Muscle Dynamics in Fish During Steady Swimming

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SYNOPSIS. Recent research in fish locomotion has been dominated by an interest in the dynamic mechanical properties of the swimming musculature. Prior observations have indicated that waves of muscle activation travel along the body of an undulating fish faster than the resulting waves of muscular contraction, suggesting that the phase relation between the muscle strain cycle and its activation must vary along the body. Since this phase relation is critical in determining how the muscle performs in cyclic contractions, the possibility has emerged that dynamic muscle function may change with axial position in swimming fish. Quantification of muscle contractile properties in cyclic contractions relies on in vivo experiments using strain and activation data collected in vivo. In this paper we discuss the relation between these parameters and body kinematics. Using videoradiographic data from swimming mackerel we demonstrate that red muscle strain can be accurately predicted from midline curvature but not from lateral displacement. Electromyographic recordings show neuronal activation patterns that are consistent with red muscle performing net positive work at all axial positions. The relatively constant cross-section of red muscle along much of the body suggests that positive power for swimming is generated fairly uniformly along the length of the fish.

INTRODUCTION

Undulatory swimming in fish involves the coordinated activation and contraction of lateral myotomes alternately down each side of the body. This muscular action, together with the physical interaction between the body and the water, results in characteristic waves of lateral deformation, or bending, that propagate along the body with increasing amplitude from head to tail. In his pioneering kinematic studies James Gray (1933) described several important features of the body undulations that are involved in the production of forward thrust. For example, in steady swimming the velocity of the wave of bending that travels posteriorly along the body, must always be greater than the forward velocity of the fish through the water, an idea also proposed by Breder in 1926. The significance of this observation is that each portion of the body has a positive angle of attack relative to the fluid flow as it crosses the path of forward motion, and thrust is generated continuously by the collection of segments throughout each tail beat cycle. In addition, Gray showed that the very accentuated waves seen in anguilliform swimmers were also present (although with higher velocities and lower amplitudes) on the bodies of other fish such as dogfish, mackerel and whiting, which, to the naked eye, appeared to move simply by transverse strokes of the tail across the path of travel (as described by Borelli in 1680 (see Videler, 1993)). These descriptive studies led researchers to investigate the activation patterns and contractile properties of the lateral muscles that produce the transmission of muscular waves along the body. In the past decade, new methods in image analysis and muscle dynamics have provided the focus for experimental and modeling studies on the action of lateral muscle in powering undulatory swimming.

In this paper we discuss the relations between muscle activation, muscle strain and...
midline kinematics in steady swimming of fish, and we present kinematic data for mackerel obtained by a new radiographic technique. Our results show that midline curvature is an accurate predictor of lateral red muscle strain. A coupled analysis of electromyographic data show activation patterns that are consistent with red muscle performing net positive work at all positions. Finally, when the axial distributions of red muscle area and peak strain are considered it seems likely that power production for swimming is generated uniformly along most of the body.

