

The effects of viscosity on the axial motor pattern and kinematics of the African lungfish (*Protopterus annectens*) during lateral undulatory swimming

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SUMMARY

Separate studies of terrestrial and aquatic locomotion are abundant, but research addressing locomotion in transitional environments (e.g. mud) is scant. The African lungfish (*Protopterus annectens*) moves in a gradation of water to mud conditions during seasonal droughts, and breathes air. Thus, the lungfish was an ideal organism for our study to determine the effects of a wide range of viscosities on lateral undulatory swimming and to simulate some of the muddy conditions early tetrapods may have encountered. Regardless of viscosity, several aspects of lungfish swimming were similar to those of other swimming vertebrates including: posteriorly propagated muscle activity that was unilateral and alternated between the left and right sides at each longitudinal location, and posterior increases in the amount of bending, the amplitude of muscle activity and the timing differences between muscle activity and bending. With increased viscosity (1–1000 cSt), significant increases occurred in the amount of lateral bending of the vertebral column and the amplitude of muscle activity, particularly in the most anterior sites, but the distance the fish traveled per tail beat decreased. The magnitude of the phase shift between EMG onset relative to bending increased by as much as 13% of a cycle with increased viscosity, so that the muscles were increasingly active during lengthening rather than shortening. Therefore, with increased viscosity the relationship between axial muscle activity and bending in the lungfish became more dissimilar rather than converging on the motor pattern used by other ectothermic vertebrates when undulating in fully terrestrial environments.

Key words: locomotion, lungfish, EMG, kinematics, swimming, viscosity, tetrapod evolution.

INTRODUCTION

Although many studies have compared locomotion on land and water (Ashley-Ross and Bechtel, 2004; Gillis, 1998a; Gillis and Blob, 2001; Jayne, 1988), how organisms move through transitional environments is poorly understood. Muds commonly occur at the interface of water and land and have a wide range of viscosities that depend on the relative concentration of substrate to water. Clay mud was probably the first terrestrial substrate the earliest tetrapods encountered (Clack, 2002). The increased viscosity of substances such as mud increases the amount of work required to overcome drag forces. Any lack of a streamlined form, such as extended appendages, could exacerbate this problem (Blake, 1983). Similarly, increased speeds of swimming also increase the amount of work needed to overcome drag forces. Consequently, just as many fishes abandon the use of paired appendages when swimming faster (Webb, 1984), moving through a high-drag muddy environment may necessitate a reliance on axial structures.

The practical difficulties of observing locomotion in substances with high viscosity such as mud are considerable, including low visibility and impeding respiration *via* gills. Unlike many other species of fish, lungfish (Dipnoi: Sarcopterygii) are able to breathe air and they frequently encounter mud in their natural habitat. For example, lungfish in the genus *Protopterus* often encounter harsh seasonal droughts that prompt burrowing into the increasingly viscous, muddy substrate (Fishman et al., 1987). *Protopterus*, like most modern lungfish, has morphological traits that are secondarily derived for burrowing, including an elongate body and diminutive

paired fins that have little function in open-water locomotion. These behavioral and morphological attributes make lungfish a well-suited taxon for studying how environmental variation affects the locomotor function of axial musculature. Surprisingly, axial muscle activity has not previously been studied for the locomotion of any lungfish.

Axial motor patterns during steady undulatory swimming in water are well documented and share many features in phylogenetically diverse ectothermic vertebrates. Common features of swimming axial motor patterns in lamprey (Williams et al., 1989), sharks (Grillner, 1974), actinopterygian fishes with diverse morphologies (Altringham and Ellerby, 1999; Coughlin, 2002), salamanders (Bennett et al., 2001; Frolich and Biewener, 1992) and snakes (Jayne, 1988) include a posterior propagation of activity, and at a given longitudinal location activity is unilateral and alternates between the left and right sides. Differences commonly occur between the speed of propagation of muscle activity and the wave of lateral bending (Grillner and Kashin, 1976; Jayne, 1988; van Leeuwen et al., 1990). These timing differences can result in the activation of muscle fibers during lengthening, which may stiffen the posterior regions of the organism as they push against the water (Long and Nipper, 1996; Wardle et al., 1995) (but see Rome et al., 1993). In addition to varying longitudinally, the timing of muscle activity relative to lengthening can vary among species or with changes in swimming speed (Coughlin, 2000; Jayne and Lauder, 1995a; Syme and Shadwick, 2002). The different waveforms observed during the undulatory swimming of vertebrates are thus a complicated

consequence of how the intrinsic stiffness of the swimmer interacts with environmental resistance (Blight, 1977).

Environmental variation affects both the nature and the magnitude of the forces that facilitate or resist movement, and for diverse types of locomotion that use lateral undulation of the axial structures the stiffness of the animal relative to the compliance of the environment is a key determinant in the relationship between muscle activity and bending. For example, ectothermic vertebrates use different axial motor patterns in aquatic and terrestrial environments. In contrast to swimming, snakes undulating on land have equal speeds of propagating muscle activity and bending that result in activity during the shortening of muscle fibers (Jayne, 1988). When salamanders walk on land epaxial muscles are activated in a standing, rather than traveling, wave pattern that results in bending the trunk towards the side of muscle activity (Frolich and Biewener, 1992). Unlike the terrestrial undulation of both snakes and salamanders, when eels move on land the timing of axial muscle activity relative to bending changes longitudinally but more muscle activity occurs during shortening than when the eels are swimming (Gillis, 2000). Hence, the timing of muscle activity and bending during the terrestrial locomotion of eels becomes more similar to that of snakes crawling on land, although the phase relationships between muscle activity and bending are not identical to those of snakes. For a hypothetical animal that is very flexible, a very viscous solution may create an environment with so little compliance that it may be nearly equivalent to when an animal pushes against certain terrestrial substrates. However, the lack of empirical data on animal locomotion in a wide range of viscosities presently impedes understanding of whether aquatic or terrestrial motor patterns are most relevant for predicting axial function in mud.

In this study, we quantified the kinematics and axial muscle activity used by the lungfish *Protopterus annectens* during lateral undulatory swimming in a wide range of viscosities to understand what may occur in muddy environments. Our primary objectives were (1) to describe lateral undulatory swimming in water in the lungfish and compare the data with those from other vertebrates, and (2) to test whether increased viscosity affects the kinematics and muscle activity of lungfish.

MATERIALS AND METHODS

Experimental subjects and protocol

We obtained specimens of the African lungfish, *Protopterus annectens* (Owen 1839), from a commercial dealer (Rehobath, Baton Rouge, LA, USA). The lungfish were housed separately in glass aquaria with a water temperature of $24 \pm 1^\circ\text{C}$, and the experiments were performed at the same temperature. We performed experiments with a total of eight lungfish and only analyzed data from the four individuals with the best behavior and with the greatest number of electrodes remaining in place after experimentation. The total lengths of these four analyzed individuals ranged from 51 to 59 cm. The numbers of precaudal and caudal vertebrae ranged from 34 to 36 and 34 to 38, respectively. To facilitate implanting electrodes, the lungfish were anesthetized with an aqueous solution of MS222 (1.5 g l^{-1}). The fish recovered for at least 12 h before experimentation. The lungfish were then placed into a 284 l ($123 \text{ cm} \times 47 \text{ cm}$) glass tank filled to a depth of 17 cm with a mixture that was ordered randomly with respect to viscosity.

