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I am submitting herwith a thesis written by Roel P. Funke entitled "Muscle Function in Carp During Locomotion". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

Lawrence C. Rome

We have read this thesis
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and Dean of The Graduate School

MUSCLE FUNCTION IN CARP DURING LOCOMOTION

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Roel P. Funke

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ABSTRACT

Animals have different muscle fiber types: slow ones with a low maximum velocity of shortening (V_{max}) and fast ones with a high V_{max}. The advantages conferred by having different muscle fiber types have been assumed solely on the basis of *in vitro* experiments. These experiments show that efficiency and power production are both functions of V/V_{max}, the ratio between the velocity of shortening of a muscle fiber *in vivo* and its maximum intrinsic velocity of shortening.

To test these ideas concerning the role of different muscle fiber types, carp were used as an experimental model. The V_{max} of their slow and fast muscle fibers was determined. V, the velocity of shortening of muscle fibers, was determined from high speed motion pictures of swimming carp. The results show that during slow swimming, where they are used exclusively, the V/V_{max} of the slow muscle fibers corresponds to a position on their force-velocity curve where efficiency and power production are at a maximum. Similarly, during the very rapid escape response the fast muscle fibers are producing maximum power. The results also provide evidence that the fiber types are not functionally interchangeable, and that carp require both of them to execute the full range of movements in their locomotory repertoire.

Temperature affects the contractile properties of muscle with a Q_{10} of about 2, and is expected to be an important environmental factor in determining the locomotory capacity of ectotherms. The experiments were repeated over a 10°C temperature range, and the results show that in spite of a 2-fold change in V_{max}, carp use their muscle fibers at velocities where efficiency and power production are optimized.

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CHAPTER I

INTRODUCTION AND OVERVIEW

This thesis consists of a paper on the effect of temperature on muscle function during locomotion. It is being prepared for submission to the Journal of Physiology.

It was preceded by a paper that has been accepted for publication in Nature ("Why animals have different muscle fiber types", see Appendix 1). The paper "Why animals have different muscle fiber types" lays the foundation for the work presented here. It argues that while it has been assumed that slow muscle fibers are efficient at slow speeds of locomotion and that fast fibers are necessary for maximal movements, experimental evidence on this point is lacking. It provides the first experimental measure of V/V_{max}, which is the main determinant of efficiency and mechanical power production of muscle fibers in vivo (V/V_{max} is the ratio between the velocity of shortening of a muscle fiber during locomotion and its maximum intrinsic shortening velocity).

The approach used combines studies on single cells (muscle mechanics) with measurements made on whole animals (determination of the velocity of shortening of muscle fibers during locomotion). In the first paper, carp are introduced as an experimental model having the necessary properties to make this approach successful. The paper contains V_{max} data for both slow and fast fibers and describes how the V of both fiber types was measured.

In brief, the paper shows that in carp, at least, the slow and

fast fibers operate over a range of V/V_{max} where their efficiency and mechanical power production are optimized. Moreover, it demonstrates that the fiber types are not functionally interchangeable: the animal cannot use slow fibers to power maximal movements, and if fast fibers were active at slow swimming speeds, their efficiency and mechanical power production would be nearly zero.

Having established this pattern, the second paper asks whether it disrupted by acute changes in temperature. Contractile properties of muscles such as V_{max} have a high thermal sensitivity (Bennett, 1985). Thus, in ectothermic animals, temperature might be expected to have a considerable effect on V/V_{max} and hence on the efficiency and mechanical power production of their muscles. V was measured at 10^{o} and $20^{o}C$ using the techniques developed in the first paper, and V_{max} was estimated from the value obtained at $15^{o}C$ using a Q_{10} of 1.8. Only the slow muscle fiber type is discussed in this paper.

The paper suggests that in spite of large changes in V_{max} with temperature, at swimming speeds where they provide all the requisite power the slow fibers of carp continue to operate on the part of their force-velocity curve where power and efficiency are optimized.

There appears to be a mechanistic link between swimming speed and muscle shortening velocity that is largely independent of temperature. At each temperature there is a maximum swimming speed the fish can sustain using their red muscle exclusively, and this speed is slower at low temperatures. At higher speeds the white muscle is recruited as well. At each temperature at the maximum sustainable swimming speed, the slow muscle fibers are shortening at a velocity where they are

producing their maximum mechanical power. If they were to shorten more quickly, their power output would actually start to decline, and thus at higher swimming speeds the white muscle must be activated to supply the additional power for locomotion. In a sense then, locomotory function in carp is integrated in such a way that the slow muscle fibers operate over a part of their force-velocity curve where efficiency and mechanical power output are optimized.

These conclusions would be supported by calculations showing that at the maximum sustainable swimming speed the power output of the total mass of slow muscle fibers is just adequate to propel the fish. Data presented in the second paper suggest that it may be difficult to extrapolate from the power output of single muscle fibers to the thrust produced by an intact fish during swimming. However, it is worthwhile to make this calculation, and most of the necessary parameters can be estimated from high speed motion pictures and muscle mechanics data.

Experimental data on the V_{max} of the slow muscle fibers at 10^{o} and $20^{o}C$ should help confirm these observations. Analysis of maximum movements at these temperatures will broaden the perspective and show whether the fast muscle fibers conform to the general conclusions reached so far in this study.

CHAPTER II

EFFECT OF TEMPERATURE ON THE RATE OF SHORTENING OF MUSCLE IN VIVO: IMPLICATIONS FOR EFFICIENCY AND MECHANICAL POWER PRODUCTION

INTRODUCTION

The presence of different muscle fibre types in animals has been well documented. It has been shown, at least in fish, that there is role partitioning between the fiber types (Bone, 1966; Rome, Loughna, and Goldspink, 1984). Slow fibres with a low maximum velocity of shortening (V_{max}) are used during slow, sustainable swimming that is mainly aerobic, whereas fast fibres are recruited at higher swim speeds. It has been assumed that role partitioning allows the different muscle fibre types to be used over a range of shortening velocities where their power production and effficiency are optimized. Power production and efficiency depend on the rate of shortening, V, of a fibre compared to its maximum rate of shortening. That is, they are not fixed properties of a muscle fibre. Power production is maximal at intermediate values of V/V_{max} (0.2-0.3; Hill, 1964) and falls to zero at $V/V_{max}=0$ and $V/V_{max}=1$. Recent experiments on dogfish myotomal muscle indicate that maximum efficiency is achieved at a V/V_{max} of approximately 0.2 to 0.7 (Curtin and Woledge, 1988).

Therefore to discover whether power production and efficiency are normally optimized, it is necessary to determine V, V_{max}, and the types of fibre active at different speeds of locomotion. Only with this

information is it possible to state whether or not muscle fibres operate over the appropriate range of values of V/V_{max}.

Rome, Funke, Alexander, Lutz, Aldridge, Scott, and Freadman (1988) used carp as an experimental model to test this hypothesis concerning the functional significance of different muscle fibre types. Previously, Rome et al. (1984) had used electromyography to monitor the activity of the different muscle types in carp and showed that at low swim speeds only the red (slow) muscle was active, while at higher speeds the white (fast) muscle was recruited as well. In Rome et al. (1988), we were able to determine the velocity of shortening of the red muscle at low swimming speeds using a combination of anatomical techniques and high speed motion picture analysis. Bundles containing 20-30 fibres were used to determine the V_{max} of the red muscle. The range of values of V/V_{max} at low swim speeds was found to be 0.16-0.35. Thus we were able to show for the first time that the red muscle is used over a range of velocities where power production and efficiency are predicted to be at a maximum.

In the following experiments we[1] further developed carp as an animal model to study the influence of temperature on muscle function and locomotory performance. Temperature has a large effect on the contractile properties of muscles: the V_{max} of vertebrate skeletal muscle has a Q_{10} value of approximately 2-3 (Bennett, 1985). Most

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[1]Throughout this chapter "we" will be used instead of "I" because the paper is being prepared for submission with multiple authors. Roel Funke will be the principal author.

cold-blooded animals are subjected to considerable acute, diurnal and seasonal changes in body temperature, and it would appear from the thermal sensitivity of their muscle V_{max} that they are faced with major problems when trying to locomote at different temperatures. If the ratio V/V_{max} during locomotion changes by the same amount as V_{max}, muscle fibres would be operating on a different part of their force-velocity curve at different temperatures. Under these conditions they might no longer be generating maximum mechanical power at peak efficiency. By including temperature as a factor in our experiments, and using previously developed techniques for measuring the rate of shortening of lateral red muscle in carp, we had an additional opportunity to test the ideas concerning the functional role of different muscle fibre types. In particular, we were in a position to ask whether the ratio V/V_{max} is affected when muscle properties are changed approximately 2-fold by acute changes in body temperature in ectothermic animals.

The thermal dependence of the properties of isolated muscles seems to have no effect on the kinematics of animals locomoting at moderate, sustainable speeds over a range of temperatures. For example, Rome (1982), found that posture and stride frequency were the same in Savannah Monitor lizards running with muscle temperatures of 28.5^{o} and 38^{o}C. Similarly, Rome et al. (1984) found that tail-beat frequency in carp was virtually independent of swimming temperature. To swim at a given speed requires nearly the same power regardless of the ambient temperature, but due to the change in V_{max} cold muscle generates less force per cross-section than warm muscle. A mechanism which enables

animals to locomote in the cold is "compression of the recruitment order". According to this theory, motor unit groups are recruited over a narrower range of speeds at low temperatures to compensate for the reduced power output of the muscle. The order of recruitment stays the same at all temperatures (Rome et al., 1984).

On the other hand, maximal movements such as the escape response, in which all the available muscle power appears to be utilized, show a temperature dependence similar to that of isolated muscle. Hirano and Rome (1984), who studied frog jumping, found that lowering the temperature decreased maximum locomotory ability with a Q_{10} of 1.6. Webb (1978) found that in the fast start response of salmon, response time and maximum acceleration were diminished at lower temperatures with an average Q_{10} of 1.8.

While temperature has not been found to influence the gross body movements of animals locomoting at moderate speeds, many studies have shown that parameters such as endurance and maximum sustainable speed are heavily temperature-dependent (Fry and Hart, 1948; Brett, 1967; Griffiths and Alderdice, 1972). The power for sustainable activity is supplied mainly by slow aerobic muscle fibres. The limitations set on this kind of actitity by temperature can be understood from the compression of the recruitment order theory (Rome et al., 1984). While the power needed to locomote at a given speed is essentially temperature-independent, each motor unit in the muscle of a cold animal will be generating less power at a given speed of shortening as compared to the motor units in a warm animal. Thus, a cold animal locomoting at a given speed will need to use a higher proportion of its

available aerobic muscle than a warm animal. The speed at which all the motor units in the aerobic muscle have been recruited, and at which the faster anaerobic fibres (in which ATP is not replenished by oxidative means to keep pace with rate at which it is being used) begin to be activated will be lower in the cold animal, causing exhaustion, and this will limit its maximum sustainable performance (Rome et al., 1984; Rome, 1986).

Since the kinematics of locomotion at moderate speeds do not apppear to be affected by temperature, it would seem fair to assume that the muscle fibres generating the required power are shortening at the same velocity (**V**) in each case. However, the $\mathbf{V_{max}}$ is drastically changed by temperature, and therefore the ratio $\mathbf{V/V_{max}}$ would be altered. If muscle fibres operate on a different part of their force-velocity curve at different temperatures, this would have important consequences for the efficiency of power production. In these experiments we used a direct method for measuring the speed of shortening of muscle fibres **in vivo** to investigate whether or not $\mathbf{V/V_{max}}$ is affected by temperature.

METHODS

The procedures were the same as those used previously to measure $\mathbf{V/V_{max}}$ in carp (Rome et al., 1988). They will be described at some more length here.

Measurement of sarcomere length

Our objective in this work was to determine the effect of temperature on the ratio V/V_{max} for slow muscle fibres by making measurements from high speed motion pictures of carp swimming at different temperatures. We will briefly restate our reasons for using carp as an experimental model.

First, it is necessary to measure the V of the appropriate kind of muscle fibres, that is, those fibres that are generating the requisite power at a certain speed of locomotion. In carp the slow and fast muscle fibre types are separated anatomically, and this enables electromyographic determination of the activity of the different muscle fibre types (figure 1). Rome et al. (1984) showed that over the range of swimming speeds studied, only the slow red fibres of carp are active. Second, the slow fibres are arranged in a thin strip parallel to the body axis just beneath the skin, which is convenient for experimental purposes because it leads to simple predictions about the relationship between sarcomere length and the shape of the body.

From geometric reasoning, it is clear that on the convex side of a bent fish muscle fibres will be stretched relative to their resting length in a straight fish, and that on the concave side muscle fibres will be shortened. Simple beam theory enabled us develop the following relationship:

$$\lambda_{convex} = {}_{o}(1+A/R) \qquad \lambda_{concave} = {}_{o}(1-A/R) \quad (1)$$

Fig.1. Longitudinal view (A), dorsal view (B) and cross section (C) of carp. The red muscle represent a thin sheet of muscle just under the skin which extends to a depth of only 10% of the distance to the backbone (N. B. the cross-section of the red muscle is exaggerated for illustrative purposes). Because the red fibres run parallel to the body axis (A,B) we were able to use simple beam theory to derive a straightforward relationship between sarcomere length on the convex and concave sides of the fish and the curvature of the spine:

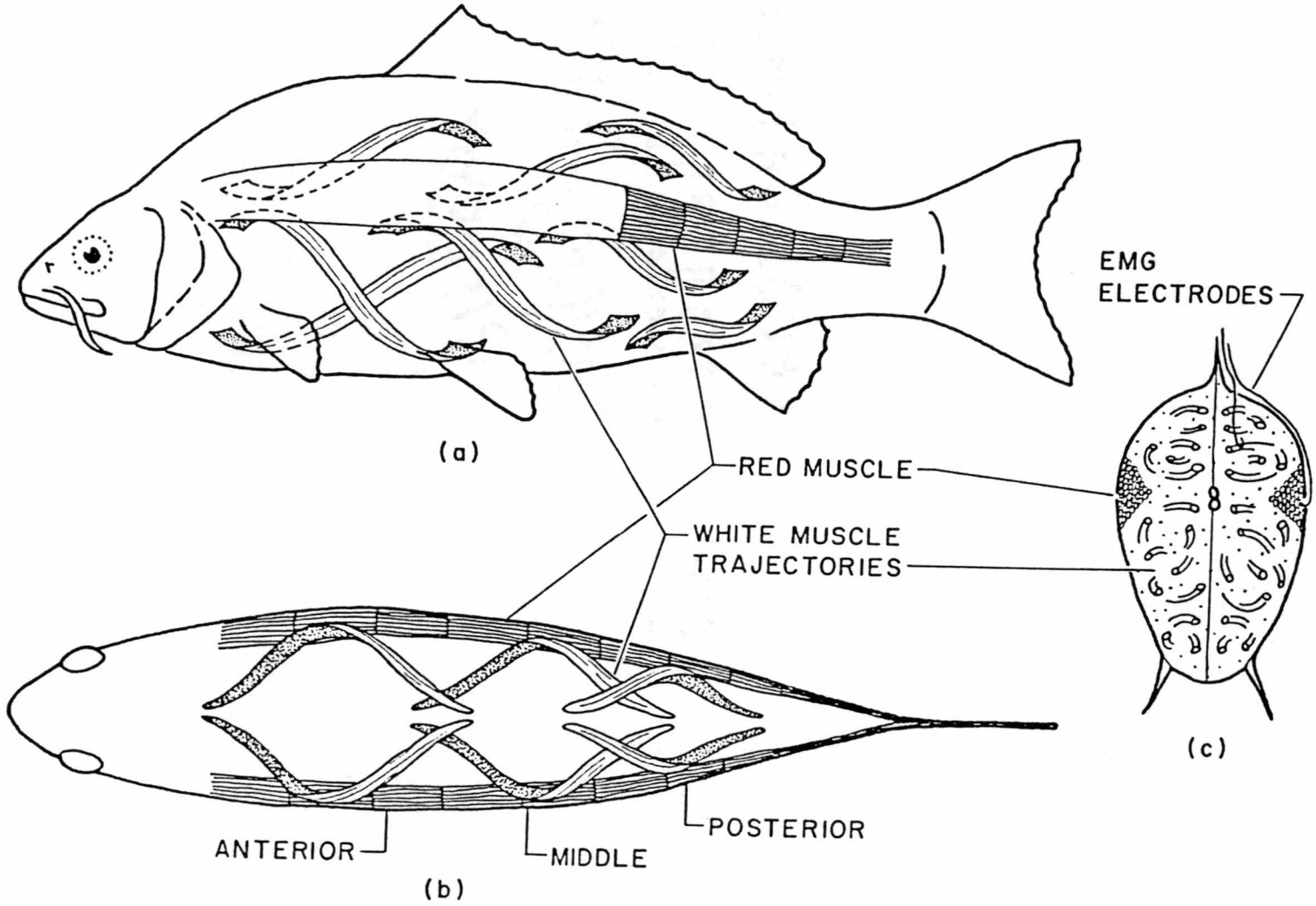
$$\lambda_{convex} = \lambda_o (R+A)/R \qquad \lambda_{concave} = \lambda_o (R-A)/R,$$

where λ is sarcomere length, λ_o the resting sarcomere length, A the half thickness of the fish, and R is the radius of curvature at the spine. We verified this relationship by measuring the sarcomere length of muscle fibres from carp fixed in shapes found during swimming.

The radius of curvature of the spine must be calculated from the average of the curvatures of the left and right sides of the fish since the spine is not visible in motion picture films. This involved digitizing the silhouettes of the left and right sides of the fish and determining each curvature with a 7-point moving curve fit. Velocity (V), the slope of the sarcomere length-time graph, was estimated with a 5-point parabolic fit.

The V of the white fibres was estimated by dividing the V of the red fibres by 4. The trajectories of the white muscle fibres shown in A and B are based on Alexander's (1969) description. The white fibres lie closer to the median plane than the red ones, and they run helically rather than parallel to the long axis of the body. Consequently, according to the calculations of Alexander (1969), they shorten only about 1/4 as much as the red ones for a given curvature change of the body. Measurements of sarcomere lengths of white fibres from fixed fish are consistent with this calculation. Sarcomere length excursions were determined at 3 places along the fishes' body. An anterior position (38% of the distance from snout to tail), a mid position (52% of the distance from snout to tail) and a posterior position (68% of the distance from snout to tail).

Placement of EMG electrodes used to determine the activity of the red and white muscle are show in C. Note that the so called "pink muscle", a thin sheet interposed between the red and white is not shown in this figure. It has been excluded from this analysis because its physiology and recruitment pattern has not been adequately characterized. Its exclusion, however, does not influence the analysis of the red and white muscle.


EMG
ELECTRODES
(a)
RED MUSCLE
WHITE MUSCLE
TRAJECTORIES
(c)
ANTERIOR
MIDDLE
POSTERIOR
(b)

where R is the radius of curvature at the backbone, λ is the sarcomere length observed for a given radius of curvature at the backbone, λ_o is the resting sarcomere length, and A is the half-thickness of the fish (positive on the convex side, negative on the concave side). For reasons of simplicity we assumed the width of the lateral red muscle strip to be negligible, and consequently A is the half-thickness measured to the skin. In measuring the velocity of shortening a correction factor was used to make allowance for the finite thickness of the muscle strip (see below).

The relationship between λ and A/R should therefore take the form of a straight line with intercept λ_o and a slope equal to λ_o.

This relationship was verified by sacrificing 7 small carp (l=10-20cm), and bending them into shapes observed during normal swimming as well as into more extreme C-shapes encountered during the escape response. The carp were allowed to go into rigor over a period of approximately 16 hours in a bath of water. They were then photographed from above to obtain a sharp silhouette of their shapes. After removing their intestines the rigid carp were placed in a bath of 10% formalin. The skin was removed to allow for better penetration of the fixative.

