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How swimming fish use slow and fast muscle fibers: implications for models of vertebrate muscle recruitment

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Abstract We quantified the intensity and duration of electromyograms (emgs) from the red and white axial muscles in five bluegill sunfish (Lepomis macrochirus) which performed three categories of behavior including steady swimming and burst and glide swimming at moderate and rapid speeds. Steady swimming (at 2 lengths/s) involved exclusively red muscle activity (mean posterior emg duration = 95 ms), whereas unsteady swimming utilized red and white fibers with two features of fiber type recruitment previously undescribed for any ectothermic vertebrate locomotor muscle. First, for moderate speed swimming, the timing of red and white activity differed significantly with the average onset time of white lagging behind that of red by approximately 40 ms. The durations of these white emgs were shorter than those of the red emgs (posterior mean = 82 ms) because offset times were effectively synchronous . Second, compared to steady and moderate speed unsteady swimming, the intensity of red activity during rapid unsteady swimming decreased while the intensity of white muscle activity (mean white emg duration = 33 ms) increased. Decreased red activity associated with increased white activity differs from the general pattern of vertebrate muscle recruitment in which faster fiber types are recruited in addition to, but not to the exclusion of, slower fiber types.

Key words Muscle · Locomotion · Recruitment · Fish Electromyography

Introduction

Most vertebrate muscle contains several different fiber types that are proposed to allow more effective movement over a wide range of speeds, much as gear systems can improve the efficiency of machines. A recurrent finding for vertebrate locomotion is that as speed of movement increases, successively faster muscle fiber types are recruited in addition to (but not at the exclusion of) slower fiber types (Grillner 1981; Armstrong 1981). Thus, slower (red) muscle fibers are used to power slow- and medium-speed movements while both slow fibers and the faster (white) fibers are used during rapid movement. This "additive model" (Fig. 1) of muscle fiber function has been supported by numerous studies of the locomotion of fishes and other ectothermic vertebrates which often have a spatial segregation of fiber types within muscles that facilitates in vivo studies of muscle fiber function (Rayner and Keenan 1967; Hudson 1973; Johnston et al. 1977; Bone 1978; Bone et al. 1978; Johnston and Moon 1980; Johnston 1983; Rome et al. 1984; Jayne et al. 1990a). Even for the remarkably fast (25 ms) escape response of a fish, the slow red axial muscles are recruited simultaneously (additively) with the fast white fibers (Jayne and Lauder 1993). This fiber recruitment during the escape response is paradoxical because mechanical considerations of force development in the slow muscle fibers suggest that such activity should contribute little or nothing to performance during very rapid locomotion (Rome et al. 1988; Rome and Sosnicki 1991).

The neuroanatomy relevant to axial function in fishes further suggests why an additive pattern of fiber recruitment might be generally expected. In fishes, the white muscle is innervated by both small (secondary) and large (primary) motor neurons whereas only small motor neurons innervate red muscle (Fetcho 1986; Westerfield et al. 1986). The size order of recruitment principle (Henneman et al. 1965) predicts that increasing levels of activity occur as a result of recruiting successively larger motor neurons. Thus, if small followed by large is the actual order of motoneuron activation during swimming as speed increases, then red and white fibers would be expected to be activated together during rapid locomotion as excitation of both types of motoneurons occurs.

By eliciting different speeds of locomotion, one can investigate how muscle activity increases both within a

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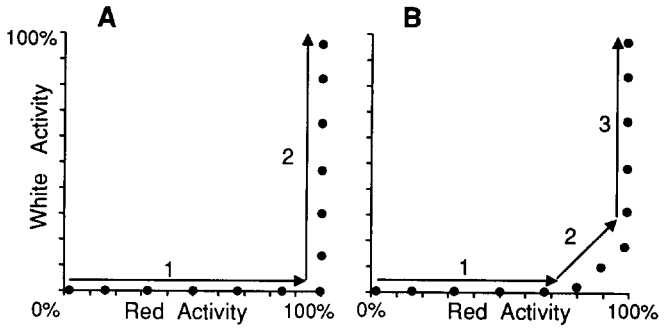


Fig. 1A, B Diagrammatic summary of the expected patterns of recruitment of red (slow) and white (fast) muscle fiber types based on current models of "additive" recruitment. Filled circles indicate arbitrarily chosen points that conform to previous models and qualitative emg studies (A) and quantitative emg studies (B) of fiber type recruitment in ectothermic vertebrate muscle. *Arrows* indicate the progression of events with increasing speed of movement

single mode of locomotion and among different modes of locomotion. For example, bluegill sunfish undulate steadily from about one to two body lengths per second, whereas faster speeds are usually attained by performing one or two rapid undulatory bursts after which the body is held straight as the fish glides forward (burst and glide swimming). Most previous electromyographic studies of fish locomotion (e.g., Bone 1966; Hudson 1973; Johnston et al. 1977; Johnston and Moon 1980; Rome et al. 1985) primarily have dealt with steady lateral undulatory swimming and have qualitatively determined the threshold for white muscle activity while varying the swimming speeds of fish. Rome (1986) recently summarized these studies and suggested that white fish muscle is activated only after the red muscle has been maximally recruited, and this would predict the pattern shown in Fig. 1A. More recent studies that have quantified the emg amplitude for ectothermic vertebrate muscle (Jayne et al. 1990a; Rome et al. 1992) have found that the intensity of red muscle activity increases with speed until a maximal level is attained and then maintained with further increases in speed. Furthermore, white muscle may be recruited slightly before maximal activity of red muscle (Jayne et al. 1990a) leading to a refined additive model of fiber type recruitment shown in Fig. 1B. Hence, there are rather clear expectations for how levels of muscle activity increase, but little is known about the details of the timing of white versus red activity and whether there are any circumstances for which activity of one fiber type actually decreases in the face of increased requirements for mechanical power. In addition, no study of fishes has focused on high-speed forward locomotion to examine patterns of fiber recruitment.