We manipulated the viscosity of the medium by using Poly-Bore (Baroid Industrial Drilling Products, Houston, TX, USA), a partially hydrolyzed polyacrylamide polymer commonly used in offshore oil drilling. Poly-Bore was the only transparent, non-toxic material we were able to locate that created viscosities likely to approach those

of mud, and the mixtures were clear enough for both lateral and ventral views of the fish. We obtained kinematic viscosities of 1 (water), 10, 100 and 1000 centi-Stokes (cSt; where $1 \text{ St} = 10^{-4} \text{ m}^2 \text{ s}^{-1}$) by increasing the concentration of Poly-Bore in a water solution and allowing ample time (4–6 weeks) to obtain a homogeneous mixture. Viscosity was measured with several different sizes of Otswald-style viscometers (Fisher Scientific, Hampton, NH, USA) to accommodate a wide range of viscosities.

A similarity between Poly-Bore and a wide variety of muds is that they are shear-thinning non-Newtonian fluids, whereas some other thickening agents such as corn starch are shear-thickening non-Newtonian fluids (Balmforth and Provenzale, 2001). For the purpose of comparison we created a mud slurry using a dry smectite clay mixture from a local pottery shop (Starbrick Clay, Nelsonville, OH, USA) that was suspended in a known volume of water. By using a rotational viscometer (Brookfield Service Company, Stoughton, MA, USA) we determined that a mixture with dry weight of clay equal to 31% of wet weight of mud had a kinematic viscosity of 929 cSt, which was nearly equivalent to our highest viscosity Poly-Bore mixture.

After we recorded 3–5 bouts of steady swimming in one viscosity, the lungfish was moved to a small tank of distilled water while the mixture in the experimental tank was filled with a mixture of a different viscosity. The total transition time to the next mixture was 20–30 min. We selected trials with average swimming speeds as similar as possible across all viscosities to reduce the confounding effects of speed. For our analyses we chose trials with steady speeds and straight trajectories, and the analyzed trials had speeds that were not significantly different among subjects and cycles. After each experiment, the lungfish were killed with an overdose of MS222 and fixed in formalin. All individuals were radiographed and dissected to confirm electrode position within the myomere and relative to a vertebra.

Kinematics

The lungfish were videotaped simultaneously from a lateral and ventral view using a two-camera NAC HSV 500 high-speed video system operating at $250 \text{ images s}^{-1}$. We used Didge Image Digitizing Software (Cullum, 1999) to digitize video images at equal time intervals with at least 20 images per cycle and 50–60 points around the outline of the fish. As described in previous studies (Jayne and Lauder, 1995a), for each digitized outline, additional software (Stereo Measurement TV, written by Garr Updegraff, San Clemente, CA, USA; garru@fea.net) reconstructed the midline and subsequently estimated the angles of lateral midline bending after partitioning the midline into lengths representing the head and individual vertebrae, which were obtained from radiographs of each individual fish (Fig. 1A).

We calculated cycle duration as the time between successive maximum displacements of the tail tip to the left side. The distance traveled per cycle and average forward swimming speeds were converted to relative units of total fish length (L). We determined the angle of lateral bending between adjacent vertebrae (β) for each of the eight longitudinal locations corresponding to the electrode sites. We also calculated the lag times between homologous kinematic events at successive longitudinal locations and converted these values to phase shifts by dividing by cycle duration.

Electromyography

After anesthesia, lungfish were implanted percutaneously with a total of 13 finewire (0.051 mm diameter) stainless steel bipolar electrodes (California Finewire, Grover Beach, CA, USA). The two strands

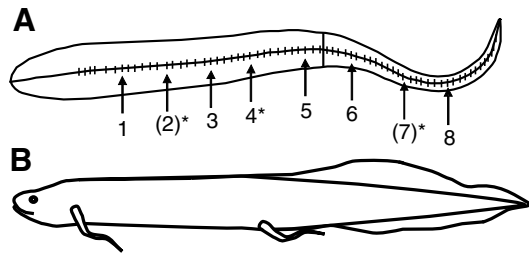


Fig. 1. Ventral view of EMG locations (A) and lateral view of fish shape (B). A ventral mirror view was used to reconstruct the lungfish outline and midline, which was then partitioned into segmental lengths determined from radiographs. The large vertical bar indicates the location of the border between the body and the tail. The numbers indicate average anatomical locations for red muscle EMG sites (see Table 1), with bilateral sites denoted by asterisks. White muscle sites are indicated parenthetically.

of each electrode were bound together with cyanoacrylate glue, and approximately 0.5 mm of insulation was scraped from the tips of the wire to construct hooks, as described previously (Jayne, 1988). Sutures sewn into mid-dorsal tissue and the medial fin affixed the electrode wires at each longitudinal location, and all electrodes were glued into a single main cable with an average diameter of approximately 1 mm. Although we did not analyze kinematics quantitatively, the movements and behaviors of lungfish without electrodes during preliminary experiments appeared similar to those with electrodes.

On the left side of the lungfish, eight EMG electrodes were implanted in superficial red muscle (Fig. 1A, Table 1), and two electrodes were placed into deeper, white musculature at sites 2 and 7. In addition, three electrodes were implanted on the right side of the fish in red muscle at locations 2, 4 and 7 (Fig. 1A). Data from all 13 channels were recorded simultaneously. Only electromyograms (EMGs) from electrodes that remained in place throughout the entire experiment were analyzed statistically for this study. We confirmed electrode position within a myomere and relative to the vertebral column by post-mortem dissections and radiographs (see Table 1).

EMGs were amplified 5000 times using Grass (West Warwick, RI, USA) model P511 K preamplifiers with low- and high-bandpass filter settings of 10 kHz and 30 Hz, respectively, and a 60 Hz notch filter. The analog EMGs were recorded with a TEAC XR-5000 FM data recorder using a tape speed of 9.5 cm s⁻¹. A 100 Hz square-wave was transmitted simultaneously to both the NAC video system and the TEAC data recorder to synchronize the video and EMG data. We converted the analog signal to digital data using an ADInstruments (Colorado Springs, CO, USA) Powerlab 16 channel

converter with an effective sampling rate of 8.8 kHz. The digital EMG data were filtered with a 50 Hz high-pass filter.

We used Chart 5 software (ADInstruments) to measure the onset, offset and duration (offset – onset) for each burst of muscle activity. Relative burst duration (or EMG duty factor) was calculated by dividing EMG burst duration by the cycle duration. EMG intensity was calculated by dividing the rectified integrated area of an EMG burst by its duration.

Four variables described the timing differences in absolute (lag times) or relative (phase shift) amounts of time between muscle activity and bending (β) that were either maximally convex (β_{\max}) or maximally concave on the side of the fish with the electrode. On- β lag equaled the time of EMG onset minus the time of β_{\max} at the same longitudinal location, and off- β lag was between the times of EMG offset and when the region was bent maximally concave. On- β shift and off- β shift were the respective lag times divided by cycle duration. Thus, values of zero correspond to muscle activity during lateral flexion (presumed muscle shortening), and negative values indicate that EMG events preceded the relevant bending at a particular location.

