3 \pm 2.0* (10)	15.1 \pm 1.2* (4)
	Warm	13.6 \pm 2.6 (5)	13.2 \pm 1.8 (5)	9.8 \pm 1.1 (17)	10.0 \pm 1.1 (7)
2.85	Cold	22.5 \pm 2.9* (3)	16.7 \pm 3.3 (4)	15.9 \pm 2.1 (5)	14.8 \pm 1.2 (3)
	Warm	13.0 \pm 2.4 (5)	12.7 \pm 1.7 (4)	10.1 \pm 1.8 (9)	10.3 \pm 4.7 (2)
3.5	Cold	19.8 \pm 3.9 (3)	18.9 \pm 3.3 (4)	14.1 \pm 2.0* (5)	14.2 \pm 1.8 (4)
	Warm	11.2 \pm 2.3 (5)	7.5 \pm 0.4 (2)	7.6 \pm 1.3 (9)	8.5 \pm 2.8 (2)
4.0	Cold	15.8 \pm 1.3 (3)	12.7 \pm 1.4 (5)	12.5 \pm 1.3* (8)	12.5 \pm 1.4 (2)
	Warm	10.0 \pm 2.0 (4)	8.9 \pm 2.7 (2)	8.7 \pm 1.1 (7)	9.1 \pm 2.8 (3)

All values are means \pm 1 s.e.m. (N).

An asterisk indicates a statistically significant difference due to acclimation (t -test, $P < 0.05$).

At all frequencies, the difference between acclimation temperatures was statistically significant by two-way ANOVA, but differences due to position were not. The P values for acclimation (acc) and position (pos) were as follows: 2.5 Hz acc, $P = 0.001$; pos, $P = 0.818$; 2.85 Hz acc, $P = 0.007$; pos, $P = 0.081$; 3.5 Hz acc, $P < 0.001$; pos, $P = 0.708$; 4.0 Hz acc, $P = 0.018$; pos, $P = 0.60$.

For an explanation of position (ANT-1, ANT-2, MID and POST), see Materials and methods.

fact, cold-acclimated muscle could generate more power under its *in vivo* swimming conditions than the maximum oscillatory

power output (i.e. from optimized work loops) of warm-acclimated muscle (compare Fig. 1 with Table 1).

