J. exp. Biol. 156, 453–466 (1991)
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SKELETAL MUSCLE DEFORMATION IN THE LATERAL MUSCLE OF THE INTACT RAINBOW TROUT *ONCORHYNCHUS MYKISS* DURING FAST START MANEUVERS

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Accepted 24 September 1990

Summary

Although there is an extensive body of information on the kinematics of the fast start response in teleosts, there is little information on the deformation of the skeletal muscle which produces the changes in body position during a fast start. This study presents preliminary information on the determination of skeletal muscle deformation with implanted ultrasonic dimension gauges in the intact fish during startle behavior. Deformation was measured in the lateral epaxial musculature of the rainbow trout *Oncorhynchus mykiss* during stage one of fast starts. The results show that ultrasound transit time dimension gauges can be implanted in the skeletal muscle of rainbow trout with minimum trauma and used to record local deformation along the length of the fish. Measurements remained stable over 4–8 h of implantation. Preliminary results show that: (1) muscle at different positions along the fish reaches its minimum length (average 9.6% shortening) at approximately the same time and coincident with the end of stage one of the fast start response; (2) the onset of concave curvature of the entire fish precedes the onset of local shortening at more caudal sites; (3) muscle on the convex side of a bend lengthens while muscle on the concave side shortens, and the two deformations follow a similar time course. These results indicate an asynchronous onset of skeletal muscle contraction in fast starts and support the hypothesis that local skeletal muscle deformation is transmitted caudally through skeletal or other structures.

Introduction

Much of our current knowledge of teleost locomotion stems from studies that span nearly five decades and have carefully documented the kinematics of locomotor movements using high-speed cinematography (Gray, 1933; Bainbridge, 1958; Weihs, 1973; Webb, 1978*a*; Eaton *et al.* 1977; Harper and Blake, 1989).

Key words: startle response, fast start, skeletal muscle, *Oncorhynchus mykiss*.

Electrophysiological and anatomical studies have demonstrated neural mechanisms that can control the sequence of activation of skeletal muscle (Bone, 1966; Eaton and Hackett, 1984; Williams *et al.* 1989) and there is a substantial body of information on the function of isolated fish skeletal muscle (Altringham and Johnston, 1982) and some information on the performance of *in situ* skeletal muscle studied *in vitro* (Johnsrude and Webb, 1985). These techniques are rarely used simultaneously and only recently has it been apparent that there is a significant phase difference between neural activation as reflected by the electromyogram (EMG) and changes in local radii of curvature during normal swimming in the rainbow trout (Williams *et al.* 1989).

Unfortunately, the complex nature of the geometric arrangement of skeletal muscle in the teleost and the varying material properties of the skin and septa that transmit forces generated by muscle contraction to the vertebrae make it unlikely that one can simply apply our current knowledge of the performance and configuration of isolated muscle to the generation of locomotor movements in the fish. What is missing from this analysis is information on the local deformation of skeletal muscle *in vivo* during normal locomotor activity and more detailed knowledge of the structures involved and their properties (Wainwright, 1983).

In this study we present preliminary results on local deformation in skeletal muscle during fast starts in the rainbow trout using high-speed cinematography and locally implanted ultrasound dimension gauges. The transit time of ultrasound between two implanted piezoelectric crystals has been used extensively in other branches of physiology to measure strains in contracting muscle (Rushmer *et al.* 1956; Villareal *et al.* 1988). We have studied stage one (Weihs, 1973) of the fast start maneuver. We chose the fast start response because there is an extensive body of kinematic data indicating that this is an easily reproduced response that should produce maximum deformation in local skeletal muscle.

The results of the study indicate that the onset of local shortening on the concave side of the fish is not uniform and the fish bends prior to the onset of local shortening. Moreover, when two dimensions were measured on the same side of the fish both reached minimum length at the same time, which was closely associated with the end of stage one of the fast start response. The results imply a substantial modification of the nearly instantaneous activation implied by Mauthner cell activation and provide for the first time direct evidence of the coupling of local shortening of skeletal muscle to a distant site.