We used β_{\max} to estimate the change in muscle length relative to resting, assuming that the superficial red fibers located at the widest part of the fish keep pace with the change in curvature of the fish during swimming (Coughlin et al., 1996; Jayne and Lauder, 1995b; Katz and Shadwick, 1998; Rome, 1990; Rome and Sosnicki, 1991; Rome et al., 1993). Muscle length change was calculated as the percentage of the ratio of the lateral to midline radii of curvature:

$$\frac{100\{[L_C / 2 \sin(\beta_{\max} / 2)] - W / 2\}}{[L_C / 2 \sin(\beta_{\max} / 2)]},$$

where L_C equals the average length of an adjacent pair of centra, and W is the body width at a particular longitudinal site.

Statistical analyses

Three to four cycles from each viscosity were analyzed for each individual. We performed analyses of variance (ANOVA) for both β_{\max} and estimated muscle strains using data from all eight longitudinal sites. The EMG data did not conform to a balanced experimental design for which a single ANOVA could be used with all viscosities and all longitudinal sites. For example, the anterior muscle sites were often inactive in lower viscosities and in one individual, electrodes at sites 1 and 8 dislodged before the experiments were complete (Table 1). Consequently, an ANOVA with all four viscosities was only possible for most EMG variables for all fish in all viscosities at sites 5–7. Because EMG intensity was equal to zero when no activity was observed, we were able to perform an ANOVA using sites 2–7. To account for variation

Table 1. Locations of EMG electrodes in ipsilateral red muscle

Longitudinal site	Fish 1	Fish 2	Fish 3	Fish 4	Mean \pm s.e.m.
1	0.18 (5)	0.21 (7)	0.21 (7)	–	0.20 \pm 0.01 (7 \pm 0.6)
2	0.25 (10)	0.29 (13)	0.32 (14)	0.29 (13)	0.29 \pm 0.01 (13 \pm 0.9)
3	0.35 (17)	0.38 (19)	0.39 (19)	0.38 (19)	0.37 \pm 0.01 (19 \pm 0.5)
4	0.47 (27)	0.45 (25)	0.45 (24)	0.44 (24)	0.45 \pm 0.01 (25 \pm 0.7)
5*	0.59 (36)	0.53 (31)	0.52 (30)	0.55 (33)	0.55 \pm 0.02 (33 \pm 1.3)
6*	0.68 (44)	0.63 (39)	0.60 (36)	0.65 (41)	0.64 \pm 0.02 (40 \pm 1.7)
7*	0.78 (52)	0.72 (47)	0.67 (45)	0.75 (50)	0.73 \pm 0.02 (49 \pm 1.6)
8	0.86 (59)	0.80 (54)	0.77 (55)	–	0.82 \pm 0.02 (56 \pm 1.3)

Locations are given as proportions of total length and intervertebral joint number (in parentheses) counting from anterior to posterior. Sites 6–8 are caudal locations. Empty cells indicate dislodged or otherwise defective electrodes. *Discernible EMG activity in all viscosities.

Table 2. Strain estimated for superficial red muscle fibers from mean morphological values and β_{\max} during steady swimming in four viscosities

Site	Body width (%L)	Centrum length (%L)	Estimated muscle strain (%L ₀)			
			1 cSt (Re=46225)	10 cSt (Re=5726)	100 cSt (Re=743)	1000 cSt (Re=81)
1	11±0.25	1.2±0.03	4.3	4.1	11.6	8.7
2	10±0.15	1.2±0.05	5.2	5.6	12.1	11.8
3	11±0.20	1.3±0.05	5.8	6.1	14.6	13.2
4	10±0.15	1.2±0.05	8.0	7.8	15.4	16.5
5	9.0±0.40	1.2±0.09	10.5	13.7	19.9	22.4
6	7.0±0.05	1.1±0.09	15.2	17.6	21.8	23.2
7	4.0±0.04	1.0±0.06	16.6	16.0	17.0	19.5
8	2.0±0.15	0.80±0.08	15.3	15.4	15.2	16.1

L₀ indicates muscle length when the midline is straight. Site indicates the longitudinal positions as described in Table 1. Body width and centrum length values are means ± s.e.m. for four individuals.

Reynolds numbers; $Re=UL/v$, where U is swimming speed, L is total length of the fish, and v is kinematic viscosity.

within and among individuals, we used three-way, mixed-model ANOVA with viscosity ($N=4$) and longitudinal site ($N=3$) as fixed, crossed factors, and individual ($N=4$) as a random factor. The error term for the main, fixed effects was the 2-way interaction term of the fixed and the random factor. All mean values are reported ±s.e.m.

RESULTS

Morphology

Lungfish are elongate fishes with highly reduced, filamentous pectoral and pelvic fins. The trunk is nearly cylindrical whereas the tail is laterally flattened (Fig. 1B). The post-cranial skeletons of lungfish are mostly cartilaginous (Arratia et al., 2001). The trunk contains 34–36 vertebrae and the tail contains 34–38 vertebrae. The lengths of the vertebrae in the trunk were nearly constant, whereas the lengths of those in the tail decreased posteriorly (Table 2) except for the terminal vertebrae posterior to the last EMG (site 8). Similarly, the width of the trunk was nearly constant, whereas the width of the tail decreased more than 4-fold (Table 2).

Kinematics

Although we selected cycles of swimming with speeds as similar as possible, the swimming speeds tended to increase with increasing viscosity (Fig. 2A). However, this variation in speed with viscosity was not statistically significant (2-way ANOVA, $F=0.87$, d.f.=3,9, $P>0.50$). Cycle duration decreased significantly with increasing viscosity (Fig. 2B; $F=5.79$, d.f.=3,9, $P<0.025$). The distance traveled per tail-beat cycle increased with viscosity but the relationship was not statistically significant (Fig. 2C; $F=2.75$, d.f.=3,9, $P>0.10$). With increasing viscosity, the distance from nose tip to tail tip usually decreased, and anterior lateral displacement increased (Fig. 3). Maximum lateral flexion increased significantly both posteriorly and with increasing viscosity (Fig. 4A, Table 3). Reynolds numbers ranged from over 40 000 in the lowest viscosity to less than 100 in the highest viscosity (Table 2).

Motor pattern

The overall pattern of red muscle activity consisted of posteriorly propagated, unilateral EMGs (Fig. 5) that alternated between right and left sides (Fig. 6) at a given longitudinal location. All individuals increased anterior muscle recruitment with increasing viscosity (Fig. 5), and with the exception of one individual, all eight sites were active in the highest viscosity. As viscosity increased, activity at white muscle sites increased (Fig. 7).

