THE INFLUENCE OF TEMPERATURE ON MUSCLE FUNCTION IN THE FAST SWIMMING SCUP

I. SHORTENING VELOCITY AND MUSCLE RECRUITMENT DURING SWIMMING

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Summary

In this study, electromyography showed that scup can swim to a maximum speed of $80 \, \text{cm s}^{-1}$ with their red muscle whereas previous results showed that carp can swim to only $45 \, \text{cm s}^{-1}$. Our aim was to evaluate the adaptations that enable scup to swim nearly twice as fast as carp. Although we anticipated that, at their respective maximum speeds, the red muscle of scup would be shortening at twice the velocity (V) of carp muscle, we found that the values of V were the same (2.04 muscle lengths s^{-1}). At *any given swimming speed*, V was higher in carp than in scup because carp had a larger sarcomere length excursion and higher tail-beat frequency. The smaller sarcomere excursion in scup is primarily associated with using a less undulatory style of swimming (i.e. with a smaller backbone curvature). This less undulatory style of swimming may be an important adaptation that not only reduces V but may also reduce drag. At their respective *maximum speeds*, however, the 28 % lower sarcomere length excursion in scup is balanced by a 26 % higher tail-beat frequency, giving an equal V to that of carp. Although the scup in this study were somewhat longer than the carp in the previous one (19.7 *vs* 13.4 cm), we believe that many of the observed differences are species-related rather than size-related.

We also found that scup swam in a kinematically similar fashion at 10°C and 20°C. However, at 10°C, the scup could swim to only $54 \, \text{cm s}^{-1}$ before recruiting their white muscle whereas, at 20°C, they could swim to $80 \, \text{cm s}^{-1}$. The difference in speed of initial white muscle recruitment, as well as information on muscle mechanics (see the following paper), suggests that the scup compress their recruitment order into a narrow speed range at low temperatures, thereby recruiting more muscle fibres. Quantitative analysis of red muscle electromyograms in this paper supports this hypothesis.

Introduction

Some animals can locomote rapidly while others can locomote only slowly. One

would expect certain adaptations in the contractile properties and anatomical arrangement of muscle fibres that would permit both slow and fast movements to be made effectively. Although the components of the muscular system can be varied, it is reasonable to ask whether this variation is constrained to satisfy definite rules.

Recently, Rome and colleagues (Rome *et al.* 1988, 1990*a,b*; Rome and Sosnicki, 1990, 1991; Sosnicki *et al.* 1991) have evaluated two such constraints: the sarcomere length–tension relationship, and V/V_{max}, where V is the velocity at which the muscles shorten and V_{max} is the maximum velocity at which the fibres are capable of shortening. They found in carp (Sosnicki *et al.* 1991; Rome and Sosnicki, 1991) that fibres are anatomically arranged so that, no matter what the movement, the active fibres shorten over the optimal portion of the sarcomere length–tension curve (i.e. where maximum force is generated). During moderate movements, carp used red fibres with a parallel orientation and low gear ratio (change of backbone curvature/change of sarcomere length). When the animals make more extreme movements, the white fibres, which have a helical orientation and a higher gear ratio, are used. They also found that during locomotion fibres are only used at V/V_{max} values where power is, and efficiency is thought to be, maximal. When the fish needs to swim rapidly, it uses its white fibres with their higher gear ratio and higher values of V_{max} (Rome *et al.* 1988). In addition, they found that, despite changes in V_{max} associated with temperature, the range of V over which the muscle is used was adjusted so that muscles are used over the same range of V/V_{max} at different temperatures (Rome and Sosnicki, 1990; Rome *et al.* 1990*a*).

Although maximum swimming speed is quite difficult to induce and quantify, electromyography makes it relatively easy to define the swimming speed at which fish start to recruit their white muscle. Rome and colleagues have shown in carp (Rome *et al.* 1984, 1985) that at low swimming speeds only the slow red fibres are used, whereas at higher swimming speeds the fast white fibres are also used. The recruitment of the white muscle results in 'burst-and-coast swimming' and rapid fatigue (Rome *et al.* 1990*a*). Hence, the recruitment of white muscle represents a change in 'gait' and has marked physiological manifestations in fish. Finally, they showed that the swimming speed at which white muscle recruitment occurs is reproducible for a given fish and is similar among carp over about a twofold range of size (Rome *et al.* 1984, 1990*a*).

In this set of papers we compare the swimming capabilities, *in vivo* muscle shortening and isolated muscle properties in scup with the previously published values in carp (Rome *et al.* 1988, 1990*a*; Rome and Sosnicki, 1990). This comparison is interesting because preliminary experiments showed that scup can swim approximately twice as fast with their red muscle as can carp before recruiting their white muscle. This will give us the opportunity to examine adaptations involved in increased locomotory capabilities and to test further the validity of the aforementioned design constraints. We also examined the influence of temperature on muscle and locomotory performance to elucidate further the

neuromuscular mechanisms that fish use to enable them to locomote at different temperatures.

Materials and methods

The description of the swimming machine and techniques for ciné photography, film analysis and electromyography have been previously described in detail for carp (Rome *et al.* 1990*a*; Rome and Sosnicki, 1991). Below, the equipment and techniques are described only briefly, except where some important modifications have been made.

Animals

Scup (*Stenotomus chrysops* L.) were obtained from the Marine Biological Laboratories in Woods Hole. The fish were housed in fibreglass tanks through which fresh sea water (20°C) flowed. Before experimentation at 10°C, the flow rate of salt water was decreased and the temperature was maintained at 10°C with a compressor for at least 24 h. The fish were fed squid every other day and kept on a 14h:10 h light:dark cycle.

Measurement of sarcomere length during swimming

Our goal was to measure the changes in sarcomere length that the red muscle underwent during swimming. This involved (1) determining the relationship between sarcomere length (SL) in a 4 mm thick transverse slice of the fish (hence called steak) and the curvature of the backbone within the steak and then (2) measuring changes in the curvature of the backbone during swimming as in Rome *et al.* (1990*a*) and Rome and Sosnicki (1991).

To determine the relationship between SL and curvature, five scup (length 17–20 cm) were killed and bent into shapes similar to those observed during sustainable swimming. Two additional fish were left unbent to measure the sarcomere length in the straight fish. The bent and straight scup were held in place and allowed to go into rigor and then frozen in liquid nitrogen. They were photographed from above and the curvature of the backbone was determined as in Rome and Sosnicki (1991). Sarcomere length was measured from frozen longitudinal sections (8 μm) of steaks taken from three positions along the length of the fish: 38 %, 52 % and 68 % of the overall length. These positions were labelled anterior, middle and posterior, respectively.

Having established this relationship, sarcomere length of the lateral red muscle during swimming was calculated from the changes in curvature of fishes' bodies filmed during the tail-beat cycle. This involved digitizing the outline of the fish and determining the curvature of the backbone in each frame as detailed in Rome and Sosnicki (1991).