Materials and methods

Eight rainbow trout *Oncorhynchus mykiss* weighing 521.3±41 g (S.D.) and with a fork length averaging 35.7±0.9 cm were obtained from a local (British Columbia) hatchery. Fish were held in a circular outdoor holding tank (1000 l, 2 m diameter) which was flushed continuously with fresh water at 15–20 °C. Fast start performance is independent of temperature over this range (Webb, 1978*b*). Trout were fed trout chow (Purina) daily.

Studies were conducted in a rectangular shallow tank (2.45 m×1.22 m×0.47 m) constructed of glass and Plexiglas. A 2 cm×2 cm grid constructed of Plexiglas was positioned on the bottom of the tank and served to calibrate film images obtained during the response. The tank was filled to a depth of approximately 20 cm, which served to limit vertical movement during the startle response. Fish were acclimated to the study tank for 24 h and were not fed during that time.

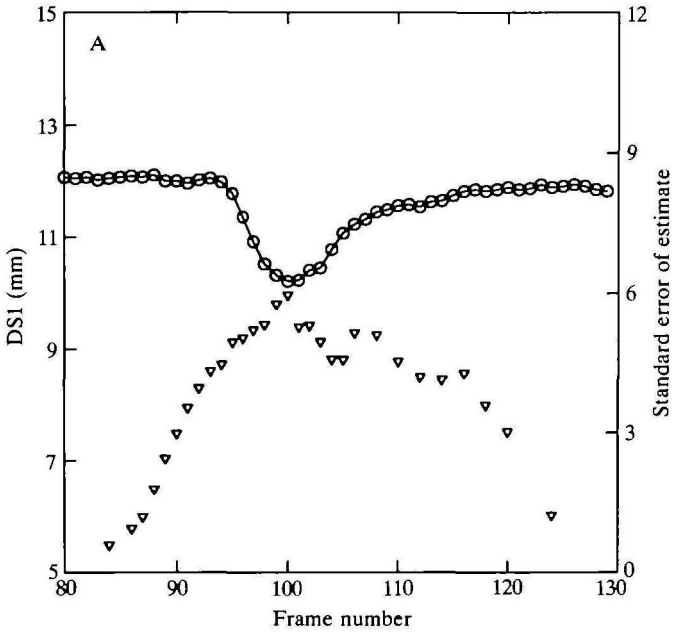
On the day of the study anesthesia was induced with tricaine methanesulfonate (MS222) (0.0002 kg l^{-1} buffered with 0.0004 kg l^{-1} Na_2CO_3) and then maintained with recirculating perfusion of one-third strength of buffered MS222. During the surgical procedure fish were maintained on a 'wet' table with the fish held upright in a sling while perfusion was maintained over the gills. After surgery fish were placed in a 7.5 cm×7.5 cm×44 cm tank and fresh water was flushed over the gills until normal opercular activity resumed. At this point the fish was returned to the large study tank and allowed to recover. Fish recovered for a minimum of 3 h and a maximum of 6 h before the study was started. The criterion for initiating the study was the presence of a 'normal' startle response initiated by thrusting a pole towards the head of the fish. Although this is primarily a visual stimulus, it probably activates the acoustic system and possibly the lateral line to varying degrees. In two responses a method for startling the fish using electric shock was employed (Webb, 1975). Even at low currents, however, there was evidence for a slightly different response (early lengthening of a segment prior to shortening) and all data illustrated in this report are from responses induced by thrusting the pole towards the fish.

Surgical preparation

Implantation of the gauges was time consuming. However, we considered it imperative to keep the anesthesia period to a minimum. Thus, the total anesthesia time was limited to 1 h. In each animal eight markers were implanted along the dorsal center line of the fish and 1–3 pairs of dimension gauges were implanted in the dorsal muscle group.

After the animal had been placed on the wet table, eight markers constructed of 1.5 mm×4 mm thin yellow latex (balloons) were sutured to the dorsal midline of the fish with 000 gauge silk and a 'tapercut' V-5 needle. One marker was positioned just anterior to the origin of the dorsal fin and formed a reference point for two of the implanted dimension gauges located on the right and left side of the fish. Three additional markers were implanted anterior (rostral) to this marker (Fig. 1). Four markers were implanted at roughly evenly spaced intervals posterior to the dorsal fin with the first being positioned at the caudal end of the dorsal fin. This latter mark served as the reference point for the site of the third gauge.