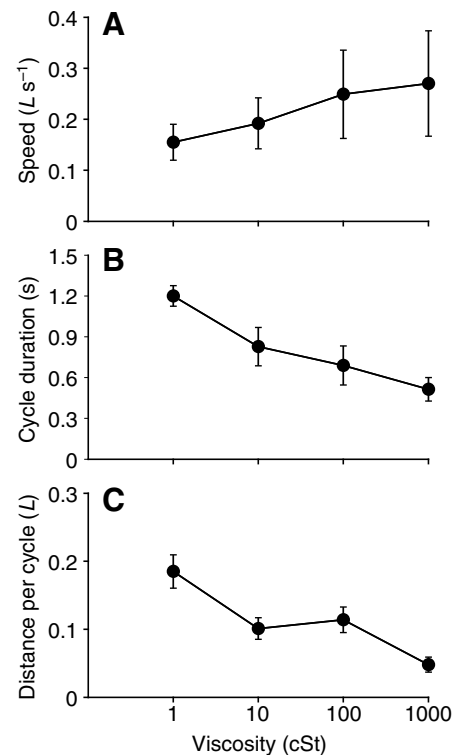


Fig. 2. Grand means of speed (A), cycle duration (B) and distance traveled per cycle (C) for each viscosity. Despite regular patterns of change in speed and distance per cycle, only cycle duration was significantly affected by viscosity (see Table 3). L , length.

Burst duration decreased with increasing posterior longitudinal location within one individual among sites 2–8, but no significant effects were evident even when bursts were pooled across individuals for sites 5–7. The absolute duration of EMG bursts decreased significantly with increasing viscosity (Table 3) in sites 5–7 among all individuals and sites 2–8 in one individual. Relative burst duration, or EMG duty factor, did not vary significantly with site or viscosity among individuals in sites 5–7, but it decreased significantly within the single subject with greatest site replication. The intensity of EMGs increased significantly with increased viscosity and for increasingly posterior location (Fig. 4B).

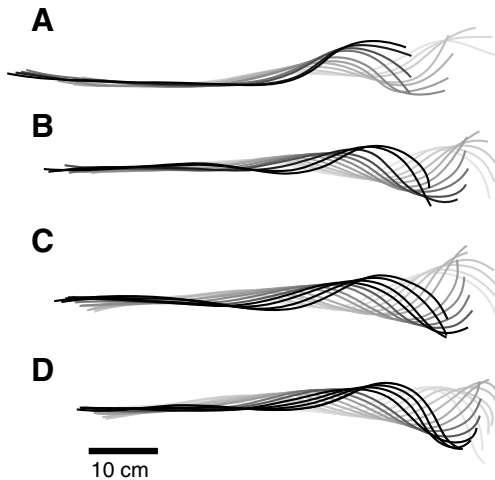


Fig. 3. Series of lungfish midlines for one cycle from the same individual with viscosities and swimming speeds of (A) 1 cSt, 0.20 L s^{-1} , (B) 10 cSt, 0.23 L s^{-1} , (C) 100 cSt, 0.14 L s^{-1} and (D) 1000 cSt, 0.15 L s^{-1} , respectively. Note that lateral displacement and lateral bending increased most conspicuously anteriorly with increased viscosity.

Muscle strain

The estimated muscle strain increased significantly with increasing viscosity, and differed significantly among sites (Tables 2 and 3). The increase in strain was greatest in sites 5 and 6, which were the sites closest to the pelvic girdle. Although site was a significant factor overall, posterior sites increased strain minimally compared with anterior sites (Table 2). Muscle strain at sites 1–5 increased 2-fold from water to the highest viscosity.

Timing of muscle activity and kinematics

The timing of EMGs relative to bending changed longitudinally in a manner that indicated the EMGs propagated posteriorly faster than the mechanical wave of bending (Figs 8–10). If muscle activity corresponded to shortening of muscle tissue, then the EMG onset would be synchronous with maximal convexity on the side of the fish with muscle activity (Fig. 9), and on- β phase shifts would equal zero (Fig. 10C,D). When present, significant differences in lag times among viscosities and among sites generally paralleled those observed for phase shifts (Table 3; Fig. 10A,B). However, large variance in cycle duration obscured some trends for lag times that were much more evident in phase shifts. As viscosity increased, the EMG onset at several longitudinal locations began to precede the timing of maximal convexity, presumably indicating muscle activity during lengthening (Figs 8–10). Thus, the phase shift of EMG onset relative to bending increased significantly (Table 3) by both viscosity and site (Figs 8–10) in sites 5–7 among all four individuals, as well as sites 2–8 in one individual. Though the interaction term was not significant (Table 3), on- β phase shifts tended to increase posteriorly with increasing viscosity (Fig. 10C). The variation in the longitudinal extent of muscle activity can also be observed in Fig. 7.

DISCUSSION

Regardless of the viscosity, the axial motor pattern of lungfish was similar to that of nearly all other vertebrates that swim with lateral undulation – muscle activity at a given longitudinal location was unilateral and alternated between the left and right sides. Increased viscosity primarily affected the longitudinal extent of muscle activity and bending as well as the timing between these two

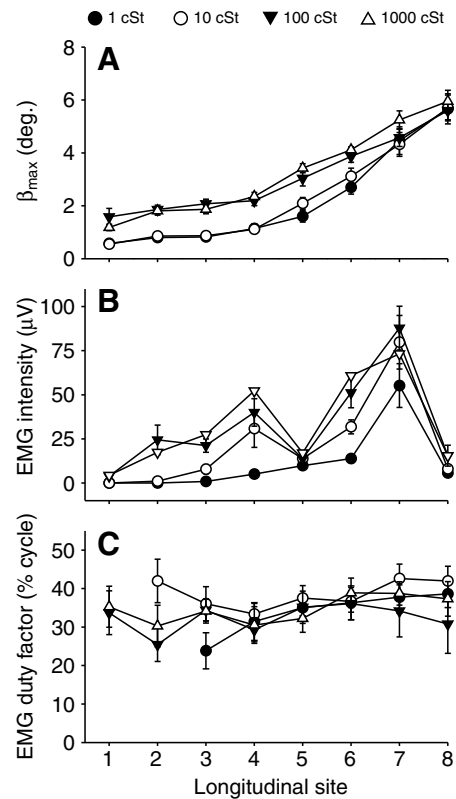


Fig. 4. Effects of viscosity and longitudinal location on the mean values of kinematics and muscle activity. (A) Lateral vertebral flexion (β_{\max}) is the maximum attained within a cycle. For a single viscosity, flexion was always greatest in posterior sites, but with increased viscosity the largest change in flexion occurred anteriorly. (B) EMG intensity increased significantly with increased viscosity and was commonly greater in more posterior locations. (C) EMG duty factor was nearly constant for all longitudinal sites and viscosities.