**Muscle Dynamics**

**Electromyography**

In a swimming fish the spinal cord generates a motor pattern that progresses along the body and causes waves of muscle contraction. Details of the activation of lateral muscle during undulatory swimming have been revealed by electromyographic (EMG) studies. For each tail beat cycle, the electrical activity proceeds like a wave from anterior to posterior, with bursts occurring alternately along each side of the body (Grillner, 1974; Grillner and Kajahn, 1976; Blight, 1976; Williams et al., 1989; He et al., 1990; van Leeuwen et al., 1990; Wardle and Videler, 1993; Jayne and Lauder, 1993, 1995b). In fish there is a distinct anatomical division between red (oxidative) and white (glycolytic) muscle fibers, the former generally occupying a superficial lateral position and the latter making up the bulk of the underlying myotomes. In some fish small amounts of intermediate ("pink," or fast oxidative glycolytic) fibers are found in a transition zone between red and white muscle (Johnston, 1983). Sustained slow swimming is powered by the activation of slow oxidative red muscle. Increased swimming speeds result from a progression to higher tail beat frequencies driven by proportionately faster and more intense waves of muscle activation, increased red muscle recruitment and eventual recruitment of fast oxidative and glycolytic fibers at intermediate and sprint speeds (Bone, 1966; Rayner and Keenan, 1967; Bone et al., 1978; Brill and Dizon, 1979; Rome et al., 1984, 1992; Tsukamoto, 1984). With increased swimming speed the EMG burst duration decreases in proportion to the decreasing tail beat period. Consequently, the duty cycle (i.e., the fraction of each tail beat period when muscle is active) at each axial location remains relatively constant at different swimming speeds (Grillner and Kajahn, 1976; Wardle and Videler, 1993; Johnston et al., 1993; Knower et al., 1993). An exception to this pattern is reported by Rome and Swank (1992) who found that red muscle EMG duty cycle (at an unspecified location) decreased with increasing swim speed. Typically, duty cycles approach 0.5 in anterior muscle, but this may vary with longitudinal position. In anguilliform fishes burst durations remain relatively constant along the body because the waves of EMG onset and offset travel at about the same speed (Grillner and Kajahn, 1976; Williams et al., 1989). In other fish there is a progressive decrease in duty cycle towards the posterior sites. In teach, bluegill and bass the changes are small (Blight, 1977; Jayne and Lauder, 1993, 1995b), while in carp, mackerel and tuna there is nearly simultaneous EMG offset all along the body, and the posterior bursts may be only half as long as anterior ones (see Fig. 1, van Leeuwen et al., 1990; Wardle and Videler, 1993; Knower et al., 1993). Comparisons of the EMG timing to the progression of the waves of curvature or lateral displacement of the midline yield further discrepancies among different species, although attempts have been made to rationalize these discrepancies on the basis of variations in body morphologies and swimming modes (Wardle and Videler, 1994; Wardle et al., 1995), or differences in experimental methodologies (van Leeuwen, 1995). In most cases it appears that the onset of the EMG signal progresses along the body faster than the wave of body deformation, which itself is assumed to reflect the cycle of muscle strain (Videler and Hess, 1984; Williams et al., 1989; van Leeuwen et al., 1990; Rome et al., 1993; Wardle and Videler, 1993; Wardle et al., 1995). This suggests the temporal relation between muscle strain and neuronal activation varies with position along the body. Furthermore, differences in the intrin-
sic contractile properties of lateral muscle which are related to axial location have been demonstrated in several species. These factors have led to the idea that the dynamic function of lateral muscle changes along the length of the body, but data from various species have resulted in different predictions about how muscle power is produced and transmitted posterosively during swimming.

The importance of muscle activation timing in cyclic contractions

Unlike terrestrial vertebrates, where muscle and ground reaction forces can be measured directly during locomotion, studies on fish muscle dynamics in vivo have been largely inferential. The performance of muscle in a swimming fish is assessed indirectly from contractile properties measured in vitro. Under conditions which attempt to recreate the in vivo muscle strain and activation patterns (e.g., Altringham et al., 1993; Rome et al., 1993; Coughlin and Rome, 1996; Coughlin et al., 1996)), these parameters are typically determined from kinematic analysis of the body deformation or minute curvature, and simultaneous EMG recordings. In this experimental "work loop" technique the force output from a bundle of isolated fibres is measured during imposed oscillatory length changes and electrical stimulation patterns that are designed to mimic the in vivo events. A large body of information is available on the in vivo dynamic mechanical properties of both red and white muscle from various fishes (reviewed in Johnson, 1991; Altringham, 1994; Rome, 1994; van Leeuwen, 1995). In general, the work per cycle and power produced are highly dependent on temperature, frequency, strain amplitude, number of stimuli per cycle, and the timing of the start of stimulation relative to the phase of muscle strain. Maximum muscle power output requires pre-stretch of activated muscle, and strain-dependent shortening enhanced deactivation is also important. In a number of studies the optimal phase of muscle stimulation was shown to occur during muscle lengthening (i.e., before 90°, as defined in Fig. 2) and to be dependent on temperature, frequency and strain amplitude (Altringham and Johnson,
Fig. 2. A summary of the effect of varying the phase of muscle activation relative to muscle strain in work loop experiments (redrawn from Johnson and Johnston, 1991). One strain cycle is indicated by the sinuoidal curve. Maximum length occurs at a phase of 90°, and minimum length is at 270°. Mean or "rest" length occurs twice in each cycle, at 0° when the muscle is being lengthened and at 180° when the muscle is shortening. Different shaped work loops are obtained by providing electrical stimulation at various phases in the strain cycle, as shown by the loops drawn along the strain axis. When activation occurs between mean and peak lengths (0–90°) the work loop is large and positive, as indicated by the counter-clockwise arrows. In contrast, when activation occurs late, either in the latter part of shortening or the early part of lengthening (180–320°), the work loop is large and negative, as indicated by the clockwise arrows.

1990a, b; Johnson and Johnston, 1991; Johnson et al., 1991; Moon et al., 1991; Rome and Swank, 1992; Curtin and Wolledge, 1993; Johnston et al., 1993; Rome, 1994). This timing allows the muscle to develop higher than isometric forces during a short period of "negative" (energy absorbing) work, to maintain relatively high forces while shortening, and to relax to much lower forces during lengthening. These conditions yield large net positive (i.e., counter-clockwise) work loops, as shown in Figure 2 (phases 5–60°). Conversely, if muscle is activated much earlier than the optimal phase then the force may remain high during lengthening than during shortening. In this situation the absorption of energy will yield large net negative (clockwise) work loops (e.g., Fig. 2: phases 180–320°).

