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THE INFLUENCE OF THERMAL ACCLIMATION ON POWER PRODUCTION DURING SWIMMING

I. *IN VIVO* STIMULATION AND LENGTH CHANGE PATTERN OF SCUP RED MUSCLE

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Summary

Ectothermal animals are able to locomote in a kinematically similar manner over a wide range of temperatures. It has long been recognized that there can be a significant reduction in the power output of muscle during swimming at low temperatures because of the reduced steady-state (i.e. constant activation and shortening velocity) power-generating capabilities of muscle. However, an additional reduction in power involves the interplay between the non-steady-state contractile properties of the muscles (i.e. the rates of activation and relaxation) and the *in vivo* stimulation and length change pattern the muscle undergoes during locomotion. In particular, it has been found that isolated scup (*Stenotomus chrysops*) red muscle working under *in vivo* stimulus and length change conditions (measured in warm-acclimated scup swimming at low temperatures) generates very little power for swimming. Even though the relaxation of the muscle has slowed greatly, warm-acclimated fish swim with the same tail-beat frequencies and the same stimulus duty cycles at cold temperatures, thereby not affording the slow-relaxing muscle any extra time to relax.

We hypothesize that considerable improvement in the power output of the red muscle at low temperatures could be achieved if cold acclimation resulted in either a faster muscle relaxation rate or in the muscle being given more time to relax (e.g. by shortening the stimulus duration or reducing the tail-beat frequency). We test these hypotheses in this paper and the accompanying paper.

Scup were acclimated to 10 °C (cold-acclimated) and 20 °C (warm-acclimated) for at least 6 weeks. Electromyograms (EMGs) and high-speed cine films were taken of fish swimming steadily at 10 °C and 20 °C. At 10 °C, we found that, although there were no differences in tail-beat frequency, muscle strain or stimulation phase between acclimation groups, cold-acclimated scup had EMG duty cycles approximately 20 % shorter than warm-acclimated scup. In contrast at 20 °C, there was no difference between acclimation groups in EMG duty cycle, nor in any other muscle length change or stimulation parameter.

Thus, in response to cold acclimation, there appears to be a reduction in EMG duty cycle at low swimming temperatures that is probably due to an alteration in the operation of the pattern generator. This novel acclimation probably improves muscle power output at low temperatures compared with that of warm-acclimated fish, an expectation we test in the accompanying paper using the work-loop technique.

Key words: scup, *Stenotomus chrysops*, electromyogram, thermal acclimation, muscle, power, duty cycle, pattern generator.

Introduction

Ectotherms move in a kinematically similar manner over a wide range of temperatures (Rome, 1982; Rome, 1990). To move at a given speed, they need to generate the same mechanical power at each temperature (Rome et al., 1984; Rome, 1990). However, it has been known for many years that temperature has a large effect on the steady-state (i.e. during constant activation and shortening velocity) power-generating capability of muscle. The Q_{10} for the maximum shortening velocity (V_{max}) and steady-state power generation varies from approximately 1.6 to 3 (see Rall and Woledge, 1990; Marsh, 1990; Rome, 1990). Thus, to generate the same mechanical power for locomotion, it has been proposed that animals must recruit more muscle fibres (and eventually faster fibre types) at lower temperatures. This has been called ‘the compression of the recruitment order theory’, and it has the important corollary that, at cold temperatures, animals will run out of aerobic fibres

to recruit at slower speeds (Rome et al., 1984; Rome, 1990). Therefore, their maximum aerobic locomotory speed would unavoidably be reduced.

Following long-term exposure to cold temperatures, some species are able to increase the total mechanical power they can generate either by increasing the mass-specific steady-state power-generating capability of their muscle (i.e. by increasing V_{max}) (Johnston et al., 1985; Langfeld et al., 1991) or by simply adding more muscle (Johnston and Lucking, 1978; Sidell, 1980; Jones and Sidell, 1982). This increase in power output has been shown to improve their steady sustainable swimming performance at cold temperatures and has been referred to as thermal acclimation (Rome et al., 1985; Sisson and Sidell, 1987).

The development of the work-loop technique (Josephson, 1985) has revealed much larger effects of temperature on muscle performance and, hence, a greater need for compensation by thermal acclimation than had been previously recognized. For instance, it has been known for many years that temperature affects the non-'steady-state' properties of muscle (i.e. the rate of activation and the rate of relaxation), but the work-loop technique has demonstrated that these effects can have a substantial influence on muscle power generation. For example, the Q_{10} for net power generation during optimized work loops is substantially larger than the Q_{10} for V_{max} (e.g. the respective Q_{10} values for scup red muscle are 2.3 and 1.8, respectively) (Rome and Swank, 1992).

Recently, more detailed attempts to assess the influence of temperature on muscle performance *in vivo* have revealed even larger effects of temperature on muscle power output than shown by optimized work loops. For instance, by driving warm-acclimated scup red muscle bundles through the same length change and stimulation patterns that they undergo during swimming at different temperatures, it was found that, in the posterior region of the fish, the Q_{10} for power production while swimming at 40–50 cm s^{-1} is between 7 and 14. However, in more anterior regions, only negative power can be generated at cold temperatures, making the Q_{10} indeterminate (Rome et al., 2000; Swank and Rome, 2000). The mechanical power output of muscle performing oscillatory contractions is greatly reduced at low temperatures, not only because the relaxation rate of the muscle has been slowed by low temperature, but also because no extra time has been allowed for the muscle to relax: during swimming, the muscle is stimulated for the same duration and works at the same oscillatory (i.e. tail-beat) frequency (Swank and Rome, 2000).

This new appreciation of the problems associated with cyclical locomotion at low temperatures raises possibilities for exploring new mechanisms by which thermal acclimation can improve muscle and swimming performance. First, it is possible that a substantial improvement in power could be achieved without changing V_{max} (or steady-state power production) by speeding up the kinetics of relaxation and possibly activation. It is known that variations in intrinsic isometric relaxation rates are possible without changes in V_{max}. For instance, in scup, isometric relaxation rate varies approximately twofold along the length of the fish despite V_{max} being independent of position (Rome et al., 1993; Swank et al., 1997).

Second, changes in the output of the nervous system, a class of thermal acclimation that has not been the subject of much investigation, could play a significant role in improving muscle and locomotory performance at low temperatures. The mechanical power output of the muscle at a given tail-beat (i.e. oscillation) frequency could be increased considerably if the nervous system simply reduced the time for which a muscle was stimulated to compensate for slower relaxation rates at cold temperature (Rome and Swank, 1992). The reduced stimulus duty cycle would permit the muscle to be more relaxed during relengthening and, hence, to generate greater net mechanical power. We have previously shown that such a reduction in the stimulus duty cycle does not occur in warm-acclimated scup during acute exposure to cold temperatures (Swank and Rome, 2000), but it is possible that it might occur in response to long-term thermal acclimation.