In this paper we test the generality of additive models of vertebrate muscle fiber function by quantifying both the intensity and timing of in vivo activity of slow and fast axial muscles in fish swimming over a wide range of speeds. We were most interested in the burst and glide mode which occurs at speeds intermediate to those of the better-studied steady swimming and escape behaviors. Current literature suggests that both fiber types are recruited obligatorily in an additive manner as the speed of movement increases. We sought to clarify whether some ectothermic vertebrates have sufficient plasticity in fiber recruitment to decouple activity of slow and fast fibers during rapid movements to avoid mechanically suboptimal patterns of force generation.

Materials and methods

Experimental subjects and protocol

All bluegill sunfish (*Lepomis macrochirus*) were captured from small ponds in southern and central California and average time in captivity before experiments approximated 3 months. All animals were housed individually in 40–80 l aquaria with a 12:12 light:dark cycle and a constant temperature (20 ±2°C) which was equal to that used during experiments. After preliminary experiments on three individuals, we then standardized our methods and only analyzed the results from 5 additional individuals which had standard and total lengths and mass that ranged from 12.7–16.0 cm, 16.0–19.5 cm, 77–110 g, respectively.

To facilitate controlling the swimming speeds of the fish, we used two calibrated flow tanks which had working sections of approximately 46 × 18 × 18 cm and 91 × 30 × 30 cm with the long dimension parallel to the flow. A mirror mounted below the transparent working section of each flow tank allowed us to videotape ventral and lateral views using a two camera high-speed video system (NAC HSV-400) operating at 200 images per second. For each of the 5 fish, we first obtained 5 consecutive cycles of steady swimming at approximately 1.6 and 2.0 total lengths (TL)/s. The transition between steady and burst and glide swimming generally occurred between speeds of 2.0 and 2.5 TL/s, and we elicited burst and glide swimming using flow rates varying from about 2.5 to 4 TL/s. During steady swimming we only analyzed sequences for which the fish was almost perfectly centered (side-to-side and top-to-bottom) in the working section of the tank, and the body of the fish was never within 3 cm of any wall of the tank or the surface of the water. The position of the fish during burst and glide swimming could not be controlled as precisely as that during steady swimming, but we were able to restrict our analysis of this behavior to sequences in which the fish were at least 3 cm away from the two sides of the working section and the surface of the water, but occasionally the ventral surface of the fish was as close as 1.5 cm to the bottom of the flow tank. For each individual fish, we selected 5 tail beats from each of two groups of burst and glide swimming which were subjectively rated as either moderate speed or rapid burst and glide swimming.

Electromyography

To quantify the patterns of fiber type recruitment, we used hypodermic needles (26 gauge) to percutaneously implant 12 bipolar fine wire electrodes (0.051 mm diameter stainless steel wire) into the red and white muscle at 3 longitudinal positions on both the right and the left sides for each of 5 individuals. Greater details of electrode construction (Jayne 1988) and implantation procedures (Jayne and Lauder 1993) are given elsewhere. The 4 portions of fish myomeres schematically resemble a W-shape, the top of which is oriented anteriorly while the middle of the W is on the horizontal septum that separates the epaxial and hypaxial musculature. We inserted electrodes into the white muscle of the epaxial portion of the myomere that was closest to the horizontal septum and at a depth midway between the skin and the mid-sagittal plane. We attempted to implant electrodes into the epaxial white muscle as close as possible to the same longitudinal location as the electrode which was located in the superficial red muscle, and we used post-mortem x-rays and dissections of all of the fish to con-

firm the longitudinal position of electrodes. Proceeding from anterior to posterior, the approximate average locations of the three longitudinal sites were: 1) in the trunk directly ventral to the seventh spine of the spiny dorsal fin, 11 vertebrae posterior to the skull; 2) in the anterior tail, directly dorsal to the first spine of the anal fin, lateral to the second caudal vertebra and 15 vertebrae posterior to the skull; and 3) in the mid-tail, directly ventral to the posterior margin of the soft dorsal fin, lateral to the sixth caudal vertebra and 19 vertebrae posterior to the skull.

During burst and glide swimming, white muscle activity can occur on one side without subsequent white muscle activity on the contralateral side; hence, we restricted our analysis to ipsilateral electrodes. Furthermore, we restricted our quantitative analysis to electrode sites that met our 4 criteria for successful implantation and could be analyzed for each and every individual. We considered a pair of red and white electrodes at a single longitudinal position successfully implanted if: (1) the longitudinal location of the red and white electrodes at a site varied by less than the length of a single vertebra, (2) high levels of red muscle activity could be observed during steady swimming without noticeable crosstalk with the white electrode; (3) substantial white muscle activity could be observed during unsteady high speed swimming; and (4) the electrodes were not pulled out before the end of the experiment. Because the middle site of each fish did not always conform to all of these conditions in each and every individual, we only quantified data from ipsilateral anterior and posterior sites in each individual. This simplified our statistical comparisons by avoiding a large number of missing values in the experimental design. However, our results from the middle longitudinal site when they were available did conform qualitatively to the result which we describe for the anterior and posterior sites.

Fig. 2A–C Electromyograms recorded simultaneously from the same side of a single fish at the most anterior and most posterior longitudinal sites. A During steady swimming of the fish at about 2 TL/s. Note the complete absence of white activity. B During a rapid burst preceding a glide. C During unsteady swimming at a speed intermediate to that in A and B. Dashed lines indicate the onset and offset of red muscle activity at each site whereas arrows indicate the onset and offset of white muscle activity. Near the threshold of white recruitment the onset times of white muscle were delayed with respect to red whereas the offset times of red and white at a single site were nearly synchronous. A–C are shown in the order in which they were recorded; hence, the decreased amounts of red activity can not be attributed to damaged electrodes. All illustrated emgs are analog traces from a digital file after processing with a finite impulse response filter as described in Materials and methods