Five scup (17–20 cm in length) were filmed from above in a recirculating water treadmill (as in Rome *et al.* 1990*a*). The fish were filmed at 200 frames s^{-1} while swimming at 20–90 cm s^{-1}. In this study, the LOCAM (Redlake Corp) high-speed

camera was fitted with a video viewfinder (custom-modified VUCAM, Redlake Corp.) and a low light sensitivity CCD video camera (Sanyo). This camera system had several advantages. First, we were able to instantly review and assess whether the fish swam steadily during filming. This was accomplished by videotaping the entire swimming sequence and identifying the period during which the ciné filming occurred by slight darkening of the videotape caused by the motion picture shutter. Second, this technique permitted us to synchronize the electromyogram (EMG) (stored on audio tracts of video tapes) to muscle length changes determined from high-speed ciné film.

Because scup have a deeper trunk than carp, special care was taken to ensure that the scup were absolutely upright during filming and gave an appropriate silhouette. This sometimes involved using lights and other visual cues.

Fish swam at 10°C and 20°C, with at least 48 h of rest between bouts of exercise. Animals were held at the new swimming temperature for 24 h before filming. Fish were placed in the swim tube and allowed to adjust for at least 30 min with the water circulating at a moderate speed $(20-30 \, cm \, s^{-1})$ prior to experimentation. Usually three tail-beat cycles were analyzed at each swimming speed. Sarcomere lengths and shortening velocity (V) were calculated at the anterior, middle and posterior positions, as in Rome et al. (1990a).

Measurement of tail-beat amplitude and tail height

For each of the sequences used to determine V, the amplitude of the tail-beat was also measured. Generally, at least 10 tail-beat cycles were examined and excursions averaged.

In addition, the fish were filmed from the side at each speed using the LOCAM $(100 \, frames \, s^{-1})$ to examine fluctuations in tail height during swimming. We found the same rhythmical fluctuations in height as in Rome et al. (1990a) and used an average value calculated over about 8–10 tail beats.

Electromyography

The swimming speed at which white muscle recruitment occurred was determined at each temperature by monitoring the EMGs of the white muscle, and in some cases both the red and white muscle, using the general procedures outlined in Rome et al. (1990a). The EMG amplifiers were equipped with cut-off filters of 10 and 3000 Hz as well as a 60 Hz notch filter. The EMGs at each swimming speed were stored on the audio tracts (bandwidth of 20 Hz to 20 kHz) of the videotape while fish swimming was filmed. The flow velocity was increased in steps of $10 \, cm \, s^{-1}$ (smaller increments of $5 \, cm \, s^{-1}$ were used near the recruitment speed). The recruitment speed was taken to be half way between the first speed at which the fish was unable to maintain position without regularly using their white muscle and the speed immediately below it (as in Rome et al. 1984, 1990a).

In one fish, which had the cleanest EMGs (see Fig. 1), we quantified several important variables of the EMG according to the scheme of Jayne et al. (1990). Bursts were divided into sequential 20 ms bins beginning with the first spike of the

burst. The number and amplitude of spikes per 20 ms bin were identified by a custom software routine following digitization at 5–10 kHz with a Nicolet 4094 digital oscilloscope. Only peaks that exceeded 12 μV were counted. In addition, we measured the integrated rectified EMG per 20 ms bin using the Nicolet.

Statistics

Statistical differences between means were established at the $P \leq 0.05$ level using paired t-tests. Statistical differences between slopes of linear regression lines were also established using the t-test. All statistical tests were performed with SPSS-PC. Differences referred to in the text are statistically significant unless otherwise stated.

Results

Electromyography

The electromyography shows that scup used only their red muscle at relatively low steady swimming speeds and recruited their white muscle at higher swimming speeds (Fig. 1). The initial speeds of white muscle recruitment were 54 ± 1.9 cm s^{-1} ($\pm$s.e., $N=6$) at 10 °C and 80 ± 2.3 cm s^{-1} ($\pm$s.e., $N=6$) at 20 °C.

Figs 1, 2A, and 2B show that both the peak and mean amplitude of the red muscle EMG spikes increased with increasing swimming speed and that, at any given swimming speed, they were larger at 10 °C than at 20 °C. Fig. 2A,B also shows that, at 10 °C, the amplitude plateaued between 50 and 60 cm s^{-1} (the white muscle recruitment speed for this fish was approximately 62 cm s^{-1}). At 20 °C, however, both peak amplitude and mean amplitude increased with increasing swimming speed up to the highest swimming speed from which we recorded red muscle activity (84 cm s^{-1}).

The number of spikes per 20 ms bin increased fairly linearly with swimming speed up to the highest speed recorded at 20 °C (Fig. 2C). At 10 °C, however, the values increased linearly below 50 cm s^{-1} and plateaued between 50 and 60 cm s^{-1}. Unlike all other measures of activity, spike number per bin at 50 and 60 cm s^{-1} was significantly higher at 20 °C than at 10 °C.

The combined effects of spike amplitude and spike number were expressed in two ways. First, the mean spike amplitude was multiplied by the number of spikes per bin. This product (Fig. 2D) increased fairly linearly with swimming speed at 10 °C and then plateaued between 50 and 60 cm s^{-1}. At 20 °C, the product of mean amplitude and spike number increased in a slightly curvilinear fashion up to the highest swimming speed recorded. At each swimming speed the product was higher at 10 °C than at 20 °C because of the larger mean amplitude. However, the maximum value at 20 °C (occurring at 84 cm s^{-1}) was almost three-quarters of the maximum value at 10 °C, because the greater number of spikes nearly balanced the lower mean amplitude.

The second way in which the spike amplitude and spike number were combined is the integrated rectified area per bin. This had a similar form at both
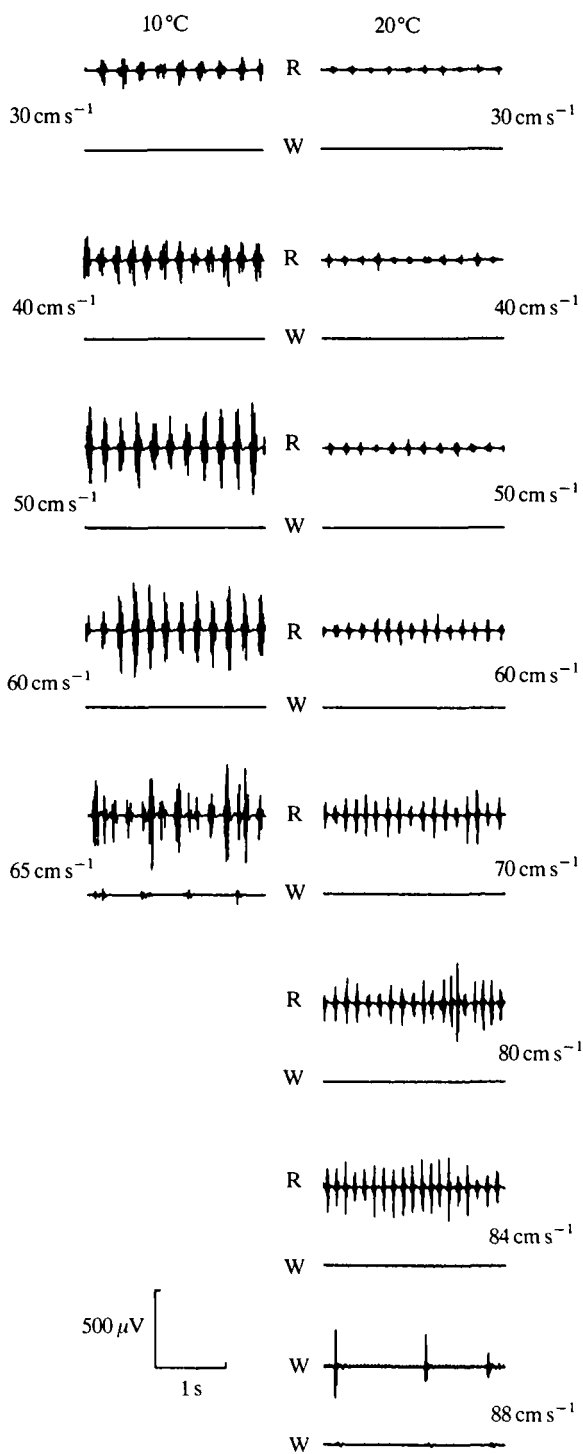
Fig. 1