Each gauge was constructed of a crystal, a lens and a wire. In this study a 3 m length of 38 gauge copper stranded wire (a single twisted pair for each crystal, Cooner Wire Chatsworth, CA no. nmvf 2/38-1650v) was soldered to 2.25 mm diameter piezoelectric crystals (LTZ-2 Transducer Products Inc. Torringon, CT) and the crystals were covered with a drop of polyester resin which stabilized the



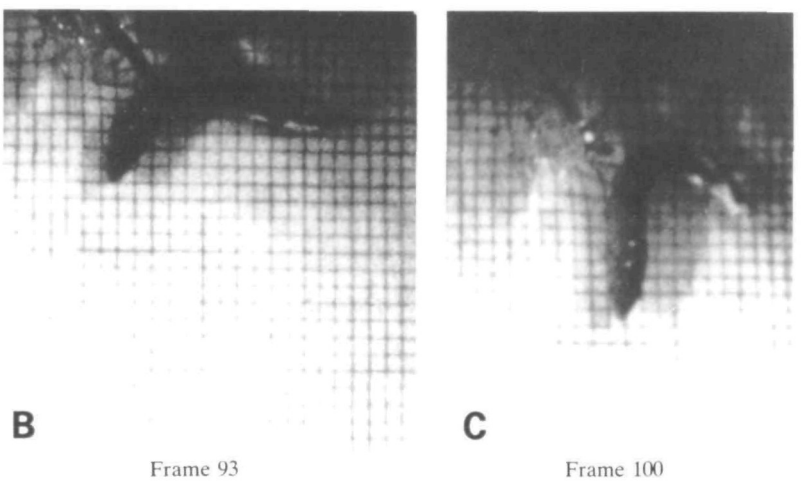


Fig. 1. (A) Plot of segment dimension (DS1) (○) from a dimension gauge located in the epaxial lateral musculature at the level of the dorsal fin and standard error of the estimate (S.E.E.) (▽), an index of the overall curvature of the fish (see text) at each frame during a single fast start in the rainbow trout. The gauge is located on the side of the fish with the concave bend. (B,C) Reproductions of the 16 mm ciné film at (B) the time of onset of shortening and (C) peak shortening.

solder joints and acted as a diverging lens for the sound. This system results in alignment errors of less than 2% (Kirkpatrick *et al.* 1972). Each gauge was implanted through a 3 mm incision in the skin, made with a number 11 blade, and a pair of crystals was implanted approximately 1 cm apart centered about the level of the marker and halfway between the lateral line and the dorsal fin. The gauge was forced 5 mm into the muscle in the direction of the spine. In each fish at necropsy the gauges were located in the inner half of the muscle and the entry site was located at the center of the epaxial lateral muscle. Of the 11 pairs of gauges reported in this study, only 4 of the 22 gauges showed grossly visible evidence of local hemorrhage near the gauge. One fish showed evidence of subcutaneous hemorrhage. The presence of small amounts of local hemorrhage did not appear to influence either the startle response or the measured dimensions. Fig. 2 shows a schematic diagram of the dimension system. A lensed piezoelectric transducer is excited with 5 MHz sound. A conical envelope of sound is transmitted to the receiving transducer and amplified. The bistable circuit is set high at the time of excitation of the transmitting gauge and reset by the onset of the sound detected at the receiving gauge. The transit time of sound between the two transducers is proportional to the duration of the pulse developed at the bistable circuit. Integration of this signal produces an analog signal proportional to the transit time between the two transducers. After implantation, the transducers were aligned so that the pattern of the received signal on the oscilloscope formed a triangular shape (Kirkpatrick *et al.* 1972). This alignment ensured that the two plates of piezoelectric material were facing each other. The skin incisions were then closed with a figure-of-eight stitch of 000 gauge silk and the wire was secured to the stitch. The two wires for each gauge were also anchored to the skin with a single suture leaving a loop of about 3 cm of wire to allow for bending of the fish. After all the