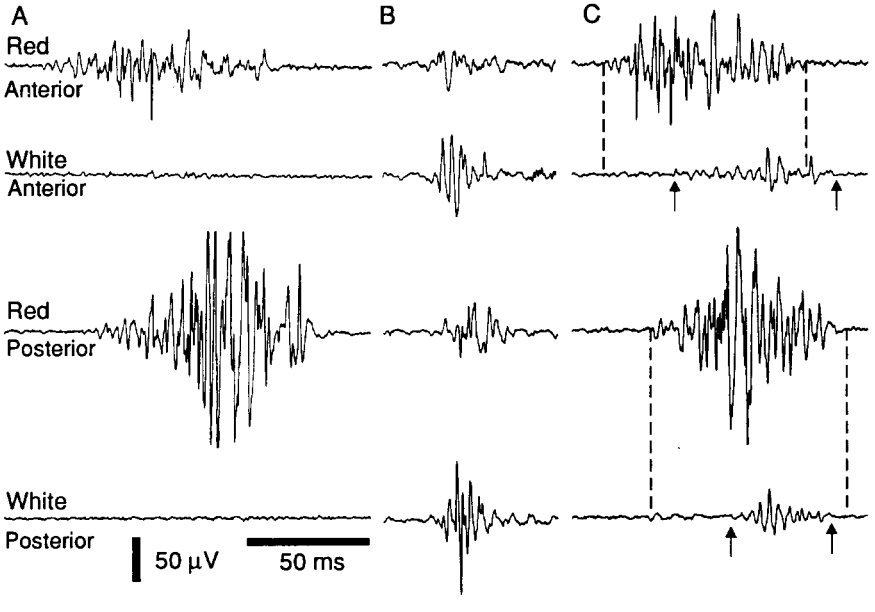
Signal conditioning and analysis

Electromyograms (emgs) were amplified 20,000 × using Grass model P511 K preamplifiers with high and low band pass filter settings of 100 Hz and 3 kHz and a 60 Hz notch filter. The emgs were recorded with a TEAC XR-5000 FM data recorder using a tape speed of 9.5 cm/s. We converted the analog emgs to digital data using a sampling rate of 8 kHz (see Jayne et al. 1990b). Because the rapid movements of burst and glide swimming can cause considerable low frequency artifacts, we filtered the digital emgs using a finite impulse response filter that reduced the portion of the signal below 100 Hz to less than 10% of its original amplitude.

Using the digital, filtered data, we used custom computer programs to determine the onset and offset time of muscle activity to the nearest ms. We also calculated the rectified integrated area for each channel using 10 ms bins. All resulting values were then normalized to a percentage of the maximal value observed for each electrode at any time during the experiments. We did not elicit escape responses during these experiments; hence the maximal values (which occurred during rapid burst and glide swimming) used to normalize intensity of white muscle recruitment could be less than that which occurs during escape responses. Values for normalizing red activity usually occurred during moderate speed burst and glides and they are also probably less than those that occur during escapes. We then used the greatest value (from one 10 ms bin) of the normalized rectified integrated area within an emg burst to indicate the intensity of fiber recruitment during a single cycle of locomotion.

Because the durations of red and white emgs at a single location within an individual were often not identical, we used the following conventions. Burst duration refers to the maximal value of emg duration observed for a pair of the red and white electrodes at a single site, and this quantity could always be determined for all of the different swimming behaviors. Because of the variable amplitude of red and white emgs it was not always possible to objectively measure the onset and offset times and calculate durations. In order to refer to the duration of emgs from only a single fiber type, we will specifically indicate either red burst duration or white burst duration.

In part, sequences were chosen subjectively for analysis based on the quality of primarily the posterior emgs. For each individual, we analyzed five bursts of muscle activity at each of the two steady swimming speeds and each of two types of burst and glide swimming. Occasionally some aspects of the anterior emgs could not be quantified even though the posterior emgs were suitable for analysis. Hence, some of the sample sizes for the anterior site are a subset of the total of the 25 observations which were analyzed for each behavior.



Results

Steady speed swimming

As shown in Fig. 2A, bluegill swim steadily by using exclusively the red axial muscle fibers for speeds of 2 TL/s and slower. Figure 3A, B shows for a single individual how the burst duration decreases with increases in both the steady swimming speed and intensity of red recruitment. At 1.6 L/s the mean anterior red burst duration was 116 ms (SD = 17; N = 25) and the red intensity relative to a maximum of 100%, was 10.8% (SD = 9.5). For the posterior site these values were 109 ms (SD = 17) and 38.2% (SD = 16.5), respectively. When swimming steadily at 2 TL/s, the anterior and posterior mean red burst durations decreased slightly to 109 ms (SD = 18) and 95 ms (SD = 13). The anterior site duration at 2 TL/s was not significantly different from 1.6 TL/s, while the posterior site duration did decrease significantly between steady swimming speeds (two-tailed paired t-tests on individual means). The corresponding mean relative intensities of red recruitment increased significantly to 24.1% (SD = 10.8) and 56.6% (SD = 12.5). Longitudinal variation in the duration of emg bursts is common for the swimming of fish (van Leeuwen et al. 1990; Williams et al.1989), and this was the reason for standardizing anatomical locations and not pooling data from different longitudinal positions. A much more detailed (7 positions, 4 speeds) analysis of the effects of speed and longitudinal position on red activity of Lepomis during steady swimming will be presented elswhere.

The red muscle activity was propagated posteriorly for both speeds of steady swimming. As indicated in Table 1, both the onset and offset of red muscle activity at the posterior site usually lagged more than 20 ms behind those of the anterior site. Furthermore, as the speed of steady swimming increased, these longitudinal lag times between the anterior and posterior sites decreased. Hence, the rate of emg propagation increased with increased swimming speed.

Bluegill had difficulty sustaining steady swimming at speeds greater than about 2.5 TL/s. The maximal intensity of the red recruitment observed during swimming at 2 TL/s was 75.5%, but values higher than this were observed occasionally during the exclusive recruitment of red at speeds between 2 and 2.5 TL/s.