The rationale for this experiment is that as the fish goes into rigor its muscle fibres shorten to lengths appropriate to the degree of bending of the body, or more precisely, to the degree of curvature at the backbone. In a similar experiment in which freshly killed rabbits were arranged in postures encountered during a galloping stride, Dimery (1982) used toothpicks as splints to prevent shrinkage of the muscle

fibres during the fixing procedure. The carp muscle fibres were fixed in situ and thus the backbone acted as a brace to prevent shrinkage in the longtitudinal direction.

After 24 hours, small blocks of red muscle were removed from several places along the length of the fish and floated in a small basin of water. Single muscle fibres were carefully teased out and transferred onto a microscope slide. Sarcomere lengths were measured using a diffraction method described by Dimery (1982), and also more directly by counting them from photographs (Julian, Rome, Stephenson, and Striz, 1986a & b). These two methods gave the same results. At least 10 fibres were measured at each position and an average sarcomere length was calculated.

2 of the fish were fixed in straight positions to measure the resting sarcomere length, λ_o. This was found to be equal to 1.92um, and also to be independent of the place along the length of the fish from which the fibers were taken.

Measurement of Curvature

At each position along the fish for which sarcomere length had been measured, the radius of curvature at the backbone was calculated. The equation for radius of curvature at a point is:

$$R=\frac{[1+(dy/dx)^2)]^{3/2}}{d^2y/dx^2} \qquad (2)$$

Because the backbone is not visible from the outside of the animal, we determined the R by taking the mean of the radii for corresponding points on the sides of the body. Corresponding skin points have the property that a line joining them makes the same angle with each side of the body and passes through the corresponding spine point. In a straight fish, corresponding points lie directly opposite each other and are joined by a line that is perpendicular to the backbone. When a fish is bent, corresponding points shift and no longer lie directly across from each other. We wrote a software routine that identified pairs of corresponding points and calculated the radius of curvature at the point on the backbone. The half-thickness A was simply half the length of the line joining the skin points.

Images of the fixed fish (magnified 2-3 times) were analysed on a 22" square digitizing tablet (Altek Model AC30) interfaced with a computer. A cursor was moved slowly along the sides of the fish and its x-y position on the tablet was communicated to the computer at a rate of 50 points per second. Sometimes fins interrupted the profile of the side of the fish, and we made an approximation by drawing through them. The resolution of the cursor was 0.001". A simple software program sorted the points into equal x intervals (0.025"), interpolating between points where necessary.

We used analytical equations from Lanczos (1964) to determine the first and second derivatives at selected points on the sides of the fish. These equations minimize random error by approximating a smooth second order parabola to the data. Different weights are given to a number of points on either side of a central value, the total number of

points used and the interval between them depending on the degree of smoothing needed. We found that the best reproducibility was obtained when we used 9 point equations for both the first and second derivatives:

$$\frac{dy}{dx} = \frac{-4y(1)-3y(2)-2y(3)-y(4)+y(6)+2y(7)+3y(8)+4y(9)}{60z} \quad (3)$$

$$\frac{d^2y}{dx^2} = \frac{4y(1)+4y(2)+y(3)-4y(4)-10y(5)-4y(6)+y(7)+4y(8)+4y(9)}{100z^2}$$

where z is the x interval and y(5) is the y-coordinate of the central value of the equation. We used an x interval of 0.175" (7 0.025").

The technique made use of a "moving strip" principle. y(4) becomes the next central value for which the radius of curvature is calculated, what was previously y(8) becomes y(9), etc. In this way values were obtained for all but four points lost at both ends of our list of data (see figure 2).

The sarcomere lengths were plotted against A/R and a simple regression analysis produced the equation $\lambda=1.92+1.68A/R$ (see figure 3). All the sarcomere lengths in the straight fish were entered as separate data points with a curvature of zero. Note that the half-thickness is considered negative on the inside of the bend.

The upper and lower 95% confidence limits on the slope are 2.00 and 1.36, respectively. According to our theoretical analysis, it is

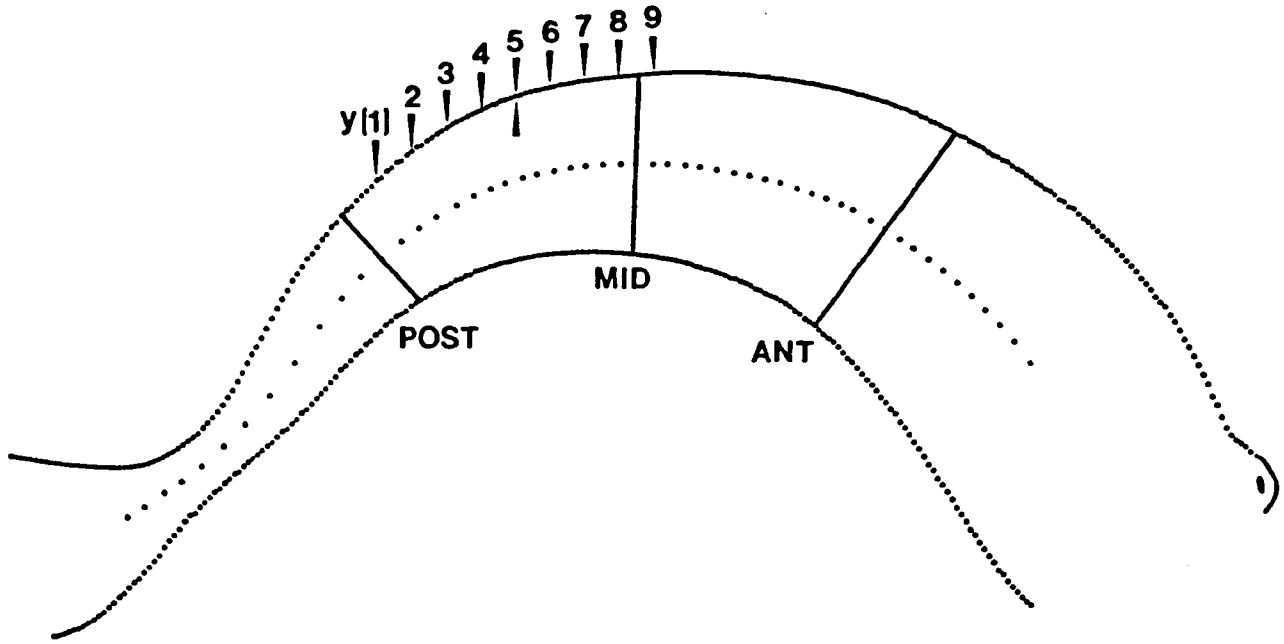


Fig.2. Plotted outline of a carp performing an escape response. Points used to calculate curvature at a central value y(5) are shown. Individual points are separated by 0.025". The position of the backbone was calculated in a software routine by taking midpoints of lines joining corresponding points on the sides of the fish. Equations used to calculate curvature are given in the text.

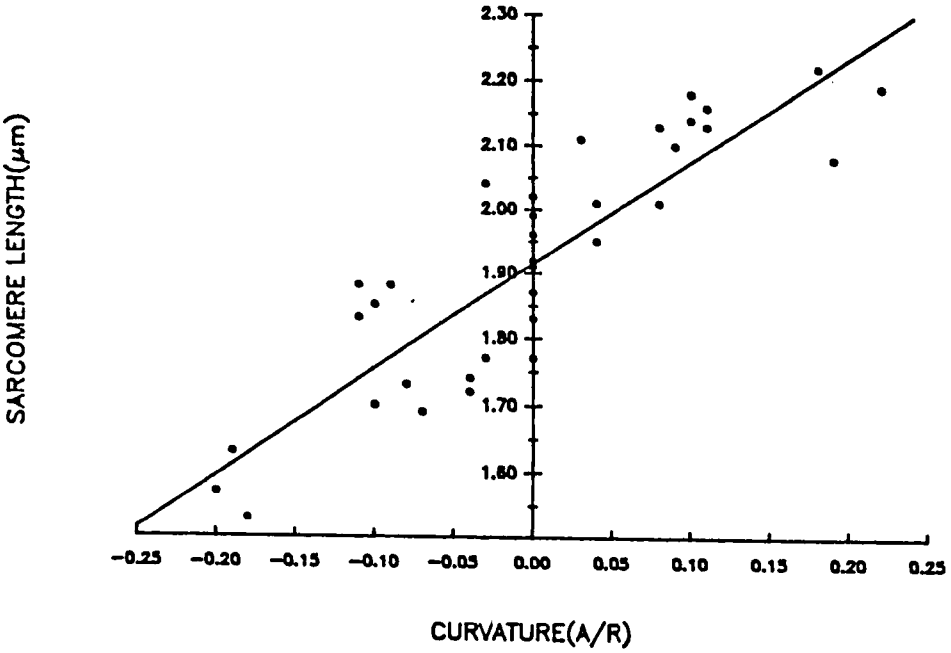


Fig. 3. Relationship of sarcomere length in the lateral red muscle to A/R, the product of the half-thickness A and the degree of curvature at the backbone, 1/R.
$\lambda = 1.9214 + 1.6779 A/R$, $r^2 = 0.76$, $p < 0.005$.

expected to be equal to 1.92. Thus the theoretical analysis supports our prediction of the relationship between sarcomere length and curvature. Therefore in the film analysis, we felt justified in using the predicted equation, with a slope and resting sarcomere length (um) equal to 1.92.

If the muscle on the concave side of the fish contracts isovolumetrically, it might be expected to increase in thickness, causing the spine to shift from the midline. In this case, our assumption that the distance to the backbone is simply the half-thickness of the fish would no longer be justified. We checked this by X-raying mildly and sharply bent fixed fish, and overlaying the plates with plotted outlines in which the position of the backbone was exactly central. Even in a sharp C-bend, the lateral movement of the spine was less than 0.5mm. This is less than 5% of the thickness of the fish, and would not make an appreciable difference in our calculations. Of course the situation in a fixed fish might not exactly duplicate the events that take place during an actual escape response, and it would be useful to repeat this experiment in vivo if the techniques were available.

Fish Swimming Experiments

Having established a relationship between sarcomere length and curvature, it was possible to measure the velocity of shortening of lateral red muscle from the rate of change of curvature of fishes' bodies during a tail beat cycle. To do this, carp (11-14 cm in length)

swimming in a recirculating water treadmill were filmed directly from above. The carp were purchased from a commercial fishery and kept in a tank in the lab (water temperature=15°C) for six weeks prior to these experiments.

The water treadmill consisted of a variable drive motor, centrifugal pump, plastic pipe sections, and a plexiglass swim tube (diameter=20 cm). The temperature of the unit was contolled to within 1°C, and the fish were confined to the swim tube with upstream and downstream honeycomb grids.

Fish were placed in the swim tube and allowed to acclimatize for at least 15 minutes with the water circulating at moderate speed (about 15 cm/s). In the first series of swims, the temperature of the water was maintained at 15°C.

4 carp were filmed from above swimming at speeds ranging from 15 to 45 cm/s (LOCAM camera, Redlake Corp.). To obtain a good silhouette of the fish in very low light levels, the bottom of the swim tube was covered with reflector material (580-10 Scotchlite, as in Wardle, 1975), which was illuminated with a ring light mounted on the camera lens. Rome et al. (1984) had found that carp will avoid bright lights and prefer to swim in areas of darkness. Thus we used low light levels and illuminated the sides and back of the swim tube with narrow-beamed lights to prevent the fish from drifting back or taking advantage of decreased flow rates near the edges.

A framing rate of 200/s was used. A small diode in the camera put a timing mark on the film every 0.1s, and these were used during the subsequent analysis to recognize and discard sequences in which the

camera had been accelerating or slowing down.

Following the first series of swims at 15°C (see Rome et al., 1988), the temperature in the holding tank was changed to 20°C. After 24 hours, when we could be reasonably certain that the muscle temperature of the fish was equal to that of the water, a second series of swims at 20°C was carried out on the same individuals.

Finally, the temperature in the holding tank was changed acutely to 10°C and after 24 hours the fish were filmed swimming at this temperature.

Measurement of V

Films were projected onto the back of the digitizing tablet via a front surface mirror placed below it on the floor at an angle of 30° to the horizontal. The tablet was also tilted at 30° so that the cone of light from the projector fell on it perpendicularly. The system was carefully aligned and periodically checked to prevent distortion of the image on the film.

The criteria for steady swimming at a certain speed were that the fish should be swimming steadily in the middle of the flume without accelerating or decelerating throughout at least 5 complete tail beat cycles.

Usually 3 tail beat cycles were analysed. At slow speeds (15-25 cm/s) sufficient resolution was achieved by analysing every 5th frame of a sequence, whereas at higher speeds it was necessary to analyze every 4th or 3rd frame. Thus depending on swim speed each sequence

contained 40-60 frames. Sarcomere lengths on the concave and convex sides were calculated for three positions along the length of the fish: 38%, 52%, and and 68% of the overall length, labelled anterior, middle, and posterior respectively. The sarcomere lengths were written to disk and subsequently plotted against time on the horizontal axis using a commercial graphics package.

The plots of sarcomere length against time looked sinusoidal at slow swim speeds, and at higher speeds were best described as triangular waves. The velocity of shortening of the muscle was estimated for the three positions on the fish at each swim speed using a 5 point analytical equation:

$$V=\frac{2y(1)-y(2)+y(4)+2y(5)}{10z} \qquad (5)$$

where y(1) etc. are the sarcomere lengths at one position on the fish at times separated by the interval z (0.015-0.025s). The equation gives the velocity at the time represented by data point y(3). Thus the highest velocities were obtained in the rising and falling parts of the waves, and the lowest velocities at the crests and troughs where the slope was changing sign. We chose sufficiently small frame intervals at each swim speed so that the equation gave accurate values for the maximal velocity i.e. so that the endpoints of the strip did not lie in the crests and troughs of the graph. **V** was calculated as the average of the 5 or 6 maximal velocities obtained from each sequence in this way.

The changes in sarcomere length taking place on one side of the

fish during a tail beat cycle are equal in magnitude but opposite in sign to the changes taking place on the other side. Therefore, the rate of shortening **V** of the muscle can be measured from either side of the fish. In these experiments we always used the sarcomere length excursions on the side of the fish that was nearer the top of the digitizing tablet.

Because the lateral red muscle strip has a finite thickness, a correction factor was used to calculate an average velocity of shortening of the fibres. Some fibres just beneath the skin would be shortening at velocities appropriate to the half-thickness A we used in the program, but the velocity of others nearer the spine would be proportionately smaller. By looking at cross-sections of carp, we estimated that a typical red fibre is located at about 10% the distance from the skin to the backbone. Thus all muscle velocities were multiplied by 0.9 to take account of the thickness of the red muscle strip.

The contribution of the tendons in the red muscle to the overall length change was considered to be negligible. In fish, the red muscle fibres are attached end to end through short tendinous sheaths. These sheaths are thin and their length change would not represent an appreciable percentage of the total change in length of the muscle-tendon complex.

Electromyography

The swim speed at which white muscle recruitment occurred was determined at each temperature by monitoring the activity of the white muscle by electromyography as in Rome et al. (1984). Electrodes were implanted in the white muscle on both sides of the fish with a hypodermic needle. During this procedure fish were held in a surgical rack while their gills were perfused with tricaine (MS222~50mg/1). Signals from the muscle were passed through AC preamplifiers (Grass no. P511), and recorded on audio tracks of simultaneous video tape of the fish swimming during the electromyography experiments.

We increased the flow velocity in steps of 5 and 2.5 cm/s (smaller increments were used near the recruitment speed), and the recruitment speed was taken to be halfway between the first speed the fish was unable to maintain without regularly using their white muscle and the speed immediately below it.

Recruitment speeds at 15°C were determined the same year subsequent to the swims at 10°, 15°, and 20°C. There was not sufficient time to do electromyography experiments at 10° and 20° as well. Recruitment speeds at these temperatures were determined one year later on carp that were slightly larger (l=18-22cm) swimming in the same flume.

Measurement of tail beat amplitude and tail height

For each of the sequences we used to determine **V**, we also measured the amplitude of the tail beat. We generally looked at a minimum of 10 tail beat cycles. To compensate for any sideways drift and unsteady swimming, we took the average excursion of the trailing edge of the tail to each extreme and subtracted one from the other.

In addition, the fish were filmed from the side at each speed to look at fluctuations in tail height during swimming. Filming from the top and from the side was not strictly synchronized, but as far as possible the cameras were turned on only when the fish appeared to be swimming steadily. We found the same rhythmical fluctuations in height as in Bainbridge (1963), and used an average value calculated over about 8-10 tail beats (figure 4). The camera was placed one meter from the swim tube and calibration marks were drawn on reflector material (580-10 Scotchlite) on the far side of the tube. With the fish swimming steadily in the middle of the tube parallax was about 5%, and the measurements were adjusted accordingly.

RESULTS

Figure 3 shows the relationship between spinal curvature and sarcomere length in the red muscle that we obtained by anatomical analysis of seven fixed carp. We used this relationship in the film analysis to determine sarcomere length from the curvature at the fishes backbone. Figure 5 is a typical record showing the changes in sarcomere

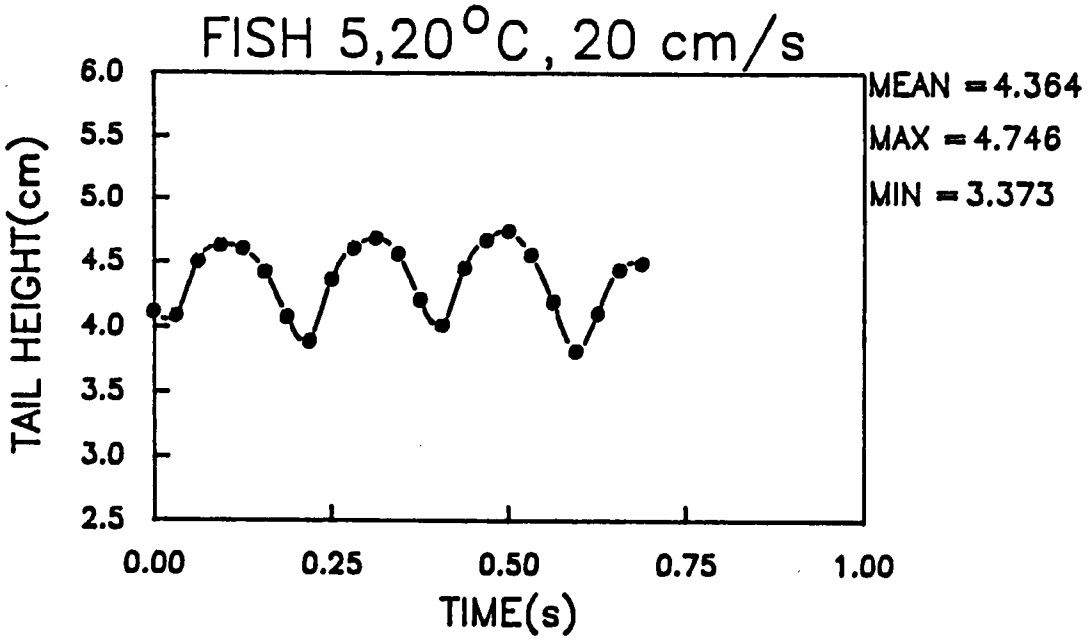


Fig.4. Representative record of fluctuating tail height during two tail beat cycles in a fish swimming at 20 cm/s. Measurements were taken at the trailing edge of the tail, and mean values were used to test the influence of swimming speed and temperature on tail height.