Fig. 1. Electrical activity of red (R) and white (W) muscle during swimming at different speeds and temperature. This scup swam first at 10°C and 48 h later at 20°C. Electrical activities of red and white muscles were recorded from the same electrodes at both temperatures. At 88 cm s^{-1} at 20°C, white muscle was recorded from both sides of the fish. At low speeds only red muscle was active (each burst corresponding to a tail beat), whereas at higher speeds the white muscle was recruited as well. When the scup was at 20°C it could swim much faster without white muscle recruitment than it could at 10°C. The speed of initial white muscle recruitment was estimated as an average between the speed at which white muscle activity was first observed and that speed immediately below it (usually 5 cm s^{-1} slower).

temperatures to the product of amplitude and spike number. At 10°C, it increased linearly and plateaued between 50 and 60 cm s^{-1}, whereas at 20°C, the values increased in a fairly linear fashion to the highest swimming speed recorded. Again, at any swimming speed, the values were higher at 10°C than at 20°C.

To assess the role of passive membrane properties on the measure of EMG activity at different temperatures, we plotted the mean rectified area per bin as a function of the product of the mean amplitude and spike number (not shown; as in Jayne *et al.* 1990). The slope at 10°C was 1.4-fold higher than that at 20°C.

Sarcomere length of red muscle as a function of backbone curvature

Sarcomere length of the scup red muscle is proportional to $1/R$ (where R is the radius of curvature of the backbone), and the slope in the anterior position was about 1.9-fold steeper than that in the posterior position (Fig. 3A) because of the 2.3-fold greater distance between the strip of red fibres and the backbone. On average ($N=5$), the distances between the backbone and red muscle (or the half-thickness of the fish, A), are 1.28±0.04 cm (±s.e.), 0.95±0.06 cm and 0.56±0.09 cm for anterior, middle and posterior positions, respectively.

The dependency of SL on distance from the backbone is further demonstrated by the overlap of the data from all three positions on the fish, when plotted as a function of A/R (Fig. 3B). Thus, sarcomere length is a function of both radius of curvature and distance from the backbone and can be calculated from a simple linear relationship:

$$SL = 2.10 + 1.36A/R \qquad (r^2=0.98). \tag{1}$$

Note that A/R and $1/R$ are considered to be positive on the outside of the bend (convex) and negative on the inside of the bend (concave). In a straight fish, the SL of red muscle was 2.10–2.11 μm and it was not dependent on position (see Fig. 3). Furthermore, in the red muscle, there was little difference in SL between pre- (i.e. frozen prior to rigor) and post-rigor sections. In both cases the SL was 2.10–2.11 μm.