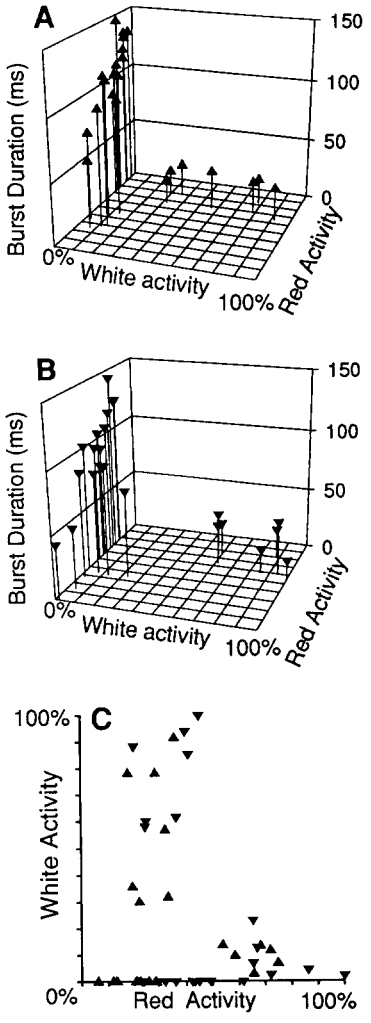


Fig. 3A–C Patterns of red and white fiber recruitment observed in this study for the most anterior (triangle) and most posterior (inverted triangle) electrode sites from the same side of a single fish. A Anterior site. B Posterior site. C Combined anterior and posterior data. Data are shown for all observations of steady swimming at both 1.6 and 2.0 TL/s, and for moderate speed and rapid burst and glide swimming. Note that decreasing burst duration indicates increased speed of movement, and the points with 0% white activity are for the two speeds of steady swimming.Also note the absence of points in the upper right hand quadrant of C

Table 1 Mean longitudinal lag times (in ms) of electromyographic events. Values indicate that the posterior site lagged behind the anterior site. Beside each mean the standard deviation is indicated parenthetically and N = sample size for the mean. The first two rows are for steady swimming at the speeds indicated, and the last two rows are for burst and glide swimming. *Note that low amplitudes of the red emgs during rapid burst and glide swimming prohibited accurate determination of lag times for the tail-beats used for the white muscle values shown below

Behavior	Red muscle			White muscle		
	N	Onset lag	Offset lag	N	Onset lag	Offset lag
1.6 TL/s	23	43.2 (12.6)	36.9 (11.2)			
2.0 TL/s	25	31.3 (13.3)	17.4 (8.7)			
Moderate	25	25.2 (14.8)	12.5 (17.2)	21	25.6 (10.1)	10.1 (5.8)
Rapid		*	*	25	7.3 (5.1)	7.7 (7.5)

Moderate speed burst and glide swimming

At swimming speeds slightly greater than about 2.5 TL/s, bluegill usually did not swim steadily, and they switched to a burst and glide mode of swimming during which fish usually had low but detectable amounts of white muscle activity in addition to high levels of red muscle activity (Fig. 2C). During this mode of swimming the red burst durations were highly variable ranging from 31 to 162 ms, and the mean values (N = 25) for red burst durations at the anterior and posterior site were 94 ms (SD = 32) and 82 ms (SD = 31), respectively. The mean intensities of red recruitment at the anterior and posterior sites were 57.4% (SD = 16.9) and 67.5% (SD = 17.3), respectively, and compared to steady swimming at 2 TL/s, this approximated a doubling of anterior activity but was only a slight increase in mean posterior red activity. However, the intensity of red recruitment during moderate speed burst and glide swimming did vary considerably, and the highest amplitude red emgs were observed for all individuals during this swimming behavior.

One novel characteristic of the motor pattern during this mode of swimming was that the white burst durations were consistently shorter than those of the red muscle at both the anterior (two-tailed paired t = 7.5, P<<0.001, N = 21) and posterior (t = 8.5, P<<0.001, N = 25) sites. White burst duration varied widely and ranged from 15 to 84 ms with mean values for anterior and posterior sites of 52 ms (SD = 15, N = 21) and 37 ms (SD = 12, N = 25), respectively. Compared to the red muscle, the intensity of white muscle activity during moderate speed burst and glide swimming was rather low, ranging from 0.4% to 32.2%. Mean values at the anterior and posterior sites were 14.8% (SD = 11.1) and 12.9% (SD = 7.6), respectively.

Another novel feature of moderate speed unsteady swimming was how the timing of muscle activity differed between the red and white fibers at the same longitudinal location (Fig. 2C). At the anterior site, the onset of white muscle activity lagged behind that of the red muscle by a mean value of 40 ms (SD = 18) which was significantly greater than zero (two tailed t = 7.1, P<<0.001, N = 21). In contrast, the offset times of anterior red and white muscle were effectively synchronous (Fig. 2C) having mean lag of −1 ms (SD = 7) which was not significantly different from zero (t = 0.7, P>0.4, N = 25). The anterior red-white lag in onset times was highly variable (−1 to 89 ms) and showed a highly significant positive correlation with red burst duration (Fig. 4A; Table 2). In contrast, the anterior red-white lags in offset time were less variable and did not correlate significantly with red burst duration (Fig. 4B, Table 2).

The timing differences between red and white at the posterior site were generally similar to those of the anterior site during moderate speed burst and glide swimming. The mean posterior red-white lag in onset was 39 ms (SD = 10) and it differed very significantly from zero (two- tailed t = 10.8, P<<0.001, N = 25). The mean posterior red-white lag in offset was only −5 ms (SD = 10) and did not have a highly significant difference from zero (t = 2.5, 0.01<P<0.02, N = 25). The posterior red-white lag in onset showed a clear and significant increase with increased red burst duration (Fig. 5A, Table 2). The combined data for 4 of the 5 individuals showed minimal variability in the posterior red-white lag in offset (Fig. 5B) which was not significantly correlated with burst duration (Table 2, row 6). For all 25 tail beats, including those from one individual with unusually long burst durations, there was a significant negative correlation between posterior red-white offset lag and red burst duration (Table 2, row 4).