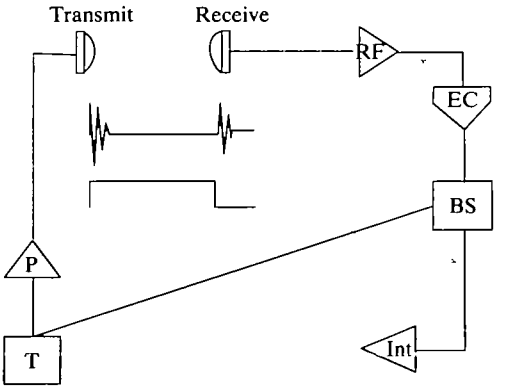


Fig. 2. Schematic diagram of the dimension gauge system showing two piezoelectric transducers and a functional diagram of the system used to determine the transit time of sound between the two transducers. Dotted arrows indicate waveforms detected after RF amplifier circuit (RF) and bistable circuit (BS) timing of the bistable current. Excitation of the transmitting transducer (P) is controlled by a timing circuit (T). Int, integrator; EC, electronic comparator.

gauges had been implanted, all the wires were fixed to the skin in such a way that the free end passed ventrally. This allowed a coil of free wire to be positioned on the bottom of the tank. The weight of the entire system including implanted dimension gauges (four pairs of crystals were included) and enough wire to allow the fish to swim to all edges of the tank was 5.7 gm. The extra pair of gauges (fourth pair) was used occasionally if transducers failed during implantation. Since the dimension measurement technique required that a cable containing eight pairs of wires remain attached to the animal during the study, and we were not sure that the gauges would stay in place over long periods, we elected to use short recovery times and study the animal on the same day. However, now that it is clear that gauges can be implanted and do not migrate within the tissue, in future studies it would be advisable to allow fish to recover overnight and to develop floats and connectors to handle the necessary long cable.

Dimension measurements

Ultrasonic dimension measurements have been used for nearly two decades in some branches of physiology (Rushmer *et al.* 1956), but have not been widely used in comparative physiology or for studies of animal locomotion. Since the velocity of high-frequency sound is relatively constant in mammalian soft tissue (Goldman and Hueter, 1956), the distance between one gauge activated with 5 MHz sound and a receiving gauge may be determined by the transit time of sound between the two gauges. We will refer to this dimension as 'segment length'. In the system employed in this laboratory (Villareal *et al.* 1988) each channel is sampled at a 1 kHz rate and an analog signal is developed which is proportional to the transit time between gauges. For this study the response of the system to a step change in dimension (time constant) was adjusted to 12 ms.

Dimension signals and shutter correlation pulses were simultaneously digitized at 1200 samples per second per channel using an 80286-based computer with 2 megabytes of memory and an A/D converter model 2821 (Data Translation, Inc., Marlborough, MA) controlled by Dataq A/D conversion software and graphics adaptor (Dataq Instruments, Inc., Akron, OH).

Photographic measurements

Fish were photographed at 200 Hz with a tripod-mounted Photec rotating prism 16 mm high-speed camera (Photonic Systems Inc., Santa Clara, CA) focused on a mirror angled at 45° and positioned over the tank. A Mamiya–Sekor fisheye ULDC 24 mm F/4 lens and/or a 75–150 mm f/4.5 zoom lens were used. Two microsecond duration shutter correlation pulses generated by the camera were shaped to 1.5 ms, 5 V square waves for correlation with recorded dimension signals. Kodak 7277 4-X reversal film was used. Processed films were analyzed on an image analyzer (Photographic Analysis Limited, Projection Analysis Unit, ZAE 76). Calibration factors were determined by repeatedly digitizing three intersection points on the grid on one frame for each response. On each frame during a fast start response the location of each of the marker points and the position along the

centerline of maximum curvature (percentage distance from the head) were determined. Thus, 10 points along the centerline of the fish were digitized. The errors involved in this analysis are of two types: errors in geometric calibration stemming from several sources, such as operator error in placing the digitizing cross hairs, spherical distortion from the camera and projector lenses and random noise in the A/D convertor; errors in frame count due to loss of frame registration in the projector. The results of this study are based on the correlation between analog measurements of deformation and the film images. They were thus quite sensitive to the latter type of error and great care was taken to ensure not only that the frame count was reproducible, which indicates that each frame is 'registered' in the projector, but also that the number of frames registered by the projector and the software that detected the shutter correlation pulses matched exactly. The effects of spherical distortion (significant with this lens) were minimized by performing the calibration in the area of the image where the fast start took place. This usually involved less than 25 % of the image area. Other geometric errors in this system have been shown to average 2.8 % (Harper and Blake, 1989).