In this first paper, we examine whether acclimation affects the *in vivo* stimulus duration, strain and stimulus phase during swimming at different temperatures in scup acclimated to low and high temperature. In the accompanying paper (Swank and Rome, 2001), we use the work-loop technique to address whether differences in these variables between cold-acclimated and warm-acclimated scup result in the increased power output of the red muscle. Further, we test whether any changes occur in the mechanical properties (e.g. $V_{max,}$ kinetics of activation and relaxation) of red muscle that might also increase power production. Finally, we explore what relevance changes in muscle power output may have for swimming performance.

Materials and methods

Many of the experimental procedures used in this study have been described previously in detail (Rome et al., 1993; Swank and Rome, 2000) and are described below only briefly.

Fish

Scup (*Stenotomus chrysops* L.), 19–23 cm fork length, were caught by hook and line in Woods Hole, MA, USA, from an ambient water temperature of 12 °C. They were acclimated to either 10 °C (cold-acclimated) or 20 °C (warm-acclimated) in flowthrough tanks for at least 6 weeks. Scup were fed daily on a mixture of food pellets and chopped clams, and were maintained on a 12 h:12 h light:dark photoperiod.

Electromyography

Electromyograms (EMGs) were recorded from the red muscle at four places along the length of the fish (ANT-1, ANT-2, MID, POST; 29 %, 40 %, 54 %, and 70 %, respectively, of fork length from the snout) and from one position in the white muscle. The anaesthesia, initial positioning of the electrodes, implantation procedure and post-experiment verification of electrode positioning were as described previously (Swank and Rome, 2000). EMGs were

recorded with Grass P511 preamplifiers fitted with high-impedance probes and recorded at 5 kHz with a PC-based A/D system (Datapac by Run Technologies; as in Swank and Rome, 2000).

Filming of scup swimming

Fish were filmed from above at 200 frames s^{-1} with a Locam (Redlake) high-speed cine camera fitted with a video viewfinder (as in Rome et al., 1992). All swimming sequences were simultaneously videotaped to provide immediate feedback about the quality of each film sequence and to provide an accurate record of the experiment.

Muscle length changes

Muscle length changes were determined as the product of the curvature of the backbone (as in Rome and Sosnicki, 1991) and a constant derived from a calibration equation of sarcomere length (*SL*) *versus* backbone curvature and distance from the backbone (Rome et al., 1992). The magnitude and phase of strain recordings obtained using this 'anatomical-cine' procedure have previously been shown to be the same as those determined by the independent technique of sonomicrometry (Coughlin et al., 1996). To determine more accurately the timing of muscle length amplitude maxima, and hence phase (Fig. 1), we used a three-step analysis procedure (including spectral analysis, digital filtering and curve-fitting with a spline routine) which has been described previously (Swank and Rome, 2000). The procedure used to filter the data did not cause any phase shift in the length maxima.

Duty cycle determination

Duty cycle was calculated from the average duration of five EMG bursts divided by the tail-beat period of the fish. Burst duration was measured from the first to the last spike of an EMG burst (see Fig. 2 for examples). This manual method of duty cycle measurement was compared with an automated method of measuring duty cycle. The on- and off-times of an EMG burst were automatically selected using a software routine based on spike amplitude after differentiating and rectifying the EMG traces. Therefore, EMG spikes were separated from background noise not only by amplitude, but also by slope. This procedure removed motion artefacts occurring at the end of the EMG burst. In addition, the number of EMG spikes per 20 ms bin was counted using custom-designed signal-processing macros (Datapac, Run Technologies) based on criteria developed previously (Rome et al., 1992).

Electrical and mechanical wave speed

Electrical wave speed is defined as the speed at which the wave front of electrical activity (i.e. the EMG onset time) passes the EMG electrodes situated along the length of the fish. The mechanical wave speed is defined as the speed at which a characteristic of the bending wave (in this case, maximal muscle length) moves past the positions along the length of the fish where muscle length was being measured.

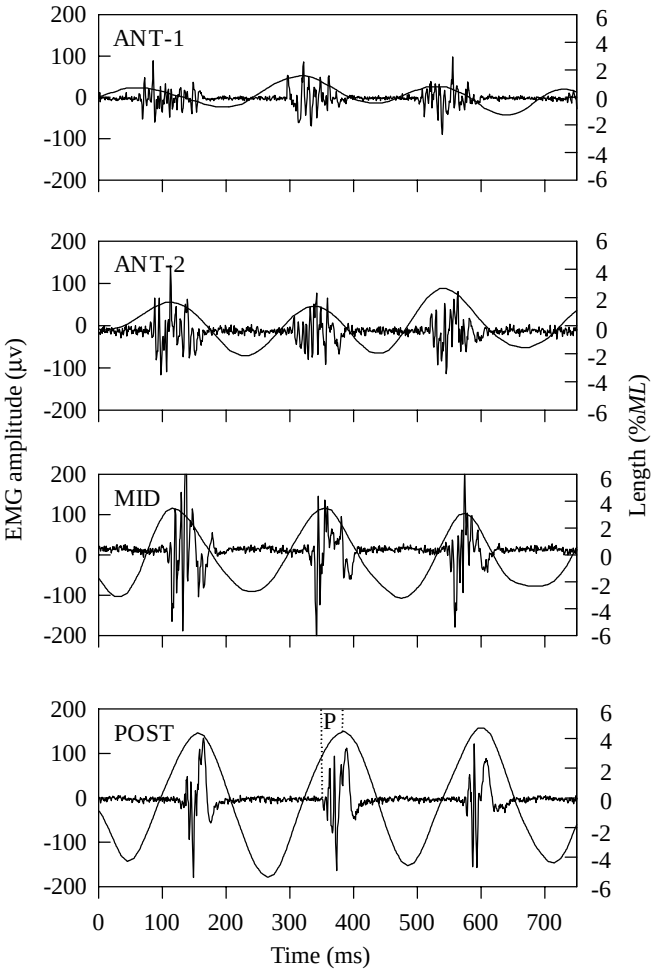

Fig. 1. Red muscle length (*ML*) change and electromyograms (EMGs) from the four longitudinal electrode positions on a cold-acclimated scup swimming at 50 cm s^{-1} at 10 °C. The length trace was produced by digitizing the outline of a swimming scup from high-speed cine films. A computer program calculated length changes from the backbone curvature and previous measurements of sarcomere lengths in frozen scup (Rome and Sosnicki, 1991; Rome et al., 1992). The length traces were smoothed with an eight-pole Butterworth filter (Matlab). The EMGs were recorded with bipolar electrodes. Length traces and EMGs were synchronized using an electronic device described previously (Rome, 1995). Phase (P) determination is shown for the POST position.