During the unsteady swimming behavior at moderate speeds, activity of both the red and white muscle was propagated posteriorly. For both the red and white muscle the mean longitudinal lag times in onset and offset were extremely similar (Table 1). The lags between anterior and posterior onset time of red and white were within 1 ms of each other and approximated 25 ms, which in-

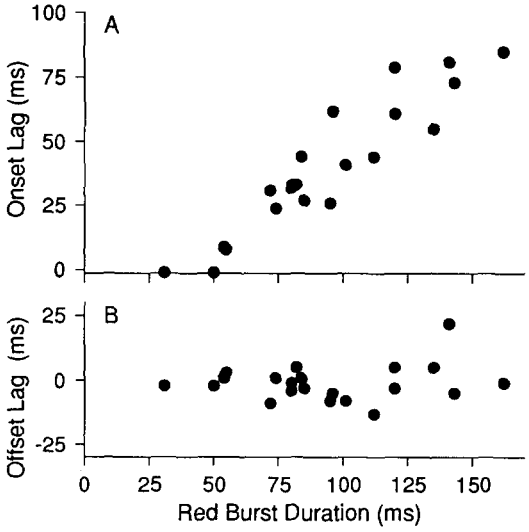


Fig. 4A, B The lag times between red and white muscle activity for all available data (N = 21) from the anterior site in 5 individuals that performed a total of 25 episodes of moderate speed burst and glide swimming. For 4 tail-beats the amplitude of white activity was too small to allow objective measurement of onset and offset times. Positive values of lag indicate that the time of white activity followed that of red activity

Table 2 Regression statistics relating lag time between red and white activity (ms) to burst duration (ms) at the anterior and posterior ipsilateral sites during moderate speed burst and glide swimming. The first 4 rows of the table use all available data for the same 25 tail beats, whereas a single individual with the 5 longest posterior burst durations was excluded in the last two rows. N = sample size

Dependent Variable	Burst indiv. N	N	r^2	P	Slope	Intercept
Ant. On Lag	21	5	0.87	<<0.001	0.72	−26.9
Ant. Off Lag	21	5	0.04	0.39	0.04	− 4.9
Post. On Lag	25	5	0.81	<<0.001	0.51	− 2.5
Post. Off Lag	25	5	0.75	<<0.001	−0.27	16.8
Post. On Lag	20	4	0.61	<<0.001	−0.62	− 9.7
Post. Off Lag	20	4	0.07	0.27	−0.07	3.7

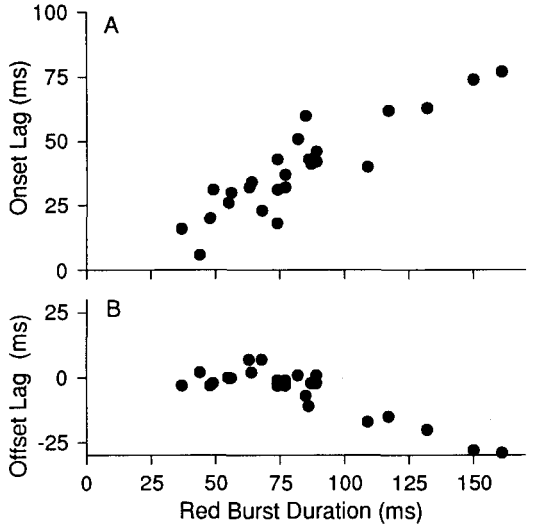


Fig. 5A, B The lag times between red and white muscle activity for all available data ($N = 25$) from the posterior site in 5 individuals and the same 25 episodes of moderate speed burst and glide swimming that were used for Fig. 4. Note that a single individual had the 5 greatest values of burst duration

dicates a slightly faster posterior propagation of muscle activity during this behavior compared to swimming steadily at 2.0 TL/s (Table 1).

Rapid burst and glide swimming

Another unexpected pattern of recruitment occurred during the highest speed burst and glide swimming which often had a large decrease in the intensity of red muscle activity (Figs. 2B, 3). The mean intensity of posterior red activity was only 27.7% (range 12.2%-50.4%, SD = 11.6) and 17 of the 25 posterior red burst intensities were less than 25%. In contrast, the highest levels of posterior white intensities were observed during this behavior with values that ranged from 21.8% to 100% and had a mean of 59.5% (SD = 23.5). Anteriorly, the intensities of red and white muscle had means of 41.7% (range 1.9%–94.9%, SD = 25.2) and 51.2% (range 11.3%–91.7%, SD = 20.6), respectively. Eight of the 25 anterior red bursts (from 3 different individuals) had intensities that exceeded the maximum value observed for all of the posterior red bursts. Consequently, there may be some subtle longitudinal variation in the speed of movement at which decreased amount of red muscle activity occurred during rapid swimming.

For this particular behavior the amplitude of red muscle activity was often so low that the onset and offset times could not be quantified objectively (for 12 of 25 emg bursts). Hence, we calculated a burst duration (used in Fig. 3 A, B) that was the maximum observed for either the red or white burst at a particular site during each cycle of locomotion. Mean anterior and posterior burst durations for the 25 observations of this behavior were 42 ms (SD = 26) and 35 ms (SD = 15), respectively. For the tail beats having quantifiable red onsets and offsets, the anterior white burst durations (mean = 33, SD = 8, N = 13) were generally shorter than those of the red muscle (mean = 57 ms, SD = 28, N = 13). For all 25 tail beats, anterior and posterior white burst durations had effectively identical means (29 ms) and standard deviations (7 ms). Posterior red burst durations ranged from 17 to 84 ms with a mean value of 39 ms (SD = 19, N = 13). Thus, muscle activity during rapid burst and glides was highly variable.

During rapid burst and glide swimming muscle activity was propagated posteriorly. As shown in Table 1, the lag time between the anterior and posterior sites approximated 7 ms; hence, the propagation of muscle activity was fastest for this group of observations compared to the other three experimental conditions.

The amplitude of red muscle activity at the anterior site did not differ significantly (two-tailed paired t-tests on individual means) between moderate and rapid burst and glide swimming (means = 58.8% and 40.3%, respectively), while the amplitude of red activity at the posterior site was significantly less at higher-speed swimming (means = 68.8% and 28.1%). White muscle activity was significantly greater at the higher burst-and glide speed for both anterior and posterior sites.