Sarcomere length changes during steady swimming

Sarcomere length during swimming was calculated from the curvature of the fishes' backbone in the films according to equation 1. Fig. 4 shows a typical record
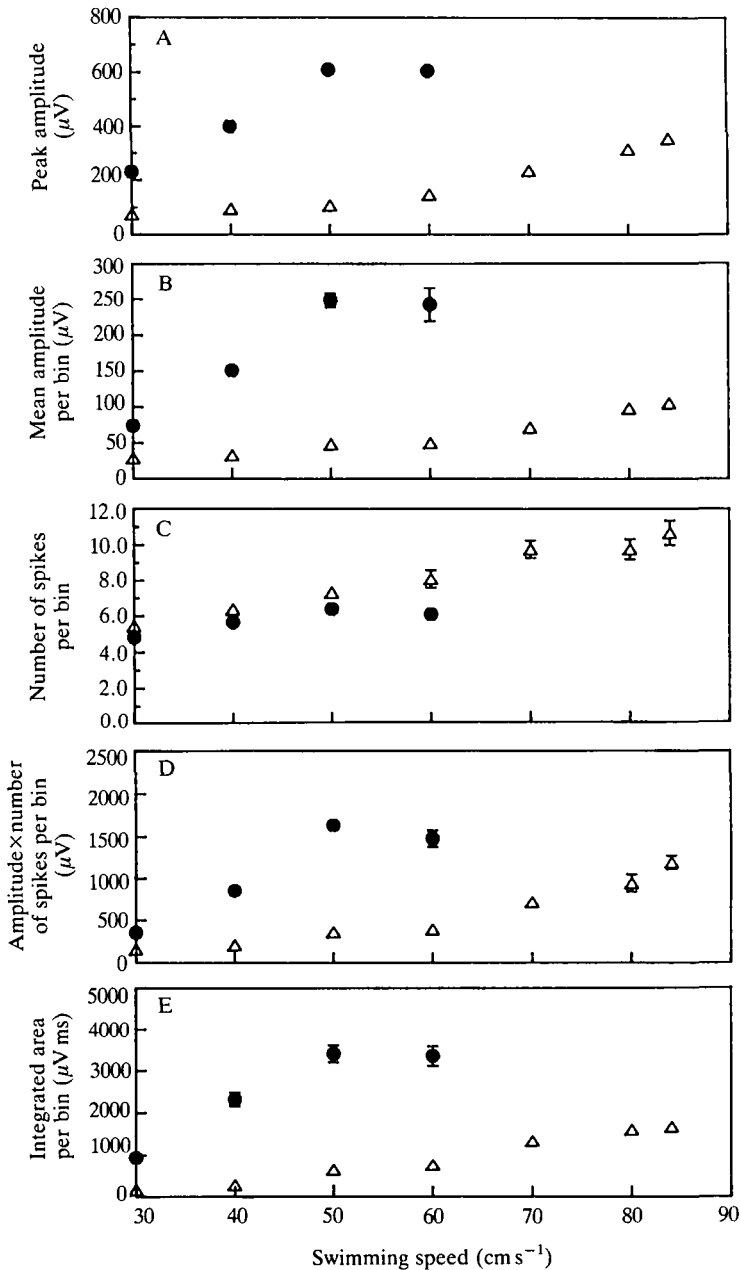
Fig. 2. Quantified electromyogram (EMG) of red muscle from one scup swimming at 10°C (●) and 20°C (△). Data points represent means of 11–29 bursts for peak amplitude (A) and integrated area (E) and means of 6–7 bursts for B–D. Each burst value (except for peak amplitude) is the average from 2–6 bins. Vertical bars represent ±1 s.e. At a given swimming speed, all the variables except number of spikes per bin were higher at 10°C than at 20°C. The maximum of all the variables at 10°C was reached between 50 and 60 cm s^{-1}, while values at 20°C increased fairly linearly up to the highest speed recorded.
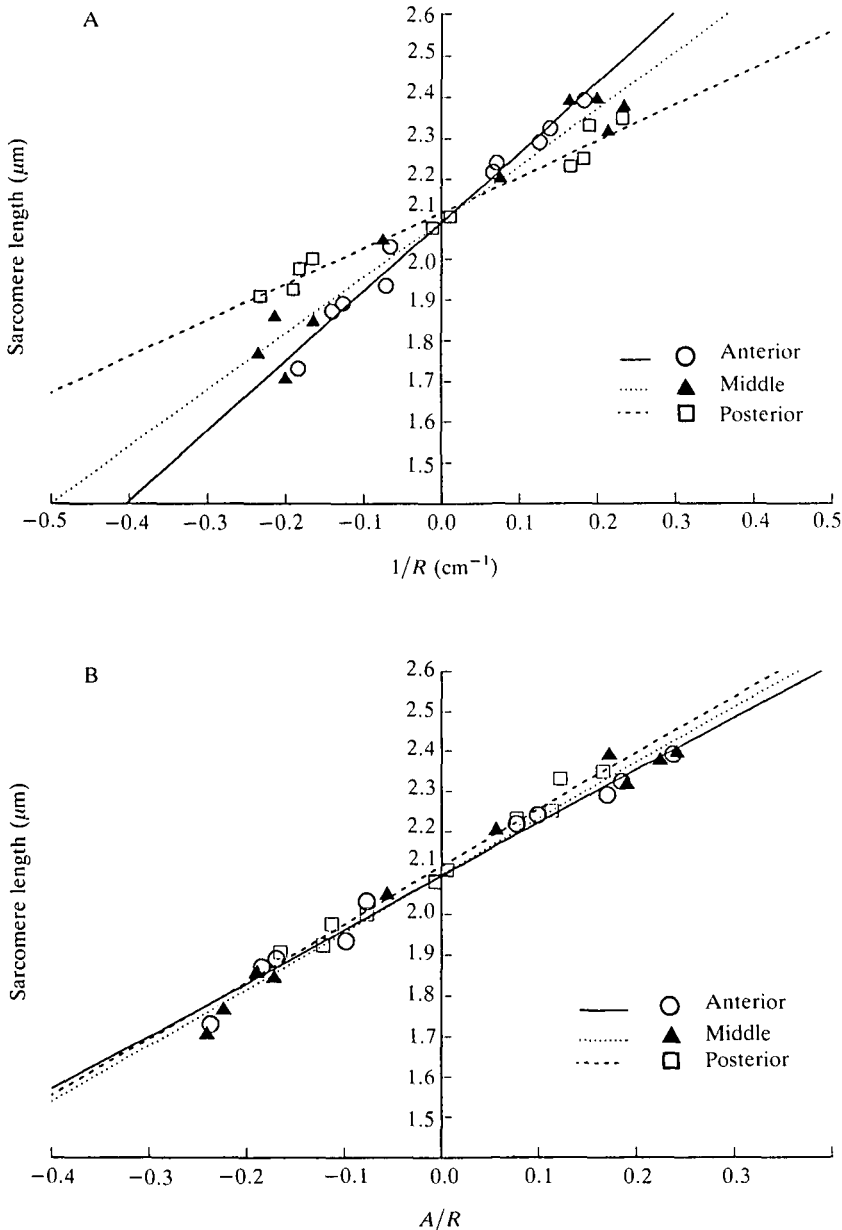
Fig. 3. Sarcomere length (SL) of red muscle as a function of curvature and distance from backbone. Sarcomere lengths were measured in frozen sections of fish allowed to go into rigor in various curved shapes. R is the radius of curvature of the backbone (in cm) and A is the distance from the backbone to the red muscle (in cm). (A) Note that the slope for the anterior points is much steeper than that for the posterior points owing to the greater distance from the backbone. Slopes are 1.71, 1.38, 0.89 μm cm, respectively, for anterior, middle and posterior points. (B) When plotted against A/R the data are nearly superimposed, showing that SL is a function of both curvature and distance to backbone. SL=2.10+1.36A/R, r^2=0.98, N=30.
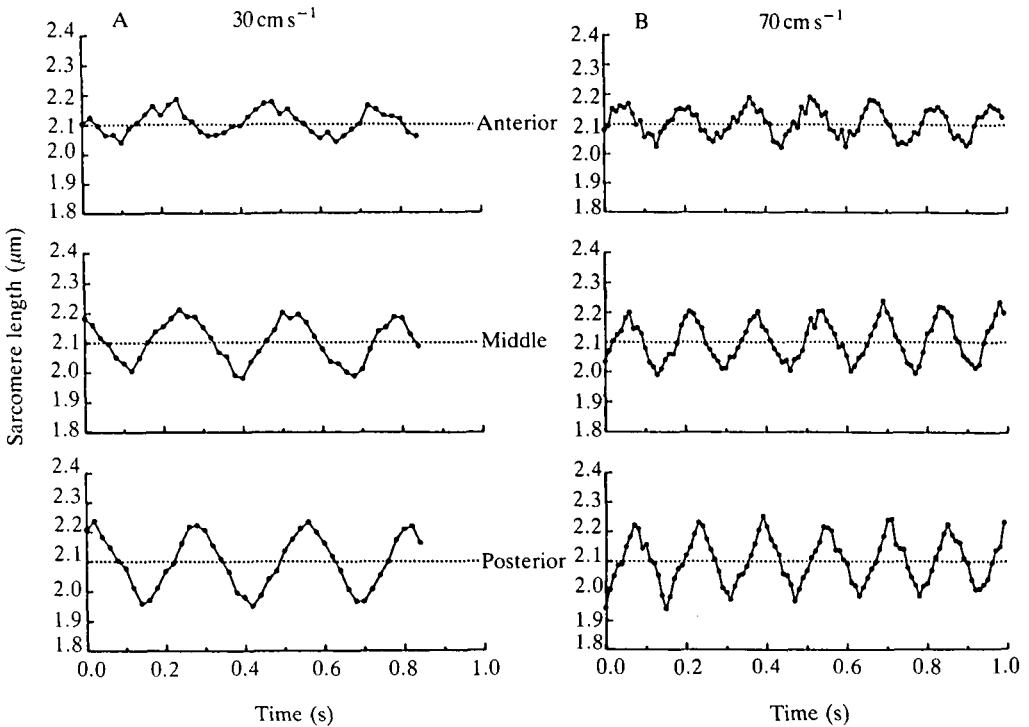
Fig. 4. Typical records showing changes in sarcomere length in anterior, middle and posterior positions of a fish swimming at $30\,\text{cm}\,\text{s}^{-1}$ (A) and $70\,\text{cm}\,\text{s}^{-1}$ (B) at 20°C. Sarcomere length excursion (average difference between shortest and longest lengths measured in a sequence) was calculated from the graph. Muscle velocity was calculated from the slope of the graph.

of the changes in sarcomere length at three places along the body of a fish swimming at 30 and at $70\,\text{cm}\,\text{s}^{-1}$.

As we found previously in carp, the sarcomere length change from the anterior position was noisier than those from the middle and posterior positions. This is because curvatures were smaller in that position and random errors (e.g. due to tracing the side of the fish with a cursor) are more apparent. In addition, the pelvic fins sometimes interrupted the profile of the side of the fish and we had to make an approximation by drawing through them. This noise made it difficult to pick out the waveform of sarcomere length with time and thus to measure accurately the amplitude (sarcomere length excursion) and slope (muscle shortening velocity) of the anterior waveform.