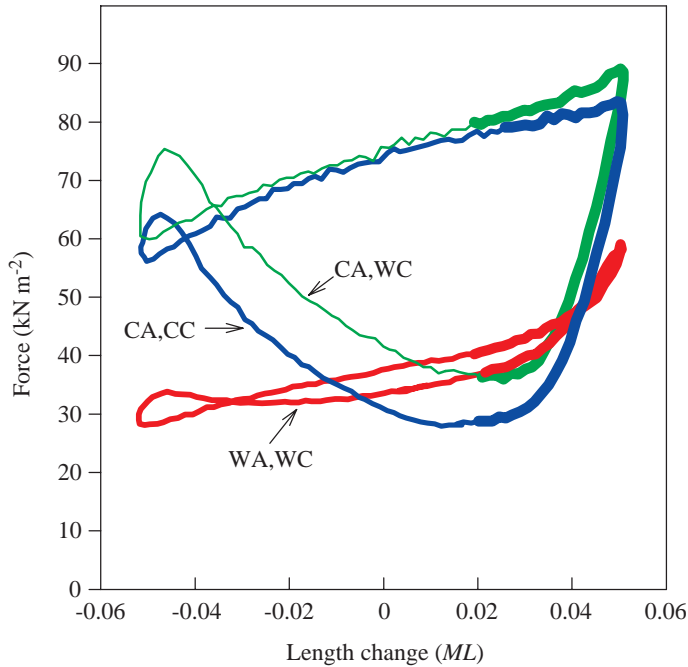


Fig. 6. Power generated by warm- and cold-acclimated muscle. The two muscle bundles are from the POST position of warm-acclimated (WA) and cold-acclimated (CA) scup and were driven through POST *in vivo* conditions for the 40 cm s⁻¹ swimming speed. The warm-acclimated conditions (WC) were 3.6 Hz, 5.4% strain, -54° phase and 85 ms stimulus duration. The cold-acclimated conditions (CC) were 3.5 Hz, 5.4% strain, -56° phase and 80 ms stimulus duration. Power generation for WA,WC was 1.4 W kg⁻¹; for CA,WC was 9.1 W kg⁻¹ and for CA,CC was 12.6 W kg⁻¹. Note that, for this particular swimming speed and anatomical position, there is only a small change in stimulus duty cycle and thus there is only a moderate enhancement in work production associated with the cold-acclimated conditions. *ML*, muscle length. The timing of the stimulus is denoted by the thicker sections of the work-loop traces.

The large magnitude of the power increase with cold acclimation can best be appreciated when compared with muscle performance at 20°C. Fig. 7 shows power output at a swimming speed of 80 cm s⁻¹ at 20°C in warm-acclimated scup (black line; data from Rome et al., 2000). Although the Q₁₀ values for power production were generally large or indeterminate for the warm-acclimated fish (red), the Q₁₀ values for the cold-acclimated fish (blue) were often modest. For instance, the Q₁₀ for MID and POST power production at 30–40 cm s⁻¹ ranged between 0.8 and 1.8. In the MID position at 30 cm s⁻¹, the cold-acclimated muscle actually appeared to generate more power during swimming at 10°C than the warm-acclimated muscle during swimming at 20°C.

Higher power output from modified muscle activation rate

To understand better the underlying mechanism for the increase in power production, we first explore in more detail the acclimatory changes that occur in the muscle. Changes in intrinsic muscle power production capabilities after cold acclimation contributed approximately 60% to the overall