Discussion

Several new findings from our study of the axial undulatory locomotion of Lepomis suggest that fishes have a much greater diversity of motor output than has been previously described. The timing of white and red muscle activity at a given longitudinal position need not be synchronous, and this was most apparent near the threshold levels for recruitment of white muscle. During moderate speed unsteady swimming, durations of white muscle emgs were consistently less than those recorded from the more superficial red locations. Furthermore, the onset of white activity consistently lagged behind that of red while the offsets of red and white activity were effectively synchronous. During the most rapid burst and glide swimming there was a pronounced decrease in the intensity of red recruitment, and this finding differs from previous additive models of fiber type recruitment (Fig. 1).

Working hypothesis for fiber recruitment in fishes

It is important to emphasize that in this study of Lepomis we examined muscle recruitment over a continuum of locomotor speeds and behaviors that excluded the startle response. The startle response involves specialized neurocircuitry (Yasargil and Diamond 1968; Zottoli 1977; Fetcho and Faber 1988; Fetcho 1991; Lee and Eaton 1991) that produces an initial motor output with no detectable (< 1 ms) longitudinal lags in the activation of the axial muscles of Lepomis (Jayne and Lauder 1993). Hence, we propose the following model of fiber type recruitment to encompass undulatory locomotion of fis-

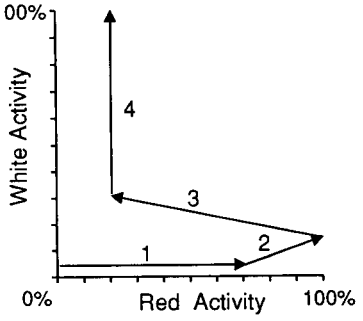


Fig. 6 Schematic summary of a current working model of fiber type recruitment based on the patterns of fiber type recruitment observed in this study (Fig. 3C). Numbers and arrows indicate the progression of events with increased swimming speed. See Discussion for further explanation

hes that involves posteriorly propagated axial muscle activity.

Figure 6 schematically summarizes our current working model for the effect of swimming speed on the pattern of fiber type recruitment based on our observations of in vivo muscle activity (Fig. 3 C). The arrows indicate the progression of events with increased locomotor speed, and burst duration (an indirect measure of locomotor speed) decreases from the beginning to the end of the progression. Initially, during steady speed swimming (Fig. 6, arrow 1) only red fibers are used with an increasing intensity to about 70% of maximal level as red burst duration decreases to about 100 ms. After the threshold for white recruitment is reached (Fig. 6, arrow 2), some further increases in red activity usually occur with low levels of white activity such that red and white intensity are positively correlated until red burst durations approximate 80–90 ms (Fig. 3 A, B). Thereafter, (Fig. 6, arrow 3), a moderate increase in the level of white activity to about 30–40% maximal is accompanied by a large decrease in the intensity of red activity from about 100% to 25% as emg duration decreases to about 50 ms. Finally, white activity increases independently of red activity which is nearly nonexistent as the white burst durations decrease from about 50 to 25 ms (Fig. 6 arrow 4; Fig. 3 A, B).

Comparisons with other studies

Previous studies of in vivo patterns of fiber type recruitment in fishes during swimming would appear to differ from the results we report here. However, several differences between our methodology and that of previous studies may help to explain why we have detected previously undescribed patterns of red and white fiber recruitment. One major difference was that studies of fiber recruitment during fish locomotion prior to 1990 simply did not systematically quantify either the timing or the intensity of both red and white muscle activity. Hence, previous additive models of fiber type recruitment (e.g., Rome 1986) were consistent with available qualitative data. The importance of quantitative analysis of emgs for clarifying patterns of recruitment in ectothermic vertebrates (Fig. 1) is discussed in more detail elsewhere (Jayne et al. 1990a). In addition, most previously published illustrations of emgs of fish muscle (Rayner and Keenan 1967; Hudson 1973; Johnston et al. 1977; Johnston and Moon 1980; Rome et al. 1984, 1985) have had extremely compressed time scales, and show from two to six seconds of emg data. At this scale virtually all emg bursts look similar in duration. Hence, it is not feasible to determine retrospectively if other studies may have observed some of the novel features of red-white motor pattern which we found could occur on a time scale of several milliseconds.

Another drawback of many published figures of fish muscle emgs is that it is difficult to evaluate whether or not movement artifacts may have contributed significantly to the observed amplitude of emgs. In our experiment, we had to take particular care in: 1) our methods of implantation and attachment, 2) not allowing the fish or electrode wires bump into the flow tank walls, 3) controlling and verifying the position of the recording electrodes after the experiment using both dissections and x-rays, and 4) choosing high-pass filter settings appropriate for dealing with the motion artifacts which could become particularly troublesome during the unsteady swimming behaviors. High-speed flows can cause low frequency oscillations of the electrode cable which may look like an emg burst if a very compressed time scale is used for illustrations (or for hardcopies of recordings which are then analyzed). (We thus recommend that analyses of rapid locomotor behaviors show emgs with a time scale sufficiently expanded to resolve individual spikes within bursts as well as lag times among bursts.)

We also used a wide range of flow rates to elicit the greatest possible diversity of undulatory swimming modes in Lepomis. Many previous studies of red and white muscle recruitment in fishes (Hudson 1973; Johnston et al. 1977: Johnston and Moon 1980; Rome et al. 1984, 1985) were mainly interested in the events leading to the threshold for recruitment of white muscle; consequently few speeds beyond the threshold of white recruitment were used. Because fish fatigue rapidly during rapid burst and glide swimming, it is indeed technically difficult to work with this particular swimming mode, but it was only during the highest speed burst and glide swimming when the substantial decrease in red emg intensity occurred.

Finally, patterns of fiber type recruitment appear to vary among different fish taxa (Johnston et al. 1977). Some of the most detailed studies of fiber type recruitment have been for the carp (Johnston et al. 1977; Rome et al. 1984, 1985) which is not closely related to Lepomis (Lauder and Liem 1983). Bone (1978) suggested that the innervation of axial muscle varies among different fish taxa and that this may partially explain some of the observed variation in fiber recruitment. Future studies will be needed to clarify whether the patterns of fiber type recruitment in Lepomis are the result of neuroanatomical differences with other fish taxa, or if such a pattern of recruitment may simply have not been detected because

of the different experimental techniques which were used with other species.