Combining EMG and standard length changes

To drive the isolated muscles accurately through the measured *in vivo* variables, it was essential to obtain very accurate synchronization of EMG and length changes. As described previously (Rome, 1995), we used a digital synchronization device developed to provide resolutions of better than one frame (±0.2 ms). Phase was defined as the on-time of an EMG burst with respect to the beginning of muscle shortening. Hence, at a phase of 0 °, the EMG started at the moment the muscle started shortening, whereas at a phase of –60 °, the EMG started one-sixth of a cycle prior to muscle shortening (Fig. 1).

Experimental protocol

Fish were given 24 h to recover from anaesthesia and 48 h between swimming bouts at different temperatures. Fish were first made to swim at their acclimation temperature and then 48 h later at the acclimation temperature of the other group. The water temperature was slowly (over 24 h) changed from the fish's acclimation temperature to the new acute swimming temperature. The fish were then given an additional 24 h to adjust prior to swimming.

Our goal was to analyze both the slowest speed and the fastest speed at which the fish could swim steadily at each temperature without recruitment of their white muscle. Scup were filmed swimming at 20, 30, 40, 50 and 60 cm s^{-1} at 10 °C and at 40, 50, 60, 70, 80 and 90 cm s^{-1} at 20 °C. Because of individual variation, to ensure that each fish could swim at the chosen speed, we analyzed swimming speeds of 80 cm s^{-1} and 50 cm s^{-1} at 20 °C and of 50 cm s^{-1} and 30 cm s^{-1} at 10 °C. Note that some of the data for warm-acclimated scup are taken from Swank and Rome (Swank and Rome, 2000).

Additional speeds were recorded on video for determination of the exact maximum steady swimming speed. These were 70 and 80 cm s^{-1} at 10 °C and 100 and 110 cm s^{-1} at 20 °C. The maximum steady swimming speed powered by the aerobic red and pink muscle was taken as the speed at which the scup could swim for 10 tail beats, holding the same position, without recruiting its white muscle (Rome et al., 1984; Rome et al., 1990).

Statistical analyses

Statistical analyses were performed using SigmaStat software (Jandel). All values are reported as the mean ± S.E.M. To compare means, *t*-tests and analysis of variance (ANOVA) were used. Two-way ANOVA was performed to make general statements about acclimation groups, and *t*-tests were also run to determine the statistical significance of differences at each position along the body of the fish. This procedure is based on our previous measurements demonstrating that muscle function is very different at different positions along the fish and, hence, that positions should be treated independently. Statistical significance was set at the 0.05 level.

Results

Swimming at 10 °C water temperature: EMG characteristics

EMG recordings from scup swimming at a water temperature of 10 °C revealed significant differences in duty cycle between acclimation groups. The duty cycles of cold-acclimated scup at the ANT-1, ANT-2 and MID positions were significantly shorter than those of warm-acclimated scup by 14–20 % at a swimming speed of 50 cm s^{-1} (Figs 2, 3A; Table 1). At 30 cm s^{-1}, duty cycle was 19–20 % shorter in cold-acclimated scup than warm-acclimated scup at the ANT-2 and MID positions (Fig. 4A; Table 1).

The differences in EMG duty cycle were due to an earlier cessation of activity because there was no change in the phase of stimulus onset time with acclimation. Controlling for position, EMG phases measured in cold-acclimated scup at 50 cm s^{-1} and 30 cm s^{-1} were not statistically different from EMG phases in warm-acclimated scup (Figs 3B, 4B).

The automated EMG analysis confirmed the differences found by manual analysis between cold- and warm-acclimated duty cycles (Table 1). This confirmation is important because the automated analysis rules out operator bias in determining EMG on- and off-times when comparing the acclimation groups. At 50 cm s^{-1}, the magnitude of the reduction in EMG durations in cold-acclimated scup was similar to that obtained using the manual method. At 30 cm s^{-1}, the automated analysis showed even larger reductions in EMG duty cycle than found using the manual method. Because of filtering, duty cycle values obtained using automated EMG analysis are systematically shorter than those obtained using the manual technique (as discussed by Rome et al., 2000).

In addition, we found that, at a swimming speed of 50 cm s^{-1}, the average number of EMG spikes per 20 ms bin was 24 % lower in cold-acclimated than in warm-acclimated scup (two-way ANOVA, P=0.001 for acclimation

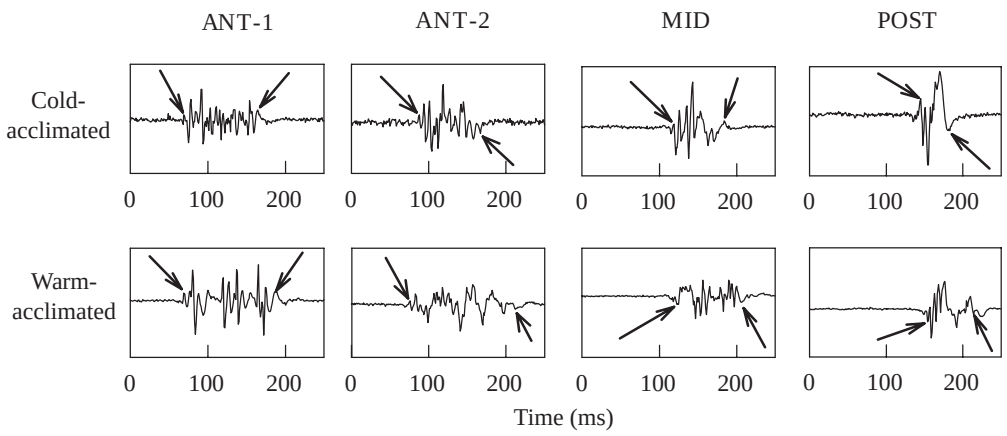

Fig. 2. Red muscle electromyograms (EMGs) from the four longitudinal electrode positions showing the longer EMG duration of warm-acclimated compared with cold-acclimated scup. The start times of the ANT-1 bursts were used to align the traces. Both fish swam at 50 cm s^{-1} at 10 °C. Arrows mark the start and stop times of the EMGs determined manually.