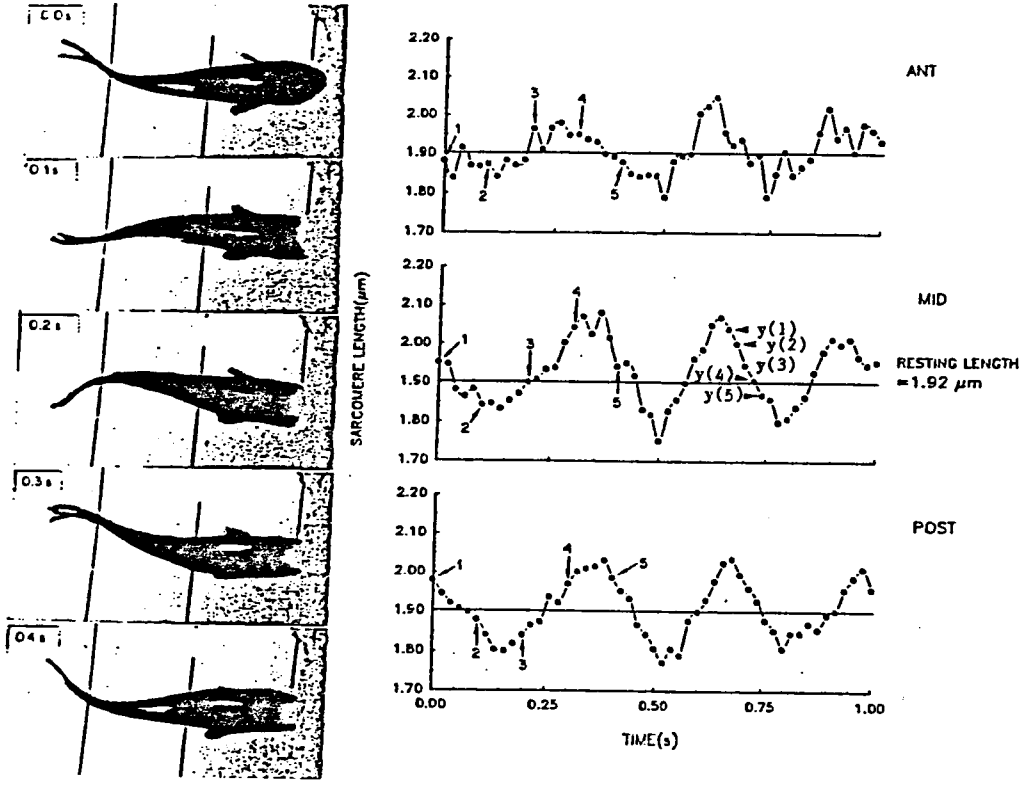


Fig.5. Typical record showing changes in sarcomere length in anterior, middle, and posterior positions of a fish swimming at 25 cm/s. 5 frames separated by 0.1s are shown and numbers on the photographs correspond to data points in the graphs on the right. Muscle velocity was calculated from the slope of the graph of sarcomere length against time. This is illustrated in the middle position. A 5-point analytical equation determines the velocity at y(3). Sarcomere length excursion (average difference between shortest and longest lengths measured in a sequence) was calculated from the amplitude of the graph.

length at three places along the body of a fish swimming at 25 cm/s.

In general, the anterior position was noisier than the middle and posterior. This is because curvatures were smaller in that position, and at small excursions random errors (eg due to tracing the side of the fish with a cursor) tended to be magnified. 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. The signal-to-noise ratio improved as the fish swam faster and sarcomere length excursions increased.

We were able to film fish swimming steadily at speeds ranging from 15-30 cm/s, 20-40 cm/s, and 20-45 cm/s at 10^{o}, 15^{o}, and 20^{o}C respectively. The fish were able to swim faster at 20^{o} than at 15^{o} or 10^{o}C because the red muscle produces more power per cross-section at a given shortening velocity at higher temperatures. This enables the fish to sustain higher speeds before activating the white muscle and beginning the burst and coast swimming mode. In burst and coast swimming, the fish utilize their white muscle intermittently to accelerate with rapid and powerful strokes of the tail. The fish did not swim steadily at 15 cm/s at 15^{o} or 20^{o}C, and a possible reason for this is that at the higher temperatures this swimming speed represents an unfavorable position on the force-velocity curve of the red fibres (see discussion, below). The fish would either not swim at all or alternate between swimming forward in the flume and drifting backwards without maintaining a steady position.

The maximum speeds at which the fish were able to swim steadily at each temperature correlated well with the speeds of white muscle

recruitment determined by electromyography. At 10^{o} we obtained an average recruitment speed of 27.1 cm/s $\pm$ 1.8 S.E (see table 1). At 15^{o} and 20^{o} the values were 36.3 $\pm$ 1.4 and 42.9 $\pm$ 3.5 cm/s. A certain amount of scatter is evident in the results. Although the fish swum in 1986 were smaller, on the whole they were able to attain higher swim speeds without recruiting their white muscle than the larger fish swum two years later. This may have been due to a difference in the condition and the size of the animals. Nevertheless the recruitment speeds we measured agree well with those of Rome et al. (1984) (26 $\pm$ 3 S.E. and 46 $\pm$ 6 cm/s at 10^{o} and 20^{o}C, respectively), and we are confident that at the speeds we analyzed the fish were swimming with their red muscle alone.

Figure 6 shows the relationship between swim speed and sarcomere length excursion at all three experimental temperatures in the anterior, middle and posterior positions. The data for this figure are presented in table 2. In the middle and posterior, excursions ranged from 0.2 to 0.3 um, centred around the resting length of 1.92 um. In these positions the excursions were essentially independent of swim speed. The excursions in the anterior were smaller than those in the middle and posterior at low swim speeds, but they increased more significantly as the fish swam faster.

Figure 7 summarizes the effect of temperature on sarcomere length excursion at each position on the body. Temperature had no influence on excursions in the middle and posterior at speeds the fish were able to swim at all three temperatures. In the anterior position, excursions appear to be larger at 10^{o} and 15^{o} than at 20^{o}C (ANCOVA, $p<0.0005$, as

TABLE 1. Speeds of Initial White Muscle Recruitment in Carp at 3 Temperatures.

CARP No.	LENGTH, cm	10°C	15°C	20°C
2a*	13.2	32	37	
4a	13.3		42	50
6a	13.7		38	
9a	14.1	37	42	
10a	12.5		33	
1	20.1	27.5		52.5
2	17.5	22.5		
3	19.3	22.5		
4	17.0	24	33.5	35
5	18.3	24		37.5
6	18.9	27.5		47.5
9	20.0		32.5	
11	17.8		32.5	
	MEAN±S.E.	27.1±1.8	36.3±1.4	44.5±3.5

* a Indicates Fish Swum in 1986. Remaining Experiments Carried Out in 1988.

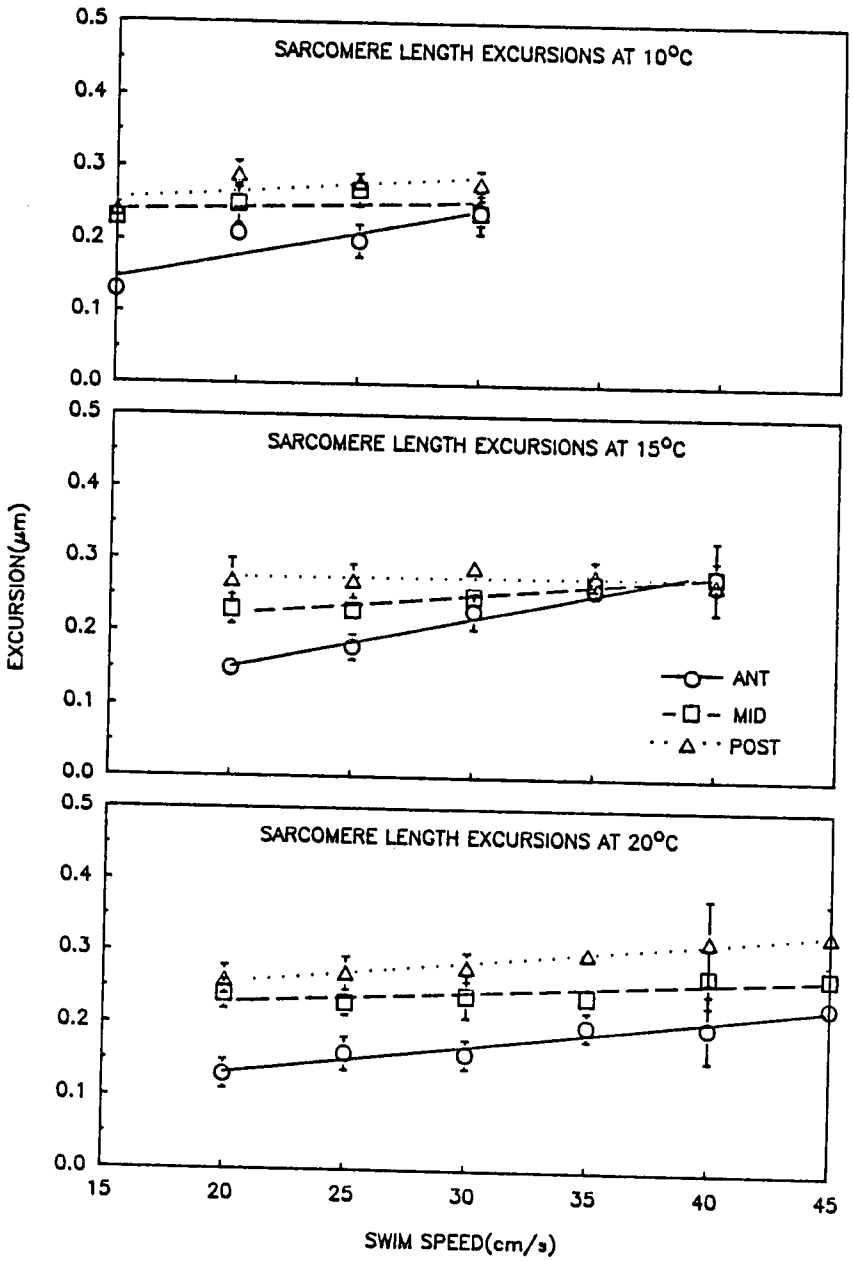


Fig. 6 . Relationship between swimming speed and sarcomere length excursion at the three experimental temperatures in the anterior, middle, and posterior positions on the fish. Points are means ± S.E. The influence of swimming speed in the middle and posterior positions was small (only at 15°C in the middle is the linear regression significant at 5%). Excursions increased more significantly with swimming speed in the anterior position: linear regressions are significant at 5% at all three temperatures.

TABLE 2. Average Sarcomere Length Excursions (um) in 3 Positions on Carp Swimming Slowly at 3 Temperatures.

SWIMMING VELOCITY, cm/s	10°C			15°C			20°C		
	ANT	MID	POST	ANT	MID	POST	ANT	MID	POST
15	0.13±0.01	0.23±0.02 n=3	0.24±0.001						
20	0.21±0.03	0.25±0.06 n=5	0.29±0.04	0.15±0.02	0.23±0.04 n=4	0.27±0.06	0.13±0.04	0.24±0.04 n=4	0.26±0.04
25	0.20±0.05	0.27±0.05 n=5	0.28±0.01	0.18±0.03	0.23±0.01 n=3	0.27±0.04	0.16±0.04	0.23±0.03 n=3	0.27±0.04
30	0.24±0.05	0.24±0.03 n=3	0.28±0.03	0.23±0.05	0.25±0.03 n=4	0.29±0.03	0.16±0.04	0.24±0.06 n=4	0.28±0.04
35				0.26±0.03	0.27±0.03 n=4	0.28±0.04	0.20±0.04	0.24±0.02 n=4	0.30±0.03
40				0.28±0.07	0.28±0.03 n=2	0.27±0.00	0.20±0.08	0.27±0.07 n=3	0.32±0.10
45							0.23±0.00	0.27±0.01 n=2	0.33±0.06

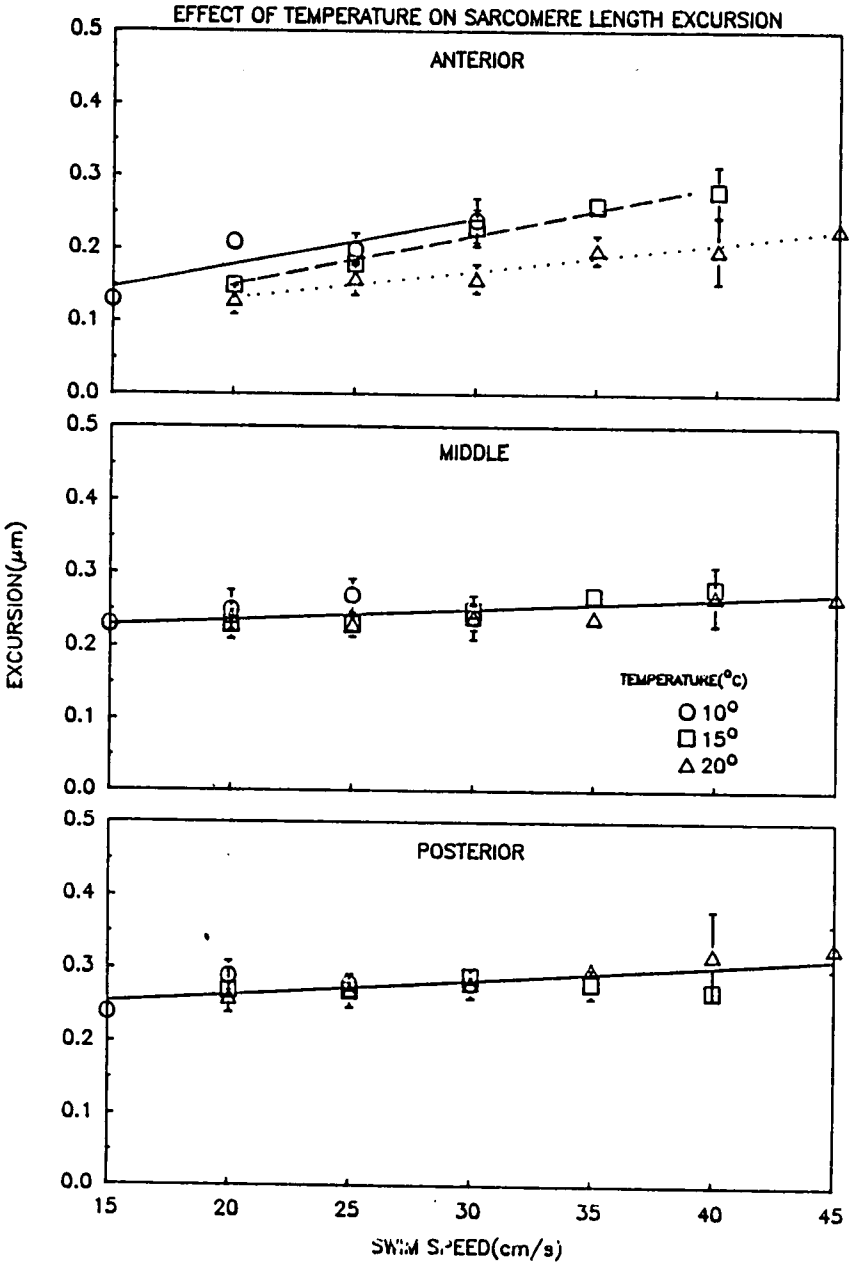


Fig. 7 . Effect of temperature on sarcomere length excursion at each position on the body. Points are means ± S.E. In the middle and posterior temperature had no effect on excursion. (ANCOVA, $p>0.25$ in both positions). Mean excursions (±S.D.) are 0.25 ± 0.04 in the middle and 0.28 ± 0.04 in the posterior position. In the anterior, excursions are significantly larger at lower temperatures (ANCOVA, $p<0.0005$).

described in Zar, 1985). This is an interesting and potentially meaningful result, but in view of the added noise in the data from the anterior position it should be treated with some caution.

Tail-beat frequency was found to increase linearly with swim speed in a manner that was independent of temperature (see fig. 8 and table 3). Thus the sarcomeres underwent the same length changes in a shorter period of time, resulting in an increase in muscle shortening velocity as is shown in figure 9. Muscle velocity data are presented in table 4.

The effect of temperature on muscle velocity is shown in figure 10. As with the sarcomere excursions, temperature had no effect on the velocity of the red muscle in the middle and posterior positions. In the anterior, velocities appeared to be lower at 20^{o} than at 15^{o} and $10^{o}C$ (ANCOVA, $p<0.0005$), but again this result should be viewed critically.

It is possible that the fish generate additional thrust at low temperatures by increasing the amplitude of the body wave in the anterior part of the body. Two other parameters that influence the amount of thrust produced are tail height and tail-beat amplitude. Since a fish must move its body when it moves its tail (Lindsey, 1978), it might be expected that the increased side-to-side movement in the anterior position would be manifested in an increase in the tail-beat amplitude at the low temperatures. Figure 11 shows that neither temperature nor swim speed had an effect on tail-beat amplitude (see table 5). Figure 12 (see also table 6) summarizes the results of our analysis of tail height. Here too, there is no effect of swim speed or temperature.

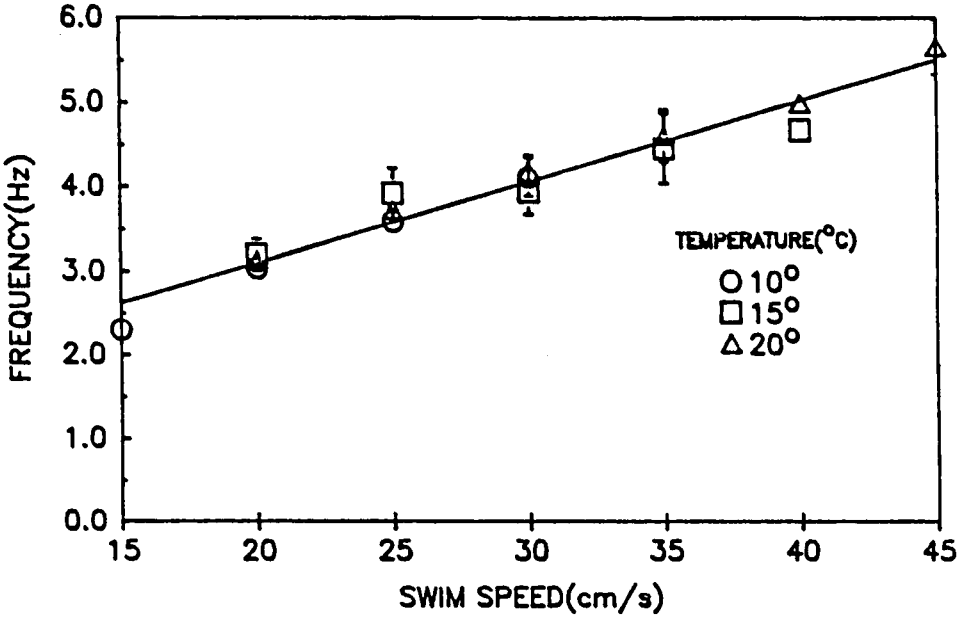


Fig. 8. Temperature had no effect on tail beat frequency (ANCOVA, $p>0.1$). Linear increase of tail beat frequency with swimming speed at all three temperatures is shown by the solid line (frequency=0.0964×velocity+1.1745, $r^2=0.98$, n=52). Points are means ± S.E.

TABLE 3. Tail-Beat Frequencies (Hz) in Carp Swimming Steadily at 3 Temperatures.