We were able to film most of the fish swimming *steadily* at speeds ranging from 30 to $50\,\text{cm}\,\text{s}^{-1}$ at 10°C and 30 to $70\,\text{cm}\,\text{s}^{-1}$ at 20°C (some scup could swim somewhat faster). The EMG recruitment speeds reported above suggest that, at the steady swimming speeds we filmed, the scup were using their red muscle fibres exclusively.

At higher speeds at each temperature the white muscle was recruited and the

fish switched to a burst-and-coast mode of swimming. At swimming speeds below the minimum for *steady* swimming, the fish used their large pelvic fins to help them locomote. Some of the fish at 10°C could swim steadily (without the pelvic fins) at 20 cm s^{-1}, whereas at 20°C, the fish could not swim steadily below about 30 cm s^{-1}.

Fig. 5A,B shows sarcomere length excursion in the middle and posterior positions at 10°C and 20°C over the range of speeds at which the fish swam steadily. Sarcomere length excursion was essentially independent of swimming speed and temperature, and was slightly greater in the posterior position than in the middle. The maximum SL excursion was 0.24 μm and occurred at 70 cm s^{-1}. Thus, the fish's sarcomeres underwent cyclical excursions between 1.99 and 2.23 μm. At slower swimming speeds, and in the middle and anterior regions of the fish, the fibres operated over a smaller range of sarcomere lengths.

Tail-beat frequency was found to increase linearly with swimming speed (Fig. 5C). Thus, as swimming speed increased, the sarcomeres underwent approximately the same length changes in a shorter time, resulting in an increase in muscle shortening velocity, as shown in Fig. 5D,E. Fig. 5D,E also shows that the velocity of the red muscle was essentially independent of temperature, except at 40 cm s^{-1} in the posterior position, where it was 22 % higher at 20°C than at 10°C.

Two variables that influence the amount of thrust transmitted to the water are tail-beat amplitude and tail height. Fig. 6A,B shows that neither temperature nor swimming speed had an effect on tail-beat amplitude or tail height.

Four of the five fish swam similarly at a given speed at 10°C and 20°C. One fish swam with high-frequency, small-amplitude tail beats at low swimming speeds at 20°C. Excluding this fish from the statistical analysis, the only significant difference with temperature was in muscle velocity at 40 cm s^{-1} (see above).

Discussion

Validity of calculation of SL changes during swimming

In this study, sarcomere length changes during swimming were calculated from the backbone curvature measured from high-speed motion pictures of swimming fish and from a calibration curve generated from frozen sections. This technique is based on the assumption that the SL measured at a given backbone curvature in the frozen fish would be the same as in a swimming fish.

Several lines of evidence suggest that this is a reasonable assumption. First, we have previously found that SL depended only on local curvature, not on how that curvature was attained (i.e. 'C' bend *vs* 'S' bend; Rome and Sosnicki, 1991).

Second, the series elasticity of the fibre–myosepta connections, which could be potentially confounding, can be shown to be quite low. We observed a series elasticity (SEL) of the whole bundle preparation under maximum isometric force of about 6 % (Rome *et al.* 1992). This value consists of three components: the intrinsic SEL of the fibres themselves (thought to be 1–2 %), the SEL of the fibre–myosepta connection, and the SEL of the connections between the servo
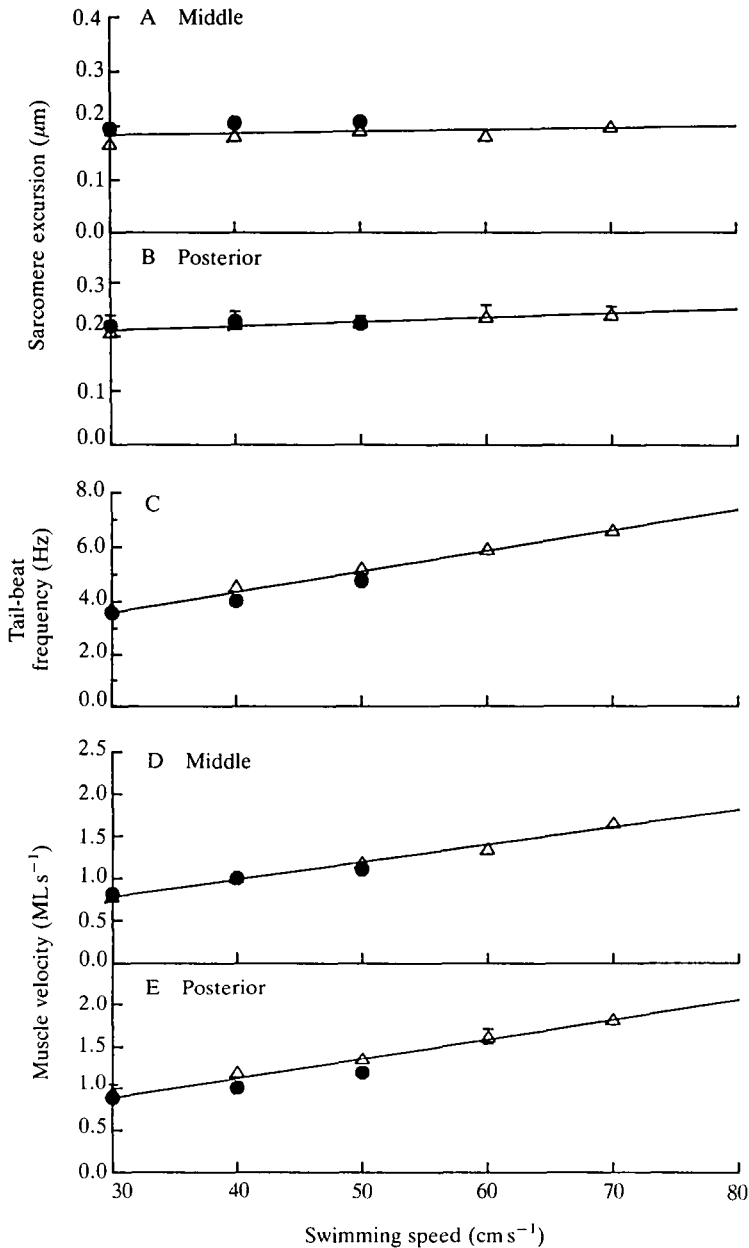
Fig. 5

system and the held tissue bundle (this includes the extra lengths of tissue external to the myosepta that are held in tying). Experimentally, it is not possible to distinguish between the last two components: as a rough estimate, half of the SEL could be allotted to each. Thus, under isometric conditions, an SEL of approximately 2–3% would lie in the fibre–myosepta connections. However, during

Fig. 5. Sarcomere length excursion, tail-beat frequency and muscle velocity as a function of swimming speed at 10°C (●) and 20°C (△) in the middle and posterior positions on the fish. Points are means±s.E. The influence of swimming speed on sarcomere length excursion was small and temperature had no effect (A,B). Temperature had no effect on tail-beat frequency (C). The linear increase of tail-beat frequency with swimming speed at two temperatures is shown by the solid line (frequency= $0.075 \times$ velocity$+1.338$, $r^2=0.85$, $N=56$). Muscle velocities (D,E) increase linearly with swimming speed at both positions ($r^2=0.98$ and 0.95 in the middle and posterior, respectively, $N=53$) owing to the increase in tail-beat frequency. Temperature generally had no effect on velocities (except in the posterior position at $40\,\mathrm{cm\,s}^{-1}$).