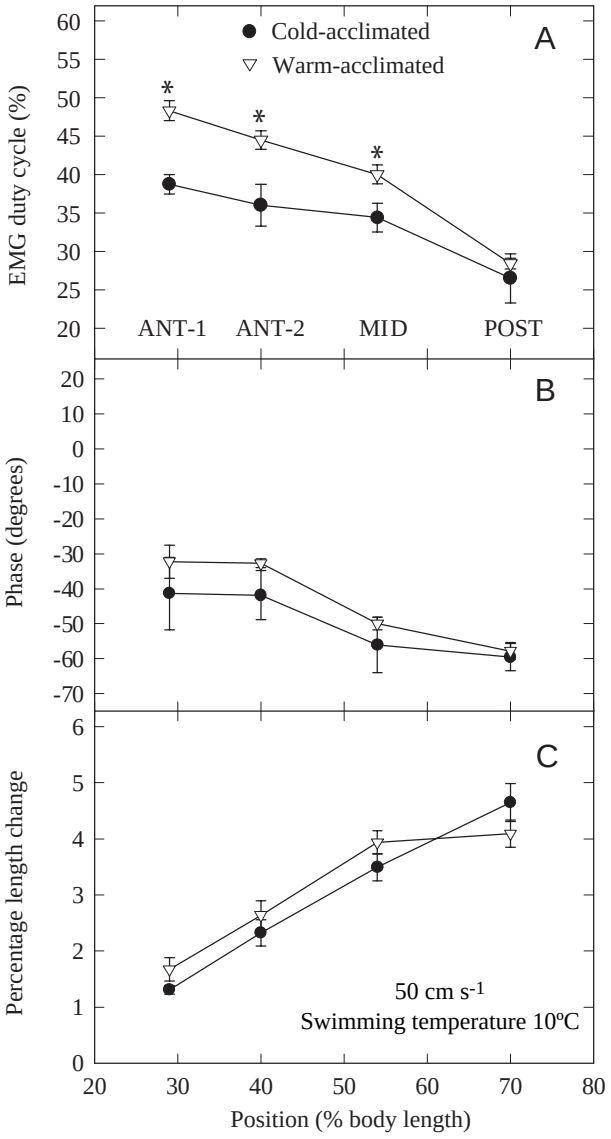

Fig. 3. EMG duty cycle, phase and percentage muscle length change (strain) for cold- and warm-acclimated scup swimming at 50 cm s^{-1} in 10 °C water. (A) Duty cycle is EMG duration divided by tail-beat period (two-way ANOVA, P<0.001 for acclimation temperature). An asterisk indicates a significant difference at a given body position due to acclimation (t-test, P<0.05). (B) Phase is measured as the on-time of the EMG burst with respect to muscle length change (a phase of 0 ° represents an EMG on-time at maximal muscle length) (two-way ANOVA, P=0.077 for acclimation temperature). (C) Strain is determined from high-speed cine films and anatomical studies (two-way ANOVA, P=0.423 for acclimation temperature). Values are means ± S.E.M. For warm-acclimated fish, N ranged from 6 to 7 and for cold-acclimated fish N ranged from 4 to 5.

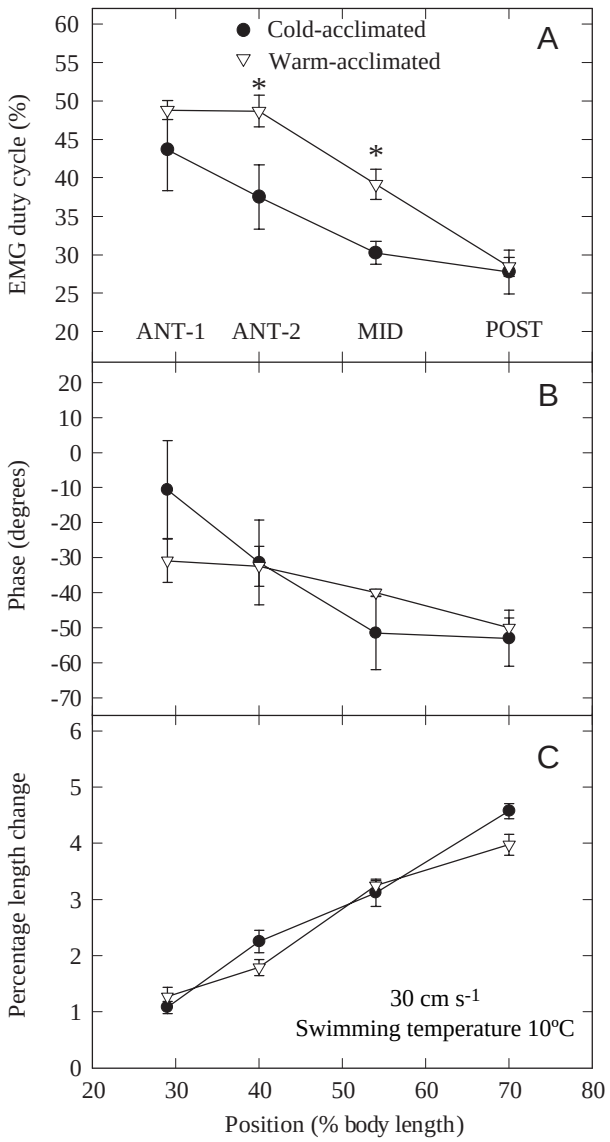

Fig. 4. EMG duty cycle, phase and percentage muscle length change (strain) for cold- and warm-acclimated scup swimming at 30 cm s^{-1} at 10 °C. Duty cycle was significantly shorter in the cold-acclimated scup (two-way ANOVA, P=0.003 for acclimation temperature). Neither phase (two-way ANOVA, P=0.88) nor percentage muscle length change (two-way ANOVA, P=0.138) was significantly affected by acclimation temperature. An asterisk indicates a significant difference at a given body position due to acclimation (t-test, P<0.05). Values are means ± S.E.M. For EMG and phase, N=5–7 for warm-acclimated fish and N=4–5 for cold-acclimated fish, except for the ANT-1 position where N=3. For strain, N=7 for warm-acclimated and N=5 for cold-acclimated fish.

temperature), except for the ANT-1 position. This finding provides further evidence for differences in the EMG pattern associated with thermal acclimation.

The EMG wave speed of the cold-acclimated scup increased with swimming speed and was faster than the mechanical wave speed, but was not significantly different from that for warm-acclimated scup for all swimming speeds at a swimming temperature of 10 °C (Table 2).

Swimming at 10 °C water temperature: kinematics and muscle length changes

Despite the significant differences in EMGs, the swimming

Table 1. *EMG duty cycles from warm- and cold-acclimated scup swimming at two different speeds in water at 10 °C*

	EMG duty cycle (%)			
	ANT-1	ANT-2	MID	POST
50 cm s^{-1}				
Manual[1]				
Cold	39±1.3* (4)	36±2.7* (5)	34±1.9* (5)	27±3.2 (4)
Warm	48±1.3 (6)	45±1.2 (6)	40±1.2 (7)	28±0.7 (7)
Automated[1]				
Cold	37±1.2* (4)	33±1.0* (4)	26±2.2* (5)	19±1.9 (5)
Warm	44±2.4 (5)	38±1.2 (5)	34±1.6 (6)	20±0.9 (7)
30 cm s^{-1}				
Manual[2]				
Cold	44±5.3 (3)	38±4.2* (4)	30±1.5* (4)	28±2.8 (4)
Warm	49±1.2 (5)	49±2.1 (6)	39±2.0 (7)	28±1.3 (7)
Automated[1]				
Cold	33±1.0* (2)	26±1.1* (4)	25±3.8* (5)	19±2.2* (5)
Warm	51±2.0 (4)	43±2.8 (4)	36±2.8 (5)	25±0.5 (5)

The two ways of measuring duty cycle, manual and automated, are described in the text.