	FISH 2			FISH 4			FISH 5			FISH 6			FISH 8			FISH 9		
SWIMMING VELOCITY, cm/s	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C
15							2.32			2.41						2.16		
20	3.30	2.79	3.24	2.93	2.98	3.12	3.40			2.95	3.32	3.10		3.67		2.98		3.09
25	4.07	3.38	3.80	3.36			3.89			3.52	3.97	3.85		4.40		3.13		3.51
30	4.56	3.65	4.65	3.81	3.65	4.15	4.00		3.72		3.76			4.74				4.22
35			5.35		4.32	4.50					3.82	3.86		5.25				4.71
40					4.53	5.17			5.08		4.80	4.74						
45						5.33												5.99

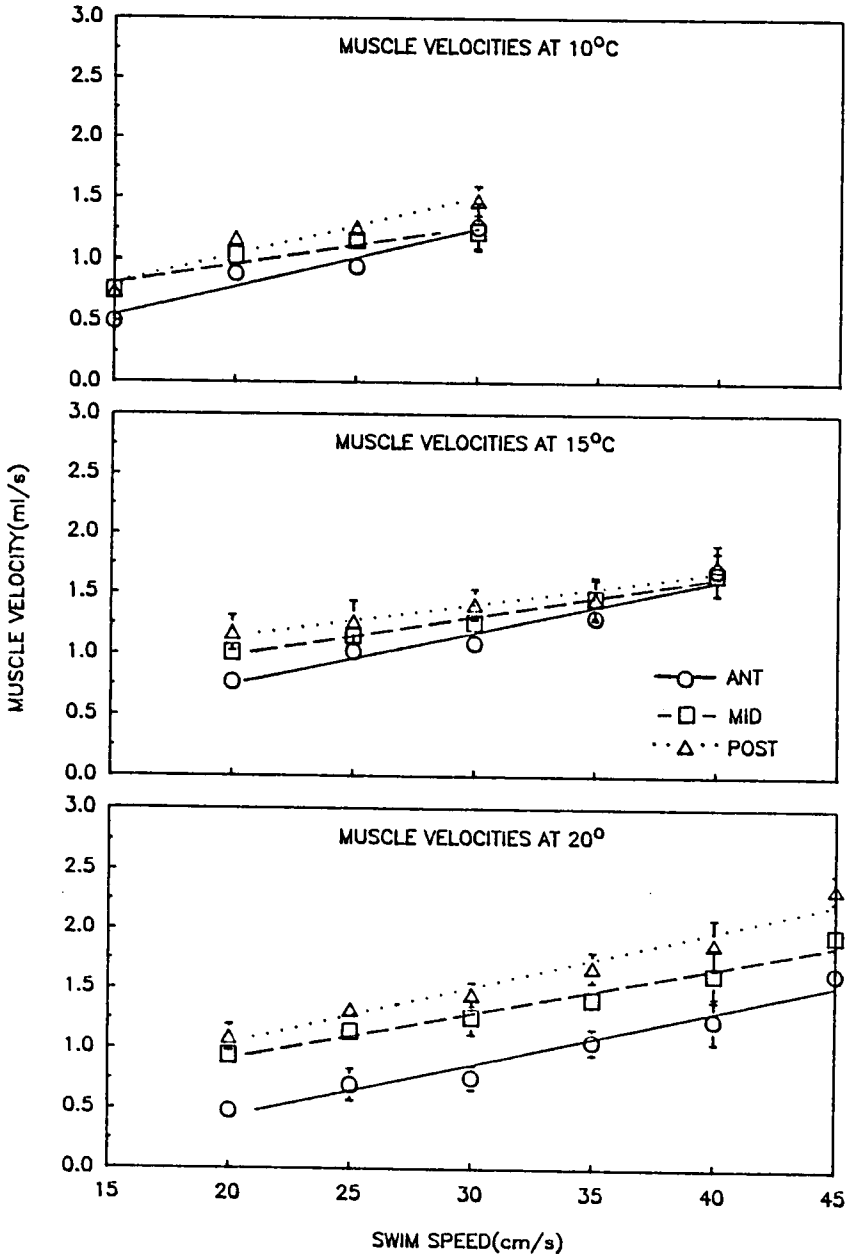


Fig. 9 . Relationship between swimming speed and muscle velocity at 10°, 15°, and 20°C in the anterior, middle and posterior positions. Points are means ± S.E.

TABLE 4. Average Red Muscle Velocities (lengths s^{-1}) in 3 Positions on Carp Swimming Slowly at 3 Temperatures.

SWIMMING VELOCITY, cm/s	10°C			15°C			20°C		
	ANT	MID	POST	ANT	MID	POST	ANT	MID	POST
15	0.50±0.07	0.75±0.02 n=3	0.74±0.07						
20	0.89±0.19	1.04±0.19 n=5	1.17±0.16	0.77±0.10	1.01±0.15 n=4	1.17±0.29	0.48±0.14	0.94±0.10 n=4	1.09±0.22
25	0.95±0.16	1.16±0.16 n=5	1.26±0.09	1.02±0.13	1.15±0.08 n=3	1.26±0.31	0.70±0.23	1.14±0.16 n=3	1.32±0.16
30	1.27±0.33	1.23±0.23 n=3	1.48±0.21	1.09±0.06	1.26±0.11 n=4	1.41±0.25	0.76±0.20	1.26±0.29 n=4	1.45±0.20
35				1.30±0.17	1.46±0.36 n=4	1.46±0.31	1.06±0.22	1.41±0.10 n=4	1.68±0.26
40				1.70±0.42	1.66±0.13 n=2	1.74±0.15	1.24±0.33	1.62±0.39 n=3	1.88±0.37
45							1.63±0.15	1.95±0.13 n=2	2.35±0.17

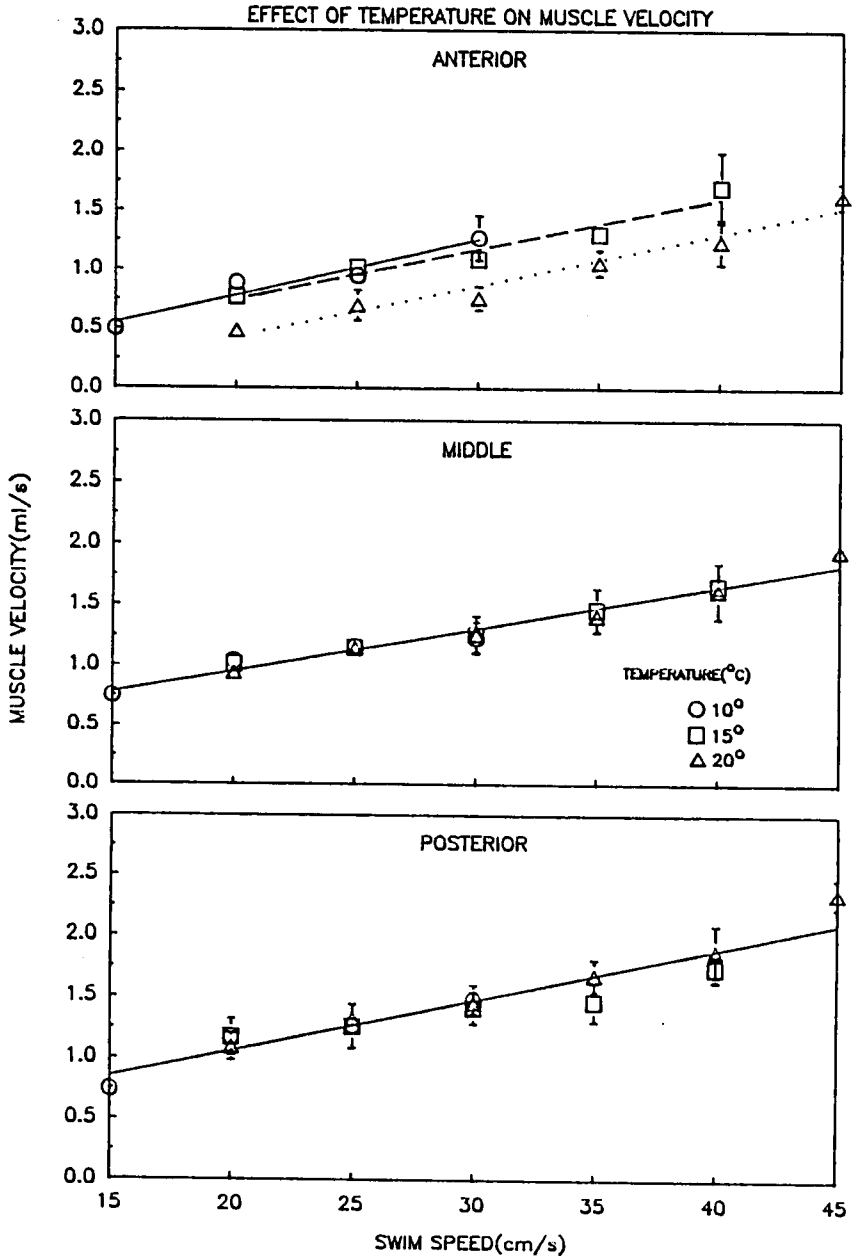


Fig.10. Effect of temperature on muscle velocities in the three positions on the body. Points are means $\pm$ S.E. Temperature had no effect on velocities in the middle and posterior positions (ANCOVA, $p>0.25$ in both positions). Due to the increase in tail beat frequency, there is a significant increase in muscle velocity with swimming speed ($r^2=0.98$ and 0.95 in the middle and posterior, respectively, $n=53$). Temperature had a significant influence on muscle velocities in the anterior position (ANCOVA, $p<0.0005$).

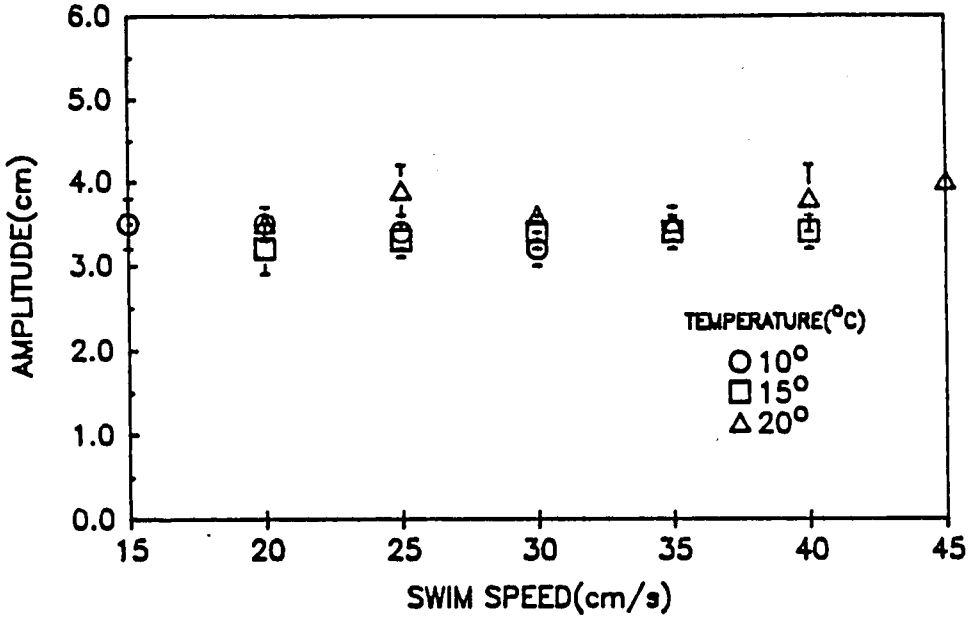


Fig.11. Neither swimming speed nor temperature had an influence on tail beat amplitude (ANOVA, $p>0.25$). Points are means $\pm$ S.E.

TABLE 5. Tail-Beat Amplitudes (cm) in Carp Swimming Steadily at 3 Temperatures.

SWIMMING VELOCITY, cm/s	FISH 2			FISH 4			FISH 5			FISH 6			FISH 8			FISH 9		
	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C
15							2.9			4.0						3.6		
20	3.2	2.8	2.9	3.8	3.8	3.4	2.9			3.6		3.7		2.9		3.8		3.8
25	3.0	3.0	3.3	3.7	3.5		3.1			3.8	3.6	3.8		3.1		3.4		4.5
30	2.9	3.1	3.2	3.6	4.0	3.4	3.2		3.5		3.6	3.8		3.0				4.0
35		3.2	3.0		3.7	3.5					3.8	3.7		2.9				3.7
40		2.9			3.5	3.9			3.1		3.9	4.4		3.3				
45						4.0												

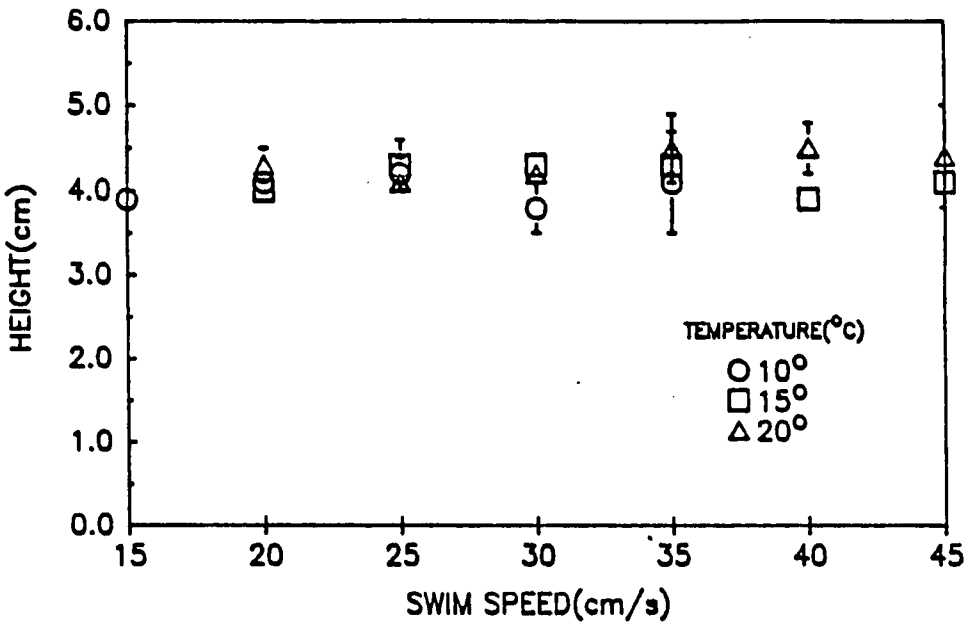


Fig.12. Neither swimming speed nor temperature had an influence on tail height (ANOVA, $p>0.25$). Points are means $\pm$ S.E.

TABLE 6. Tail Heights (cm) in Carp Swimming Steadily at 3 Temperatures.

	FISH 4			FISH 5			FISH 6			FISH 9			FISH 10		
SWIMMING VELOCITY, cm/s	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C	10°C	15°C	20°C
15							3.9								
20	4.2			3.9	4.1	4.4	4.0	4.3	3.8	4.3	3.8	4.6		3.8	
25	4.6	4.7		3.9	4.4	4.1		4.5		4.0		4.1		3.4	
30		4.5			4.1			4.7		4.1	4.2	4.2	3.5	4.0	
35	4.6	4.6			4.2	4.1					4.1	4.8	3.5		
40					3.9	4.2						4.7			
45					4.0				3.8		4.2	4.9			

In summary, these results show that at low temperatures the maximum sustainable swim speed is reduced but that at a given swim speed, temperature has no effect on the rate of shortening of the lateral red muscle. The muscle in the anterior position presents a possible meaningful exception to this generalization.

DISCUSSION

Temperature affects the rate of biochemical processes by a factor of 2-3 for every change of 10°C. The contractile properties of muscles are similarly affected by temperature. In fish and other ectothermic animals the decrease in V_{max} in the cold means that for a given rate of contraction, their muscle will produce less force per cross-section than it would at a higher temperature. By comparison temperature has virtually no effect on the amount of power needed to swim at a certain speed (temperature-dependent changes in the viscosity of water will be discussed below). Thus it is expected that temperature should have drastic effects on the swimming performance of fishes. In particular, we may ask whether determinants of thrust such as tail height, tail-beat amplitude, and tail-beat frequency are affected. Furthermore, this study addresses the question of whether the V/V_{max} of the red myotomal muscle is changed in response to large changes in V_{max}.

Temperature effects on the kinematics of swimming

There are no reports in the literature concerning the effects of temperature on tail height or tail-beat amplitude. A number of studies have been done on the relationship between swim speed and tail-beat amplitude (Bainbridge, 1958; Hunter and Zweiffel, 1971; Videler and Hess, 1984). Bainbridge demonstrated that at slow swimming speeds fish modulate both tail-beat amplitude and frequency, while at high speeds they modulate only frequency. Hunter et al. stated that fish do not modulate amplitude at all and produce more thrust at higher swim speeds solely by increasing tail-beat frequency. Over the range of speeds we studied, we found no changes in tail-beat amplitude with swim speed. Tail height also remained constant at all swim speeds. Neither of these parameters appeared to be influenced by temperature.

The effect of temperature on tail-beat frequency has been the subject of a several studies. Rome et al. (1984) reported as we do here that temperature has no effect on the relationship between swim speed and tail-beat frequency in carp. Smit, Amelink-Koustaal, Vijverberg, and von Vaupel-Klein (1971) stated that in goldfish tail-beat frequency was also independent of temperature. On the other hand, Stevens (1979) reported that in trout frequencies were lower at higher water temperatures, whereas in bass the opposite was true: in these fish tail-beat frequency decreased with decreasing temperature. The changes Stevens reported were small, however (on the order of 0.2 Hz or 10% for every 10^{o}C change in temperature), and were significant in only 10 out of 18 comparisons. Sisson and Sidell's study of striped bass (1987)

concurred with Stevens' findings on largemouth bass. This suggests that in these species the relationship between tail-beat frequency and stride length (distance moved per tail beat) changes with temperature.

Sidell and Moerland (1988) examined the possibility that in striped bass stride length is greater in the cold. If it is true that tail-beat frequency is lower and that the mean thrust is approximately the same at decreased temperatures, more thrust should be be provided per stroke to achieve the same swimming speed. From their own data, and using trailing edge kinematics, they estimated that for every $10^{o}C$ decrease in temperature, a 30% increase in tail beat amplitude or depth of the trailing edge or a combination of the two would suffice to produce equivalent thrust at the lower temperature. There is no evidence that this happens, and although different species may have different temperature compensation mechanisms, our results show that there is no necessity for fish to redistribute the production of thrust when the water temperature changes. In carp, at least, tail-beat frequency, tail height, and tail-beat amplitude are not influenced by temperature.

On the basis of these findings, the fish appeared to be producing the same average amount of thrust at each experimental temperature. However, the viscosity (μ) of the water depends on temperature, being about 30% greater at 10^{o} than at $20^{o}C$. Since the drag on a fish is approximately proportional to $1/\mu^{0.3}$, this causes the fish to experience about 10% more resistance to forward motion at the lower temperature. Thus we would expect some locomotory parameter to be changed to provide the additional thrust at low temperatures. From our

results, it appears that the carp might be doing this by increasing the amplitude of the body wave near the front of trunk at low temperatures.

Fish are able to produce thrust all along their trunk because as the propulsive wave moves towards the tail, any body segment that has a positive angle of attack exerts a force against the water. Gray (1933) estimated that the trunk contributes as much as 60% of the total thrust. The amount of thrust produced by a particular segment is proportional to W, the wetted surface area of the segment, and S^2, where S is the speed of transverse movement of the segment (Bainbridge, 1963). Thus it is plausible that at low temperatures, carp increase the speed of transverse movement of the anterior segments of their body to offset the increased viscosity of the medium. It is not certain that the higher muscle velocities we measured in the anterior position increase the speed of transverse movement of that part of the body, and additional film analysis is necessary to settle this question. The 10% change in viscosity is, in any case, small compared to the nearly 2-fold change in muscle V_{max} that the fish undergoes in the same temperature range.