Under optimal conditions the power output in cyclic contractions increases nearly linearly with strain up to amplitudes of 20°, and power optimization requires fewer stimuli as cycle frequency increases (Johnson and Johnston, 1991). This is likely comparable to the situation in vivo, since EMG burst duration decreases with increasing tail beat frequency. The ability of muscle to do useful contractile work as cycle frequency increases is limited primarily by activation and relaxation kinetics. Because of its shorter force rise and relaxation times, white muscle can perform positive work at higher cycle frequencies than can red muscle. This mechanical behavior matches with the physiological recruitment of white muscle for fast burst swimming (Altringham, 1994).

Models of muscle function in swimming
A dynamic analysis of swimming in saïxus first suggested that muscle function should vary along the body. Hess and Viderer (1984) predicted that force development in lateral muscle would be simultaneous along the body, while muscle shortening would proceed as a wave. Consequently, muscle in the anterior and middle of the fish would produce positive power but more posterior muscle would produce increasing amounts of negative power. These authors also predicted that physiological differences in muscle might occur along the body. Since then evidence has accumulated to show that the intrinsic contractile properties of lateral muscle vary as
a function of axial position in some fish, but no consistent pattern has yet emerged. For example, in situ measurements of twitch time (i.e., the time from stimulus onset to maximal force) of white muscle show increases of up to two-fold from rostral to caudal positions in cod, saithe, salmon, mackerel and tuna. (Wardle, 1985; Wardle et al., 1980; T: Knower and R. Shadwick, unpublished). Similarly, activation and relaxation times increase caudally in isolated fibers from cod and saithe white muscle (Davies and Johnston, 1993; Altringham et al., 1993), and scup red muscle (Rome et al., 1993). In contrast, no significant difference in muscle twitch times was observed for white muscle of sculpin (Johnston et al., 1993) or for red muscle of mackerel (He et al., 1990) and yellowfin tuna (T. Knower and R. Shadwick, unpublished).

Following on the work of Hess and Videler (1984), Wardle (1985) and Wardle and Videler (1994) proposed that the major function of posterior muscles (i.e., at >0.65L) were to act as stiff transmitters of force to the tail. Their studies of lateral (red and white) muscle in mackerel and saithe predicted that, relative to the onset of muscle shortening, activation begins earlier in more posterior locations. Wardle et al. (1995) postulated that positive force from anterior and mid level muscles is transmitted to the tail via posterior muscles which are stiff while actively being stretched. In this scheme the peak in power output from anterior muscle occurs coincident with the maximum stiffness of the posterior muscle. Videler (1993) suggested that the activation pattern of posterior muscle in mackerel and saithe will produce net negative work, but the data in Altringham et al. (1993) show this is not the case. Their work loop studies show a large negative work component for posterior muscle as it is lengthened, but a high residual force (due to slow relaxation) that produces positive work during shortening, and results in slightly net positive work per cycle. In a study on cap van Leeuwen et al. (1990) showed that all axial muscle aces some negative work because activation begins during lengthening (as predicted by Hess and Videler, 1984). However, the calculated net work per cycle was positive for all locations anterior to the anus. Caudal to this (i.e., >0.7L) net work was expected to be negative. Recently van Leeuwen (1995) revised this analysis by using more realistic values of muscle relaxation times and incorporating pre-stretch force enhancement. The result was an increase in the positive work component at all axial positions, and only a small net negative work output at the caudal peduncle (0.84L). This prediction remains uncorroborated since no other studies have measured muscle properties at this extreme caudal position where most fish have virtually no red muscle. Stiffening of the body by posterior muscles as a mechanism to facilitate increases in swimming speed has also been proposed (Long and Nipper, 1996; McHenry et al., 1995). An alternative model of muscle dynamics is based on data from swimming scup (Rome et al., 1993; Coughlin and Rome, 1996). These authors found that net positive work was performed (at 20°C) by anterior, mid and posterior red and white muscle under conditions that mimic in vivo activation and strain. In this fish the anterior muscle apparently undergoes very small strains (±0.015) and consequently generates relatively little power, while muscle located posterior to 0.5L undergoes larger strain (≥0.025) and thus generates much more mass-specific power. Since the cross-sectional area of red muscle is relatively constant along the body of scup, Rome and his colleagues concluded that the majority of swimming power was derived from the posterior muscle, although the function of the anterior muscle was not defined. Johnston et al. (1994) demonstrated that posterior muscle in bass also produces large net positive work loops under conditions that simulate in vivo activation and strain.