Values are means ± S.E.M. (*N*).

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

[1]Significantly different due to acclimation temperature (two-way ANOVA, $P<0.001$) and also significantly different by position (two-way ANOVA, $P<0.001$).

[2]Significantly different due to acclimation temperature (two-way ANOVA, $P=0.003$) and also significantly different by position (two-way ANOVA, $P<0.001$).

ANT-1, ANT-2, MID and POST are the recording sites along the fish (see Materials and methods).

kinematics of the two acclimation groups were not different at 10 °C. The tail-beat frequency of cold-acclimated scup swimming at 50 cm s^{-1} was 4.05 Hz, and tail-beat frequency was 2.89 Hz at 30 cm s^{-1} (Table 2). These values were not statistically different from warm-acclimated scup tailbeat frequencies of 4.0 Hz and 2.85 Hz at 50 and 30 cm s^{-1} respectively (*t*-test, $P=0.793$ for 50 cm s^{-1} and $P=0.691$ for 30 cm s^{-1}). In addition, although mechanical wave speed increased with swimming speed, there was no significant difference between cold-acclimated and warm-acclimated scup (Table 2). Finally, muscle strain was nearly identical at all positions for the two acclimation groups at swimming speeds of 50 cm s^{-1} and 30 cm s^{-1} (Figs 3C, 4C). The strain amplitude values were somewhat lower than we had reported previously using unfiltered data (Rome et al., 1992) because those raw tracings appeared to have less noise than those reported here.

Table 2. *Tail-beat frequency, EMG wave speed and mechanical wave speed of warm- and cold-acclimated scup swimming at 10 °C and 20 °C*

Acclimation	Swimming speed (cm s^{-1})	Tail-beat frequency (Hz)	Mechanical wave speed (body lengths s^{-1})	EMG wave speed (body lengths s^{-1})
Swimming at 10 °C				
Cold	30	2.89±0.07	3.4±0.7	3.7 ±.07
Warm	30	2.85±0.06	2.8±0.2	3.5±0.4
Cold	50	4.05±0.20	5.8±0.6	7.3±0.9
Warm	50	4.00±0.08	4.7±0.2	6.0±0.3
Swimming at 20 °C				
Cold	50	4.76±0.24	4.4±0.4	5.6±0.2
Warm	50	4.69±0.28	4.8±0.4	6.1±0.2
Cold	80	6.75±0.16	8.5±0.8	9.1±0.9
Warm	80	6.38±0.28	8.6±0.8	8.9±0.4

Values are means ± S.E.M. N=5–6 for each value.

No statistically significant differences due to acclimation temperature were found for any of the values (*t*-test).

EMG wave speed is faster than mechanical wave speed ($P=0.046$, two-way ANOVA).

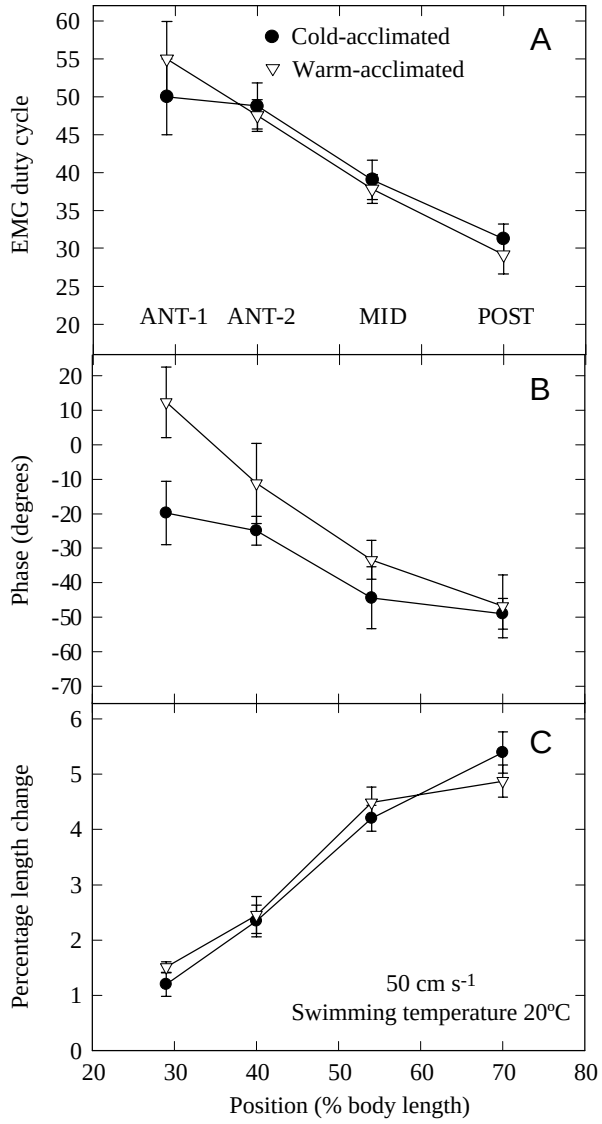

Fig. 5. EMG duty cycle, phase and percentage muscle length change (strain) for cold- and warm-acclimated scup swimming at 50 cm s^{-1} at 20 °C. Neither duty cycle (two-way ANOVA, P=0.349 for acclimation temperature) nor strain (two-way ANOVA, P=0.815 for acclimation temperature) was significantly different between the cold-acclimated and warm-acclimated scup. Excluding the ANT-1 data (see text and Swank and Rome, 2000, for rationale), phase was not significantly different either (two-way ANOVA, P=0.19 for acclimation temperature). Values are means ± S.E.M. For EMG and phase, N=5–6 for warm-acclimated fish and N=4–5 for cold-acclimated fish, except for the ANT-1 position where N=2 for cold-acclimated fish. For strain, N=6 for warm-acclimated and N=5 for cold-acclimated fish.