Table 3. Summary of *F*-values from 3-way ANOVAs performed separately on each variable for four individuals across four viscosities

Dependent variable	Viscosity	Site	Viscosity \times site
β_{\max} (deg.)	10.4* (3, 9)	14.2* (7, 21)	3.9* (21, 63)
Muscle strain (% L_0)	13.5* (3, 9)	6.7* (7, 21)	3.7* (21, 63)
EMG burst duration (ms)	4.4* (3, 9)	2.3 (2, 6)	0.8 (6, 18)
EMG duty factor (% cycle)	0.5 (3, 9)	1.7 (2, 6)	1.3 (6, 18)
On- β shift (cycles)	4.4* (3, 9)	7.3* (2, 6)	2.3 (6, 18)
Off- β shift (cycles)	4.9* (3, 9)	3.4 (2, 6)	3.4* (6, 18)
On- β lag (s)	1.6 (3, 9)	11.9* (2, 6)	0.8 (6, 18)
Off- β lag (s)	7.9* (3, 9)	1.3 (2, 6)	0.8 (6, 18)
EMG intensity (μV) [†]	13.5* (3, 15)	2.9* (5, 15)	0.9 (15, 45)

Degrees of freedom (numerator, denominator) are indicated parenthetically after each *F*-value for the effects of viscosity, site and viscosity \times site.

* $P < 0.05$.

[†]EMG data were from sites 5–7 except for EMG intensity, which was analyzed for the sites (2–7) present in all individuals in all viscosities.

posteriorly propagated events. For many vertebrate taxa these phase relationships between muscle activity and bending can be affected by phylogeny, morphology, behavior, the speed of travel and environment. Thus, we sought to determine how unusual the patterns of muscle activity and bending were for lungfish swimming in water compared with those of other vertebrates, as well as to

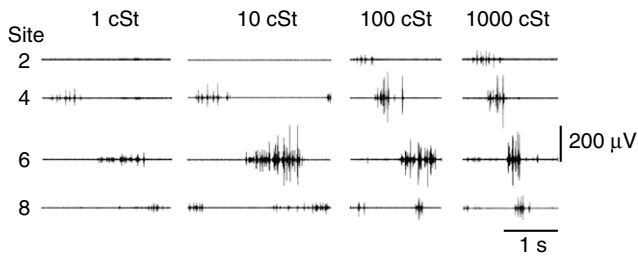


Fig. 5. EMGs from superficial red muscle from four ipsilateral longitudinal sites from a single lungfish. EMG amplitude increased most conspicuously with increased viscosity in anterior sites.

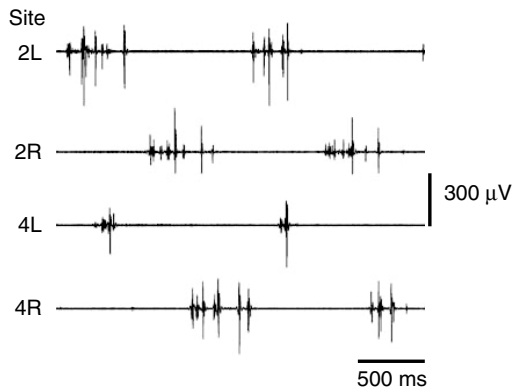


Fig. 6. EMGs from superficial red muscle from three longitudinal sites with both left side (L) and right side (R) showing unilateral and alternating motor pattern. As a result of having EMG duty factors less than 50% of a cycle, each longitudinal location briefly lacks muscle activity on either the left or right side of the body.

compare the patterns in water *versus* a highly viscous solution to obtain some insight into the demands that mud was likely to impose on the locomotion of the ancestors of tetrapods.

Shape, segmentation and axial bending

The anguilliform, sub-carangiform, carangiform and thunniform modes of swimming *via* lateral undulations of the axial structures form a continuum of behavioral variation that has some large scale correlations with axial morphology (Breder, 1926; Lindsey, 1978; Sfakiotakis et al., 1999). At one extreme, anguilliform swimmers undulate their entire body, creating more than one complete wave, and they have a relatively uniform longitudinal distribution of surface area, low values of maximal body depth divided by total length (snakes: 3%; eels and lampreys 6–7%) and high numbers of total (body + caudal) vertebrae (snakes 180–300; eels and lampreys 100–200) (Jayne, 1986; Jayne, 1985; Wardle et al., 1995; Ward and Azizi, 2004). At the other extreme, thunniform swimmers undulate only the posterior portion of a narrow caudal peduncle and fin, usually with much less than one wave visible along the length of the body. Non-anguilliform swimmers have relative high values (20–30%) of relative body depth (Jayne and Lauder, 1994a; Wardle et al., 1995), and except for the trout (*Oncorhynchus mykiss*), which has 61–65 total vertebrae, most other species of bony fishes (including tuna) for which EMGs are available have from 34 to 41 total vertebrae (Donley and Dickson, 2000; Jayne and Lauder, 1995b; Syme and Shadwick, 2002; Ward and Azizi, 2004; Webb and Johnsrude, 1988). With a relative body depth slightly exceeding 10% (Fig. 1B) and 68–74 total vertebrae, the lungfish in our study

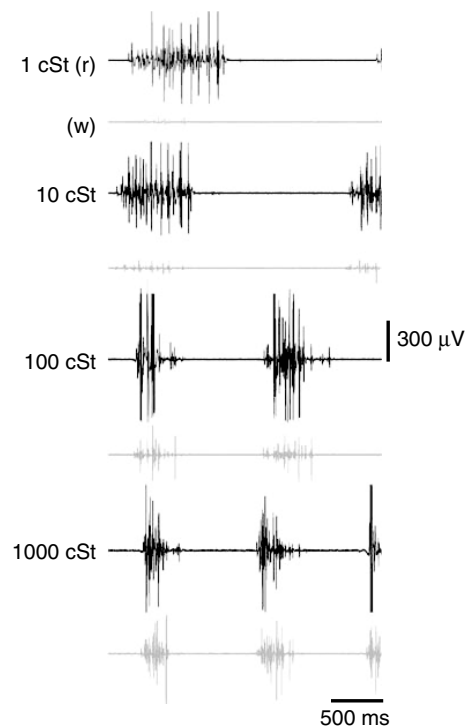


Fig. 7. Comparison of red (r) and white (w) muscle activity in 1 cSt (0.13 L s^{-1}), 10 cSt (0.08 L s^{-1}), 100 cSt (0.13 L s^{-1}) and 1000 cSt (0.16 L s^{-1}) from a single longitudinal location (site 7) in an individual lungfish.

are morphologically intermediate to the classic examples of anguilliform swimmers and most sub-carangiform/carangiform swimmers.

Although vertebral numbers among diverse taxa do tend to be correlated to swimming mode, the functional consequences of variation in segmentation are also mediated by body width and depth, patterns of muscle activity and the mechanical properties of tissues within the body and of the environment that may resist bending (Blight, 1977; Jayne, 1985; Long, 1998; Long et al., 1996; Long and Nipper, 1996; Wainwright, 1983). For example, the larger surface area per unit length that is associated with greater body depth will increase the resistance the animal encounters as its body pushes against the water. Hence, for a given internal bending force when all other factors are equal, one would expect more lateral bending with decreased body depth (Blight, 1977). As is the case for nearly all undulatory swimmers, the amount of lateral vertebral flexion in the lungfish was greatest in the most posterior regions where the body is most shallow and narrow (Fig. 4A).

The limited data available suggest that the amount of lateral vertebral bending during steady undulatory swimming rarely approaches the maximal capacity for *in vivo* lateral flexion. For example, the lateral flexion during the steady swimming of some Centrarchid fishes is approximately one-third that observed during escape locomotion (Johnson et al., 1994). Similarly, the steady swimming of lungfish in this study in water had lateral vertebral flexion that was only one-half that of the flexion in the most viscous solution over a large portion of the anterior body (Fig. 4A). In addition, the lateral flexion of the lungfish even in the most viscous solution was less than that observed in preliminary experiments during escape responses (A.M.H. and B.C.J., personal observation).