Because the timing of local muscle activation with respect to strain is critically important in determining the properties of the work loop, precise matching of the in vivo experimental conditions with in vivo strain and activation patterns is essential in order to formulate predictions about muscle function.
How is muscle strain calculated from kinematics?

The most common approach for determining in vivo muscle strain is to treat the fish body as a homogeneous beam, and calculate local strain as a function of the local curvature (or an approximation of it) of the neutral axis (the body midline) and the lateral distance from this axis (see Fig. 3). This has been done in studies on saithe (Videler and Hess, 1984), carp (van Leeuwen et al., 1990; Rome and Sonnicki, 1991), scup (Rome et al., 1993; Coughlin and Rome, 1996), bass (Johnson et al., 1994; Jayne and Lauder, 1995a, b), and gur (Long et al., 1996). Among the studies where EMG recordings were also made, all showed that activation precedes peak muscle length. Based on the results of work loop experiments (e.g., Fig. 2), this timing should result in the development of high forces during shortening and net positive work, in most cases (van Leeuwen, 1995).

In other kinematic studies the timing of muscle strain has been inferred, not from curvature, but from lateral deflection of the midline (Williams et al., 1989; Wardle and Videler, 1993), resulting in the predictions that muscle in posterior sites is active only during lengthening, and probably performing net negative work (Videler, 1993). This method may lead to an erroneous estimation of the timing of peak strain, because midline curvature precedes lateral deflection at any point along the body, with a phase difference that increases posteriorly (Videler and Hess, 1984; Katz and Shadwick, 1997). It is important, therefore, to determine whether muscle strain is in phase with midline curvature or with lateral deflection. Recently Coughlin et al. (1996) showed, with sonoemetric measurements, that strain in lateral muscle can indeed be accurately calculated from midline curvature. In the following section we reach a similar conclusion from experiments using a radiographic technique to make measurements of muscle deformation in swimming mackerel.

EXPERIMENTAL METHODS

Direct measurements of body deformation by videoradiography

Pacific mackerel (Scomber japonicus) 22–29 cm total length (L) and 110 to 185 g mass were captured at the Scripps pier with baited barbless hooks, transferred to holding tanks with well oxygenated flow-through sea water, and fed daily on chopped squid. These fish were used for energetics and kinematics experiments (R. E. Shadwick and J. E. Steffensen, unpublished), as well as for videoradiography and EMG experiments, some of which will be described here. The videoradiography technique is based on one used to measure 3-dimensionalt deformation of mammalian cardiac muscle (Waldman et al., 1985). For surgery 3 fish were anesthetized and ventilated in 1/10,000 MS222 (tricaine methanesulfonate) in sea water at 15°C. Columns of 0.5 mm gold beads were inserted horizontally into lateral muscle, just above the horizontal septum, at 5 axial sites separated by intervals of about 1.4 cm (Fig. 4) by using a uroc made from an 18G syringe needle. Beads within each column were separated by approximately 3.5 mm. The body thickness determined the number of beads in each column: these at the three anterior sites and two at the posterior sites. At each
site the hole in the skin was sutured closed and a 1.5 mm diameter drilled lead bead was anchored with 4-0 silt suture. A short length of 1 mm diameter mercury-filled sliastic tubing was sutured to the dorsal midline in the region of the beads. The fish were returned to the holding tanks for three days and then each was placed in a small water tunnel treadmill to swim at steady speeds of 1-3 Lsec^{-1}. A biplane X-ray source projected dorsal and lateral images of the swimming fish onto fluoroscope screens. Two synchronized CCD video cameras recorded these images at 60 Hz on VHS tapes. In the present study we analyzed only the motion of the implanted markers in the horizontal plane (i.e., the dorsal view). Video fields were digitized with a RasterOps video interface board, and the x-y coordinates of each marker in each image were determined with NIH image software. From this data the lateral displacement of any marker or the segment length between markers in adjacent columns could be followed in time. The position of a fixed calibration marker in the video images could be measured to within 0.1 mm. Thus the error associated with calculations of segment length is <2%. Time dependent position and segment length data were smoothed minimally with no phase shifts by the use of digital low-pass finite impulse response filters (15-20 Hz cutoff) available in AcqKnowledge (Biopac Systems Inc.) software.

EMG recordings

Three fish were anesthetized, as described above, and implanted with paired copper EMG hook electrodes at sites ranging from about 0.4L to 0.78L. The bare wire tips were placed in the lateral red muscle from an entry point near the dorsal midline, using a 26G syringe needle. Each wire was sutured to the skin at the point of entry, and the wires were collected into one bundle which was anchored dorsally in two places. After recovery from anesthesia each fish was placed in a water tunnel respirometer, with a working section of 14.5 x 14.5 x 47 cm, for swimming bouts at speeds varying from 1.0 to 3.0 Lsec^{-1}. EMG signals were amplified and analog filtered (100 to 500 Hz) with an A-M Systems AC amplifier, and collected digitally at 2 kHz on an 80386 computer with Axotape software (Axon Instruments). Post-acquisition digital filtering of EMG data was carried out by using finite impulse response filters in the AcqKnowledge (Biopac Systems Inc.) software. This allowed us to determine onset and offset timing using thresholds (as in Wardle and Videler, 1993).