Liu and Westerfield (1988) have suggested that large white muscle emg amplitudes (in the zebrafish) were the result of activation by primary motoneurons, whereas small amplitude emgs from the same recording site might indicate activation by only the secondary motoneurons. Interestingly, the clearest timing differences between red and white muscle in Lepomis occurred when the white emgs were relatively uniform with low amplitude, whereas the diminished activity of red occurred only when white emgs were of short duration and high amplitude. In addition, it is possible that some of the extremely low intensity of emgs in the red muscle (< 10%) which we observed in Lepomis during high amplitude white activity resulted from crosstalk with the underlying white muscle, and if this were the case, then during some high speed swimming red muscle may not be activated at all. Thus, our results for Lepomis suggest two intriguing possibilities which might be examined in the future. (1) Does the motor pattern with differential onset of red and white muscle occur only when secondary motoneurons are activating both fiber types? (2) Does the decrease (or cessation) of red activity occur only when the white muscle is activated by the primary motoneurons? Our results for rapid burst and glide swimming may indicate that the circuitry associated with activation of the primary motoneurons innervating white muscle ultimately provides inhibitory input to the secondary motoneurons innervating red fibers. Such inhibition might occur via interneurons, or by activation (at rapid swimming speeds) of a pathway that inhibits secondary motoneurons to red fibers while providing excitatory input to primary motoneurons innervating white fibers.

Among vertebrate taxa other than fishes it is apparently rare to have activation of fast muscle with a simultaneous lack of slow fiber activity. Two studies of intact endothermic vertebrates are notable for having described such a phenomenon. Smith et al. (1977) found that the paw shaking behavior of cats only involved activation of fast fiber types, whereas even during such fast locomotor behaviors as jumping, cats utilized both slow and fast fiber types. Using a specialized force platform attached to the foot of humans, Nardone et al. (1989) also found some limited circumstances during which fast fiber types might be recruited at the exclusion of slow fibers. Compared to endothermic vertebrates, fiber type recruitment in ectothermic terrestrial vertebrates has received minimal study. However Jayne et al. (1990a) found that the pattern of intensities of red and white fiber recruitment in a thigh muscle of a lizard during running and walking was consistent with the additive model shown in Fig. 1B; however, this study did not document activity over the entire range of locomotor speeds that the lizards could attain. At the threshold of recruitment, the irregular nature of the white muscle bursts complicated determining the onset and offset times, but it is clear from illustrations (Jayne et al. 1990a, Figs. 1, 3) that the duration of white fiber activity can be shorter than of the red fiber burst.

It is interesting to consider the extent to which fast and slow fiber emgs might be decoupled from each other because of the considerable differences between fiber types in their rates of force production and relaxation. Although unknown for Lepomis, the times to peak twitch tension and to one-half relaxation for muscle from a closely related species of centrarchid fish (the bass, Micropterus) at the same temperature are 11 and 18 ms for white muscle and 45 and 70 ms for red muscle, respectively (Johnson et al., 1994). Hence, it is intriguing that for rapid burst and glide swimming, red activity of Lepomis effectively ceased when emg burst duration was less than the time to peak twitch tension for red, and that red levels of activity also diminished for emg durations less than the time to one-half relaxation during red twitch. It is difficult to predict the precise time course of force of muscle for activation other than a simple twitch. However, it is possible that some of the observed lags between onset of red and white activity (mean = 39 ms, range = 6–77 ms; Fig. 2C) could contribute to synchronizing force production in red and white muscles at the transition to burst and glide swimming. Since the time to peak tension for white muscle is about 34 ms shorter than red muscle, coactivation of both fiber types would likely result in asynchronous peak force generation. It seems quite unlikely that the synchronous offset of red and white muscle that was observed in Lepomis would facilitate synchronized relaxation. Instead, the variable onset time of red and white fibers and the nearly synchronous offset times may reveal more about the constraints imposed by the axial motor pattern generators rather than indicating any necessary biomechanical advantages.

Although steady swimming, burst and glide swimming and the escape response fall along a continuum of increasing speed, it is important to emphasize that there may be enough qualitative differences to regard these types of axial movements as three different behaviors. Hence, the extent to which pattern generators and other neural circuitry are shared by different modes of swimming still needs clarification.

The neural circuitry involved in the unsteady swimming behaviors that we have described has not yet been analyzed. However, our observations of burst and glide muscle activity clearly differed from the rhythmic patterns of steady swimming which have been studied and modeled extensively for other fishes. The escape behavior of fishes is an unsteady form of swimming that involves a well-studied neural network (Yasargil and Diamond 1968; Zottoli 1977; Eaton et al. 1982; Fetcho and Faber 1988; Fetcho 1991; Lee and Eaton 1991) that simultaneously (within 1 ms) activates all the muscles on one side of the fish (stage 1, Jayne and Lauder 1993), but this is unlike our recordings of high amplitude white muscle emgs during high-speed swimming which had a short but distinct posterior lag in the onset time. Furthermore, the low amplitude red muscle activity during rapid burst and glides contrasts with the high amplitude stage 1 red emgs during the escape response (Jayne and Lauder 1993). It is less clear how different rapid burst and glide muscle activity is from that of the stage 2 emgs of

the escape response. Jayne and Lauder (1993) did not quantify the intensity of the stage 2 red emgs of Lepomis, but they did mention the technical difficulties of quantifying stage 2 emgs which could have been exacerbated by low amplitude red activity. It does seem counter-intuitive that the fiber recruitment during a rapid burst and glide might differ from stage 2 of an escape because there are indeed similar mechanical demands during these times, but with current data we can not further resolve this issue. Future comprehensive understanding of fiber type recruitment as well as the neural control of the axial movements of fishes should account for all of the diverse motor patterns that have now been observed.