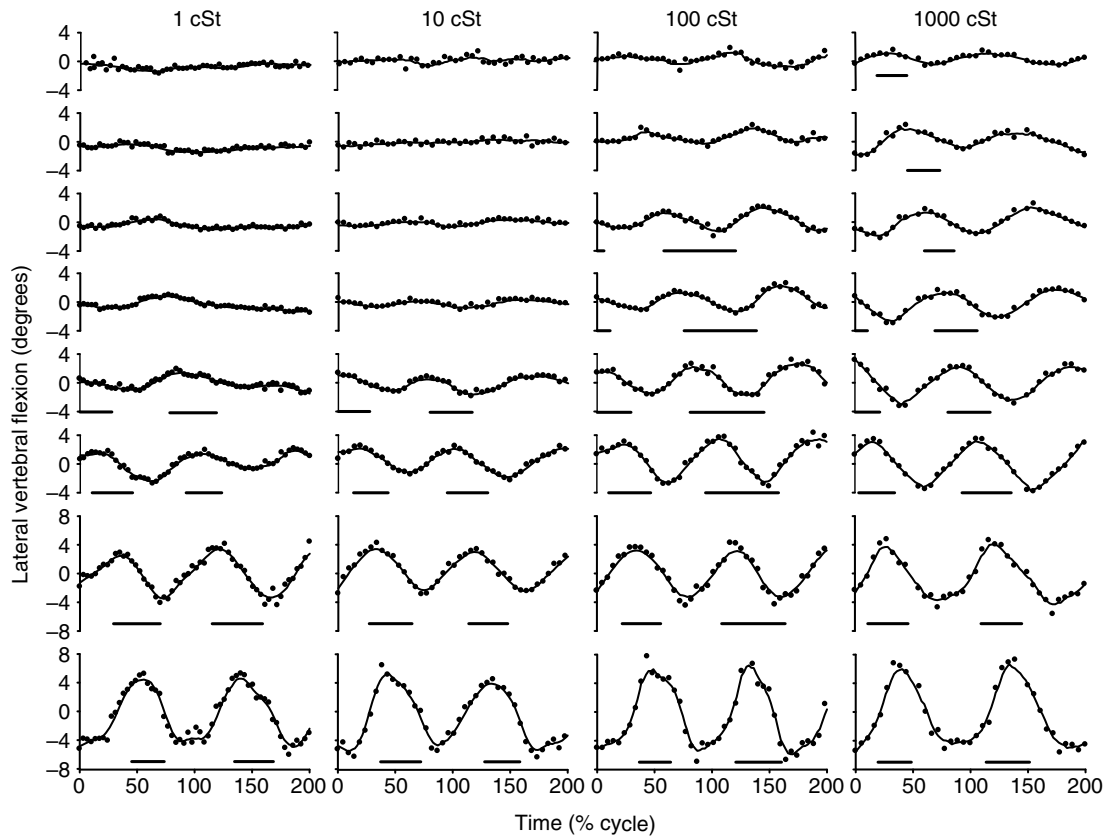


Fig. 8. Lateral vertebral flexion (β) and red muscle activity (horizontal bars) from sites 1 (top) to 8 (bottom) versus time for two cycles of an individual lungfish swimming in viscosities of 1, 10, 100 and 1000 cSt, respectively. Bending convex to the left is represented by positive values of β . The circles are raw data, and the lines represent a two-point running average of the raw data. All EMG sites were on the left side of the fish.

Longitudinal variation during steady swimming

In addition to the lungfish in this study having significant longitudinal variation in morphology, lateral bending, muscle strain (as estimated by bending), EMG intensity, and the phase shifts between muscle activity and bending also varied significantly with longitudinal position. A very general feature of non-anguilliform swimming is that EMG duty factor decreases posteriorly (Altringham and Ellerby, 1999), but the motor pattern of the lungfish in our study more closely resembled that of eels (Gillis, 1998b) and snakes (Jayne, 1988), in which the EMG duty factor lacks obvious longitudinal variation.

During steady swimming, lateral bending increased posteriorly, which is a nearly universal feature of the undulatory swimming of vertebrates including fish swimming (reviewed in Altringham and Ellerby, 1999; Coughlin, 2002), salamanders (Frolich and Biewener, 1992) and snakes (Jayne, 1988). When the lungfish were swimming steadily in the lower viscosities, lateral flexion was barely discernible in the anterior locations, which closely resembles the slow steady swimming of eels (Gillis, 1998b). In addition to the strain in axial muscles increasing with increased lateral flexion, strain also increased with increased distance from the midline. Thus, even when vertebral flexion is large, muscle strain may be small if the body is laterally compressed.

Another nearly universal feature of steady undulatory swimming is that muscle strain in phylogenetically diverse anguilliform and non-anguilliform bony fishes commonly doubles from a location near mid-body to a posterior location, but strain in the most posterior locations rarely exceeds 10% (Coughlin, 2002). The posterior

increase in muscle strain of lungfish swimming in water resembles that in many other species. However, the lungfish had an unusually large longitudinal variation in strain (nearly 4-fold), and the magnitudes of the estimated muscle strain in the three most posterior sites were nearly twice those reported in the most posterior locations of other bony fishes swimming steadily in water. The large caudal strains of lungfish swimming in water occurred despite the fact that these regions were a mere one-fifth of the width of the trunk of the fish (Table 2).

For the slowest speeds of steady undulatory swimming, both eels (Gillis, 1998b) and lungfish lack detectable muscle activity in the more anterior sites. In other species of undulatory swimmers such as snakes (Jayne, 1988) and largemouth bass (Jayne and Lauder, 1995a), muscle activity is present anteriorly during slow speed sustainable swimming, but the amplitude of activity is smaller than that in the more posterior locations. This lack of slow speed anterior muscle activity contradicts some definitions of anguilliform swimming (Breder, 1926; Gray, 1953a; Wardle et al., 1995) that suggest the entire length of the body actively contributes to propulsion.