Body kinematics

Simultaneous VHS video recordings of dorsal views were made at 60 Hz and synchronized to the EMG recordings by a flashing red diode, visible in the image plane, whose excitation voltage was recorded with the EMG signals. Video fields were digitized as described above. For each image in a swimming sequence the x-y coordinates of a series of 30 points defining the body midline were collected, and 4th order polynomial curves fit to each (see Katz and Shavwick, 1997). From this sequence of midline curves, the lateral deflection and the curvature \kappa (defined as the inverse of the radius of curvature) at any position on the body could be calculated as a function of time. Superficial muscle strain was then calculated as \kappa, where \kappa is half the body thickness (see Fig. 3). Midline curvature and displacement data were smoothed digitally with a 20 Hz low-pass filter in the Acknowledge software.

RESULTS AND DISCUSSION

Muscle deformation determined by videoradiography

The x-ray technique allows us to visualize motion of reference points within the muscle mass of a swimming fish. This same approach has been valuable in mapping cardiac muscle strain in mammals, but has not previously been used to study skeletal muscle in fish. The method of placement of the radio-opaque markers is detailed in Figure 4. The lateral motion of markers at any depth demonstrates the traveling wave of deformation on the body, characterized by a progressive delay in peaks and increase in amplitude from anterior to posterior sites (Fig. 5). At each axial location all markers, regardless of depth, moved in synchrony.
with each other and with the body midline. 

Instantaneous segment strain was deter-

mined from the axial distances between pairs of radio-opaque markers, with the assump-
tion that this represents local muscle strain (see below). A comparison of the strain at the most anterior and most posterio-

locations is shown in Figure 6, where peak strain occurs earlier at AB than at DE, due to the traveling wave of muscle deforma-
tion. Strain amplitude of the superficial muscle does not vary greatly between anterior and posterior locations because, al-
though curvature and displacement ampli-

Fig. 4. Diagram of a dorsal view and two transverse sections to illustrate the placement of radio-opaque markers in swimming muscle on the right side of a 23 cm mackerel. Slices of one-half mm diameter gold beads were implanted at five locations A–E, equivalent to 0.4L, 0.44L, 0.52L, 0.68L, and 0.85L in a horizontal plane just dorsal to the horizontal septum. At each location a 1.5 mm diameter lead bead was sutured tightly to the skin. A short length of 1 mm diameter mercury-filled plastic tube was sutured to mark the dorsal midline in the region A–E. The transverse sections at 0.4L and 0.65L show the positions of the beads, the location and shape of the lateral muscle wedges (stippled), and the location of the backbones (hatched). The red muscle dimensions w and d. To calculate muscle cross-sectional area in Figure 10, are shown indicated.

Fig. 5. Plots of lateral displacement of marker beads for four tail-beats of 23 cm mackerel swimming at 3.75 Hz (1.73 L/sec). Data were collected by videomicrography at intervals of 1/600. For clarity, thin lines joining data points and not the individual points are shown. The upper panel shows motion of the lead superficial bead at positions A through E as shown in Figure 4. The middle panel shows motion of the deepest gold beads at the same 5 locations. The lower panel shows the lateral displacement of the myeline marker at location E. The traveling wave of deformation is characterized by the progressive delay in peaks and increase in amplitude from anterior to posterior sites. Comparison of these panels shows that, at each location, the surface and deep markers move laterally in phase with each other.
tude increase posteriorly, the body thins and the lateral distance to the neutral bending axis (i.e., the backbone) decreases (see Fig. 4). At each location, strains at all depths occur in synchrony, as illustrated by the curves for superficial and deep strays at DE (Fig. 6). This means the passive white fibers (steady swimming at these speeds is powered only by red muscle) which make up the nested myotomal cores are undergoing cyclic length changes that are in phase with those of the active superficial red muscle. Figure 6 also illustrates our finding that strain amplitude at each axial location decreases with closer proximity to the backbone. Taken together, these observations support the idea that, to a first approximation, the fish body can indeed be modeled as a homogeneous bending beam during slow swimming (i.e., when red muscle is active and white muscle is passively deformed).