Effects of temperature on V/V_{max}

We have shown that in the middle and posterior positions, the velocity of shortening of the red muscle at a given swim speed is the same at all three test temperatures. However, due to the lower V_{max} of the muscle in the cold, the power output of the aerobic fibres at $10^{o}C$ is about 1/2 of that which can be produced at $20^{o}C$. Therefore more

fibres must be used at the low temperature, and the anaerobic muscle mass must be activated at a lower swim speed, as shown by the results of our electromyography experiments.

For carp of the size used in these experiments, red muscle V_{max} data from single fibre mechanics experiments are available only for 15°C. The value at this temperature is 4.65 muscle lengths s^{-1} (Rome et al., in press). The V_{max} at the other temperatures can be estimated using a Q_{10} of 1.8 (Rome, pers. comm.), and are calculated to be 3.47 and 6.24 muscle lengths s^{-1} at 10° and 20°C, respectively. With regard to this nearly two-fold change in V_{max}, the question arises whether the ratio V/V_{max} is affected by temperature. This is important in the energetics of animal locomotion, because V/V_{max} is the principal determinant of efficiency and mechanical power output of muscle fibres.

The range of values of V at each temperature is defined by the muscle velocities at the lowest and highest speeds the fish could swim at using their red muscle exclusively. The highest speed was taken as the average speed at which white muscle was initially recruited. Determining the lowest speed was more subjective: in general we found that at 10°C the fish would swim steadily at slower speeds than at 15° or 20°C (see Results section, above).

Table 7 shows the range of shortening velocities obtained in the red muscle at each temperature. The corresponding range of V/V_{max} is also shown. We concluded from the analysis at 15°C that the range of V/V_{max} of red muscle (0.16-0.35) represented maximal efficiencies, and that at the highest swim speed (40 cm/s) the fibres were operating on a part of their force-velocity curve where they would be generating

TABLE 7. Ranges of Shortening Velocities and V/V_{max} in the Red Muscle During Swimming at Slow Speeds at 3 Temperatures.

TEMPERATURE	RANGE OF V	V_{max}	RANGE OF V/V_{max}
10°C	0.50-1.48 ls^{-1}	3.47 ls^{-1}	0.14-0.43
15°	0.77-1.74	4.65	0.16-0.37
20°	0.48-2.35	6.24	0.08-0.38

maximum mechanical power (Rome et al., in press). The range of velocities observed at 10°C represents a very similar range of V/V_{max} (0.14-0.43). At 20°C at the highest swim speeds where red muscle is used exclusively V/V_{max} was 0.38, which is near the peak of mechanical power production. The lower limit of V/V_{max}=0.08 occurred in the anterior position at the slowest swim speed (20 cm/s).

V/V_{max}=0.08 is a position on the force-velocity curve where efficiency and power production are less than optimal. At the same swim speed, V/V_{max} was 0.15 in the middle position and 0.17 in the posterior, which is similar to the values obtained at 10° and 15°C. At 20°C at slow swimming speeds sarcomere lengths in the anterior position fluctuated so erratically that it may not be possible to obtain realistic estimates of V from the graphs of sarcomere length against time. Velocities in this position do appear to be somewhat smaller at 20° than at 15° and 10°C, where the data were not as noisy (see Results). At lower temperatures the muscle in the anterior portion of the trunk may be used more intensively to overcome additional drag due to changes in the viscosity of the water. However, EMGs from this position indicate that the muscle is active at 20° as well as 10°C (Rome, pers. comm.).

The Q_{10} for the velocity of the red muscle at the recruitment swimming speed (30 cm/s at 10° and 45 cm/s at 20°C) is 1.6. This is similar to the Q_{10} value of 1.8 we used to calculate the V_{max} of the red muscle at these temperatures. These values would be expected to be the same, since the muscle velocities correspond to the position on the force-velocity curve where power output is maximal at each temperature

(that is, the same value of V/V_{max} at each temperature).

Thus it appears that in spite of large changes in V_{max} with temperature, the lateral red muscle continues to operate over virtually the same part of its force-velocity curve (see fig. 13). That is, over a range of values of V/V_{max} where efficiency and mechanical power production are optimized see. Swimming speed and muscle shortening velocity are coupled mechanically in a way that is independent of temperature. As the V_{max} of the red muscle fibres increases with temperature, they can shorten more quickly and produce more power without detriment to their efficiency, and fish are able to sustain higher swimming speeds. The same shortening velocity at a $10^{o}C$ lower temperature, where the V_{max} is reduced nearly two-fold, represents an unfavorable position on the force-velocity curve of the red muscle fibres. As a result, the fish cannot sustain high swimming speeds. A corollary of this is that low muscle velocities corresponding to slow swimming speeds may be inefficient at $20^{o}C$ (where V/V_{max} would be very small), while still lying on a favorable part of the force-velocity curve at $10^{o}C$. This may explain why we were able to analyze sequences of fish swimming steadily at 15 cm/s at 10^{o} but not at 15^{o} and $20^{o}C$.

These are important findings which suggest that although their overall capacity for movement is diminished in the cold, ectothermic animals can locomote over a wide range of temperatures while maintaining maximal efficiency of power production.

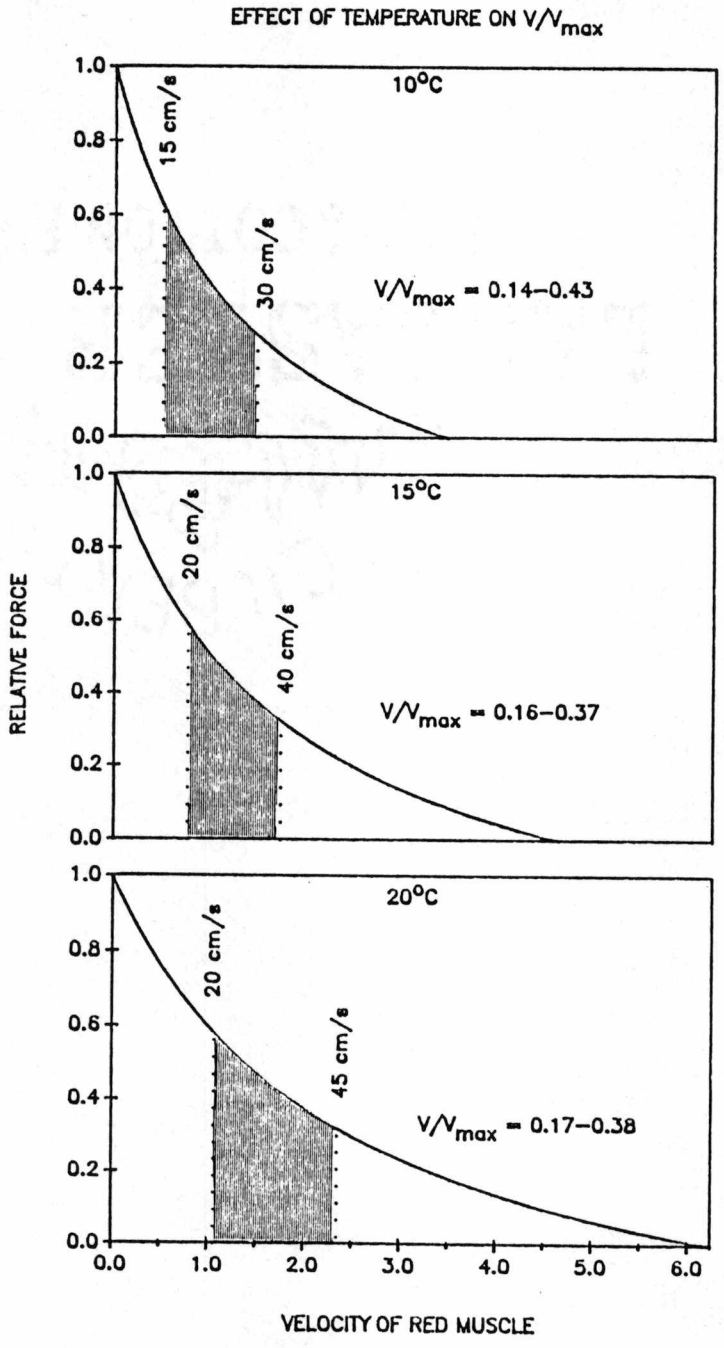


Fig. 13. Effect of temperature on **V/Vmax**. Figure shows the range of shortening velocities over which the red muscle is active exclusively at each experimental temperature. Although the **Vmax** of the muscle is reduced nearly 2-fold for every 10°C decrease in temperature, the range of **V/Vmax** is the same at each temperature, corresponding to velocities at which power production and efficiency are optimized.

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APPENDICES

APPENDIX 1

WHY ANIMALS HAVE DIFFERENT MUSCLE FIBER TYPES[2]

INTRODUCTION

It has been recognized for many years that animals have different muscle fiber types: slow ones with a low maximum velocity of shortening (V_{max}) and fast ones with a high V_{max}. It is generally accepted that slow fibers are more efficient at low speeds of locomotion, and that fast fibers are necessary for locomotion at maximal speeds because the slow fibers cannot shorten fast enough (Goldspink, 1977).

The problem with these concepts is that force generation, mechanical power production and efficiency are not singular values for a given fiber type. Rather they are all functions of "where on the force-velocity curve fibers are operating" (or more precisely V/V_{max} where V is the velocity at which the muscle is shortening during locomotion and V_{max} is the maximum velocity of shortening). For instance, both mechanical power and efficiency are equal to zero at $V/V_{max}=0$ and $V/V_{max}=1$ and reach a maximum at $V/V_{max}=0.2$-0.3 (Hill, 1950, 1964; Kushmerick and Davies, 1969). Thus, to test these theories one must determine the V/V_{max} of the slow and fast fibers both during slow speeds of locomotion and during maximal movements. Measurements of

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[2]Accepted for publication by Nature (Rome, L.C., Funke, R.P., Alexander, R.M., Lutz, G., Aldridge, H.D.J.N., Scott, F., Freadman, M. In the Press).

V/V_{max} require separate determination of 1) the V at which the muscle fibers shorten during locomotion, 2) the V_{max} of the different fiber types, and 3) which muscle fiber types are active at a given speed of locomotion.

Of the three, it is the last which most limits the selection of an animal model. Carp, (Cyprinus carpio), because of the anatomical separation of their different muscle fiber types (figure 1), provide an excellent model. We have previously shown that the activity of the different fiber types in carp can be monitored by electromyography (Rome, Loughna, and Goldspink, 1984, 1985). Electromyography shows that at low speeds, the red muscle fibers alone are active, and at higher speeds, the white muscle fibers are recruited as well. Thus, by knowing the speed of swimming, we know which muscle fiber types are active.

METHODS

Determination of V_{max}

Bundles of 20-30 red muscle fibers were removed from above the carp's midline and were maximally activated by electrical stimulation (200 pulses/s) in the presence of 1mM caffeine and 10^{-5}M eserine. This provided an exceptionally stable preparation, which on some occasions developed more than 100 tetani with less than a 2% drop in force. The force-velocity relationship was measured with a computer-controlled servo system using the force clamp method (Julian, Rome, Stephenson,

and Striz, 1986a) over approximately the same sarcomere length range (2.05- 1.82um) that the muscle underwent during swimming. Figure 14 shows the force-velocity curve for one preparation. The mean V_{max} for 5 preparations was 4.65 muscle lengths/s ($\pm$SE=0.55, n=5) at 15°C.

V_{max} was measured on single "skinned" white muscle fibers using the "slack test" method (Altringham and Johnston, 1982; Julian et al., 1986b). A V_{max} of 12.88 muscle lengths/s ($\pm$SE=0.5, n=6) was found.

Determination of V

The sarcomere length and velocity of shortening of the red and white muscle were determined from high speed motion pictures taken at 200 frames/s (see figure 1 legend).

To determine V of the fibers during slow locomotion, high speed motion pictures were taken of 4 carp swimming in a Brett-type respirometer at speeds (20-40 cm/s) and at a temperature (15°C) where only the red muscle fibers were active. Sarcomere length was calculated at 3 places along the body (see figure 1).

To investigate the effectiveness of the red muscle to power maximal movements, we photographed the "escape reponse", elicited by a 100ms, 150Hz sound pulse delivered through an underwater speaker.

RESULTS

We determined that during swimming at 40 cm/s, sarcomeres in the red fibers underwent an excursion of about 0.27um (SE=$\pm$0.01, n=9)

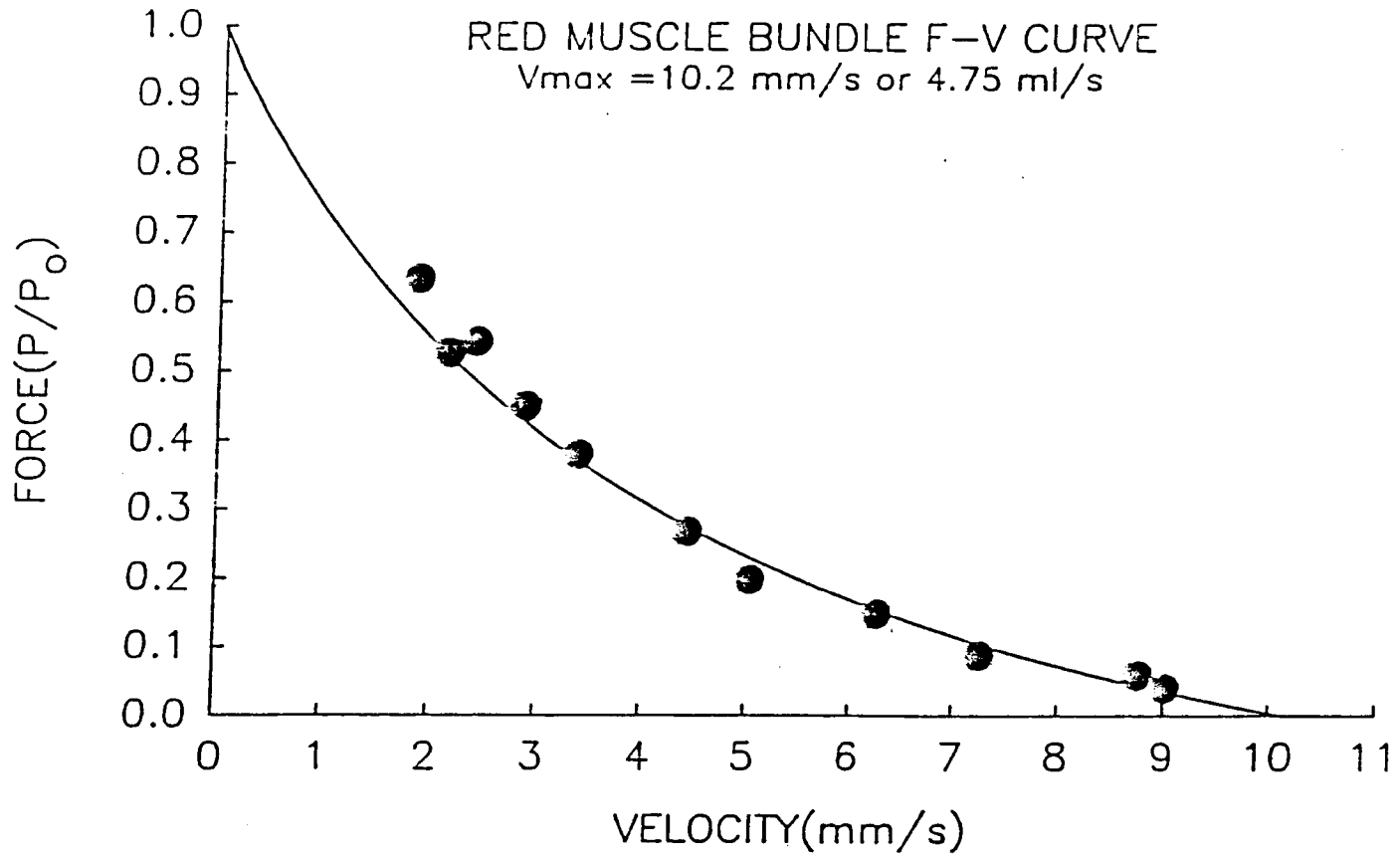


Fig.14. Force-velocity curve of red muscle bundles. Bundles were allowed to shorten against various forces (force-clamp method) while the velocity of shortening was measured. The curve was fit by an unbiased Hill equation (Julian et al, 1986; V_{max}=10.12 mm/s, a/P_o=0.40.

centered around the resting length of 1.92um (i.e. from 1.76 to 2.06um). Figure 15 shows that the sarcomere length excursion was nearly independent of swim speed in the middle and posterior regions of the fish, but was lower in the anterior region of the fish at slow swim speeds.

As swim speed increased from 20 to 40 cm/s, we found that tail-beat frequency increased as well. Thus the fishes' muscles had to undergo the same sarcomere length change in a shorter period of time, resulting in an increase in the muscle shortening velocity. On average, there was no difference in the shortening velocity (1.67 muscle lengths/s, SE=$\pm$0.13, n=4) between the anterior, middle and posterior positions of the fish at the highest speed the fish could swim at prior to recruiting their white muscle. At slow swim speeds, however, the velocity of the anterior fibers was significantly smaller than that of the middle and posterior, because of their smaller sarcomere excursion (at 20 cm/s, anterior fibers shortened at 0.73 muscle lengths/s, SE=$\pm$0.04, n=4 vs. 1.08 for the middle and posterior fibers). Thus during normal swimming, the red muscle shortens at velocities from 0.73 to 1.67 muscle lengths/s. As can be seen in figure 16, this corresponds to a velocity range at which maximum power is generated by the muscle. At these same swimming speeds, the white muscle, if it were active, would be shortening at only 0.18 to 0.42 muscle lengths/s (this is due to the different orientation of the white muscle fibers, as explained in figure legend 1).