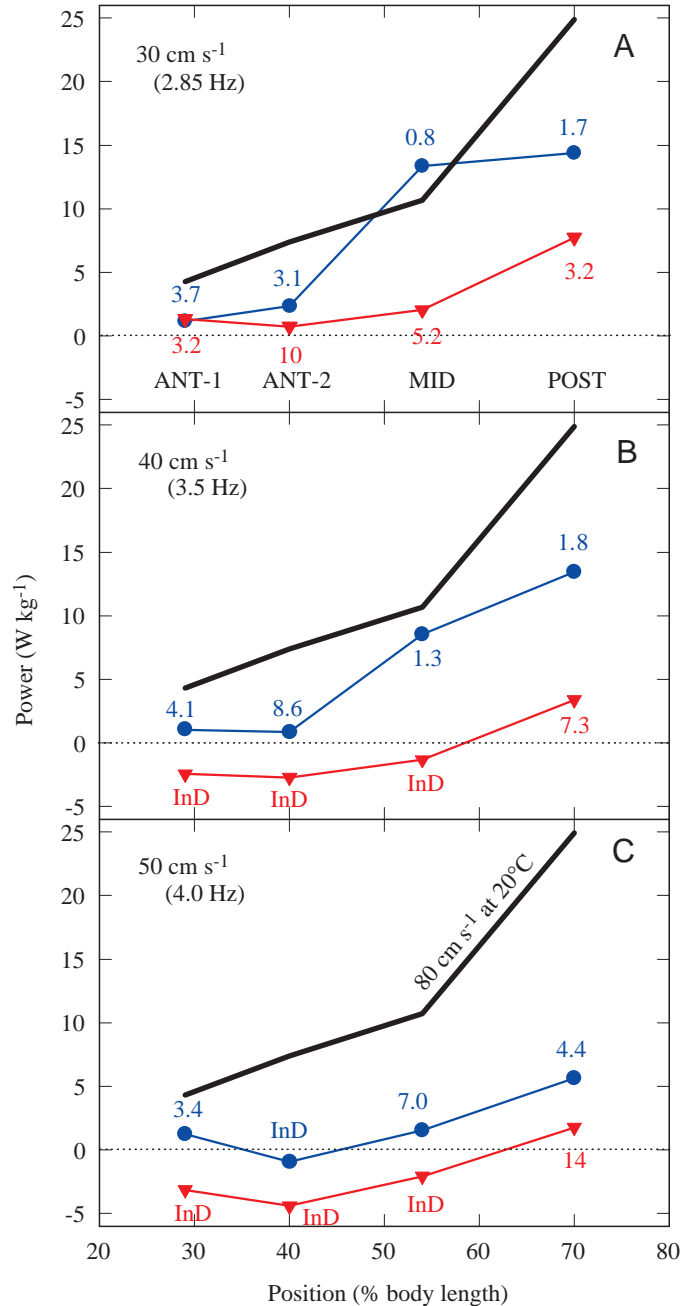


Fig. 7. The temperature-dependence of power output during swimming. Muscle power output at 30, 40 and 50 cm s⁻¹ at 10°C in cold-acclimated (blue circles) and warm-acclimated (red triangles) fish is compared with power output during swimming at 80 cm s⁻¹ at 20°C in warm-acclimated fish (black line; data from Rome et al., 2000). Q₁₀ values (power at 20°C/power at 10°C) are given in the appropriate colour for each position and swimming speed. InD signifies that the Q₁₀ is indeterminate, i.e. that the power output at 10°C is negative.

increase in power. The increase in power-generating capability was demonstrated both when cold-acclimated bundles were driven through the warm-acclimated *in vivo* conditions (Figs 2, 6) and when stimulus and length change conditions were optimized for power production at four different oscillation

frequencies (Table 1). This increase in power-generating ability does not appear to be due to changes in steady-state muscle characteristics because V_{\max} and isometric force generation did not change with acclimation. Further, the relaxation rates did not change significantly either. In contrast, the activation rate increased dramatically in response to cold acclimation (Figs 4, 5). Hence, by elimination, it appears that the increased activation rate is responsible for the increase in power associated with thermal acclimation of the muscle.

The faster activation rate in cold-acclimated muscle appears to increase power production by enabling force to increase to a higher level before the beginning of shortening, while avoiding an increase in negative work during lengthening. For instance, as shown in Fig. 6, having a faster activation rate permits force to rise very rapidly to a high level by the beginning of shortening. Thus, the cold-acclimated muscle can generate much more positive and, hence, net work. The potential importance of the faster activation rate is also illustrated by the substantially higher force level in the isometrically contracting cold-acclimated muscle 40 ms following stimulation (Fig. 4, inset; note that 40 ms is the approximate time between the first stimulus and the start of shortening in the work loop illustrated in Fig. 6).

It should be noted that the large magnitude of the increase in work in cold-acclimated muscle reflects to some extent that

in vivo muscle stimulation occurs at the same point (phase) of the work loop in cold- and warm-acclimated fish. It might be assumed that the slower activation rate of warm-acclimated muscle could be compensated for simply by starting the stimulus earlier. As shown in Fig. 8, when the start of the stimulus of a warm-acclimated muscle work loop (loop A, Fig. 8) is shifted so that it starts earlier (i.e. a more negative phase, loop B), it results in the force reaching a higher level and thus generating more positive work during the first portion of shortening. However, the higher positive work is achieved at the cost of having much more negative work during the last portion of lengthening (area 1, Fig. 8). By contrast, in a work loop from a faster-activating muscle, high force at the beginning of shortening can be achieved without as much negative work because the stimulus can start relatively late in the cycle (i.e. with a small negative phase; Fig. 6). This late stimulus results in less negative work (i.e. there is a smaller area under the work loop trace during lengthening), thus leading to a large increase in net power.

The underlying molecular mechanism for the faster activation rate has not yet been determined. Experiments on toadfish *Opsanus tau* have shown that Ca^{2+} transient duration, the off-rate of Ca^{2+} release from troponin and the crossbridge kinetic rate constants are three major determinants of twitch speed (Rome et al., 1996; Rome et al., 1999). It is possible that the observed faster activation rate (without a concomitant speeding up of V_{\max}) might reflect a faster crossbridge attachment rate constant. This faster rate would permit the observed faster rise in force but, by itself, would not lead to a faster V_{\max} (which is dependent on crossbridge detachment rate constant). Ultimately, biophysical measurements must be performed to determine the mechanism responsible for the faster activation rate.