We can now address the question: how are local curvature and lateral displacement related to red muscle strain? We observed that the peaks in strain precede the peaks in lateral displacement at each axial location. This point is illustrated in Figure 7 (upper panel), where superficial muscle strain and lateral displacement at 0.62L are plotted. These data are derived from videoradiography, and represent the segment length DE (as in Fig. 6) and the lateral displacement of a point midway between D and E, calculated by averaging the displacement traces of markers at D and E (as in Fig. 5). Here we see that local strain precedes lateral displacement by about 0.15 cycles, or 54°. Also shown, in the lower panel, are the midline curvature and lateral displacement at 0.62L, derived from polynomial curves fit to midlines of normal video images of another fish swimming at the same speed as that in the upper panel. This combination was necessary because midline kinematics could not be measured on fish swimming in the X-ray apparatus. For direct comparison between the two fish the time scale for each is normalized to tail beat cycles, and the two panels are synchronized by aligning the displacement traces (open squares) in each. This reveals that the midline curvature and red muscle strain are essentially in phase. This result concurs with a recent report by Coughlin et al. (1996) in which red muscle strain in swimming skip, measured directly by sonomicrometry, was found to be synchronous with strain calculated from midline curvature. Thus, local curvature is a good index of the timing of red muscle strain. However, local lateral displacements, which echo curvature and strain (by about 54° at this axial location) would not provide an accurate representation of the timing of muscle strain. These measurements verify the prediction by Katz and Shadwick (1998) that local strain of superficial red muscle (and passive white muscle) should be in phase with local curvature but not with lateral displacement. Interestingly,
Covell et al. (1991) showed by sonomicrometry that the onset of local concave curvature preceded the onset of local shortening of active white muscle in fast starts of trout. This suggests that white muscle may passively deform to phase with the adjacent red muscle, the anisotropic connections of the white muscle fibers to the backbone and skin are likely important in transmitting forces posteriorly when active.

Why are curvature and lateral displacement not synchronous?

If the waveform of deformation on the fish body was described by a constant amplitude sinusoid, then we would expect the peaks of curvature to coincide with the peaks of lateral displacement. This may approach the situation in some types of locomotion, e.g., terrestrial undulation of rails (Gillis, 1988) or nematodes (Gray and Lissmann, 1964), but in all aquatic undulators examined the increasing amplitude of lateral deformation along the length of the body results in phase shifts between curvature and displacement. In their kinematic analysis of satieh Videler and Less (1984) provide data for lateral deflection and curvature of the midline that indicate a significant phase shift between these two parameters of up to 0.15 cycles (curvature leading). Jayne and Lauder (1995a) compared lateral displacement to flexion angles between arbitrary segments of the body midline and found phase shifts of 0.17–0.25 cycle. This phenomenon has been studied analytically by Katz and Shadwick (1998) who showed that the midline curvature wave travels posteriorly faster than the midline displacement wave, and that this is a direct and unavoidable consequence of having a displacement wave that increases in amplitude in the direction of propagation. This point is illustrated in Figure 8. Body midlines of a swimming sequence are shown, with the peaks of local curvature and displacement at 0.7L and 0.8L indicated. This demonstrates that maximum curvature to one side occurs several video fields prior to maximum displacement at these locations, and that the phase lead of curvature increases posteriorly, reaching values in excess of 60° in a fish such as the mackerel. In fact, in this fish the axial speed of the curvature wave is nearly equal to the speed of the muscle activation wave (see..
Fig. 8. Illustration of the phase difference between local curvature and lateral displacement. Polynomial curves were fit to the digitized midline of a series of consecutive video fields of a 27 cm mackerel swimming at 3.03 \( \text{ft} \text{/sec} \) (1.75 \( \text{Ls}^{-1} \)). The time interval is 1.00 sec. Curve 4 overlays the video image from which it was derived. The perpendicular lines on each curve indicate the magnitude and direction of local curvature at 0.7L (dashed lines) and 0.8L (solid lines), as calculated from the polynomial coefficients. Maxima curvatures (+) are found in field 4 for 0.7L and in field 5 for 0.8L, but maximum lateral displacement (+) for these two locations occurs in fields 7 and 9 respectively. Thus, the phase lead of curvature increases from approximately 50 \( \text{ms} \) (0.15 cycle) at 0.7L to 67 \( \text{ms} \) (0.22 cycles) at 0.8L, in accord with the prediction that curvature-displacement phase difference should increase posteriorly as the amplitude of the body wave increases (Kats and Shaw, 1997).

Fig. 9), which leads to the prediction that there should be no significant change in muscle activation phase along the body.