Experimental protocol

Four to fifteen startle responses were induced in each of the five fish. Prior to initiating the startle, the A/D converter and then the camera were turned on. Then the fish was startled by driving a rod towards the head. After the fish had tired or it was clear that responses had been obtained both towards and away from the sides with two implanted gauges, the fish was killed by prolonged immersion in the induction concentration of MS222. The fish was then measured and weighed and the location of the gauges determined.

Data analysis

This study was designed to investigate the correlation in time of local skeletal muscle deformation and motion of the fish during the startle response. Thus, it was necessary to quantify the position of the dimension gauges at the time of each ciné frame exposure. This was accomplished by identifying the leading edge of the A/D-converted shutter correlation pulse and setting the matching dimension to the average of the five points (4 ms of data) about that point. Determining an index of overall motion of the fish was more difficult and a more arbitrary solution was used. Since we were most interested in the time at which the fish deviated from the resting (straight stretched) configuration, we elected to express an index of the curvature of the fish as the deviation of the digitized points along the centerline of the fish from a straight line. Visually the standard error of the estimate (S.E.E.) increases with an increase in overall curvature of the fish. Quantitatively this was expressed as the standard error of the estimate (S.E.E.) for the least-squares fit of a line through the 10 digitized points in each frame. Visually (Fig. 1B) increases in the S.E.E. corresponded well with the onset of stage one of the startle response. This index would, of course, be insensitive to the location and complexity of the curvature of the fish.

Although preliminary films showed no direct relationship between the magnitude of local shortening and the local radius of curvature of the fish, as might be predicted from electromyographic information, it still seemed important to establish at least an estimate of the head-to-tail location of the area of maximum curvature in each frame. Although the yellow markers were usually visible through an entire response, shadows and turbulence interfered with identification of the edge of the fish and prevented accurate determination of the local radius of curvature. However, an attempt was made to estimate the location of the maximum radius of curvature visually on each frame. This location was expressed as percentage distance from the head of the fish to the site of the maximum curvature. Data are presented as mean±standard deviation of the response in each animal ($N=5$). Where more than one response was determined in each animal the responses were averaged. Tests of significance were made by paired two-tailed t-test (again $N=5$) and were performed only on data where each animal served as its own control. Results achieving $P<0.05$ are reported as significant.

Results

Data from five of the eight preparations are presented here. In two studies image quality was technically unsatisfactory and in a third the fish quickly tired and startle responses were never successfully captured on film. In all remaining fish, dimension gauge pairs were successfully implanted on the right side at the level of the dorsal fin. Two of the fish had two pairs of working gauges implanted at and below the dorsal fin on the right side and two had all three gauges successfully implanted. Seven startle responses in these five fish were analyzed.

Fig. 1 shows a representative startle response. The gauge (DS1) is on the concave side of the bend at the level of the fourth marker. The first S.E.E. point, frame 84, represents the stretched straight configuration in this and other figures. Frame 100 is one frame after the end of stage one and the fish again reaches the reference configuration (straight stretched) at frame 124. Typically the fish bends rostral to the gauge first, reflected by a significant increase in the S.E.E. (triangles, Fig. 1A) prior to the onset of shortening of the gauge. This bend is evident in the reproduction of frame 93 (Fig. 1B). S.E.E. was significantly greater than the reference value at the start of deformation at the anterior gauge in all seven fast starts (Fig. 3) in all five animals. The onset of shortening at the gauge site coincided with the development of a bend at that site. In the startle response shown in Fig. 1 the bend at the gauge site was first detectable at frame 93. In all seven responses the onset of shortening did not differ significantly from the first detection of a bend at that site. The time between onset of shortening and local detection of a bend at that site averaged 0.83±15 ms.