Regardless of the exact mechanism of thermal acclimation, our work has demonstrated an impressive acclimation effect on contractile properties. It shows that the rate of activation is an important determinant of power output during oscillatory contractions and that a change in the activation kinetics can result in substantial increases in power generation *in vivo*. It has been assumed by some authors that V_{\max} and relaxation rate are the major determinants of power output (e.g. Marsh, 1990; Rome and Swank, 1992). However, it has previously been recognized that, at relatively high oscillation frequencies, slow activation rates can in fact limit power production because force cannot rise to a sufficient level in the reduced time prior to shortening (e.g. Josephson, 1993; Lutz and Rome, 1994; James et al., 1996; Curtin et al., 1998). Our study provides the first empirical demonstration of which we are aware of a large increase in power output associated with the increased rate of activation alone.

More power from changes in the muscle stimulation pattern

Not all the increase in power is due to a change in the contractile properties of the muscle. The 20% reduction in stimulus duty cycle measured in cold-acclimated scup compared with warm-acclimated scup in the accompanying paper (Rome and Swank, 2001) resulted in an up to twofold

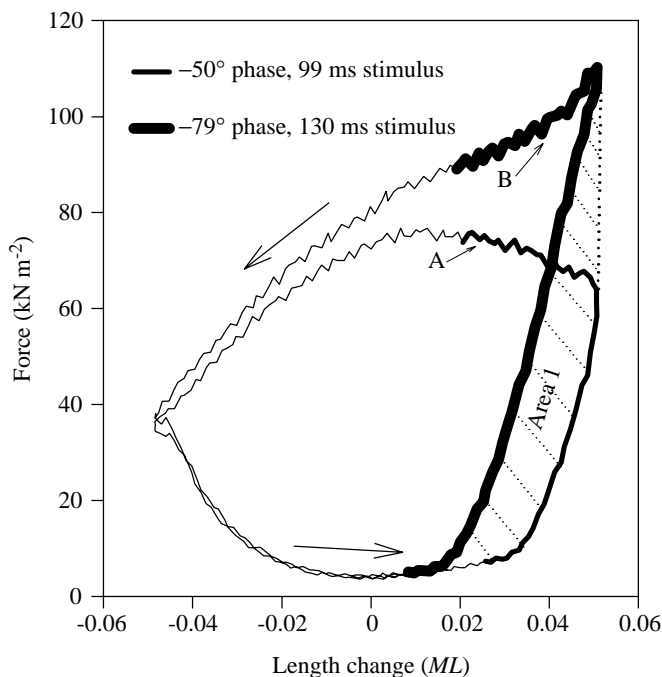


Fig. 8. Changes in power due to shifting phase (stimulus start time) in a POST muscle work loop. Loop A conditions are the same as warm-acclimated POST muscle at 30 cm s^{-1} at 10°C . Loop B has the same conditions except that the stimulus has been shifted to start 30 ms earlier. Note the higher force levels in loop B at the start of shortening. Area 1 is the work that would be gained by having a faster activation rate instead of shifting the start of the stimulus to a more negative phase. *ML*, muscle length. The timing of the stimulus is denoted by the thicker section of the work-loop trace.

increase in power production (Fig. 3). Power increased primarily in muscle from the MID and POST positions, although greater differences in duty cycle were measured at the ANT-1 and ANT-2 positions. There are two factors that contribute to the smaller increase in power output in the anterior positions despite the larger reduction in stimulus duty cycle. First, the anterior muscle needs a larger change in duty cycle to produce an increase in power output because the *in vivo* duty cycle measured from warm-acclimated anterior muscle is so much longer than optimal. For instance at 40 cm s^{-1} , the optimal stimulus duty cycle is 20% (Rome and Swank, 1992), but the *in vivo* duty cycle is 48%. Second, the ability to produce power at the anterior muscle positions is limited by the low *in vivo* strain of 1.5–3%. Thus, no matter how the stimulus duty cycle and phase are adjusted, the muscle will be unable to generate very large powers. In contrast, warm-acclimated *in vivo* duty cycles for the MID and POST positions are much closer to optimal (see Fig. 6 in Swank and Rome, 2000), and the strains are much larger and approach the optimal value for power production (5.6% for 40 cm s^{-1} ; Rome and Swank, 1992). Therefore, a small change in duty cycle for the MID or POST muscle results in a substantial increase in power. It should be noted that, at 30 cm s^{-1} , the cold-acclimated conditions did not lead to a significant increase in power production. For the POST position, this is because there was no difference between the warm- and cold-acclimated *in vivo* conditions. In the MID position, in contrast, there was an approximately 50% increase in mean power production; however, the large level of variation in the data for this position prevents this increase from being statistically significant.