**Timing of muscle activation and strain**

The results of our videoradiographic study support the calculation of local muscle strain from midline curvature, as several researchers have done previously. Combining this kinematically derived strain data with simultaneous EMG recordings gives us an index of the phase of muscle activation along the body. Figure 9 is an example of local muscle strain and electrical activity recorded in a swimming mackerel at three axial locations, and Table 1 summarizes the strain and activation timing. Peak strains ranged from about ±0.06 anteriorily to ±0.09 posteriorily. At all locations the onset of muscle activation precedes maximum strain (i.e., peak muscle length), and the offset occurs while the muscle is shortening. The average phase of activation onset (relative to rest length being 0°, as defined in Fig. 2) was 64.3° at the anterior position, and 43.6° and 41.1° at the two posterior locations. Offset phases averaged 253.3°, 153.6° and 148.8°. No significant differences in activation phase were observed for swimming bouts in the speed range of 1–3 \( \text{Ls}^{-1} \). Thus, although EMG duty cycle decreases posteriorly, there are no qualitative differences in activation patterns of muscle along the body. Reference to Figure 2 indicates that activation prior to peak length generally leads to large net positive work in sinusoidal contractions. For the most posterior location the lateral displacement of the midline is also plotted in Figure 9 to show that, if displacement was taken as the indicator of strain timing, one might conclude that this muscle was only active during lengthening, and that its primary function was likely to produce negative power (e.g., see Fig. 8.6 in Videler, 1993).
Muscle distribution and function along the body

The in vivo muscle strain and activation data obtained here should be used to define the operating parameters for work loop experiments on isolated muscle, in order to make specific predictions about muscle power output in the swimming mackerel. Without such work loop data at this time, however, we will make provisional predictions about muscle function based on previous studies on other fish. He et al. (1990) reported twitch times (i.e., the time to peak force after activation begins) for mackerel red muscle of 40-80 ms at 12°C, with no significant variation related to axial location. Based on this, we estimate an mean time to peak force of 15°C of 50 ms and assume the relaxation time is about twice this (Attridgeh et al. 1993; van Leeuwen 1995; Coughlan and Rome, 1996), or 100 ms. Taking 400 ms as a typical tail beat period (i.e., a tail beat frequency of 2.5 Hz) for steady swimming in our mackerel, a muscle activation time of 50 ms means that peak force should occur 50400 = 0.125 cycles, or 45°, later than the EMG onset. Based on the phase of muscle activation shown in Table 1, the peak force should occur at 64° + 45° = 109° anteriorly, and 41° + 45° = 86° posteriorly. Similarly, a relaxation delay of 100 ms = 90° relative to the EMG offset would have the force reach its minimum at about 233° + 90° = 323° and 149° + 90° = 239°, respectively, at these same positions. Although simplistic, these estimates suggest that muscle force is likely to rise late in the extension phase and fall

Table 1. Calculated peak muscle strain and phase of muscle activation at three axial positions in a mackerel swimming at 2.5 Hz.1,2

<table>
<thead>
<tr>
<th>Axial position</th>
<th>Peak strain (%)</th>
<th>Phase EMG onset</th>
<th>Phase EMG offset</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45</td>
<td>0.063 (+0.015)</td>
<td>64.3 (+13.9)</td>
<td>233.3 (+0.6)</td>
</tr>
<tr>
<td>0.68</td>
<td>0.077 (+0.016)</td>
<td>43.6 (+13.9)</td>
<td>153.6 (+7.0)</td>
</tr>
<tr>
<td>0.75</td>
<td>0.087 (+0.044)</td>
<td>41.1 (+28.7)</td>
<td>148.9 (+11.7)</td>
</tr>
</tbody>
</table>