The end of stage one in the startle response shown in Fig. 1 occurred at frame 100. We have taken the end of stage one as the first frame in which the tail moves in a caudal direction. In all animals the time at which a segment achieved minimum length (maximum shortening) was closely associated with the end of stage one. On

average, minimum length was achieved within two frames of the end of stage one (6.4±8.3 ms). In the response shown in Fig. 1 the segment shortened by 15.4 % at frame 100. In all responses maximum shortening at the anterior gauge averaged 9.64±3.9 % (range 4.5–15.4 %). At the time of peak shortening the midline of the fish near the gauges tended to be rather straight (frame 100, Fig. 1). The duration of stage one in these seven responses averaged 75±16.4 ms (range 50–100 ms).

After the end of stage one the responses became much more variable. In general, the local segment did not return to the reference length until the fish reached the stretched straight configuration. Deformation during the relaxation phase was variable. For example, in the response shown in Fig. 4, in which there are two gauges on the concave side of the bend, the relaxation phases at the two sites are quite different.

Deformation at two sites

Dimension changes at two sites on the same side of the fish (at the level of the rostral and caudal ends of the dorsal fin) were successfully recorded in four responses in three fish. In the response shown in Fig. 4 the centers of the gauge pairs were located 6.6 cm apart at 45 % and 64 % of body length (measured from the tip of the nose).

At both sites prior to the onset of shortening there is considerable bending and S.E.E. has increased before shortening begins. Of the four responses studied, two were 'classic' C-starts and two were similar to the fast starts recently categorized by Eaton (Eaton *et al.* 1988), which are of shorter duration and smaller turning angle. Fig. 4 shows an example of a C-start with a large turning angle and long duration. It is difficult to detect a time difference in the onset of shortening at the two sites in both C-starts with large turning angles. Interestingly, both gauges achieve

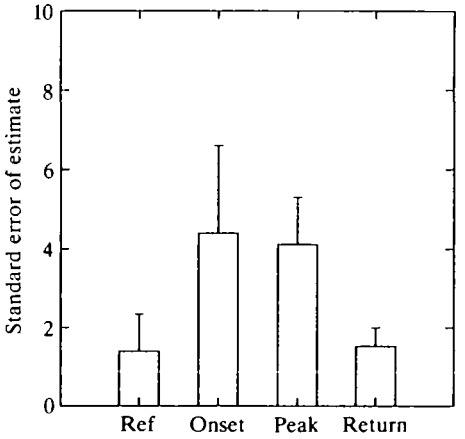


Fig. 3. Average (mean+S.D.) S.E.E. (standard error of the estimate) at four intervals in seven fast starts in five animals. Ref, resting (straight stretched) configuration; onset, time of onset of shortening at the rostral gauge located on the side of the concave bend; peak, time of peak shortening; return, time of return to the resting configuration.

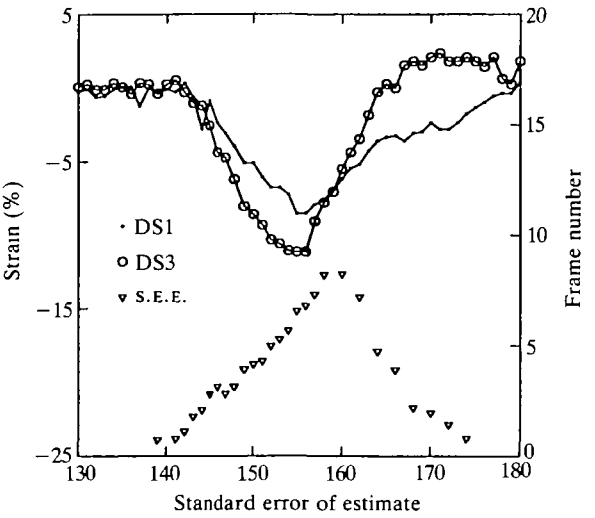


Fig. 4. Shortening expressed as percentage change from the reference configuration (strain) in a fish with an anterior gauge (circles DS3) and posterior gauge (squares DS1) located on the side of the concave bend. The standard error of the estimate (S.E.E.) is an index of the curvature of the fish (see text).

minimum length at approximately the same time and again this is closely associated with the end of stage one (in this case frame 152). In the two other responses of shorter duration and smaller turning angle the onset of shortening in the anterior gauge led that in the posterior gauge by 15 ms and 40 ms, although both achieved minimum length at approximately the same time.