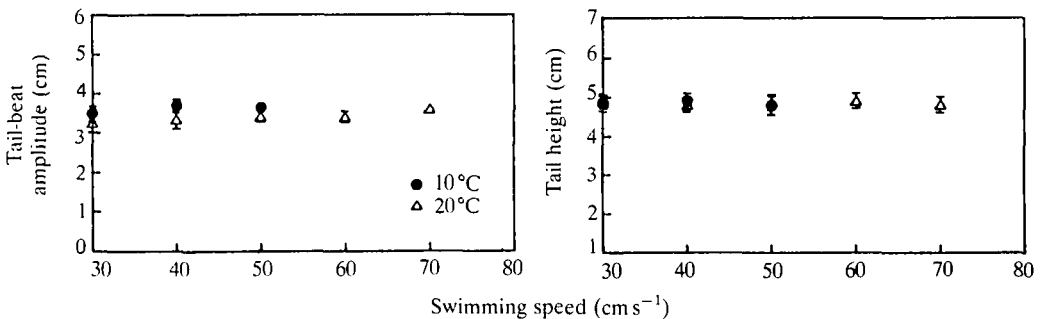Fig. 6. Neither swimming speed nor temperature influenced tail-beat amplitude or tail height. Points are means±s.E., $N=5$.

shortening (which is our main interest) at the maximum velocity at which the red muscle is used, the force generated is only about one-third of that under isometric conditions. Thus, the actual stretch of the SEL of the fibre–myosepta connection would only be approximately one-third of that at isometric tension, or about 1 % (approximately $0.02\,\mu m$).

Third, our technique of putting the fish into rigor tends to correct for any stretching of the SEL. As pointed out in Rome and Sosnicki (1991), muscle generates 30–40 % of isometric force during rigor (which is about what it generates during swimming) and it should stretch the SEL similarly. Thus, the calibration curve used should correct for any stretching of the SEL. The fact that we did not observe any difference between pre-rigor and post-rigor SL in the red muscle supports the conclusion that the SEL in the red muscle is quite small.

Fourth, the form of our calibration curve agrees with mathematical modelling of SL (i.e. SL depends on A/R; Rome *et al.* 1988).

Finally, other types of measurements support our calculation. Covell *et al.* (1991) have put sonimicrometers into the white muscle of trout during the startle response. Their results suggest that muscle 'deforms' by about 9 % in the anterior region and 10 % in the posterior region. If this measurement of muscle 'deformation' is a measure of muscle shortening, then their results agree very well with

our calculation of 6 and 10 % strain, respectively, in carp white muscle during the startle response (Rome and Sosnicki, 1991). However, in their paper, they compared their results with the amount that we would predict that red muscle should shorten *if* it were powering the escape response (this is a much greater strain) and they concluded that the techniques do not agree. This comparison was inappropriate for a number of reasons: (1) their gauges were in the white muscle rather than the red, (2) the red muscle does not power the escape response, and (3) the red muscle probably does not even shorten by more than about 10 % (its V_{max} is too low; Rome *et al.* 1990*a*). Another technique, direct sarcomere length measurement by laser diffraction in the glass catfish (Kashin *et al.* 1991), also supports our procedure. During steady swimming with the red muscle, we predict that the inactive white muscle would undergo passive sarcomere length changes of about $0.06\,\mu$m. This is the precise value that Kashin *et al.* (1991) obtained for white muscle sarcomere length excursion (presumably passive) in the swimming catfish.

The influence of temperature on kinematics of swimming in scup

The kinematics of swimming in scup are essentially independent of temperature over the range of steady swimming speeds, although some minor differences were evident due to one scup, as mentioned above. Previous findings on carp (Rome *et al.* 1990*a*) showed that there were no kinematic differences between 10 °C, 15 °C and 20 °C.

The temperature independence of the kinematic variables in scup shows that, despite the large change in muscle contractile properties with temperature, the scup is capable of recruiting its muscle fibres so that movement at a given speed is the same at all temperatures. This supports the notion that the kinematics of locomotion is set by anatomical characteristics of the animal and physical properties of the environment, which change little with temperature (Rome *et al.* 1990*a*; Rome, 1990).

Recruitment pattern

How do fish maintain similar kinematics of locomotion despite the large changes in the mechanical properties of the muscle with temperature? Rome and colleagues (Rome *et al.* 1984, 1985; Rome, 1990) have suggested that this is done by 'compressing the recruitment order' at low temperatures. Thus, at a given speed of locomotion, more motor units (sometimes including faster fibre types) are recruited at the low temperature to compensate for their reduced power output. Until recently, the main evidence for this theory was that fish recruited their white muscle at a slower swimming speed at low temperatures (as in this study). This was interpreted to mean that the fish had sequentially recruited all its red muscle at a slower swimming speed at low temperatures. To prove that more red muscle motor units are actually recruited at a given speed at the low temperature, however, has been problematic.

In one recent approach, Rome and colleagues (Rome and Sosnicki, 1990; Rome *et al.* 1990*a*) predicted that carp recruit 1.53-fold greater cross section at 10°C than at 20°C, on the basis of the shape of the force–velocity curve of red muscle and the *V* at which it shortens during swimming. In the following paper (Rome *et al.* 1992), we calculate that scup recruit 1.97-fold greater cross section at 10°C than at 20°C. These calculations hold only for actively shortening muscle and are based on the reasonable (but unproved) assumption that increased force generation is provided by increased recruitment of maximally activated fibres rather than by increased activation of submaximally activated fibres.

In another recent approach, Jayne *et al.* (1990) concluded, from quantitative EMGs of lizards locomoting with different muscle temperatures, that lizards used more red motor units at the low temperature. Although the main goal of our electromyography was to determine the swimming speed corresponding to white muscle recruitment, we also used the approach of Jayne *et al.* (1990) to see if this analysis would provide an independent assessment of increased recruitment at low temperatures in scup.

The changes of red muscle EMG properties with temperature and locomotion speed were similar to those reported for lizards. Essentially all properties (spike amplitude, spike number, spike number×spike amplitude and integrated area) increased with increasing swimming speed until a plateau was reached after which they remained constant (Fig. 2). In addition, values for all properties (except spike number per bin) were higher at 10°C than at 20°C. The observation that the fivefold increase of integrated EMG (spike number×spike amplitude) with swimming speed at 10°C was the product of a fourfold increase in mean spike amplitude and only a 20% increase in spikes per bin suggests that as swimming speed increases additional motor units are recruited in a synchronous manner. Increased stimulation frequency of already recruited motor units or the asynchronous recruitment of additional motor units with increasing swimming speed would both result in a large increase in the spikes per bin and a negligible increase in spike amplitude: this is the opposite of what was observed.