For effectively all studied species of undulatory swimmers, the speed of posterior propagation of muscle activity along the length of the animal exceeds that of lateral bending of fish (reviewed in Altringham and Ellerby, 1999; Coughlin, 2002), salamanders (Frolich and Biewener, 1992) and snakes (Jayne, 1988), with the exception of some thunniform swimmers (Shadwick et al., 1999). Consequently, a progressive, posterior increase occurs between the timing of muscle activity and lateral bending (or muscle strain). In

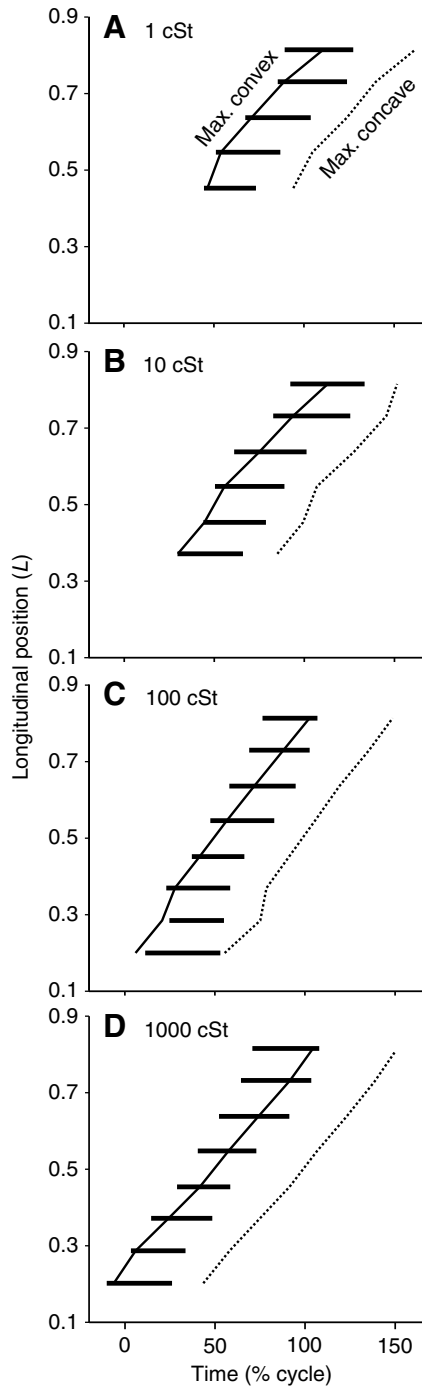


Fig. 9. Summary of bending and red muscle activity for mean values of four lungfish in viscosities of 1 (A), 10 (B), 100 (C) and 1000 cSt (D). The time of all graphs is standardized to 50% of a cycle when the body at 0.5 L was bent maximally convex towards the side of muscle activity. The horizontal bars indicate the average timing of muscle activity relative to bending at a particular longitudinal location. Muscle activity between maximum convexity and maximum concavity correlates with activity during contractile tissue shortening.

several species the timing of anterior muscle activity is nearly coincident with shortening of the muscle fibers. In the most posterior location of the lungfish swimming in water in this study, EMG onset preceded the beginning of muscle shortening by 18% of a cycle (Fig. 10C), which is more than twice that of eels (7.5%

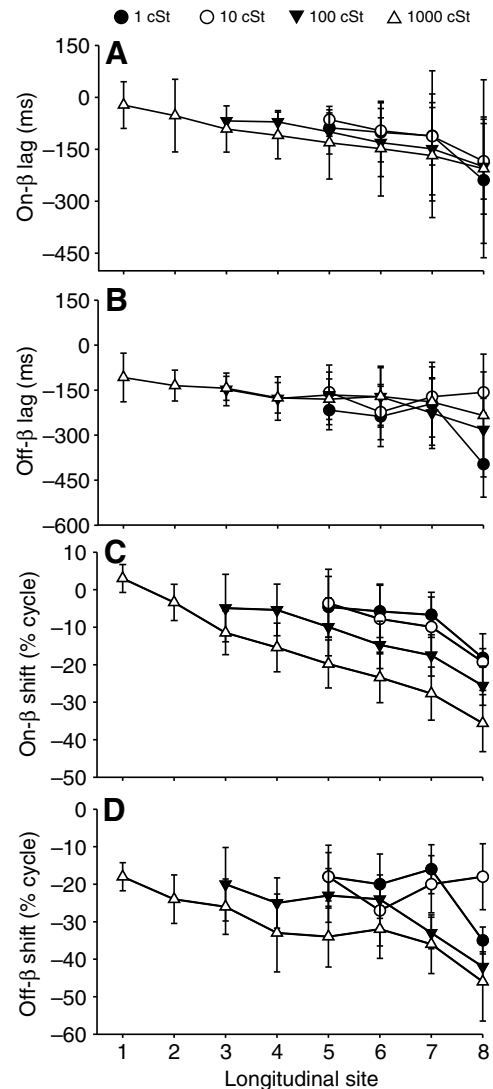


Fig. 10. (A) Lag time between EMG onset and when lateral flexion was maximally convex on the side of muscle activity. (B) Lag time between EMG offset and when lateral flexion was maximally concave on the side of muscle activity. (C) Phase shifts between EMG onset and when lateral flexion was maximally convex on the side of muscle activity. (D) Phase shifts between EMG offset and when lateral flexion was maximally concave on the side of muscle activity. Values of zero for both phase shifts indicate muscle activity during the entire time of fiber shortening. Note that the magnitude of both phase shifts increased significantly with increased viscosity.

of a cycle) (Gillis, 1998b) but within the range of values reported for swimming snakes (18–30%) (Jayne, 1988) and a variety of non-anguilliform fishes (Altringham and Ellerby, 1999; Coughlin, 2002). Activating muscle earlier in the strain cycle can provide a mechanism for increasing power production, or, if it is activated much earlier relative to shortening, the activity is well-suited for stiffening the body (Altringham et al., 1993; Johnson et al., 1994; Long et al., 1994; Rome et al., 1993). Since many of the forces most important for the propulsion of undulatory swimmers are near the trailing edge of the animal, stiffening this region may be particularly important for improving the transmission of propulsive forces.

Effects of speed and environment on locomotion

Experimental manipulations of environment are scant compared with those addressing the mechanical properties within the body of a swimming organism. For vertebrates that laterally undulate their axial structures during locomotion, the primary insights gained from manipulating the environment have been from comparing swimming in water to locomotion on land. Unlike swimming, the axial muscle activity of eels (Gillis, 2000), snakes (Jayne, 1988) and salamanders (Frolich and Biewener, 1992) moving on land is nearly confined to when muscle fibers shorten. In these two environments, however, some important details of axial function differ among these taxa. On land, eels have a significant longitudinal phase shift between the timing of muscle activity and bending (Gillis, 2000), whereas snakes and salamanders lack such a phase shift (Frolich and Biewener, 1992; Jayne, 1988). The waves of bending and muscle activity of snakes and eels are propagated posteriorly while moving both in water and on land, whereas salamanders on land have a standing wave pattern of bending and muscle activity (Frolich and Biewener, 1992). However, lungfish swimming in increasingly viscous solutions had increasingly greater amounts of muscle activity during lengthening rather than shortening.

Our 1000-fold manipulation of viscosity greatly exceeded that of any previous experimental study on the effects of viscosity for the aquatic locomotion of animals, including a 64-fold range in viscosity for swimming snakes (Gray, 1953b) and an approximately 3-fold range in viscosity for swimming fishes (Fuiman and Batty, 1997; Johnson et al., 1998). Although none of these previous studies of the effects of viscosity on aquatic vertebrate locomotion determined muscle activity, some aspects of kinematics were quantified. For example, in a solution 3.4 times more viscous than water the maximum velocities attained by guppies during escape responses were 14% less than those attained in water, but no statistically significant differences were evident between water and a solution 1.6 times as viscous as water (Johnson et al., 1998).