Deformation on the convex side of the bend

Deformation was recorded on the convex side in three responses in two fish. Fig. 5 shows the typical response; DS1 is the gauge on the convex side of the bend, and DS3 is on the concave side. Note again that there is significant change in shape prior to the onset of deformation in both segments. The maximum deformation occurs near the end of stage one (frame 152) at both sites in this response.

Discussion

Although there is an extensive body of information on the kinematics of the fast start response in teleosts (Webb, 1975; Harper and Blake, 1989; Nissanov and Eaton, 1989), the present study is the first to measure local skeletal muscle deformation during fast start locomotion. The results show that ultrasound transit time dimension gauges can be implanted in the skeletal muscle of rainbow trout with minimum trauma and used to record local deformation along the length of the fish. Measurements remained stable over 4–8 h of implantation. Preliminary results show that: (1) muscle at different positions along the fish reaches it

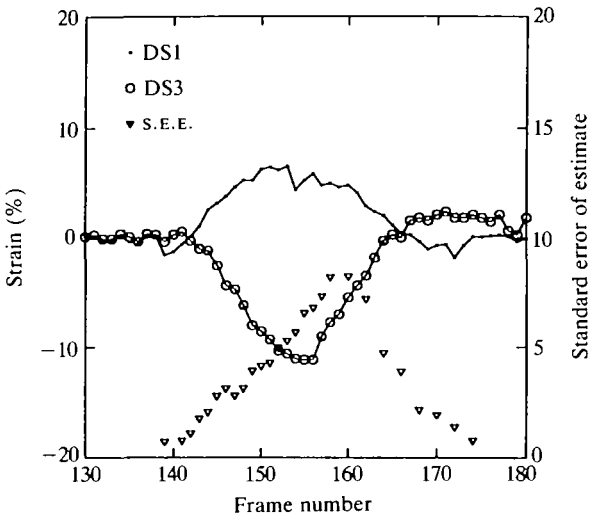


Fig. 5. Shortening of gauges located on the side of the fish with the convex bend (DS1) and on the concave side (DS3). The standard error of the estimate (S.E.E.) is an index of curvature of the fish (see text).

minimum length (average 9.6 % shortening) at approximately the same time and coincident with the end of stage one of the fast start response; (2) the onset of concave curvature of the entire fish precedes the onset of local shortening at several sites; (3) muscle on the convex side of a bend lengthens while muscle on the concave side shortens, and the two deformations follow a similar time course. These results indicate an asynchronous onset of skeletal muscle contraction in fast starts and support the hypothesis that local skeletal muscle deformation is transmitted caudally through skeletal or other structures.

The mechanism by which local skeletal muscle contraction curves the spine in teleost fish has long interested physiologists. Several anatomical studies have demonstrated the complex arrangement of overlapping cones of muscle, and Nursall (1956) and Alexander (1969) have proposed lever arm type models which assume essentially that deformation in skeletal muscle is transmitted to the nearby vertebrae through the local myosepta. In the present study, minimum length was not achieved at a local site until the location of maximum bending had progressed down the fish. Moreover, acute bending at the site of the measurement was associated with only small amounts of shortening (Fig. 1). These results imply that the transmission is over considerably longer distances. In startle responses invoked by thrusting the pole towards the fish we never observed initial lengthening at a gauge site associated with bending of the fish anterior (cephalad) to the gauge. This argues that transmission of the deformation may involve structures such as the skin (Wainwright *et al.* 1978) or at least structures not contiguous with the adjacent muscle. We were unable to implant gauges successfully below the level of the caudal end of the dorsal fin. If we assume that muscle in the caudal half of the

fish also reached a minimum length at the time of the end of stage one of the fast start, then either the onset of contraction or its duration must be different in that region. Although one could explain these differences by proposing complex alterations in the activation sequence or loading of muscle in the caudal half of the fish, which might produce this motion, it is tempting to speculate that shortening deformation in the rostral half of the fish is transmitted to the caudal half by other structures, such as the skin and median or longitudinal septa.