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References

Armstrong RB (1981) Recruitment of muscles and fibres within muscles in running animals. Symp Zool Soc Lond 48: 289–304

Bone Q (1966) On the function of the two types of myotomal muscle fibre in elasmobranch fish. J Marine Biol Assoc UK 46: 321–349

Bone Q (1978) Locomotor muscle. In: Hoar WS, Randall DJ (eds) Fish physiology vol. 7. Locomotion. Academic Press, New York, pp 361–424

Bone Q, Kicenuik J, Jones DR (1978) On the role of the different fibre types in fish myotomes at intermediate swimming speeds. Fish Bull 76: 691–699

Eaton RC, Lavender WA, Wieland CM (1982) Alternative neural pathways initiate fast-start responses following lesions of the Mauthner neuron in goldfish. J Comp Physiol 145: 485–496

Fetcho JR (1986) The organization of the motoneurons innervating the axial musculature of vertebrates. I. Goldfish (Carassius auratus) and mudpuppies (Necturus maculosus). J Comp Neurol 249: 521–550

Fetcho JR (1991) Spinal network of the Mauthner cell. Brain Behav Evol 37: 298–316

Fetcho JR, Faber DS (1988) Identification of motoneurons and interneurons in the spinal network for escapes initiated by the Mauthner cell in goldfish. J Neurosci 8: 4192–4213

Grillner S (1981) Control of locomotion in bipeds, tetrapods, and fish. In: Brooks VB (ed) Handbook of physiology, sec. 1, Brookhart JM, Mountcastle VB (eds) vol. 2, Motor control. American Physiological Society, Bethesda, pp 1179–1236

Henneman E, Somjen G, Carpenter DO (1965) Functional significance of cell size in spinal motoneurons. J Neurophysiol 28: 560–580

Hudson RCL (1973) On the function of the white muscles in teleosts at intermediate speeds. J Exp Biol 58: 509–522

Jayne BC (1988) Muscular mechanism of snake locomotion: An electromyographic study of lateral undulation of the Florida banded water snake (Nerodia fasciata) and the yellow rat snake (Elaphe obsoleta). J Morphol 197: 159–181

Jayne BC, Bennett AF, Lauder GV (1990a) Muscle recruitment during terrestrial locomotion: how speed and temperature affect fibre type use in a lizard. J Exp Biol 152: 101–128

Jayne BC, Lauder GV (1993) Red and white muscle activity and kinematics of the escape response of the bluegill sunfish during swimming. J Comp Physiol A 173: 495–508

Jayne, BC, Lauder, GV, Reilly, SM, Wainwright, PC (1990b) The effect of sampling rate on the analysis of digital electromyograms from vertebrate muscle. J Exp Biol 154: 557–565

Johnson TP, Syme DA, Lauder GV, Bennett AF (1994) Modeling red muscle power output during steady and unsteady swimming in largemouth bass (*Micropterus salmoides*): a comparison of *in vitro* and *in vivo* function. Am J Physiol (Regulatory Integrative Comp Physiol) (in press)

Johnston IA (1983) Dynamic properties of fish muscle. In: Webb PW, Weihs D (eds) Fish Biomechanics. Praeger Publishers, New York, pp 36–67

Johnston IA, Moon TW (1980) Endurance exersize training in the fast and slow muscles of a teleost fish (Pollachius virens). J Comp Physiol 135: 147–156

Johnston IA, Davison W, Goldspink G (1977) Energy metabolism of carp swimming muscles. J Comp Physiol 114: 203–216

Lauder, GV, Liem, KF (1983) The evolution and interrelationships of the actinopterygian fishes. Bull Mus Comp Zool Harvard 150: 95–197

Lee RKK, Eaton RC (1991) Identifiable reticulospinal neurons of the adult zebrafish (*Brachydanio rerio*). J Comp Neurol 304: 34–52

Liu DW, Westerfield M (1988) Function of identified motoneurons and co-ordination of primary and secondary motor systems during zebra fish swimming. J Physiol (Lond) 403: 73–89

Nardone AC, Romano M, Schieppati (1989) Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. J Physiol (Lond) 409: 451–471

Rayner MD, Keenan MJ (1967) Role of red and white muscles in the swimming of the skipjack tuna. Nature 214: 392–393

Rome LC, Sosnicki AA (1991) Myofilament overlap in swimming carp II. Sarcomere length changes during swimming. Am J Physiol 260 (Cell Physiol):C289-C286

Rome LC, Loughna PT, Goldspink G (1984) Muscle fiber activity in carp as a function of swimming speed and muscle temperature. Am J Physiol 247:R272-R279

Rome LC, Funke RP, Alexander RM, Lutz G, Aldridge H, Scott F, Freadman M (1988) Why animals have different muscle fibre types. Nature 335: 824–827

Rome LC (1986) The influence of temperature on muscle and locomotory performance. In: Heller HC, Musacchia XJ, Wang LCH (eds) Living in the cold: Physiological and biochemical adaptations: Proceedings of the Seventh International Symposium on Mammalian Hibernation. Elsevier, New York, pp 485–495

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

Rome LC, Loughna PT, Goldspink G (1985) Temperature acclimation: improved sustained swimming performance in carp at low temperatures. Science 228: 194–196

Smith JL, Betts B, Edgerton VR, Zernicke RF (1977) Rapid ankle extension during paw shakes: selective recruitment of fast ankle extensors. J Neurophysiol 43: 612–620

Van Leeuwen JL, Lankheet MJM, Akster HA, Osse JWM (1990) Function of red axial muscles of carp (Cyprinus carpio): recruitment and normalized power output during swimming in different modes. J Zool (Lond) 220: 123–145

Westerfield M, McMurray JV, Eisen JS (1986) Identified motoneurons and their innervation of axial muscles in the zebrafish. J Neurosci 6(8):2267–2277

Williams TL, Grillner S, Smoljaninov VV, Wallén P, Kashin S, Rossignol S (1989) Locomotion in lamprey and trout: the relative timing of activation and movement. J Exp Biol 143: 559–566

Yasargil GM, Diamond J (1968) Startle-response in teleost fish: An elementary circuit for neural discrimination. Nature 220: 241–243

Zottoli SJ (1977) Correlation of the startle reflex and Mauthner cell auditory responses in unrestrained goldfish. J Exp Biol 66: 243–254