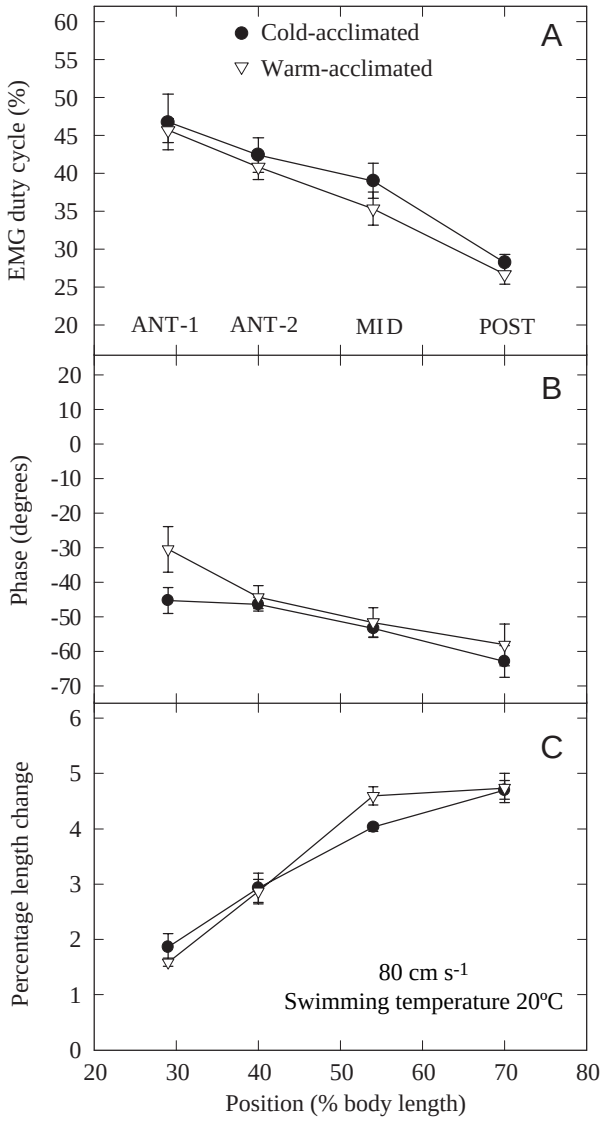

Fig. 6. EMG duty cycle, phase and percentage muscle length change (strain) for cold- and warm-acclimated scup swimming at 80 cm s^{-1} at 20 °C. Duty cycle (two-way ANOVA, P=0.176 for acclimation temperature), phase (two-way ANOVA, P=0.088 for acclimation temperature) and strain (two-way ANOVA, P=0.655 for acclimation temperature) were not significantly different between cold-acclimated and warm-acclimated scup. Values are means ± S.E.M. For EMG and phase, N=6–7 for warm-acclimated fish and N=4–5 for cold-acclimated fish. For strain, N=6–7 for warm-acclimated and N=5 for cold-acclimated fish.

Thus, in the accompanying paper (Swank and Rome, 2001), we also measure power production using the strains found by Rome et al. (Rome et al., 1992).

Swimming at 20 °C water temperature: EMG characteristics

Although significant differences were observed while swimming at 10 °C, there were no differences in EMG characteristics between warm- and cold-acclimated scup at a swimming temperature of 20 °C. At 50 cm s^{-1} and 80 cm s^{-1}, the EMG duty cycles for cold-acclimated fish were nearly identical to those for warm-acclimated fish (Figs 5A, 6A). At 50 cm s^{-1} and 80 cm s^{-1}, there was no difference in phase between acclimation groups (Figs 5B, 6B) excluding the ANT-1 position at 50 cm s^{-1}. As noted previously (Swank and Rome, 2000), the values at this position and speed may depart from the general pattern (i) because at 20 °C 50 cm s^{-1} represents a

gait transition speed for scup and (2) because the low magnitude of EMGs and strain at this speed and temperature makes determination of phase prone to considerable error. No change was found in EMG wave speed due to acclimation (Table 2).

Swimming at 20 °C: kinematics and muscle length changes

As we observed at 10 °C, muscle kinematics did not differ between acclimation groups at a water temperature of 20 °C. Tail-beat frequencies and mechanical wave speeds were nearly identical between acclimation groups at 50 cm s^{-1} and at 80 cm s^{-1} (Table 2). The muscle length excursion that the cold-acclimated red muscle underwent at the four positions was the same as in warm-acclimated scup (Figs 5C, 6C).

Initial speed of recruitment of white muscle

The maximum speed at which cold-acclimated scup could swim steadily without recruiting their white muscle at 10 °C (63±1.0 cm s^{-1}, N=7) was not significantly faster than that for warm-acclimated scup (58±0.4 cm s^{-1}, N=5; t-test, P=0.11). Similarly, at 20 °C, cold-acclimated scup did not swim significantly faster prior to recruitment of their white muscle (86±0.8 cm s^{-1}, N=7) than warm- acclimated scup (81±0.5 cm s^{-1}, N=5; t-test, P=0.054).

Discussion

Previous analysis of the *in vivo* stimulation conditions and mechanics experiments on isolated scup red muscle led Rome and Swank to predict that the slowed muscle relaxation rate at low temperatures would greatly reduce net power output if the stimulus duration remained constant (Rome and Swank, 1992). However, if the nervous system were capable of reducing the stimulation duration during swimming at low temperatures, this could lead to a large increase in mechanical power output compared with the case where duty cycle is unchanged. Swank and Rome, however, found that, in warm-acclimated scup, the nervous system does not make any compensatory changes in stimulation duration (i.e. EMG duration) in response to acute (i.e. approximately 24 h) decreases in temperature; the stimulation durations were nearly the same at 10 °C and 20 °C (Swank and Rome, 2000). Thus, as predicted, this results in very low power output of the red muscle during swimming at 10 °C. It appears that, except for the slowest swimming speed (30 cm s^{-1}), the only way that warm-acclimated scup can power swimming at 10 °C is by recruiting their pink muscle, which has a faster relaxation rate and appears to be stimulated for a shorter time than the red muscle (Rome et al., 2000).

In this study, by contrast, we have found that, after acclimation to cold temperatures, the EMG pattern of the scup's muscle is altered in a manner that should significantly increase the power output of the red muscle at low temperatures. We first discuss possible mechanisms for the change in the EMG pattern and then briefly discuss its functional significance in terms of muscle power generation.

EMGs are a complex electrical waveform arising from depolarization of the muscle cells surrounding the electrodes and are not, therefore, a direct measure of the motor nerve input to the muscle. Although motor unit recruitment by the nervous system is a major determinant of EMG patterns, in theory, some aspects of the EMG pattern can be altered by changes in the electrical properties of the muscle or by neuromuscular transmission (Jayne et al., 1990).

Nonetheless, it seems very likely that the reduction in EMG duration that we observed represents an important alteration in the functioning of the pattern generator during cold acclimation. We arrive at this conclusion both by exclusion of other possible mechanisms and from observations in other systems supporting this possibility. In theory, a failure at the motor endplate (either pre- or postsynaptic) or a failure in the conduction of action potentials in the muscle cells could result in a reduction in EMG duration for a given action potential train from a motor neuron. This seems unlikely in the case of cold-acclimated scup, however. First, if one examines the EMG durations in the ANT-2 position at 50 cm s^{-1}, for instance, it seems unlikely that the reduction in EMG duration from 111 to 90 ms in the cold-acclimated fish is due to some time-dependent neuromuscular transmission or action potential failure because one can easily increase the EMG duration of the cold-acclimated fish to 132 ms simply by slowing the swimming speed to 30 cm s^{-1}. Second, although there have been observations of neuromuscular transmission failure at low temperatures, acclimation to the cold has been shown to improve (e.g. Lnenicka and Zhao, 1991), not to impede, synaptic transmission at low temperatures; thus, it seems unlikely that the cold-acclimated fish would have degraded neuromuscular transmission compared with warm-acclimated fish.