Although no previous study has directly examined deformation in skeletal muscle during fast starts, Rome *et al.* (1988) used a modeling approach to estimate sarcomere dynamics during fast starts in the perch based on cinematography and estimates of muscle deformation from Alexander's model (Alexander, 1969). The large differences in the magnitude of shortening calculated from Rome's model (40 %) and our data (9.7 %) indicate that the model may not be a good reflection of muscle performance in the rainbow trout. Moreover, the gradient of shortening along the fish predicted by Rome's model is not substantiated by data in this study. Maximum shortening at the anterior gauge averaged 9.4 % and at the more caudal gauge 11.2 %.

The onset and maintenance of shortening in isolated skeletal muscle can be influenced by both preload and afterload as well as by the duration of tetanic stimulation. Thus, interpretation of differences in the onset and duration of shortening without a knowledge of the local loading conditions is problematical. However, there is no evidence for regional variations in load. If one assumes that there are no substantial variations in load during the fast start, then the variations in time of onset and duration of shortening observed in this study strongly support the recent data of Eaton (Eaton *et al.* 1988; Nissanov and Eaton, 1989), which indicate that the form of stage one of the fast start is variable and correlates with the motion of the fish during phase two. Our data, like those of Eaton, imply that the onset and maintenance of activation of skeletal muscle are carefully controlled by the reticulospinal system and the fast start is not simply due to the uniform and relatively instantaneous activation of skeletal muscle implied by the existence of the Mauthner cell system.

Critique of the methods

Although studies utilizing simultaneous cinematography and 'hard-wired' physiological measurements are not new (Eaton *et al.* 1981; Williams *et al.* 1989; Harper and Blake, 1989), several aspects of the instrumentation and surgical procedures in this study are unique. Fish were subjected to substantial surgery and were studied on the same day as the procedure. Moreover the wire 'harness' used in this study was larger than those previously employed. Although these stage one startle responses appeared normal and had an average duration similar to that previously observed in this species, it seems likely that the procedure itself may have had some effect. Thus, extension of these results to the free-ranging 'normal' fish must be made with some caution. Moreover, the data in this study must be considered preliminary and confirmation of these conclusions awaits a more

systematic approach with higher frame rates, better resolution of ciné data and simultaneous EMG recordings.

The ultrasonic dimension gauge technique has been used extensively in cardiovascular physiology over the past two decades. Both the spatial and temporal resolution of the system seem adequate for this task (Kirkpatrick *et al.* 1972). The greatest potential source of error in this technique is in the coupling of the gauges to the local muscle. Following acute implantation in cardiac muscle, dimensions measured with this technique are identical to dimensions computed by high-speed radiography of the crystals or of adjacent radio-opaque markers which do not have attached wires (Waldman *et al.* 1985). The extent of tissue damage in these studies did not appear grossly different from that found in our fish. However, the myocardium is a very different muscle with a tight progression of fiber directions through the wall and a very shallow pitch angle. Cardiac muscle is also very tightly bound by collagen and has a much less compliant passive stress–strain relationship. These factors make extrapolation of the results in cardiac muscle dangerous. The most convincing argument that the gauges were coupled to the muscle was the stability of the measurements. Most of these fish were monitored for over 8 h and over this period the gauge dimensions at rest (straight fish) did not change. Following each fast start they returned to the control dimensions and the envelope of received sound did not change, indicating that the gauge had not rotated. It is possible that the slow frame rate and 12 ms time constraint of the dimension gauge system may have influenced the results.

Since the skin may well be involved in transmission of the deformation, it is possible that anchoring the gauges to the skin may have influenced the results. This, however, also seems unlikely since one would not expect the gauge distance to remain reproducible if skin deformation was influencing the gauge position. However, it is clear that the extensive surgery and the presence of relatively large crystals and attached wires may have influenced the results.

The authors would like to acknowledge the advice and support of Dr John M. Gosline of The University of British Columbia and the support of Dr Jeff Graham of the University of California, San Diego, who helped conduct the pilot studies for this project. This study owes a unique debt to Professor Fred N. White (retired) of the University of California, San Diego, whose teachings, advice and encouragement led directly to these studies. This study was conducted in the Department of Zoology at The University of British Columbia while JWC was on sabbatical leave from the Department of Medicine at the University of California at San Diego. Supported in part by NHLBI H132583 and by a grant to RWB from Natural Sciences and Engineering Research Council of Canada.

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