As can be seen in figure 17, the fish undergoes a sharp "C" bend in a short period of time during the escape reponse. Muscle shortening

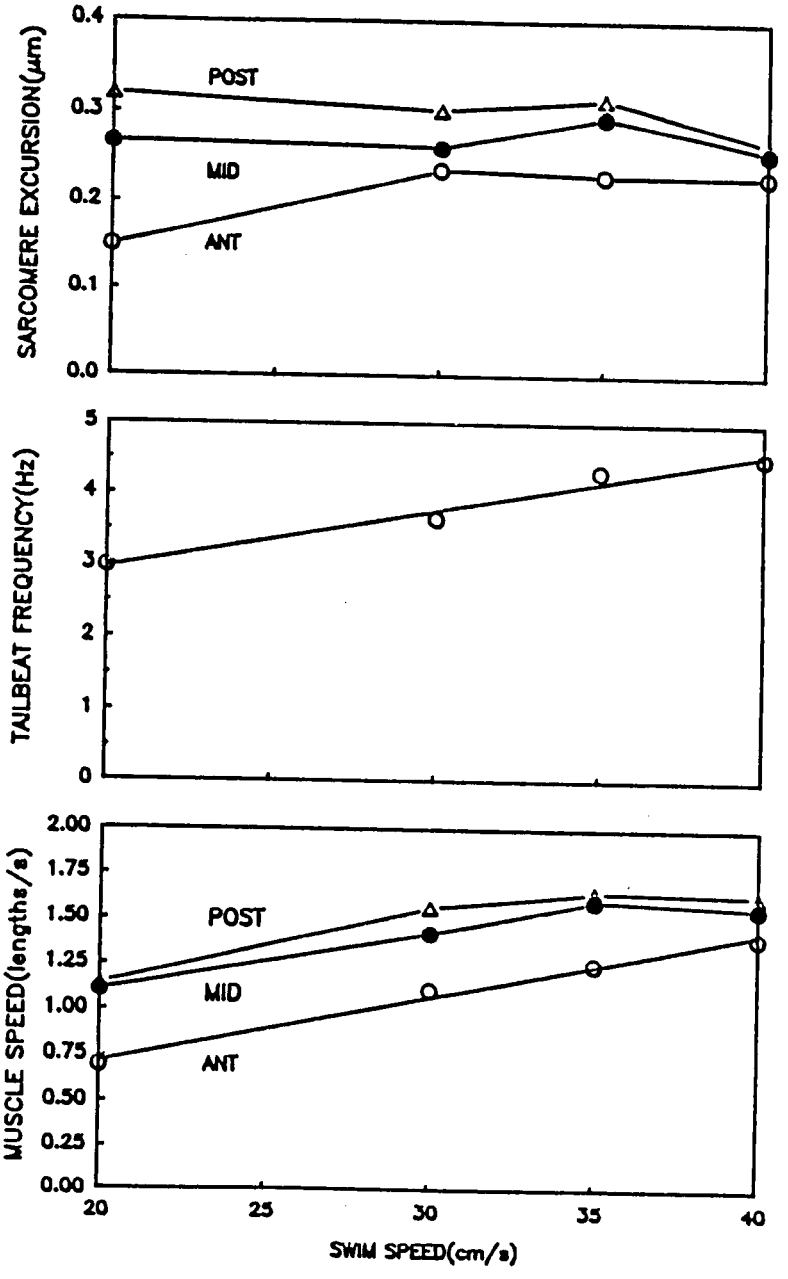


Fig.15.Sarcomere excursion, tail-beat frequency, and velocity of muscle shortening, V, as a function of swim speed. This figure shows data for one of the 4 fish examined in this study. At 20 cm/s the sarcomere length excursion was smaller in the anterior than in the middle and posterior regions of the fish. As speed increased, the sarcomere excursion of the anterior fibers increased while that of the middle and posterior stayed the same. At the maximum speed the fish can swim before recruitment of the white muscle (40 cm/s), the sarcomere length excursion is the same at the 3 positions on the fish.

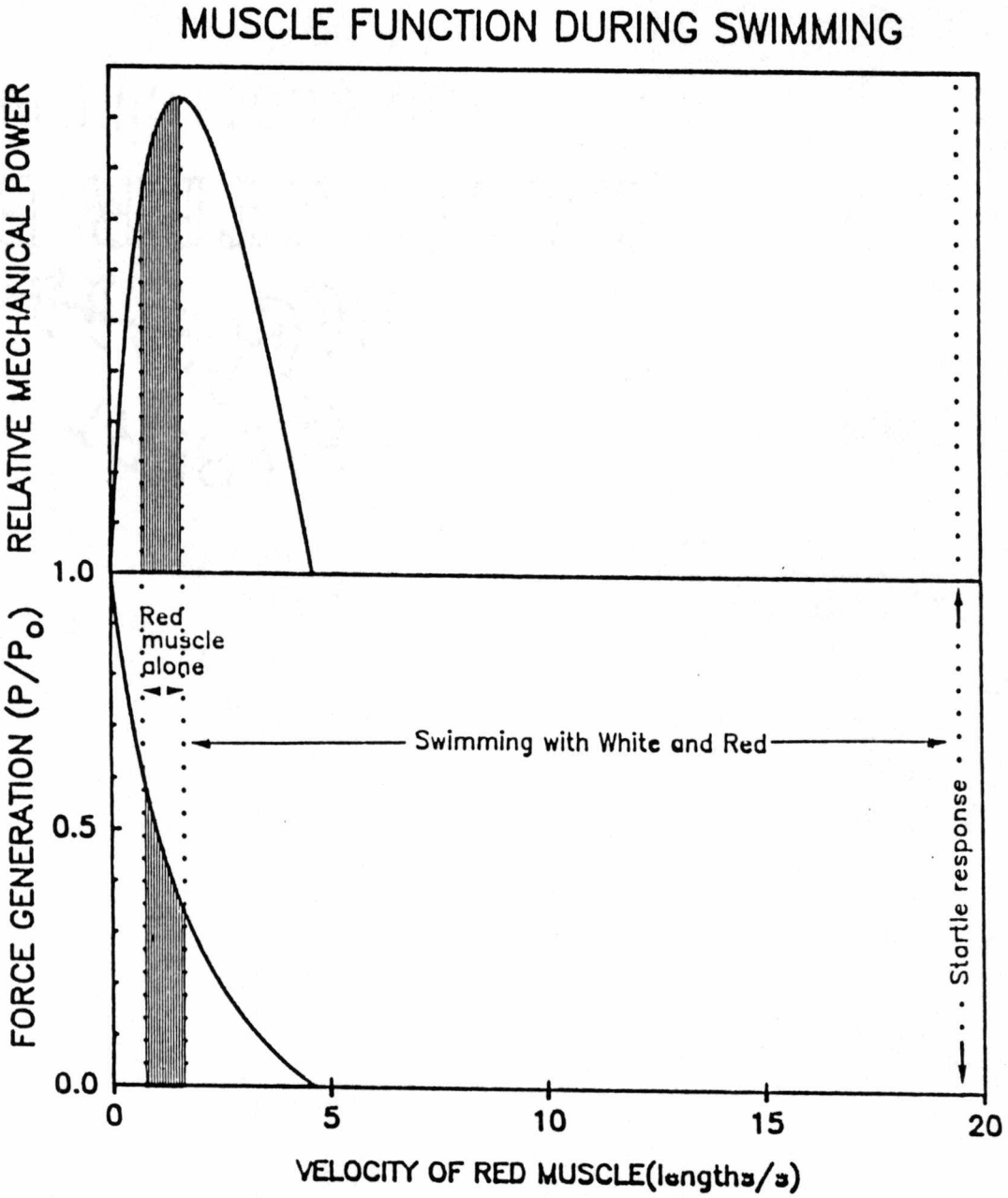


Fig.16. Muscle function during locomotion. The average force-velocity (Hill equation constants: V_{max}=4.65 muscle lengths/s, a/P_o=0.40) and mechanical power curves obtained from the red muscle bundle experiments are graphed. The velocity of shortening at the position of the red muscle during slow swimming and maximal movements are shown on the graphs. The dashed line shows the velocity the red muscle fibers must shorten to generate the "escape response".

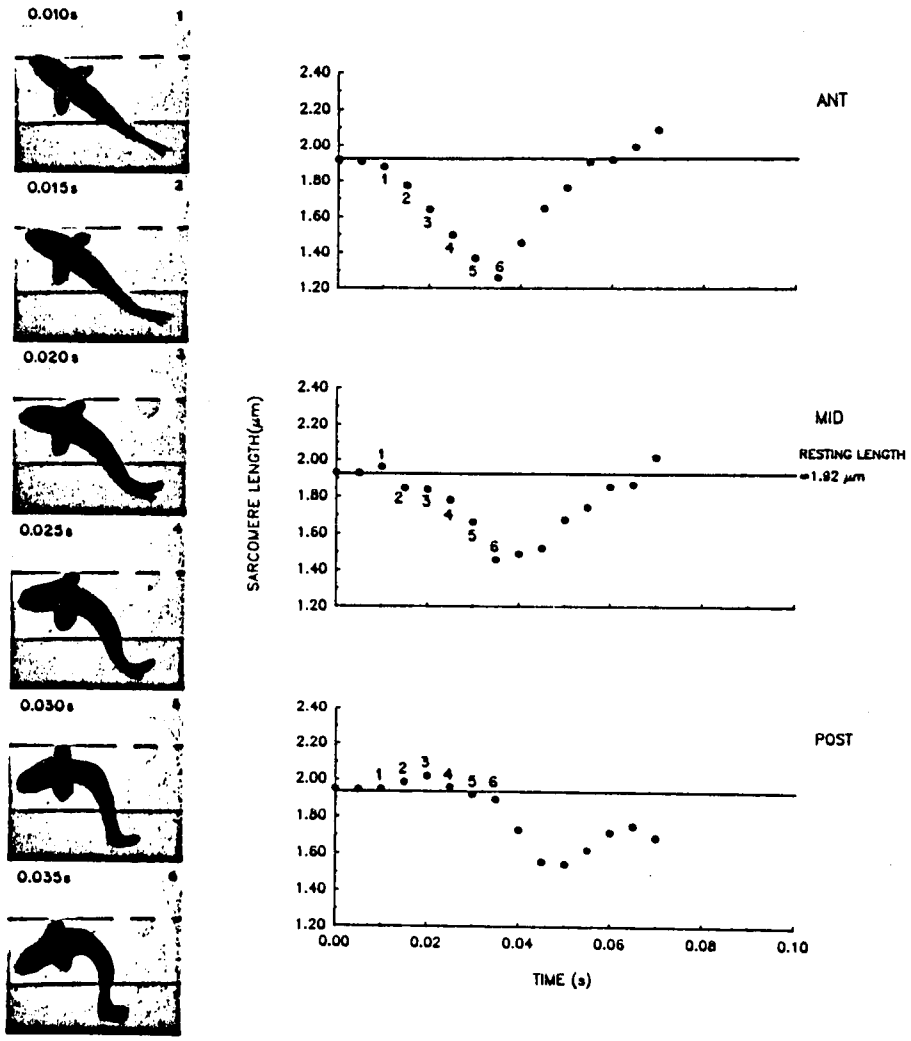


Fig.17 The "escape response" of a carp. A resting carp received a 100ms, 150Hz sound pulse through a hydrophone in the aquarium about 30 cm from the fish. The response was filmed at 200 frames/s and six consecutive frames (separated by 5ms) are numbered and shown on the left. The numbers and times given on the photographs correspond to data points on the graphs of sarcomere length of red muscle as a function of time shown on the right. Sarcomere lengths are shown for three positions along the fish. As can be seen, the sarcomere shortening in the anterior region precedes that in the middle and posterior. Fibers appear to shorten to extremely short lengths of about 1.27um. On average, the velocity of shortening (the slope of the sarcomere length-time graph) was the same for all three positions, however, the sarcomere excursion was greater in the anterior and middle regions than in the posterior.

is far greater than that undergone during normal swimming, and is far more rapid. Red muscle fibers shorten to a sarcomere length of 1.28um (SE=$\pm$0.02, n=15), while on the convex side they are stretched to about 2.56um. This occurs in approximately 20 ms. On average, the velocity of shortening of muscle in the position of the red was 19.6 muscle lengths/s (SE =+0.84, n=15), which far exceeds the V_{max} of the red muscle (figure 16). The white muscle, because of its different orientation, need shorten at only 4.85 muscle lengths/s during a startle response.

DISCUSSION

Figure 16 shows how the red muscle is used during locomotion. The shaded portion represents the velocities at which fibers shorten while the fish is swimming between 20-40cm/s (speeds at which red muscle is used exclusively). At higher swim speeds we have observed that carp recruit their white muscle and suggested that the reason for this is that the red muscle cannot generate sufficient mechanical power for these high swim speeds (Rome et al., 1984). The mechanical power needed for swimming increases with $(\text{speed})^{2.5}$. As can be seen, at the maximum speed the fish can swim prior to recruiting their white muscle, the red muscle is shortening at a velocity where it is already generating its maximum mechanical power. As the fish swims faster, its red muscle would have to shorten at a higher velocity and at a higher shortening velocity, the muscle would actually generate less mechanical power. To generate the additional mechanical power, the fish needs to recruit its

white muscle, which results in rapid fatigue because of the low aerobic capacity of the white muscle.

As the fish swims faster it recruits both its white and red muscle. The dashed line in figure 16 represents the shortening velocity in the position of the red muscle during the escape response. If the slow red muscle alone were to power the escape response, it would have to generate the mechanical power for the movement while shortening at a velocity greater than its V_{max}. The red muscle clearly cannot do this.

It is known, however, that fast white muscle fibers are active during the startle response (Zottoli, 1977; Eaton, Lavender, and Wieland, 1981). Their V_{max} of 12.88 muscle lengths/s would still not be fast enough to enable fibers in the position of the red muscle to generate the power for the escape response. Because of their different orientation, however, the white muscle fibers need shorten at only 4.85 muscle length/s, which is about 0.38 V_{max}. This is a position on its force-velocity curve, where the white muscle would be expected to generate close to its maximum mechanical power. It should be noted that if the red muscle were placed in the position occupied by the white, it would not be able to power the "startle response" because it would still have to generate mechanical power at a velocity (4.85 muscle lengths/s) which exceeds its V_{max} (4.65 muscle length/s). Therefore, fish need to have the fast white muscle fibers to power maximal movement, and it is both the higher V_{max} and the different orientation of the white muscle that permit maximal movement.

Although placing the white muscle in a helical orientation enables

it to power a startle response, it makes it very ineffective at powering moderate swim speeds. Recent information on dogfish myotomal muscle shows that maximum efficiency is achieved at a V/V_{max} of about 0.2-0.7 (Curtin and Woledge, 1988). This means that the range we found for V/V_{max} of red muscle (0.16 to 0.35) during swimming at speeds (20-40 cm/s) where red muscle is used exclusively would represent fairly high efficiencies. On the other hand, at these swim speeds, the white muscle would be shortening at 0.18 to 0.42 muscle lengths/s. For a muscle with a V_{max} of 12.88 muscle lengths/s, this corresponds to nearly isometric contractions (V/V_{max}=0.01 to 0.03). At this V/V_{max}, the mechanical power generation and efficiency of the white muscle would be nearly zero. Thus, we can be reasonably certain that the red muscle can power slow swimming far more efficiently than white muscle can.

In conclusion, our results are consistent with the view that slow muscle is used at low speeds of locomotion where it is most efficient, and show clearly, for the first time, that animals need fast muscle fiber types because the slow ones cannot shorten fast enough to generate maximal movement.

APPENDIX 2

SARCOMERE LENGTH EXCURSIONS IN THE WHITE MUSCLE

INTRODUCTION

To calculate the muscle velocity, **V**, of the white muscle fibers in carp during normal swimming and during the escape response, we have used the assumption that for a given degree of curvature at the backbone the sarcomere length excursion of the white fibers is about 1/4 that of the red ones. This assumption is based on Alexander's (1969) study of the arrangement of muscle fibers in the myomeres of teleosts.

Alexander (1969) showed that the orientation of the white muscle fibers is not parallel to the median plane of the fish. Instead, they are arranged in a complicated three-dimensional pattern as described in fig. 1. Since the fiber trajectories are inclined at an angle to the backbone, their sarcomere length excursion when the fish bends is expected to be smaller than if they ran longtitudinally, as the red fibers do. Moreover, the white muscle fibers run at different angles at different distances from the median plane. Mathematical analysis suggested that this would produce the same sarcomere length excursions and strain rates regardless of the distance of a muscle fiber from the backbone. This is advantageous because all the white muscle fibers would be operating on the same part of their force-velocity curve during locomotion. Thus during the escape response and during burst and coast swimming, where they are known to be active, all the white muscle fibers would be producing the same (near maximum) power.

The purpose of this study was to test the predictions that 1) sarcomere length excursions in the white muscle are independent of the distance from the backbone, and 2) white muscle sarcomere length excursions are about 1/4 the red muscle excursions for a given degree of curvature at the backbone.

METHODS

Preliminary anatomical analysis showed that with small curvatures at the backbone (as with moderate bends encountered during slow swimming), sarcomere length excursions in the white muscle were very small. In some cases the excursions were of the same order of magnitude as the standard error of the measurements. Therefore in our analysis we used extreme curvatures as encountered during the escape response.

The procedure for fixing fish was as described in chapter II. 2 fish were fixed in sharp C-bends duplicated from film of carp performing escape responses. In addition, one fish was fixed in a straight position to measure the sarcomere length in the muscle.

The fixed fish were photographed and slide negatives were projected onto a digitizing tablet and analyzed as described in chapter II (see **Measurement of curvature**). From this analysis we obtained the radius of curvature at the backbone at selected positions on the body (anterior, middle, and posterior) as well as the predicted sarcomere lengths in the red muscle layer.

Muscle fibers were dissected from the superficial and deep white muscle mass in positions on the fish where where spinal curvature and

the predicted red sarcomere length excursion were large. Red fibers were also removed from those positions. The fibers were photographed and sarcomere lengths determined by counting as in Julian et al. (1986a & b).

RESULTS

Table 8 shows shows the results of the analysis of sarcomere lengths in the white muscle. In fig. 18 the white muscle sarcomere lengths are shown plotted against 1/R, the reciprocal of the radius of curvature at the backbone (negative on the concave side of the bend, positive on the convex side). The linear regressions are significant for both the superficial and the deep white muscle (ANOVA, $p<0.001$).

Sarcomere lengths in the white muscle are predicted to be independent of the distance to the backbone. Therefore it is expected that for a given degree of curvarure at the backbone, there should be no difference between sarcomere lengths in the superficial and deep muscle. This is found to be the case,- neither the slopes nor the intercepts of the lines in fig. 18 are significantly different (t tests, $p>0.5$), and the mean quotient (superficial length/deep length) was equal to 1.004 ± 0.01 S.E.

To examine the prediction that excursions in the white muscle are approximately 1/4 of those in the red muscle, the white muscle sarcomere lengths were plotted against a/R, where a is the half thickness of the fish (fig. 19 and table 9). a/R was taken to be the curvature at the skin points corresponding to the places on the fish

TABLE 8. Sarcomere Lengths in Red Muscle and in Superficial and Deep White Muscle

Predicted Red		White Concave		White Convex		
concave	convex	superficial	deep	superficial	deep	1/R (inches X 1000)$^{-1}$
1.44 um	2.40	1.69 ± 0.15	1.77 ± 0.02	1.99 ± 0.07	2.00 ± 0.10	6.81×10^{-4}
1.53	2.29	1.68 ± 0.07	1.74 ± 0.09	1.96 ± 0.07	1.96 ± 0.15	7.3×10^{-4}
1.44	2.39	1.73 ± 0.05	1.69 ± 0.03	1.94 ± 0.06	1.92 ± 0.07	5.67×10^{-4}
1.54	2.30	1.78 ± 0.07	1.69 ± 0.06	1.93 ± 0.08	1.88 ± 0.09	6.96×10^{-4}

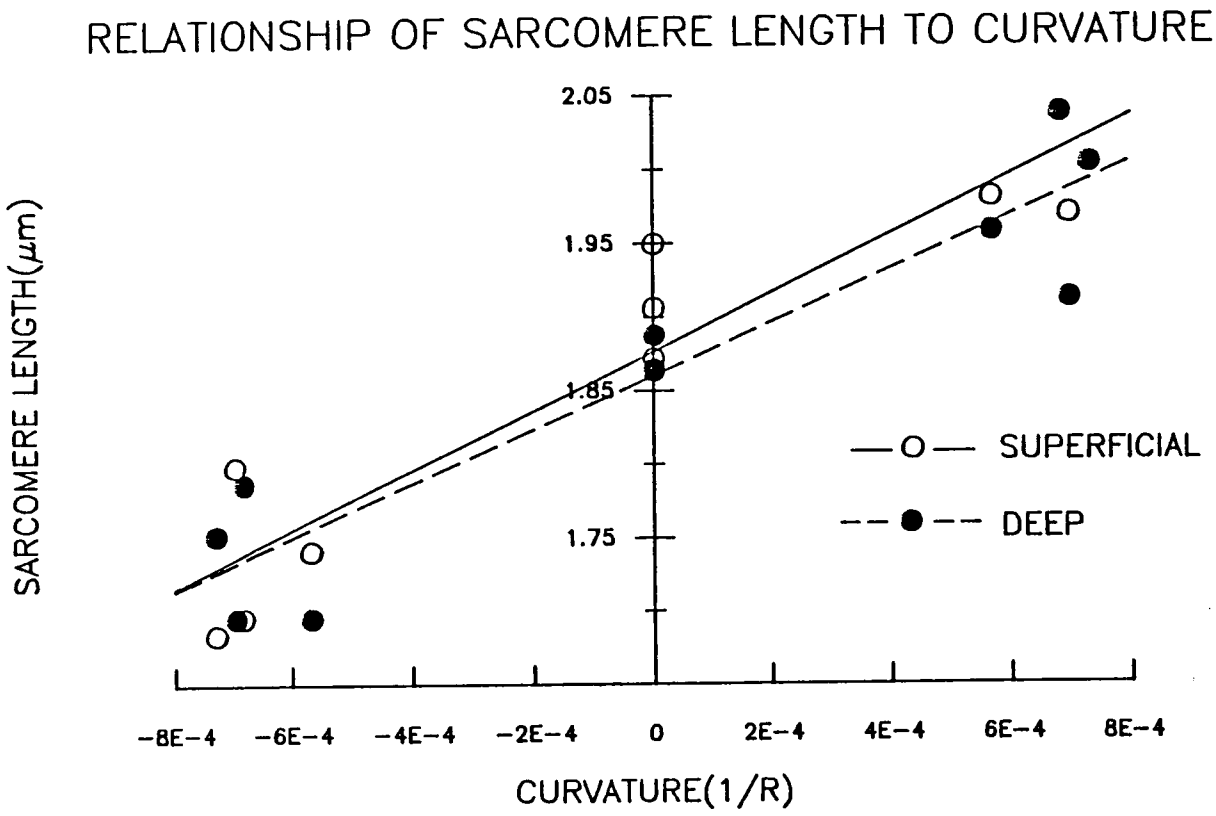


Fig. 18 . Superficial and deep white muscle sarcomere lengths plotted against 1/R. There is no difference between the lines, and the whole graph regression is $\lambda = 1.83 + 159/R$, $r^2 = 0.88$.