Fig. 9. The relative total power output of the red musculature as a function of swimming speed at 10°C . The blue curve represents the power output of the red musculature from cold-acclimated fish and the red curve represents the power output from warm-acclimated fish. The relative power output of the red musculature was determined by integrating the power output of the red muscle along the length of the fish and then normalizing to the integrated power output of the red+pink muscle of fish swimming at 80 cm s^{-1} and 20°C (as outlined by Rome et al., 2000). We chose power output at 80 cm s^{-1} and 20°C as an estimate of the maximum power output that the combined red and pink musculature can generate during swimming. This seems reasonable because 80 cm s^{-1} is close to the swimming speed of initial white muscle recruitment at 20°C , and we know that both pink and red muscle are recruited at this swimming speed (Coughlin and Rome, 1999). The relative power required for swimming at slower speeds (black curve) was calculated on the basis of the assumption

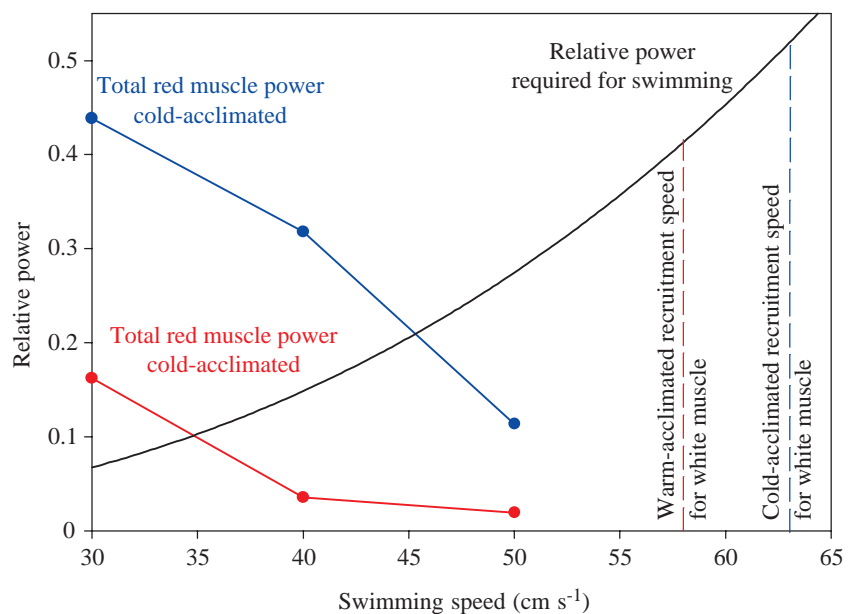
that the power required is proportional to the cube of swimming speed (Webb, 1978): relative power required $\propto (\text{speed}/80)^3$. This figure shows that there is a very large increase in total red muscle power output associated with thermal acclimation, which probably permits the cold-acclimated fish to swim at 40 cm s^{-1} using red muscle alone, whereas the warm-acclimated fish cannot. However, at swimming speeds of $58\text{--}63\text{ cm s}^{-1}$ (the initial speeds of white muscle recruitment for warm- and cold-acclimated fish), the red musculature generates very little power, only a small proportion of the total power required to swim at that speed.