Snakes swimming in water have a large increase in wave amplitude posteriorly (Gray, 1953b; Jayne, 1985), which is a very general feature of the undulatory swimming of vertebrates. However, in a solution 64 times more viscous than water the amplitude of the anterior wave greatly exceeds that of the posterior wave, and the maximum amplitude along the entire length of the snake is only about one-half that of the snake in water (Gray, 1953b). Similar to snakes, with increased viscosity the amplitude of the anterior waves of lungfish increased. In both snakes (Gray, 1953b) and lungfish (Fig. 2C) the distance traveled per cycle decreases with increased viscosity. Unlike snakes in the highest viscosity, the amplitude of anterior waves in lungfish never exceeded that of posterior waves regardless of viscosity, and the amplitude of the most posterior wave changed little with increased viscosity (Fig. 3). Overall, for an equivalent range of viscosities the changes in waveform for swimming lungfish were small compared with those observed in snakes. The different effects of increased viscosity on the waveforms of snakes and lungfish may result from snakes being much more flexible anteriorly.

Some additional details of the undulation of snakes and eels led us to expect that high viscosities might generate similar phase relationships between muscle activity and bending to that of terrestrial lateral undulation. Matching of locomotor speed to wave speed and path following are characteristic of the type of terrestrial lateral undulation in snakes in which the phase relationships between muscle activity and lateral bending are constant along the length of the body and the timing of activity is concurrent with muscle fiber shortening. Furthermore, the same trend was observed in eels during

terrestrial lateral undulation in that they tended to path-follow while on wet packed sand (Gillis, 1998a), and the phase relationship between white muscle motor activity and lateral bending was more similar to that of snakes on land than to eels in water (Gillis, 2000). Whether or not such a motor pattern would occur in lungfish in viscosities higher than we used, or indeed for any elongate vertebrate at any viscosity higher than water, remains an open question.

The earlier onset of muscle activity relative to flexion (lower values of $\text{on-}\beta$ shift) and presumed fiber shortening observed for lungfish with increased viscosity resembles previously described changes for increased swimming speed of fishes. For example, with a nearly 4-fold increase in swimming speed in the largemouth bass, $\text{on-}\beta$ shift of a posterior site is -17% of a cycle, which is 10% of a cycle earlier than for the slowest speed (Jayne and Lauder, 1995a). With a 3-fold increase in swimming speed in the rainbow trout, the onset of muscle activity in a posterior site may occur 25% of a cycle before fiber shortening, which is 10% of a cycle earlier than for the slowest speed (Coughlin, 2000). For the most posterior site of lungfish swimming in the most viscous solution, $\text{on-}\beta$ shift was -34% of a cycle and 13% of a cycle earlier than the value for water (Fig. 10). Thus, the magnitude and directionality of the change in $\text{on-}\beta$ shift with increased viscosity are similar to those reported for increased speed in other species, but the timing of muscle activity in lungfish occurs very early in the bending cycle compared with the values reported for many other species of fish studied using similar methods (Altringham and Ellerby, 1999). Remarkably, the offset of muscle activity in the most posterior site in lungfish in the highest viscosity often occurred near or slightly before the beginning of flexion towards the side having muscle activity, indicating that muscles ceased motor activity prior to shortening.

An undulating swimmer theoretically could coordinate its muscle activity to compensate for the lag time between electrical activation and time to peak twitch tension so that peak twitch force remains coincident with a key event in the strain cycle – such as maximal stretch – even when cycle duration changes. Using such a constant lag time to preserve the absolute time course of some events would in turn cause a phase shift between EMG and strain whenever cycle duration changed. However, such a situation does not appear sufficient to explain the large phase shifts associated with increased viscosity for the swimming lungfish as values of $\text{on-}\beta$ lag in the most viscous solution consistently had the greatest magnitude (Fig. 10A) even though the large variance of this variable precluded detecting a statistically significant main effect of viscosity. For aquatic undulatory locomotion both increased speed and increased viscosity increase the power required to overcome drag, as well as increasing the tendency of the body to bend when it is pushing against the fluid (Schultz and Webb, 2002). Activating muscle earlier in the strain cycle, as was observed in the lungfish with increased viscosity, is consistent with the expectation of an increased role in stiffening structures or serving as a force transmitter (Blight, 1977), and under such conditions muscle theoretically could perform no mechanical work or even negative work. However, available data indicate that even when the posterior muscles have considerable early activity while lengthening, they usually produce large amounts of positive work per cycle (Coughlin, 2000; Hammond et al., 1998; Rome et al., 1993). Rather than viewing the function of fish muscle as conforming to mutually exclusive categories of stiffeners or power producers, posterior muscle activity such as that in the lungfish in high viscosities may provide a good example of functioning as a stiffener (early in each cycle) while retaining substantial power production.

The white muscle deep in the posterior region of the lungfish was commonly active even though swimming was steady and relatively slow, and the threshold for recruitment for the anterior white muscle was near a viscosity of 100 for the speeds we studied. In contrast to the lungfish, the threshold for white muscle activity in many fish in water is near the transition between rapid steady swimming and irregular rapid tail beats associated with the burst and coast mode of swimming (Jayne and Lauder, 1994b; Rome et al., 1985). When eels undulate slowly on land they also have large amounts of white muscle activity that would otherwise be absent while swimming at a similar speed in water (Gillis, 2000). Unlike lungfish in the more viscous solutions, eels undulating on land have more intense recruitment of white muscle anteriorly than posteriorly (Gillis, 2000). These examples indicate how the *in vivo* relationships between recruitment, force and the speed or frequency of movement can be altered by the environment, which suggests a greater diversity of motor pattern and control than would have been detected by only studying steady swimming in water.

Very high viscosities may create a situation for lung fish similar to the minimum flight speed required by aerial organisms to overcome drag (Lighthill, 1974). Despite repeated trials within and among subjects in the highest viscosities, the subjects simply did not or could not swim slowly at the highest viscosity. In the most viscous solution, the first few tail beats of the lungfish often resulted in no forward displacement, but then when forward movement commenced the minimal forward speeds of swimming usually exceeded those we observed for swimming in water. The observed threshold effect may be related to the nature of non-Newtonian fluids. When shear force is applied to a non-Newtonian fluid such as mud, the viscosity decreases in a non-linear fashion (Balmforth and Provenzale, 2001). Although this weakens our ability to predict the effects of viscosity as precisely as would be ideal, our experimental fluid was also shear-thinning (Ryan Collins, Baroid IDP Chemist, personal communication) and so responds to shear force in a comparable manner.

In summary, moving through a viscous medium evidently cannot be considered an intermediate between aquatic and terrestrial locomotion, nor does it seem likely that neuromuscular control changes dramatically in very viscous solutions. Instead, increased viscosity dramatically amplified the timing differences between EMGs and bending that: (1) typify aquatic undulatory swimming, (2) are the most pronounced in the posterior regions of fish, and (3) occur to a greater extent with increased swimming speed. Early tetrapods seem likely to have encountered viscous mud before dealing with fully solid surfaces lacking the buoyancy provided by water. Whether mud firm enough to support body weight would elicit a more terrestrial pattern of axial muscle activity in lungfish remains unresolved but very interesting in the light of the pivotal position this taxon has in tetrapod evolution.

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