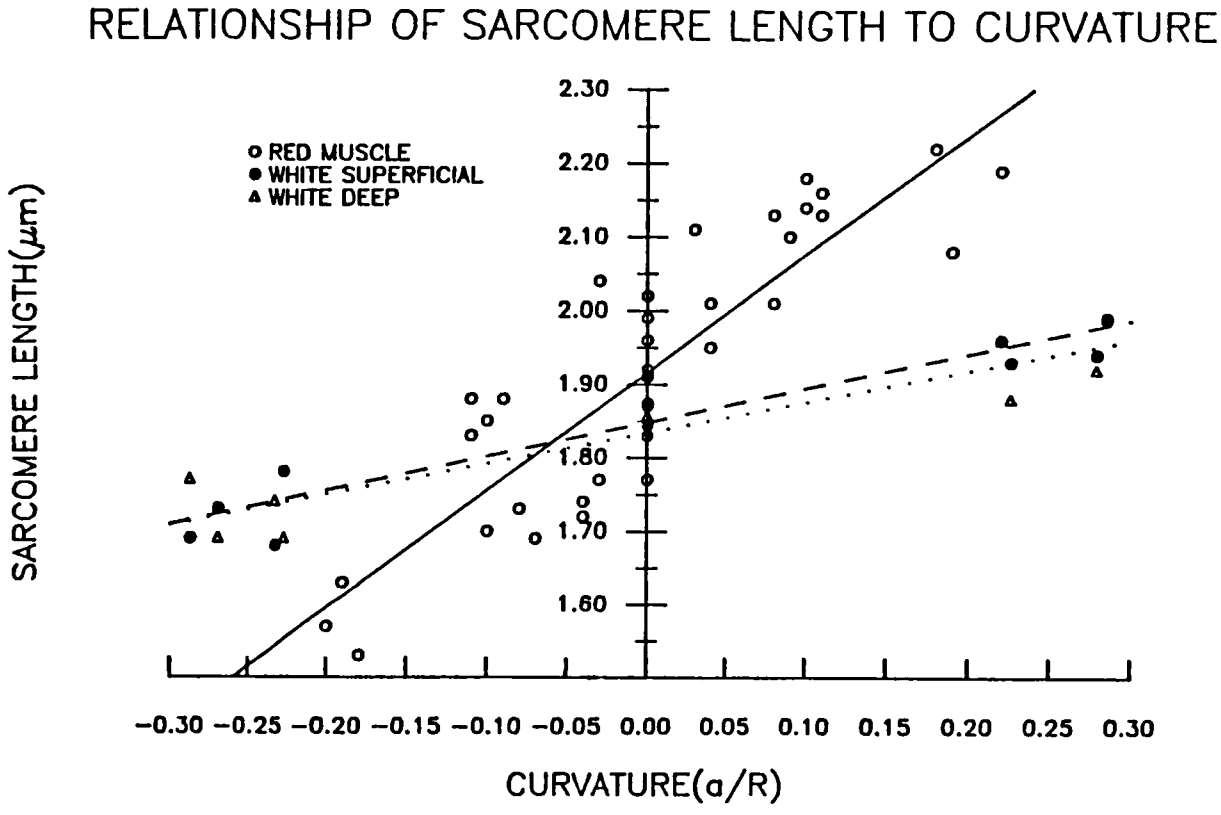


Fig. 19. Superficial and deep white sarcomere lengths plotted against a/R, shown on the same graph as the data for the red muscle (fig. 7). For the superficial and deep white lengths pooled, $\lambda = 1.841 + 0.44a/R$, $r^2 = 0.88$.

TABLE 9. Sarcomere Length Excursions in Red Muscle Compared With Excursions in Superficial and Deep White Muscle.

Predicted Excursion in Red (a)	Superficial White Excursion (b)	Deep White Excursion (c)	$\frac{b}{a}$	$\frac{c}{a}$
0.48	0.17	0.09	0.35	0.19
0.39	0.18	0.12	0.46	0.31
0.48	0.13	0.13	0.27	0.27
0.37	0.10	0.10	0.27	0.27
0.47	0.08	0.06	0.17	0.13
0.48	0.13	0.17	0.27	0.35
0.38	0.08	0.17	0.21	0.45
0.38	0.07	0.02	0.18	0.05
		MEAN $\pm$ S.E.	0.27 $\pm$ 0.03	0.25 $\pm$ 0.04

from which the white muscle was removed (i.e. as it was defined for the red muscle analysis, chapter II, **Measurement of sarcomere length**). This is an effective way to visualize the data, but it is not strictly correct since white muscle sarcomere lengths are predicted to be independent of distance to backbone. A linear regression through all the white muscle points yields the equation =1.84+0.44a/R, r^2=0.88. Since white muscle sarcomere length excursions are predicted to one-quarter of the red muscle excursions, the slope of the line is expected to be one-quarter of that of the line for the red muscle (=1.92+1.68a/R), which is also shown in fig. 19. The slope (with 95% confidence limits) is equal to 0.44 $\pm$ 0.07. This is not significantly different from one-quarter of the actual or the theoretical slope of the red muscle regression (0.42 and 0.48, respectively, see chapter II, **Measurement of curvature**; t test, p>0.5). This seems to justify our assumption that sarcomere lengths and shortening velocities in the white muscle are about 1/4 the values for the red muscle.

The results of these experiments may be subject to error due to shrinkage of the tissue during the fixing procedure (Franzini-Armstrong, pers. comm.). To investigate this possibility, we teased out fibers from the myomeres of a freshly killed fish and mounted them in skinning solution. The fibers were photographed and sarcomere lengths measured as described above. The resting sarcomere length in muscle tissue that was not fixed was found to be 2.04 $\pm$ 0.3 S.E., n=22. In the fixed tissue a value of 1.84um was obtained, indicating that shrinkage due to fixing was about 10%. This is a problem that needs closer study, but it does not invalidate these

results, which are concerned with excursions and not absolute sarcomere lengths.

CONCLUSION

In summary, these results agree with Alexander's (1969) findings, and suggest that the white muscle in carp are arranged in such a way that all the fibers in a myomere shorten by the same amount when the fish bends. This ensures that all the fibers operate on the same part of their force-velocity curve during locomotion. Moreover, due to the helical pattern of the white muscle trajectories, the fibers shorten by about 1/4 as much as the red ones for a given curvature change of the body.

APPENDIX 3

ACINT1 FISH DIGITIZING PROGRAM

```
10 DIM A$(1000),XL(500),YL(500),XR(500),YR(500),XB(500),YB(500),SMALLPOINT(500),
   PT(3),C(3),XLC(3),YLC(3),XRC(3),YRC(3),SARCIN(3),SARCOUT(3),THICK(3),K(3),
   XBP(500),YBP(500)
```

A$- string representing a point from the tablet. We used ASCII format 1.

The string is composed of x and y components as follows:

FbsXXXXXbsYYYYYc and takes up 17 bytes.

X(L),Y(L),X(R),Y(R),X(B),Y(B)- x and y coordinates of points on the

left side, right side and backbone of the fish.

SMALLPOINT- used as a counter in identifying corresponding points on

the right side of the fish. The point on the right side that gives

the smallest difference in external angles (smang and smangl).

PT- anterior, middle, and posterior points at which the program calculates

curvature. See 100.

XLC, YLC, XRC, YRC- corresponding points on the left and right sides of

the fish that will be used to calculate spinal curvature at the three

designated length points: anterior, middle, and posterior. See 1800-10.

SARCIN, SARCOUT- sarcomere lengths on the inside and outside of the fish

at the three length points.

THICK- thickness of the fish in the anterior, middle, and posterior

positions. Calculated 1970.

K- curvature, calculated 1960.

XPB, YBP- this is what the points on the backbone are called once they

have been sorted into equal x-intervals for the purpose of calculating

curvature from them. See 2100-2220.

```
20 OPEN "com1:9600,0,7,2,cs0,ds0" AS #1   Communications port opened, file
 receiving is #1.
30 INPUT "FISH#,TEMP,SPEED";FS$,T$,S$
42 F$="B:F"+FS$+T$+S$+".BAS":PRINT F$
50 INPUT "DO YOU WANT TO START NEW FILE(1) OR APPEND OLD(2)";NAP
60 IF NAP=1 THEN 80
70 OPEN F$ FOR APPEND AS #2
```

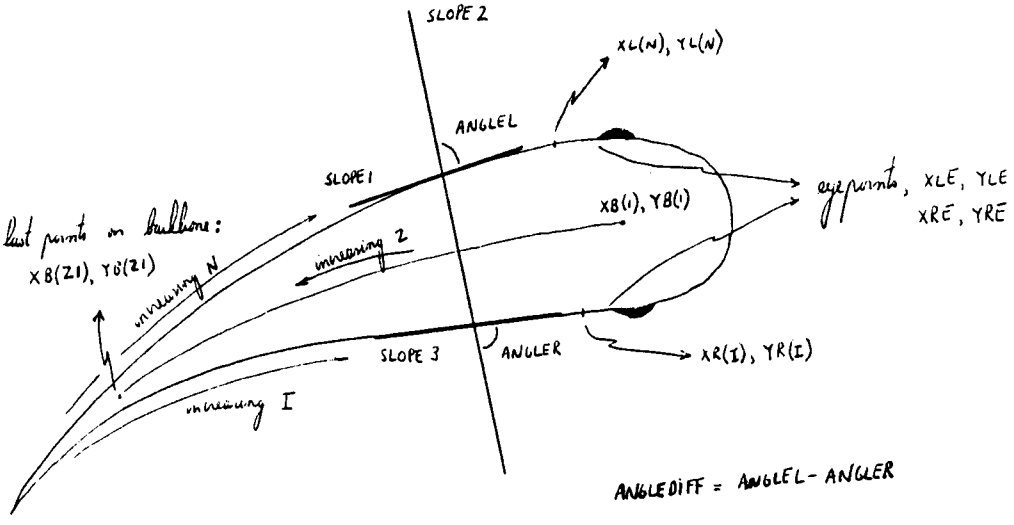
```
74 INPUT "STARTING TIME";TIME
78 GOTO 90
80 OPEN F$ FOR OUTPUT AS #2
90 INPUT "FISH LENGTH";FL:INPUT"MAGNIFICATION FACTOR";MAG:INPUT "BEGINNING FRAME #";
   FRAME
95 INPUT "FRAME INCREMENT";FRAMEINC:INPUT "TIME INCREMENT";TIMEINC

100 PT(1)=300*FL*MAG:PT(2)=450*FL*MAG:PT(3)=600*FL*MAG  Defining length points

    anterior, middle and posterior.

105 xxint=8:v=4  xxint is used in finding points on the backbone, see 1069;

    v is the set interval between points on the left side of the fish for

    which a search for the corresponding point on the right side is made.

110 REM DETERMINES POINTS FOR LEFT SIDE OF FISH

112 Width "lpt1:",140:lprint chr$(15)  Control code to printer putting it

    in compressed mode.

115 LPRINT " FISH# ";FS$,"TEMP ";T$, "SPEED ";S$, "FRAME# ";FRAME
117 LPRINT " Z     LENG      XB      YB      XL      YL      XR      YR      XINT
    K       LR       RR       BR      SARIN    SAROUT     BSI      BSO"
120 N=0:X=0:Y=0:YMAX=0

130 BEEP:BEEP:BEEP
135 PRINT FRAME

    Before starting to trace, must put cursor in "stream" mode by hitting "s"

    on the keyboard. We could not do this in the program itself.

140 B$=INKEY$ 'check for keyboard input

150 IF B$="Q" THEN 5000   Can quit program, abort the current tracing, or
160 IF B$="A" THEN 450
170 IF B$="F" THEN 220    tell computer you have finished tracing this side.


180 IF LEN(B$)<>0 THEN PRINT#1,B$  Take action if there is keyboard input

190 IF EOF(1) THEN 140  If no data in buffer then keep checking

200 PRINT LOC(1)  Location in file printed on screen, stream of numbers

                  incrementing by 17 since this is the length of A$ in bytes.

                  When the file is empty, its contents is "1".
```

```
210 GOTO 140

220 Z=LOC(1)/17   See no purpose for this. Probably a relic.


230 INPUT#1, A$              Take first point from buffer (FIFO, or first
240 X2=VAL(MID$(A$,4,5))
250 Y2=VAL(MID$(A$,11,5))    in, first out) and break into x,y components.


260 FOR X = 0 TO 25000 STEP 25    Find point with lowest x-coordinate, i.e.
270 IF X>X2 THEN 290
280 NEXT X                        the point at which you began tracing.
290 M=X


300 FOR X=M TO 25000 STEP 25
310 X1=X2:Y1=Y2
315 IF X<X1 THEN BEEP
320 IF LOC(1) = 1 THEN 500          this routine sorts the points into equal
330 INPUT#1, A$
340 X2=VAL(MID$(A$,4,5))            x-intervals (0.025"). 315 causes the computer
350 Y2=VAL(MID$(A$,11,5))
360 IF X2 < X THEN GOTO 310         to beep if points coming from the buffer are
370 IF X2=X1 THEN 310
380 Y=Y1+((X-X1)/(X2-X1))*(Y2-Y1)   separated by more than 0.025". This is
390 N=N+1
400 XL(N)=X:YL(N)=Y                 a warning that you are tracing too fast.
410 IF YL(N)> YMAX THEN YMAX=YL(N)
420 NEXT X                          In 380 y-values are calculated by interpolation.


430 REM ABORT ROUTINE
440 GOTO 500
450 INPUT #1, A$
460 IF LOC(1)>1 THEN 450
470 GOTO 110
480 INPUT #1, A$
490 IF LOC(1)>1 THEN 480


500 REM DETERMINES POINTS FOR RIGHT SIDE OF FISH   Same routine as for left side
510 I=0:X=0:Y=0:YMIN=25000
520 BEEP:BEEP:BEEP
530 B$=INKEY$
540 IF B$="Q" THEN 5000
550 IF B$="A" THEN 480
560 IF B$="F" THEN 610
570 IF LEN(B$)<>0 THEN PRINT#1,B$
580 IF EOF(1) THEN 530
```

```
590 PRINT LOC(1)
600 GOTO 530
610 Z=LOC(1)/17
620 INPUT#1, A$
630 X2=VAL(MID$(A$,4,5))
640 Y2=VAL(MID$(A$,11,5))
650 FOR X = 0 TO 25000 STEP 25
660 IF X>X2 THEN 680
670 NEXT X
680 M=X
690 FOR X=M TO 25000 STEP 25
700 X1=X2:Y1=Y2
705 IF X<X1 THEN BEEP
710 IF LOC(1) = 1 THEN 860
720 INPUT#1, A$
730 X2=VAL(MID$(A$,4,5))
740 Y2=VAL(MID$(A$,11,5))
750 IF X2 < X THEN GOTO 700
760 IF X2=X1 THEN 700
770 Y=Y1+((X-X1)/(X2-X1))*(Y2-Y1)
780 I=I+1
790 XR(I)=X:YR(I)=Y
800 IF YR(I)< YMIN THEN YMIN=YR(I)
810 NEXT X
815 CLOSE #5
820 GOTO 860


830 REM ABORT EYEPOINTS
840 INPUT #1, A$
850 IF LOC(1)>1 THEN 840


860 REM DETERMINES COORDINATES OF BACKBONE   Next feed in first coordinate on
870 REM EYEPOINTS                            backbone, which is midway between
880 BEEP:BEEP:BEEP                           the eyes. Use posterior edge of eyes
890 B$=INKEY$                                to enter "eyepoints"
900 IF B$="Q" THEN 5000
910 IF B$="A" THEN 830
920 IF B$="P" THEN 990

930 PRINT#1,"P"; 'put cursor in point mode

940 IF LEN(B$)<>0 THEN PRINT#1,B$
950 IF EOF(1) THEN 890
960 PRINT LOC(1)
970 GOTO 890
980 Z=LOC(1)/17
```

```
990 INPUT#1, A$
1000 XLE=VAL(MID$(A$,4,5))
1010 YLE=VAL(MID$(A$,11,5))  Take left and right eye points from buffer and
1020 INPUT#1, A$
1030 XRE=VAL(MID$(A$,4,5))   break into x and y components.
1040 YRE=VAL(MID$(A$,11,5))

                                                    Interpolate to get coordinates of
1050 XB(1)=(XLE+XRE)/2 : YB(1)=(YLE+YRE)/2
                                                    first point on backbone.

1060 REM OTHER POINTS ALONG BACKBONE
```

The following finds corresponding points on the left and right sides of the fish and identifies the backbone point as lying midway between them (see smang and smangl). The criterion used is that a line (slope 2) joining corresponding points makes the same angle with both sides of the fish:



Slopes 1 and 3 are centered on points on the left and right sides of the fish. From slopes 1, 2, and 3 the program calculates the correct external angles. How this calculation is performed depends on a number of conditions delineated in 1200-1380. The difference between the external angles on the left and right sides of the fish (anglediff) is calculated in 1390. Basically, for a given point on the left side, the program scans the right side until it identifies the point which gives the smallest angle difference.

Range2 and rangel find the second backbone point only (z=2 in 1069). After gosub 1170 the program returns from 1390 with an angle difference. In range2 anglediff is calculated cyclically as the program takes relatively big steps towards the peduncle on the right side (I1=I1-3). It continues to do this until the anglediff returned stops decreasing in magnitude (if angdiff2>angdiff1 then goto rangel). Rangel backtracks in smaller steps (I1=I1+1) until it finds the point on the right side for which the external angle is minimized. Having done this, it skips to smang and calculates the coordinates of the second backbone point.