As there was no significant difference in any of the other *in vivo* variables, the reduction in duty cycle appears to be solely responsible for the portion of increased power due to changes in *in vivo* conditions brought about by cold acclimation. This was demonstrated by the substantial increase in power output when a muscle was driven through work-loop conditions that were identical except that the stimulus duty cycle was appropriately reduced.

Thus, we have identified an impressive acclimation of the stimulation pattern. We have shown previously that the power output of red muscle from warm-acclimated scup is greatly reduced when the fish are placed at low temperatures (for approximately 24 h) and made to swim. This is because there was no reduction in stimulation duration (or tail-beat frequency) to compensate for the slowing of the muscle relaxation rate (Swank and Rome, 2000). By contrast, in the accompanying study (Rome and Swank, 2001), we found that stimulus duty cycle is reduced during cold acclimation. By driving muscles under their *in vivo* conditions, we were able to demonstrate that these changes do, in fact, lead to a substantial increase in the mechanical power output of the fish's musculature during swimming. This is the first report, of which we are aware, of this type of thermal acclimation.

Relevance to swimming performance

Cold acclimation appears to increase significantly the power output of the red muscle during swimming at cold temperatures, particularly in the MID and POST regions of the fish. Hence, we might expect a significant gain in swimming performance. Interestingly, in the preceding report (Rome and



Swank, 2001), we observed that the mean swimming speed of initial white muscle recruitment was only slightly (approximately 10%) higher in the cold-acclimated fish, but this was not statistically significant ($P=0.11$ for 10°C and $P=0.054$ for 20°C). How do we reconcile large power increases at the muscle level with apparently little or no increase in this particular measure of whole-animal swimming performance?

To assess better the effects of thermal acclimation on overall power generation during steady swimming, we determined the power generated by the red musculature by integrating along the body of the fish the product of power per kilogram and red muscle mass at each position (as outlined in Rome et al., 2000). By comparing these power values with the approximate power required for swimming (black curve, Fig. 9), we conclude that it is unlikely that warm-acclimated scup (red curve, Fig. 9) can power swimming at 40 or 50 cm s^{-1} at 10°C with just their red muscle (Rome et al., 2000; Coughlin and Rome, 1999). For instance, at 50 cm s^{-1} and 10°C , the fish's red musculature can generate a relative power of only 0.02, yet the fish needs to generate a value of approximately 0.27 to power swimming. Similarly, at 40 cm s^{-1} , the red muscle power outputs falls substantially below that required for swimming at this speed. In contrast, if the red muscle were fully recruited at 30 cm s^{-1} , it could generate a value of 0.16, which is higher than the power required (0.07). This analysis suggests that warm-acclimated fish would be capable of swimming at only 30 cm s^{-1} at 10°C with their red muscle alone.

Cold acclimation increases power generation considerably. Using the same normalization procedure, we calculated that, at 30, 40 and 50 cm s^{-1} , the red musculature of cold-acclimated fish (blue curve, Fig. 9) could generate relative power values of 0.44, 0.32 and 0.11, respectively. This corresponds to 2.7, 8.9 and 5.8 times more total power, respectively, than that in warm-acclimated fish. On the basis of the power required for swimming, these data suggest that, unlike warm-acclimated fish, the red muscle in cold-acclimated fish should be able to power swimming to at least 40 cm s^{-1} (i.e. the fish only needs to generate a power value of 0.15, whereas it is capable of generating up to 0.32; Fig. 9). However, this analysis also suggests that the red muscle of cold-acclimated fish would still be unable to power swimming at 50 cm s^{-1} .

This analysis has several important implications for swimming performance. First, pink muscle must be recruited to swim at 50 cm s^{-1} at 10°C in both acclimation groups. As discussed previously (Rome et al., 2000), pink muscle, because of its much faster activation rate, relaxation rate and V_{max} , can generate more mechanical power under *in vivo* conditions at cold temperatures (Coughlin et al., 1996; Coughlin and Rome, 1996). Hence, although its volume is only approximately one-third of that of the red muscle, it generates an increasing proportion of the power for swimming as the fish swims faster. Second, electromyographic experiments on warm-acclimated fish (Coughlin and Rome, 1999) have shown that pink muscle is substantially recruited by 40 cm s^{-1} , as would be predicted from Fig. 9. As the cold-acclimated fish apparently generates enough power with its red muscle to power swimming at

40 cm s^{-1} , we predict that, during cold acclimation, the speed at which pink muscle is initially recruited would be shifted to higher speeds, between 40 and 50 cm s^{-1} .