In contrast, there are many observations of EMG burst duration being coupled to the duration of the action potential train from innervating motor neurons (e.g. Wallen and Williams, 1984). Furthermore, in lamprey *Petromyzone marinus*, the motor neurons have been shown not to be part of the burst generator *per se* (Wallen and Lansner, 1984); instead, they transmit the action potential trains from the pattern generator to the muscle fibres. Finally, extensive experimental manipulations and modelling suggest that burst duration is malleable and is controlled in part by the effects of neuromodulators on the pattern generator (e.g. Lansner et al., 1998). Thus, we believe that the most likely mechanism for the reduction in EMG duration at low temperature is a modification in the operation of the pattern generator during thermal acclimation which, in turn, reduces the duration of action potential trains to the muscle at a given swimming speed.

If the change in EMG duration is indeed due to a change in the output of the pattern generator, it would mean that, over the long term (6 weeks in this study), the nervous system is flexible enough to adjust to colder temperatures. As discussed below, a reduced duty cycle in response to cold acclimation probably results in improved muscle power output during locomotion. There have been reports of the thermal

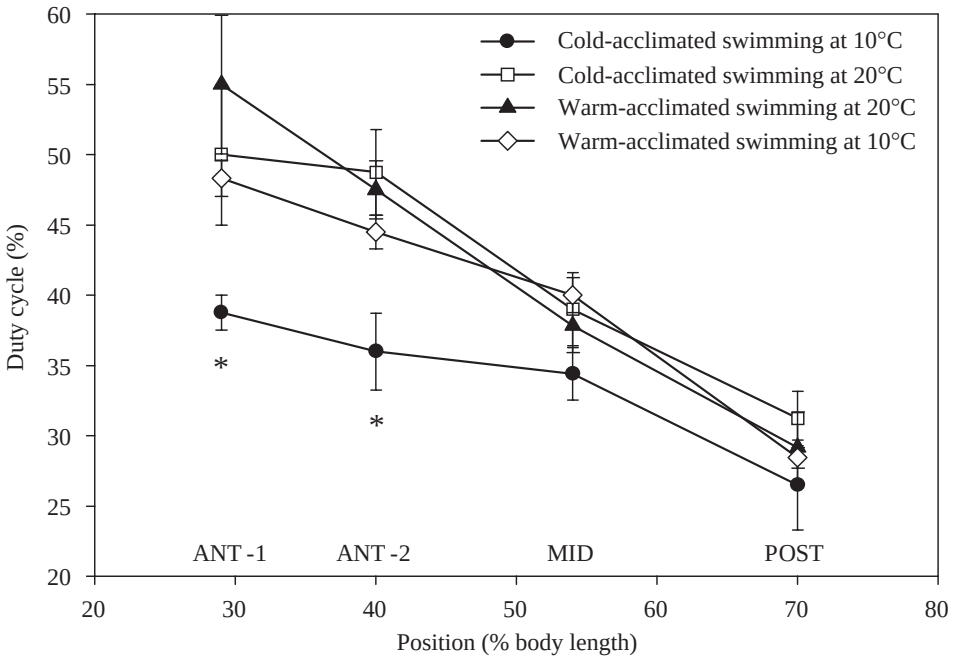

Fig. 7. Stimulus duty cycle during swimming at 50 cm s^{-1} for the four acclimation-temperature and acute-temperature conditions. For the warm-acclimated 10 °C and 20 °C swimming conditions and the cold-acclimated 20 °C swimming conditions, the stimulus duty cycles were nearly the same (there were no statistical differences). There was, however, a significant reduction in duty cycle in the cold-acclimated 10 °C swimming conditions (significant at ANT-1 and ANT-2 but not significant at MID and POST; see legend to Fig. 3).

dependence and thermal acclimation of molecular components of the nervous system and of the integrated nervous system (e.g. Jensen, 1972; Lagerspetz, 1974; Prosser and Nelson, 1981; Montgomery and Macdonald, 1984; Matheson and Roots, 1988; Harper et al., 1989; Montgomery and Macdonald, 1990; Tsai and Wang, 1997). This is the first report, of which we are aware, of a reduction in stimulation duration without a change in overall motor pattern (i.e. burst frequency) that potentially leads to a large change in performance.

Improving power output

What is most compelling about the observed thermal acclimation is the strong and direct effect it should have on mechanical power production. At 10 °C, the EMG duty cycles of warm-acclimated scup are far longer than optimal for power production (Rome et al., 2000). The muscles do not have sufficient time to relax, leading to very low power production. Cold acclimation results in approximately 20 % earlier cessation of the muscle stimulus (i.e. a 20 % shorter duty cycle), which gives the muscle more time to relax. This should reduce negative work and, thereby, increase net power production of scup red muscle at cold temperatures (Rome and Swank, 1992; Rome et al., 2000). The magnitude of this increase can only be determined using the work loop technique, and this is performed in the accompanying paper (Swank and Rome, 2001).

At a swimming temperature of 20 °C, there were no statistically significant differences in any of the *in vivo* characteristics between the acclimation groups. Furthermore, in warm-acclimated fish, the stimulus and length change pattern were the same at a swimming temperature of 10 °C as at 20 °C (Fig. 7). By contrast, in cold-acclimated fish, the stimulus duty cycle was significantly shorter at 10 °C than at 20 °C. Thus, of the four possible combinations of acclimation and swimming temperatures, the stimulus and length change pattern is the same in three cases (warm-acclimated at swimming temperatures of 10 and 20 °C and cold-acclimated at 20 °C). Only the cold-acclimated fish at a swimming temperature of 10 °C have a different (lower) stimulus duty cycle (Fig. 7). One interpretation is that the stimulus pattern for swimming at 20 °C represents a ‘default condition’. This may reflect the fact that scup spend much of their most active time at warmer temperatures (Neville and Talbot, 1964). Perhaps, the stimulus duty cycle for warm-acclimated fish at 10 °C does not differ from this ‘default’ because warm-acclimated scup spend very little time at cold temperatures. Only the cold-acclimated nervous system working at 10 °C differs from this ‘default’. This may be crucial during the long migrations of scup at cold temperatures (Neville and Talbot, 1964).

In summary, thermal acclimation to low temperatures appears to cause a significant change in the duration of the nervous system’s stimulation of the muscle during swimming. This reduction in stimulus duty cycle is predicted to result in a sizeable increase in power generation by the muscle, and this will be assessed empirically by work loop experiments in the accompanying study (Swank and Rome, 2001). Furthermore, there may also be changes in the contractile properties of the muscle during thermal acclimation. In the accompanying study, this will be determined together with its contribution to power output during swimming at low temperatures.