Determination of the other backbone points is done in an analogous fashion (range2 and smangl). The routine is efficient because the program does not search the whole right side of the fish for a corresponding point. Instead, it begins its search for the next point right where it located the previous one

```
     (the counter I1 is not reset before each new search).

     REM FINDING THE SECOND BACKBONE POINT ONLY

                                    Do not go out of range on right side (see1180).
1069 I1=I-2*xxint
     z=2                            I1 counter for coordinates on right side.


                                    W counter for coordinates on left side.
1090 W=N-3-2*xxint-V*(Z-1)          As z is incremented, every 4th point
1095 IF W-2*xxint<1 THEN 1150
1096 IF W+2*xxint>N THEN go to znxt  (v=4) going towards the peduncle is used.
                                    1150 if out of range at peduncle; increment
                                    z if out of range near eyepoint.

1100 SLOPE1=(-2*YL(W-xxint*2)-YL(W-xxint)+YL(W+xxint)+2*YL(W+2*xxint))/(10*xxint*25)
          IF I1-2*xxint<1 THEN 1150
          IF XL(W)=XR(I1) THEN GOTO range2   Prevents division by zero
                                             in 1170.
            go sub 1170                      Go get an angle difference.
            angdiff1=180
            angdiff2=anglediff

     range2:    i1=i1-3                                  Move towards peduncle
                IF I1-2*xxint<1 THEN go to smang
                IF I1+2*xxint>I THEN go to smang         on right side in big steps
                IF XL(W)=XR(I1) THEN GO TO range2
                gosub 1170                               so long as anglediff
                angdiff1=angdiff2
                angdiff2=anglediff                       returned keeps getting
                if angdiff2 > angdiff1 then go to rangel
                go to range2                             smaller. If not, rangel.


     rangel:    i1=i1+1                                  Backtrack in smaller steps
                IF I1-2*xxint<1 THEN go to smang
                IF I1+2*xxint>I THEN go to smang         until anglediff returned
                IF XL(W)=XR(I1) THEN GO TO rangel
                gosub 1170                               stops getting smaller. Sk
                angdiff1=angdiff2
                angdiff2=anglediff                       to smang.
                if angdiff2 > angdiff1 then go to smang
                go to rangel
```

```
Smang:      smallangle=angdiff1                              Point giving the smallest
            smallpoint(z)=i1-1: s=smallpoint(z)
            XB(Z)=(XL(W)+XR(S))/2:YB(Z)=(YL(W)+YR(S))/2      anglediff must have been
                                                             previous one (I1-1). Define
                                                             XB(2), YB(2).

      REM FINDING THE REST OF THE BACKBONE POINTS

1070 FOR Z=3 TO (N-10)\V
          w=N-3-2*xxint-V*(Z-1)
          IF w-2*xxint<1 THEN 1150
          IF W+2*xxint>N THEN go to znxt
     SLOPE1=(-2*YL(w-2*xxint)-YL(w-1*xxint)+Yl(w+xxint)+2*YL(W+2*xxint))/(10*xxint*25)

        IF I1-2*xxint<1 THEN 1150
        IF XL(W)=XR(I1) THEN GOTO rangel1

          go sub 1170
          angdiff1=180
          angdiff2=anglediff


     rangel1:    i1=i1-1                                     Principle used is same
          IF I1-2*xxint<1 THEN go to smangl
          IF I1+2*xxint>I THEN go to smangl                  as above except use small
          IF XL(W)=XR(I1) THEN GO TO rangel1
          gosub 1170                                         steps only (I1=I1-1).
          angdiff1=angdiff2
          angdiff2=anglediff
          if angdiff2 > angdiff1 then go to smangl
          go to rangel1

     Smangl:     smallangle=angdiff1
                 smallpoint(z)=i1+1: s=smallpoint(z)
                 XB(Z)=(XL(W)+XR(S))/2:YB(Z)=(YL(W)+YR(S))/2

     ZNXT:       NEXT Z  Having found the corresponding point, get the next

                         point on the left side.

1150  Z1=Z : go to 1470    Z1 defines last point on backbone, nearest peduncle.


      REM 1170-1390 CALCULATES EXTERNAL ANGLE

1170 SLOPE2=(YL(W)-YR(I1))/(XL(W)-XR(I1))
1180 SLOPE3=(-2*YR(I1-xxint*2)-YR(I1-xxint)+YR(I1+xxint)+2*YR(I1+2*xxint))/(10*xxint*25)
1190 IF SLOPE2 < 0 THEN 1310
```

```
1200 REM CONDITIONS WHERE SLOPE2 IS POSITIVE
1210 IF SLOPE1>SLOPE2 THEN 1240
1220 ANGLEL=(ATN(SLOPE2)-ATN(SLOPE1))*180/3.14159
1230 GOTO 1250
1240 ANGLEL=(ATN(SLOPE2)+3.14159-ATN(SLOPE1))*180/3.14159
1250 IF SLOPE2<SLOPE3 THEN 1280
1260 ANGLER=(3.14159-(ABS(ATN(SLOPE2))-ATN(SLOPE3)))*180/3.14159
1270 GOTO 1290
1280 ANGLER=(ATN(SLOPE3)-ATN(SLOPE2))*180/3.14159
1290 GOTO 1390

1300 REM CONDITIONS WHERE SLOPE2 IS NEGATIVE
1310 IF SLOPE1<SLOPE2 THEN 1340
1320 ANGLEL=(ATN(SLOPE2)+3.14159-ATN(SLOPE1))*180/3.14159
1330 GOTO 1350
1340 ANGLEL=ABS(ATN(SLOPE1)-ATN(SLOPE2))*180/3.14159
1350 IF SLOPE2>SLOPE3 THEN 1380
1360 ANGLER=(ABS(ATN(SLOPE2))+ATN(SLOPE3))*180/3.14159
1370 GOTO 1390
1380 ANGLER=3.14159-(ABS(ATN(SLOPE3))+ATN(SLOPE2))*180/3.14159
1390 ANGLEDIFF=ABS(ANGLEL-ANGLER)
1400 return

1470 REM SCREEN GRAPHICS OF FISH

1480 SCREEN 2
1490 FOR A=1 TO N-1
1500 XD1=INT((XL(A)-XL(1))*(200/(YMAX-YMIN)))
1510 YD1=INT(199-((YL(A)-YMIN)*(200/(YMAX-YMIN))))
1520 XD2=INT((XL(A+1)-XL(1))*(200/(YMAX-YMIN)))          Left side
1530 YD2=INT(199-((YL(A+1)-YMIN)*(200/(YMAX-YMIN))))
1540 LINE (XD1,YD1)-(XD2,YD2)
1550 NEXT A
1560 FOR A=1 TO I-1
1570 XD1=INT((XR(A)-XL(1))*(200/(YMAX-YMIN)))
1580 YD1=INT(199-((YR(A)-YMIN)*(200/(YMAX-YMIN))))
1590 XD2=INT((XR(A+1)-XL(1))*(200/(YMAX-YMIN)))          Right side
1600 YD2=INT(199-((YR(A+1)-YMIN)*(200/(YMAX-YMIN))))
1610 LINE (XD1,YD1)-(XD2,YD2)
1620 NEXT A
1630 FOR Z=1 TO Z1-1
1640 XD1=INT((XB(Z)-XL(1))*(200/(YMAX-YMIN)))
1650 YD1=INT(199-((YB(Z)-YMIN)*(200/(YMAX-YMIN))))
1660 XD2=INT((XB(Z+1)-XL(1))*(200/(YMAX-YMIN)))          Backbone
1670 YD2=INT(199-((YB(Z+1)-YMIN)*(200/(YMAX-YMIN))))
1680 LINE (XD1,YD1)-(XD2,YD2)
1690 NEXT Z
```

1700 REM MEASURMENT OF LENGTH AND MEASUREMENT POINT.

The following routine moves down the backbone progressively until the cumulative LENGTH is equal to the anterior length point (PT1, see 100) It defines the corresponding points on the sides of the fish at this LENGTH (1800-1810). Then it proceeds to calculate the curvature and inside and outside sarcomere lengths in this position (1830-1982). From 2100-2220 the program sorts points on the backbone into equal x-intervals for the purpose of calculating curvature directly at the backbone (2290-2459). Sorting goes only as far as PT1 when C=1 (2130). In 2190 the y-coordinate is interpolated. Sarcomere lengths calculated from the sides and from the backbone are written to disk Note that the loop incrementing xint (which determines the length of the strip used to calculate slope and acceleration) is nested within the loop incrementing C (2460, 2500). C is incremented only after the program has cycled through the values for xint as in 1831.

```
1710 BEEP:BEEP:BEEP
1720 LENGTH=0:LENG=0
1730 C=1
1740 FOR Z=2 TO Z1
1750 LENG=SQR((XB(Z)-XB(Z-1)) 2 + (YB(Z)-YB(Z-1))^2)
1760 LENGTH=LENGTH+LENG
1770 IF LENGTH<PT(C)THEN 2520    Next z until LENGTH = or > greater than PT(C)
1780 S=SMALLPOINT(Z)  Counter on right side corresponding to length point PT(C)
1790 W=N-3-2*xxint-V*(Z-1)  Counter on left side corresponding to PT(C)
1800 XLC(C)=XL(W):YLC(C)=YL(W)  Specifying the corresponding points on the sides
1810 XRC(C)=XR(S):YRC(C)=YR(S)  of the fish at XB(Z)
1820 LPRINT Z TAB(6) INT(LENGTH) TAB(14) INT(XB(Z)) TAB(21) int(YB(Z)) TAB(28) XL(W)
     TAB(35) int(YL(W)) TAB(42) XR(S) TAB(49) int(YR(S))
```

```
1830 REM CURVATURE X INTERVAL SET BY XINT

1831 FOR XINT=5 TO 9 STEP 2   Incremented 2460


1832 IF (W+XINT*4)>N THEN 2030
1834 IF (W-XINT*4)<1 THEN 2030   In case SMOOTH and D equations out of range
1836 IF (S+XINT*4)>I THEN 2030
1838 IF (S-XINT*4)<1 THEN 2030


1840 SMOOTH=4*YL(W+XINT*4)+4*YL(W+XINT*3)+YL(W+XINT*2)-4*YL(W+XINT)-10*YL(W)
     -4*YL(W-XINT)+YL(W-2*XINT)+4*YL(W-3*XINT)+4*YL(W-XINT*4)
1850 B=100*(25*XINT)^2
1860 P=SMOOTH/B
1870 D=-4*YL(W+XINT*4)-3*YL(W+XINT*3)-2*YL(W+XINT*2)-YL(W+XINT)+YL(W-XINT)
     +2*YL(W-2*XINT)+3*YL(W-XINT*3)+4*YL(W-4*XINT)
1880 E=60*XINT*25
1890 F=D/E
1900 LRAD=MAG*P/((1+(F^2))^1.5)
1910 SMOOTH=4*YR(S+XINT*4)+4*YR(S+XINT*3)+YR(S+XINT*2)-4*YR(S+XINT)-10*YR(S)
     -4*YR(S-XINT)+YR(S-2*XINT)+4*YR(S-3*XINT)+4*YR(S-XINT*4)
1920 P=SMOOTH/B
1930 D=-4*YR(S+XINT*4)-3*YR(S+XINT*3)-2*YR(S+XINT*2)-YR(S+XINT)+YR(S-XINT)
     +2*YR(S-2*XINT)+3*YR(S-XINT*3)+4*YR(S-4*XINT)
1940 F=D/E

1950 RRAD=MAG*P/((1+(F^2))^1.5)   REM MAG=magnification factor.

1960 K(C)=(LRAD+RRAD)/2   Curvature at backbone average of left and right

                          sides

1970 THICK(C)=(SQR((YL(W)-YR(S))^2+(XL(W)-XR(S))^2))/(2*MAG)
1980 SARCIN(C)=1.9214-(1.6779*K(C)*THICK(C))
1982 SARCOUT(C)=1.9214+(1.6779*K(C)*THICK(C))
2020 GO TO 2100
2030 SARCIN(C)=99999:SARCOUT(C)=99999:LRAD=1/99999:RRAD=1/99999:K(C)=1/99999

2100 REM SPACING OF BACKBONE POINTS AROUND THE CENTRAL VALUE AT PT(C)

2110 COUNTER=0:N1=0
2120 X2=XB(Z1):Y2=YB(Z1)
2125 IF X2>XB(Z)-(4*XINT*50) THEN 2400
2130 FOR X = XB(Z)-(4*XINT*50) TO XB(Z)+(4*XINT*50) STEP 50
2150 X1=X2:Y1=Y2
2155 COUNTER=COUNTER+1
2157 IF COUNTER > (Z1-2) THEN 2400
2160 X2=XB(Z1-COUNTER):Y2=YB(Z1-COUNTER)
2170 IF X2 < X THEN GOTO 2150
```

```
2180 IF X2=X1 THEN 2150
2190 Y=Y1+((X-X1)\(X2-X1))*(Y2-Y1)
2200 N1=N1+1
2210 XBP(N1)=X:YBP(N1)=Y   Specifying points (in array of equally spaced points)

                           on backbone

2220 NEXT X


     REM CALCULATING CURVATURE DIRECTLY AT THE BACKBONE

     We had decided that bsarcin and bsarcout were not all that useful

     and did not correct some bugs that appear in this part of the program.

     Bsarcin is always returned as 9999, whereas the values for

     bsarcout agree suspiciously well with sarcout every time. Could be

     problem with calculation, print statement ...


2290 MID=(4*XINT+1)
2300 SMOOTH=4*YBP(MID+XINT*4)+4*YBP(MID+XINT*3)+YBP(MID+XINT*2)-4*YBP(MID+XINT)
     -10*YBP(MID)-4*YBP(MID-XINT)+YBP(MID-2*XINT)+4*YBP(MID-3*XINT)+4*YBP(MID-XINT*4)
2310 B=100*(50*XINT)^2
2320 P=SMOOTH/B
2330 D=-4*YBP(MID+XINT*4)-3*YBP(MID+XINT*3)-2*YBP(MID+XINT*2)-YBP(MID+XINT)
     +YBP(MID-XINT)+2*YBP(MID-2*XINT)+3*YBP(MID-XINT*3)+4*YBP(MID-4*XINT)
2340 E=60*XINT*50
2350 F=D/E
2360 BRAD(C)=MAG*P/((1+(F^2))^1.5)
2370 BSARCIN(C)=1.9214-(1.6779*BRAD(C)*THICK(C))
2380 BSARCOUT(C)=1.9214+(1.6779*BRAD(C)*THICK(C))
2390 GO TO 2450
2400 BRAD(C)=1/99999:BSARCIN(C)=99999:BSARCOUT(C)=99999
2450 IF XINT <>5 THEN 2453
2451 SARCIN5(C)=SARCIN(C):SARCOUT5(C)=SARCOUT(C):K5(C)=K(C):
     BSARCIN5(C)=BSARCIN(C):BSARCOUT5(C)=BSARCOUT(C)
2452 GO TO 2457
2453 IF XINT <>7 THEN 2456
2454 SARCIN7(C)=SARCIN(C):SARCOUT7(C)=SARCOUT(C):K7(C)=K(C):
     BSARCIN7(C)=BSARCIN(C):BSARCOUT7(C)=BSARCOUT(C)
2455 GO TO 2457
2456 SARCIN9(C)=SARCIN(C):SARCOUT9(C)=SARCOUT(C):K9(C)=K(C):
     BSARCIN9(C)=BSARCIN(C):BSARCOUT9(C)=BSARCOUT(C)
2457 K$=str$(K(C)):L$=MID$(K$,1,4):R$=MID$(K$,9,5):SARI$=str$(SARCIN(C)):
     SARO$=str$(SARCOUT(C)):BSARI$=str$(BSARCIN(C)):BSARO$=str$(BSARCOUT(C))
2458 K1$=L$+R$:SARI$=MID$(SARI$,1,5):SARO$=MID$(SARO$,1,5):BSARI$=MID$(BSARI$,1,5):
     BSARO$=MID$(SARO$,1,5)
```

The following LPRINT statement does not work properly. LRAD, RRAD, and BRAD do not appear at all. K1$ always has a -1 tagged onto it. Again, location of these bugs not certain...

```
2459 LPRINT TAB(58) XINT TAB(64) K1$ TAB(73) INT(LRAD) TAB(81) INT(RRAD) TAB(89)
     INT(BRAD) TAB(98) SARI$ TAB(107) SARO$ TAB(118) BSARI$ TAB(126) BSARO$
2460 NEXT XINT

2500 C=C+1
2510 IF C>3 THEN 2530
2520 NEXT Z

2530 FOR C=1 TO 3
2540 XD1=INT((XLC(C)-XL(1))*(200/(YMAX-YMIN)))
2550 YD1=INT(199-((YLC(C)-YMIN)*(200/(YMAX-YMIN))))
2560 XD2=INT((XRC(C)-XL(1))*(200/(YMAX-YMIN)))
2570 YD2=INT(199-((YRC(C)-YMIN)*(200/(YMAX-YMIN))))
2580 LINE (XD1,YD1)-(XD2,YD2)
2610 NEXT C

2620 WRITE #2, TIME;SARCIN5(1);SARCOUT5(1);SARCIN5(2);SARCOUT5(2);SARCIN5(3);
     SARCOUT5(3);SARCIN7(1);SARCOUT7(1);SARCIN7(2);SARCOUT7(2);SARCIN7(3);SARCOUT7(3
     SARCIN9(1);SARCOUT9(1);SARCIN9(2);SARCOUT9(2);SARCIN9(3);SARCOUT9(3);FRAME
3000 BEEP:BEEP:BEEP
3010 B$=INKEY$
3020 IF B$="Q" THEN 5000
3030 IF B$="G" THEN 4000
3040 IF B$="N" THEN 3060
3050 GOTO 3080
3060 FRAME=FRAME+frameinc:TIME=TIME+timeinc
3070 GOTO 110
3080 GOTO 3010

4000 REM HP PLOTTER ROUTINE FOR FISH OUTLINE
4010 CLOSE #1
4020 OPEN "COM1:9600,S,7,1,RS,CS65535,DS,CD" AS #3
4030 PRINT #3,"IN;"
4040 FOR A=1 TO N-1
4050 PRINT #3, "PU";XL(A)*(254/250),YL(A)*(254/250);"PD;PU;"
4060 NEXT A
4070 FOR A=1 TO I-1
4080 PRINT #3, "PU";XR(A)*(254/250),YR(A)*(254/250);"PD;PU;"
4090 NEXT A
4100 FOR Z=1 TO Z1-1
4110 PRINT #3, "PU";XB(Z)*(254/250),YB(Z)*(254/250);"PD;PU;"
4120 NEXT Z
4130 FOR C=1 TO 3
4140 PRINT #3, "PA";XLC(C)*(254/250),YLC(C)*(254/250);"PD";XRC(C)*(254/250),YRC(C)
     *(254/250);"PU;"
```

Draw in lines joining corresponding points

Write to disk then can either quit, get hard copy of screen graphics, or go on to next frame

```
4150 NEXT C
4160 CLOSE #3
4170 OPEN "com1:9600,0,7,2,cs0,ds0" AS #1
4180 GOTO 3000

5000 CLOSE #2
5010 END
```

VITA

Roel Peter Funke is a Dutch national who was born in Alkhobar, Saudi Arabia on November 10, 1966. He attended elementary schools in Indonesia and Japan and graduated with an International Baccalaureate diploma from the United World College of South East Asia in Singapore. In 1984 he entered the University of Leeds, England, and received his Bachelor of Science degree in Biophysics/Zoology from there in 1987.

As a result of a collaboration that began during his final year as an undergraduate, Mr. Funke joined Dr. Rome's muscle physiology laboratory at the University of Tennessee in September 1987. He received his Masters Degree in December 1988.

After graduation Mr. Funke hopes, among other things, to earn a Ph.D. in plant molecular biology.