Finally, quantitative analysis of red muscle and pink muscle power output explains why we did not observe a larger change in the speed of initial white muscle recruitment in the cold-acclimated scup. At 50 cm s^{-1} , the red musculature from cold-acclimated fish generates approximately 45% of the total power needed to swim at that speed, whereas the red muscle from the warm-acclimated fish can generate only approximately 10%. The remainder of the power must be generated by the pink muscle, which is capable of generating considerable power at high swimming speeds (Coughlin et al., 1996; Coughlin and Rome, 1996). Between 58 and 63 cm s^{-1} (the swimming speeds at which white muscle is initially recruited in the warm-acclimated and cold-acclimated fish, respectively), we predict that the contribution by the red musculature to the total power needed for swimming will be far smaller than at 50 cm s^{-1} . The reason is that the power required for swimming increases substantially (Fig. 9), while the power output of the red musculature probably drops dramatically. The power output of the red musculature from cold-acclimated fish drops fourfold between 30 and 50 cm s^{-1} (Fig. 9) and will probably continue to decrease rapidly with increasing swimming speed, largely because of an increased tail-beat (muscle oscillation) frequency (Rome and Swank, 1992; Rome et al., 2000). Hence at 58– 63 cm s^{-1} , the power output of the red muscle would be quite small for both acclimation groups [probably less than 10% of the total power (red muscle+pink muscle) required for swimming]. Thus, even though the red musculature of cold-acclimated fish generates many times the power of the red musculature of warm-acclimated fish, the fact that the red muscle makes such a small contribution to the total power generated at 58– 63 cm s^{-1} explains why there is little or no increase in the initial speed of white muscle recruitment.

Despite not increasing the white muscle recruitment speed, the ability of red muscle in cold-acclimated fish to generate higher power probably results in several important improvements in cold-temperature swimming performance at moderate cruising speeds. First, because the red muscle of cold-acclimated fish is able to generate far more power during swimming, it is almost certainly more efficient (i.e. it generates more mechanical work for a given metabolic energy input). Although we did not measure the efficiency of red muscle directly, the higher efficiency in cold-acclimated fish is readily apparent during swimming at a speed of 40 cm s^{-1} . At this speed, the efficiency (expressed as mechanical power output/metabolic power input) of most of the red muscle in the warm-acclimated fish must be less than zero because the power output is negative. In contrast, the red muscle in the cold-acclimated fish generates positive work and thus must have a positive value for efficiency. Because the mechanical power required for swimming at a given speed is the same in both cold- and warm-acclimated fish, the higher efficiency probably results in the energetic cost of swimming at a given speed being significantly

lower in cold-acclimated fish. Second, red muscle has a higher mitochondrial density than pink muscle (Johnston et al., 1977; Johnston, 1983) and, therefore, a higher aerobic capacity and greater fatigue-resistance. Being able to generate more power with the highly aerobic red muscle, and thereby requiring less power output from the less aerobic pink muscle, may prolong the duration of steady swimming in cold-acclimated fish.

In conclusion, we have observed dramatic increases in the power output of red muscle during swimming associated with acclimation to the cold. One mechanism, a reduction in stimulus train duration (i.e. EMGs), is the first noted acclimation of this type and leads to significant increases in power generation. Furthermore, the unanticipated increase in the rate of activation of the muscle increases power output as well. We hypothesize that, at moderate cruising speeds, these changes enable the cold-acclimated fish to swim with a lower energy cost and with reduced reliance on the less fatigue-resistant pink muscle. Both these abilities may increase the swimming speed at which prolonged aerobic muscle activity can occur and, thus, reduce the travel time for the long seasonal migrations (several hundred kilometres) in which scup engage.

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