1Phases are defined as in Figure 2, i.e. peak muscle length occurs at 90° and peak shortening occurs at 270°. Each value is a mean for five tail beats ± one standard deviation.
during shortening to a minimum value below the muscle rest length, thus generating large positive work loops at all axial locations, such as those in Figure 2. If the red muscle activation and/or relaxation times increased causally, as in some fish, then the rise and/or fall in force would be delayed in the more posterior locations making these muscles even less likely to perform negative work (see van Leeuwen, 1995). Altringham et al. (1997) showed that instantaneous power output peaked in cyclic contraction when a muscle was shortening through its mean length (*i.e.*, 180°), and they predicted this would occur in anterior (0.35L) muscle while posterior (0.65L) muscle was stiff due to active lengthening. The data in Figure 9 support this model. While confirmation of our predictions awaits direct measurement, it is worth noting that the activation phase and duty cycle for the anterior and posterior locations in our study are comparable to those measured in the carp (positions 2 and 6 respectively in van Leeuwen et al., 1999) which, according to the model of van Leeuwen (1995), should produce large net positive work loops. As tail beat frequency increases the activation and relaxation times will represent a larger fraction of the cycle period, and the ability of the muscle to produce useful power will be compromised (*e.g.*, Johnson et al., 1994), eventually requiring the recruitment of faster fibers for faster swimming. If muscle at all longitudinal positions produces net positive work (as predicted above), the absolute contribution to swimming power by muscle at each position ought to be a function of its cross-sectional area and peak strain (Rome, 1994). In mackerel lateral red muscle occurs along the body from about 0.2L to 0.85L. We used muscle depth and width to calculate cross-sectional area by treating the muscle wedge as a triangle (see Fig. 4). The longitudinal distribution of these dimensions is shown in Figure 10, as well as the maximum curvature and calculated peak muscle strains, plotted as functions of axial position. Note that the change in peak strain along the body is not as great as the increase in curvature because h, the distance to the backbone, decreases. In fact, the peak strain at 0.4L and 0.85L are virtually equal even though the curvatures differ by fourfold. Between 0.4L and 0.7L, where the bulk of red muscle lies, cross-sectional area is relatively constant and peak strain varies by a factor of only 1.4, from about ≥0.06
to ±0.083 (as compared to a factor of >3 in scup; Rome et al., 1993). The effect of this longitudinal variation in peak strain on power output in mackerel red muscle is unknown but it may be insignificant; in studies on white muscle work per cycle increased nearly linearly with strain amplitude, but reached a plateau that extended over strains of about ±0.05 to ±0.08 (Altringham and Johnston, 1990b; Johnson and Johnston, 1991; Johnston et al. 1993). In scup red muscle the optimal peak strain for power output was about ±0.05 to ±0.06 for cyclic contractions at physiological frequencies, while power output was nearly equal at strains of ±0.063 and ±0.084 (Rome and Swank, 1992). It seems reasonable to conclude that, while posterior muscle may stiffen the body as anterior muscle is shortening, net positive power for steady swimming in the mackerel is produced by red muscle all along the body, and the contribution from anterior and posterior regions is likely fairly similar. Therefore, the transmission of the wave of undulation along the body is not comparable to a mechanical wave traveling in an elastic energy absorbing beam. Rather, all parts of the body have muscle that contributes positive power to the undulatory wave that progresses from head to tail.

CONCLUSIONS

Superficial red muscle

1. Local strain is lateral red muscle appears to be in phase with local midline curvature; muscle strain can be predicted from midline kinematics. This is supported by new videoradiographic data on mackerel and a previous sonomicrometry study on scup (Coughlin et al., 1996). The same conclusion holds for the white muscle when inactive.

2. The peak in muscle strain precedes the peak in lateral displacement of the midline, and this phase shift increases posteriorly along the body. Lateral displacement is therefore not a good indicator of muscle strain.

3. In swimming mackerel, red muscle activation onset occurs during the lengthening phase and offset occurs during shortening at all axial locations. Posterior muscle appears to be active while anterior muscle is shortening. These features suggest that posterior muscle may be developing force while anterior muscle is still contracting, but net positive power production should occur all along the body.

4. The bulk of red muscle occurs from 0.4L to 0.7L. In this region the muscle cross-sectional area is relatively constant and peak strains vary from about ±0.06 to ±0.08. Based on work loop studies of other fish muscle we expect that red muscle all along the body contributes to positive power production during steady swimming. These conclusions should be verified with direct measurements of muscle dynamic properties using strain and stimulation patterns determined in vivo.

Muscle in tested cones

The result that superficial red muscle shortening and local curvature are phase locked is not surprising. Nor is it unexpected that the muscle in the tested myotomal cones deforms passively in phase with the midline curvature. However, the complex anatomy of the cones, which includes nesting of myotomes and connective tissue linkages to the skin and backbone, makes it difficult to predict the phase relation between muscle strain and body kinematics when this muscle is actively contracting. Since the cones are comprised of white muscle in most fish it is harder to study the dynamics of this muscle in vivo because it is generally not recruited during steady swimming. The sonomicrometry study by Covell et al. (1991) gave evidence that local shortening of white muscle during an escape response caused bending of the backbone more caudally, thus implicating tendinous linkages as important force transmitters to the posterior regions. In this case local curvature would not be a good indicator of white muscle strain, and thus we expect that red and white muscle at the same axial location might contract out of phase with each other when both are active. Future studies of muscle dynamics should address this problem. If a fish with internalized red muscle that occupies some of the myotomal cones (such as tinca) is used then the me-
charzcal properties of this muscle may be studied during steady swimming (Knowler et al., 1993). Preliminary studies using somatic motility on yellowfin and skipjack tuna in our laboratory do indeed show that deep red (i.e., cone) muscle shortens with a significant phase lag compared to both superficial red fibers and body curvature at the same axial location. This work represents the next step in understanding how the cyclic work of single muscle cells in the complex anatomical arrangement of myotomal cones is converted into a coordinated wave of undulation on the body to power swimming.

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