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References

Coughlin, D. J., Valdes, L. and Rome, L. C. (1996). Muscle length changes during fish swimming: a comparison of sonomicrometry and anatomical high-speed ciné techniques. *J. Exp. Biol.* **199**, 459–463.

Harper, A. A., Shelton, J. R. and Watt, P. W. (1989). The temperature dependence of the time course of growth and decay of miniature end-plate currents in carp extraocular muscle following thermal acclimation. *J. Exp. Biol.* **147**, 237–248.

Jayne, B. C., Bennett, A. F. and Lauder, G. V. (1990). Muscle recruitment during terrestrial locomotion: how speed and temperature affect fibre type use in a lizard. *J. Exp. Biol.* **152**, 101–128.

Jensen, D. W. (1972). The effect of temperature on transmission at the neuro-muscular junctions of the sartorius muscle of *Rana pipiens*. *Comp. Biochem. Physiol.* **41**A, 685–695.

Johnston, I. A. and Lucking, M. (1978). Temperature induced variation in the distribution of different types of muscle fibre in the goldfish (*Carassius auratus*). *J. Comp. Physiol.* **124**, 111–116.

Johnston, I. A., Sidell, B. D. and Driedzic, W. (1985). Force–velocity characteristics and metabolism of carp muscle fibres following temperature acclimation. *J. Exp. Biol.* **119**, 239–249.

Jones, P. L. and Sidell, B. D. (1982). Metabolic responses of striped bass *Morone saxatilis* to temperature acclimation. Alterations in metabolic carbon sources and distributions of fiber types in locomotory muscle. *J. Exp. Zool.* **219**, 163–171.

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

Lagerspetz, K. Y. H. (1974). Temperature acclimation and the nervous system. *Biol. Rev.* **49**, 477–514.

Langfeld, K. S., Crockford, T. and Johnston, I. A. (1991). Temperature acclimation in the common carp: force–velocity characteristics and myosin subunit composition of slow muscle fibres. *J. Exp. Biol.* **155**, 291–304.

Lansner, A., Kotaleski, J. H. and Grillner, S. (1998). Modeling of the spinal neuronal circuitry underlying locomotion in a lower vertebrate. *Ann. N.Y. Acad. Sci.* **860**, 239–249.

Lnenicka, G. A. and Zhao, Y. (1991). Seasonal differences in the physiology and morphology of crayfish motor terminals. *J. Neurobiol.* **22**, 561–569.

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

Matheson, D. F. and Roots, B. I. (1988). Effect of acclimation temperature on the axon and fiber diameter spectra and thickness of myelin of fibers of the optic nerve of goldfish. *Exp. Neurol.* **101**, 29–40.

Montgomery, J. C. and Macdonald, J. A. (1984). Performance of motor systems in Antarctic fishes. *J. Comp. Physiol.* A **154**, 241–248.

Montgomery, J. C. and Macdonald, J. A. (1990). Effects of temperature on the nervous system: implications for behavioral performance. *Am. J. Physiol.* **259**, R191–R196.

Neville, W. C. and Talbot, G. B. (1964). The fishery for scup with special reference yield and their causes. *U.S. Dept. Int. U.S. Fish Wildl. Serv., Spec. Sci. Rep. Fish.* **459**, 61.

Prosser, C. L. and Nelson, D. O. (1981). The role of nervous systems in temperature adaptation of poikilotherms. *Annu. Rev. Physiol.* **43**, 281–300.

Rall, J. A. and Woledge, R. C. (1990). Influence of temperature on mechanics and energetics of muscle contraction. *Am. J. Physiol.* **259**, R197–R203.

Rome, L. C. (1982). The energetic cost of running with different muscle temperatures in savannah monitor lizards. *J. Exp. Biol.* **97**, 411–426.

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

Rome, L. C. (1995). A device for synchronizing physiological data to cine film. *J. Biomech.* **28**, 333–338.

Rome, L. C., Choi, I., Lutz, G. and Sosnicki, A. A. (1992). The influence of temperature on muscle function in fast swimming scup. I. Shortening velocity and muscle recruitment during swimming. *J. Exp. Biol.* **163**, 259–279.

Rome, L. C., Funke, R. P. and Alexander, R. McN. (1990). The influence of temperature on muscle velocity and sustained performance in swimming carp. *J. Exp. Biol.* **154**, 163–178.

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. (1991). Myofilament overlap in swimming carp. II. Sarcomere length changes during swimming. *Am. J. Physiol.* **260**, C289–C296.

Rome, L. C. and Swank, D. (1992). The influence of temperature on power output of scup red muscle during cyclical length changes. *J. Exp. Biol.* **171**, 261–281.

Rome, L. C., Swank, D. and Corda, D. (1993). How fish power swimming. *Science* **261**, 340–343.

Rome, L. C., Swank, D. and Coughlin, D. J. (2000). The influence of temperature on power production during swimming. II. Mechanics of red muscle fibres *in vivo*. *J. Exp. Biol.* **203**, 333–345.

Sidell, B. D. (1980). Responses of goldfish (*Carassius auratus*, L.) muscle to acclimation temperature: Alterations in biochemistry and proportions of different fiber types. *Physiol. Zool.* **53**, 98–107.

Sisson, J. E. and Sidell, B. D. (1987). Effect of thermal acclimation on muscle fiber recruitment of swimming striped bass (*Morone saxatilis*). *Physiol. Zool.* **60**, 310–320.

Swank, D. and Rome, L. C. (2000). The influence of temperature on power production during swimming. I. *In vivo* length change and stimulation pattern. *J. Exp. Biol.* **203**, 321–333.

Swank, D. and Rome, L. C. (2001). The influence of thermal acclimation on power production during swimming. II. Mechanics of scup red muscle under *in vivo* conditions. *J. Exp. Biol.* **204**, 419–430.

Swank, D., Zhang, G. and Rome, L. C. (1997). Contraction kinetics of red muscle in scup: mechanism for variation in relaxation rate. *J. Exp. Biol.* **200**, 1297–1307.

Tsai, C.-L. and Wang, L. H. (1997). Effects of thermal acclimation of the neurotransmitters, serotonin and norepinephrine in the discrete brain of male and female tilapia, *Oreochromis mossambicus*. *Neurosci. Lett.* **233**, 77–80.

Wallen, P. and Lansner, A. (1984). Do the motoneurones constitute a part of the spinal network generating the swimming rhythm in the lamprey. *J. Exp. Biol.* **113**, 493–497.

Wallen, P. and Williams, T. L. (1984). Fictive locomotion in the lamprey spinal cord *in vitro* compared with swimming in the intact and spinal animal. *J. Physiol., Lond.* **347**, 225–239.