An assessment of greater motor unit recruitment at 10°C than 20°C is confounded by differences in passive EMG properties at different temperatures. The larger EMG amplitude and larger integral of the rectified signal per bin at the low temperatures we observed does not prove greater recruitment. As pointed out by Jayne *et al.* (1990), a larger amplitude and integral are expected at the low temperature simply because of the longer time constant of the action potential. This effect on the integral is demonstrated by a steeper slope at 10°C in the integral *vs* spike number×amplitude plot. Spectrum analysis (fast Fourier transforms) of scup EMGs (not shown) further shows a shift to slower frequencies at 10°C. The observations that there is a shift to slower frequencies and that a substantial amount of the power of the signal occurs below 50 Hz may also explain the apparently contradictory lack of quantitative differences in the amplitudes of carp EMGs at 10°C and 20°C (Rome *et al.* 1984, 1985). In those studies, a recording system with a low-frequency cut-off of 50 Hz was used.

The plateau of EMG activity occurring at a slower swimming speed at 10°C than at 20°C is suggestive of greater recruitment at 10°C (as predicted by the 'compression of the recruitment order theory'). Because EMG activity increased continuously with increased locomotion speed, the plateauing of activity can be interpreted as showing that all the fibres have been recruited (Jayne et al. 1990). At 10°C, the plateau in red muscle activity occurred close to the speed of initial recruitment of white muscle. We did not demonstrate the plateau in activity at 20°C, because we did not record EMGs in the red muscle up to the speed of white muscle recruitment.

One difference between our results and those of Jayne et al. (1990) is in the effect of temperature on spike number per bin. In lizards, spike number was higher at low temperatures, whereas in scup, we found that the number of spikes per bin was independent of temperature at low speeds and larger at 20°C at higher speeds. It should be emphasized that higher spike frequency at 20°C does not imply recruitment of a greater number of motor units, because spike frequency also reflects the rate at which the motor neurones are stimulated. The following paper (Rome et al. 1992) shows that scup muscle needs to be stimulated more frequently at 20°C to generate a given force than at 10°C. Hence, one might expect that a motor unit, when recruited at 20°C, will be recruited at a higher stimulation frequency.

The quantitative analysis of red muscle EMGs can be interpreted to support greater recruitment of motor units at low temperatures in accord with the 'compression of the recruitment order theory.' As such, it provides an additional assessment, independent of qualitative EMG, and analysis of the force–velocity curve. The translation from the EMG signal to the recruitment and stimulation frequency of a specific number of motor units, however, is not unique. Thus, in our opinion, the quantitative EMG evidence taken by itself is not definitive.

Kinematics of scup versus carp

As scup can swim twice as fast with their red muscle as can carp, we anticipated that, at the swimming speed of initial white muscle recruitment, the V of the scup red muscle would be about twice that of carp muscle. We observed quite a different result. At a given temperature, the maximum velocity at which the red muscle shortened in vivo was nearly the same (2.04 ML s^{-1} at 45 cm s^{-1} at 20°C in carp and 2.05 ML s^{-1} at 80 cm s^{-1} at 20°C in scup; see Table 1).

How does the muscle shorten with the same V during swimming at 80 cm s^{-1} in scup as it does in carp swimming at half that speed? Fig. 7 shows how several important variables in the posterior position of the scup and carp vary with swimming speed at 20°C. Fig. 7D shows that the V of the carp at a given swimming speed is much higher than that of the scup. Although the intercepts of the relationships are similar, the slope for the carp is about 1.55-fold greater than that for the scup. In what follows we will discuss (1) why the slope for carp is higher than that for scup and (2) at their respective swimming speeds of initial white

Table 1. *Kinematic and anatomical parameters for carp and scup at the respective white muscle recruitment speeds at 20°C*

Variable	Carp at 45 cm s^{-1}	Scup at 80 cm s^{-1}	Carp/Scup
Muscle velocity (ML s^{-1})	2.04 (0.12, 2)	2.05	1
SL excursion (μm)	0.308 (0.03, 2)	0.24	1.283
Tail-beat frequency (Hz)	5.99 (0.15, 2)	7.56	0.792
Shape*	1.14	1.17	0.974
Slope, SL *vs* A/R† (μm)	1.53	1.36	1.125
1/R (cm^{-1})	0.26 (0.03, 2)	0.147	1.77
Thickness, A (cm)	0.387 (0.03, 4)	0.597	0.65

For scup, values are extrapolated from lower speeds. For carp, actual values are given (from Rome *et al.* 1990*a*; Rome and Sosnicki, 1991). The carp values are somewhat higher than regression values in Fig. 7 (i.e. they fall above the regression line).

Where appropriate, the s.e. and N values are given.

* A is the half-thickness of the fish and R is the radius of curvature of the backbone. Shape describes the sarcomere length–time graph and is calculated by the following $[V/(2\times\text{EXCUR}\times\text{FREQ})]$; where V is muscle velocity, EXCUR is sarcomere length excursion and FREQ is tail-beat frequency. As it is calculated from the first three terms, no statistics are given.

† For carp, this is the slope of a regression line with 52 points described in Rome and Sosnicki, 1991.

muscle fibre recruitment (45 cm s^{-1} for carp and 80 cm s^{-1} for scup), what factors result in the V being the same.

Muscle velocity is the product of three factors:

$$V = 2 \times \text{EXCUR} \times \text{FREQ} \times \text{SHAPE}, \qquad (2)$$

where EXCUR is SL excursion, FREQ is tail-beat frequency and SHAPE, $V/(2\times\text{EXCUR}\times\text{FREQ})$, is a measure of the shape of the SL–time plot which relates the measured V to a given EXCUR and FREQ. For instance, if the SL–time plot is sinusoidal, the maximum V will be 1.57-fold larger than $2\times\text{EXCUR}\times\text{FREQ}$ and thus SHAPE=1.57. Alternatively, if the SL–time plot is a triangle wave, V will be equal to $2\times\text{EXCUR}\times\text{FREQ}$ and thus SHAPE=1. Fig. 7C shows that, unlike all other variables, SHAPE decreases with increasing swimming speed.
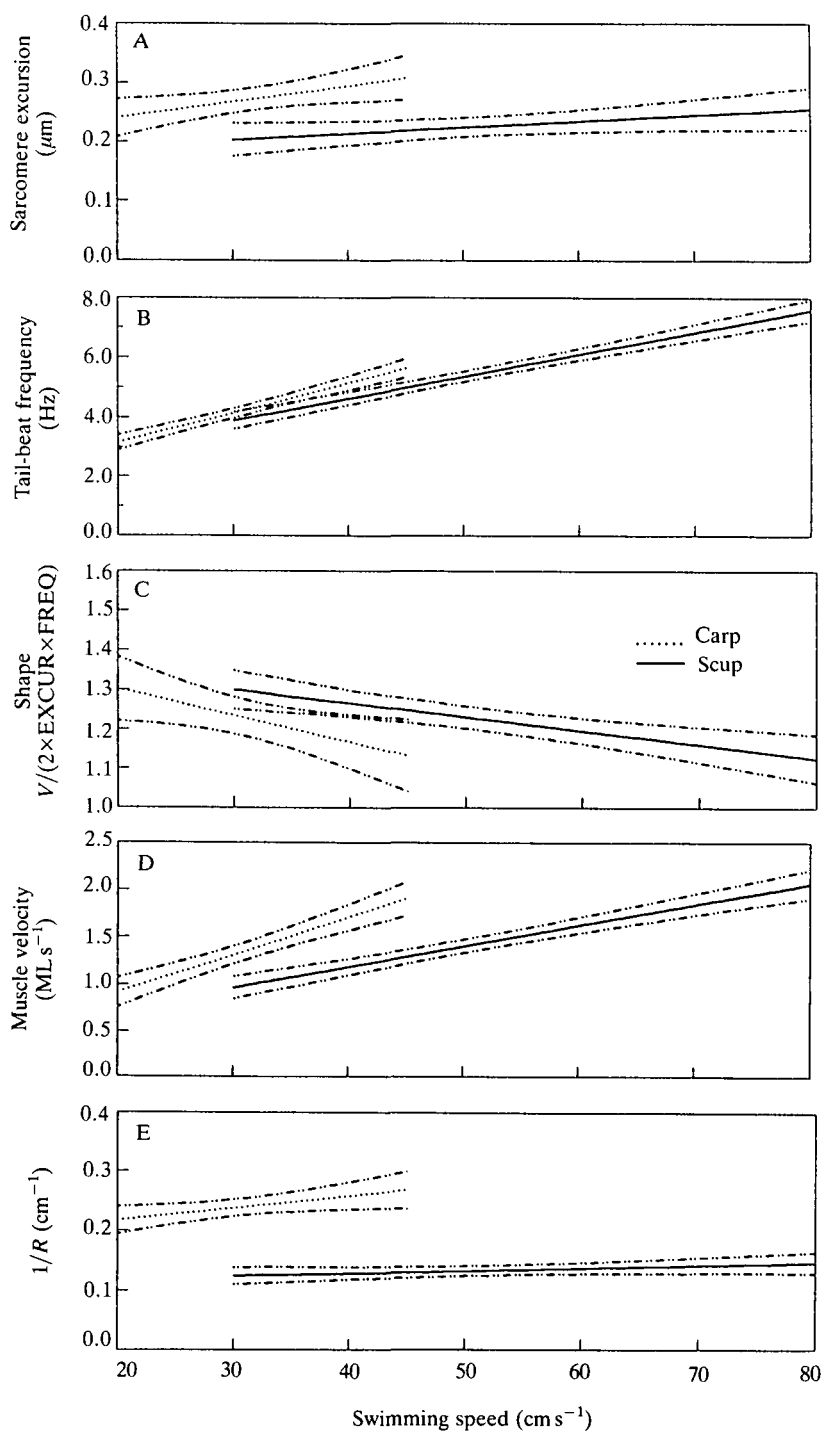
Fig. 7

Fig. 7. Sarcomere excursion, tail-beat frequency, shape, muscle velocity and back-bone curvature ($1/R$) as a function of swimming speed at 20°C in carp and scup. The carp data (represented by dotted lines) are taken directly from Rome *et al.* (1990*a*) or have been calculated from that data set. The regression lines at 45 cm s^{-1} fall below the actual values at this speed given in Table 1. The scup data are represented by solid lines. The regression lines beyond 70 cm s^{-1} are extrapolated from data below this speed. Both sets of data are surrounded by 95 % confidence limits.

Thus, formally:

$$\frac{dV}{dSPEED} = 2 \times EXCUR \times SHAPE \times \left(\frac{dFREQ}{dSPEED}\right) + 2 \times FREQ \times$$

$$SHAPE \times \left(\frac{dEXCUR}{dSPEED}\right) + 2 \times EXCUR \times FREQ \times \left(\frac{dSHAPE}{dSPEED}\right). \quad (3)$$

The first term is the most important. For instance, in scup swimming at 40 cm s^{-1}, it is 0.04 compared to 0.013 and -0.007 for the second and third terms, respectively. At 40 cm s^{-1}, the value for the first term for the carp is 1.55-fold greater than that for the scup. This is accounted for by the 1.3-fold greater slope of the tail-beat frequency *vs* swimming speed relationship, the 1.36-fold greater SL excursion (0.296 μm *vs* 0.217 μm) and the 1.11-fold smaller SHAPE factor in carp than in scup. The second and third terms are 2.4-fold and 3.3-fold, respectively, greater in carp than scup, primarily because of a 2.5-fold greater dEXCUR/dSPEED and a 2.3-fold greater dSHAPE/dSPEED. Because these terms are relatively small and opposite in sign, the first term accounts for approximately 90 % of the total $dV/dSPEED$ for both species and thus V increases linearly with swimming speed, rather than in a more complex manner.

To understand further the mechanism for the 1.36-fold larger SL excursion in carp than scup, it is useful to examine the components of this term. SL excursion is the product of three variables, the distance of the red muscle from the backbone (A), the maximum amount of curvature of the backbone during the tail-beat cycle ($1/R$), and the constant relating SL and A/R. At 40 cm s^{-1}, the backbone curvature is 1.92-fold larger (0.249 *vs* 0.13 cm^{-1}) in the carp than in the scup (Fig. 7E). In addition, the constant relating SL to A/R is 1.125-fold higher in the carp than in the scup. The ratio of sarcomere length excursion in the carp to that in the scup would be much larger than the 1.36 observed except that the thickness of the scup in the posterior region is considerably greater (0.6 cm *vs* 0.38 cm) than in the carp. This is because the scup in this study are longer than the carp used in the previous study (see below). Thus, it appears that the SL excursion in the scup is reduced primarily by adopting a less undulatory swimming style (smaller $1/R$).

Having established that the slope of V with swimming speed is steeper for carp than scup, we now must address why the V is maintained approximately the same at the respective recruitment speeds in scup and carp. Table 1 shows pertinent variables for the carp at 45 cm s^{-1} and the scup at 80 cm s^{-1} while swimming at 20°C. At 80 cm s^{-1} the scup has a 1.26-fold higher tail-beat frequency than the carp

at $45\,\mathrm{cm\,s^{-1}}$ (7.56 *vs* 5.99 Hz) but its sarcomere excursion is about 1.28-fold less. Thus, these factors nearly compensate for each other, giving the same V (note, the shape factors are nearly equal). The underlying mechanism for the lower sarcomere length excursion in the scup, in spite of its greater thickness, is the 1.77-fold smaller backbone curvature and the 1.125-fold lower SL *vs* A/R constant.

A possible confounding factor to the analysis above is that the scup were longer than the carp (Rome *et al.* 1990*a*). Thus, formally, the analysis above refers to 19.7 cm scup *vs* 13.4 cm carp. This does not invalidate the analysis, nor its importance, it just brings into question how much of the difference observed is due to species differences and how much to size differences. We believe that many of the important effects are primarily due to species differences. For instance, there is little effect of body size (over the narrow range considered) on the speed of initial white muscle fibre recruitment in carp. Recruitment at 10°C and 20°C occurred at the same swimming speeds in large carp (16–20 cm; Rome *et al.* 1984) as in small carp (11–14 cm; Rome *et al.* 1990*a*). Thus, the observation that scup can swim faster than carp with their red muscle is probably a species rather than a size difference. However, we cannot be certain how other variables (e.g. V) are affected by body size.

Thus, despite the fact that scup can swim twice as fast as carp with their red muscle, the V with which the red fibres shorten at the initial speed of white muscle recruitment is nearly the same. This is a remarkable finding that is largely explained by a less undulatory style of swimming as well as by an anatomical difference (gearing of red muscle). The independence of kinematic parameters from temperature further shows that animals keep their movements constant and our EMG evidence suggests that this is achieved by recruitment of additional fibres at low temperatures.

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