

PATTERNS OF WHITE MUSCLE ACTIVITY DURING TERRESTRIAL LOCOMOTION IN THE AMERICAN EEL (*ANGUILLA ROSTRATA*)

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Summary

Eels (*Anguilla rostrata*) are known to make occasional transitory excursions into the terrestrial environment. While on land, their locomotor kinematics deviate drastically from that observed during swimming. In this study, electromyographic (EMG) recordings were made from white muscle at various longitudinal positions in eels performing undulatory locomotion on land to determine the muscle activity patterns underlying these terrestrial movements. As during swimming, eels propagate a wave of muscle activity from anterior to posterior during terrestrial locomotion. However, the intensity of EMG bursts is much greater on land (on average approximately five times greater than in water). In addition, anteriorly located musculature has higher-intensity EMG bursts than posteriorly located muscle during locomotion on land. EMG duty cycle (burst duration relative to undulatory cycle time) is significantly affected by longitudinal position during terrestrial locomotion, and duty cycles are significantly greater on land (0.4–0.5 cycles) than in water

(0.2–0.3 cycles). Finally, as in swimming, a phase shift in the timing of muscle activity exists such that posteriorly located muscle fibers become activated earlier in their strain cycle than do more anteriorly located fibers. However, fibers become activated much later in their muscle strain cycle on land than in water. Therefore, it is clear that, while eels propagate a wave of muscle activity posteriorly to generate backward-traveling waves that generate propulsive thrust both in water and on land, the specific patterns of timing and the intensity of muscle activity are substantially altered depending upon the environment. This suggests that physical differences in an animal's external environment can play a substantial role in affecting the motor control of locomotion, even when similar structures are used to generate the propulsive forces.

Key words: terrestrial, locomotion, muscle, electromyography, behaviour, eel, *Anguilla rostrata*.

Introduction

Animals must exert forces against and exchange energy with their external environment in order to move relative to it. The nature and efficiency of this energy exchange depend upon the physical properties of the external environment, and thus different environments tend to place quite different demands on the structure and function of organismal locomotor systems (Wessells, 1980). It is interesting then that we find organisms that utilize the same anatomical structures to propel themselves *via* grossly similar modes of locomotion in different physical environments. For example, the use of wings in a variety of avian taxa to 'fly' through water and through air (Kovacs, 1997; Meyers et al., 1992) and the use of limbs in various crustaceans to 'walk' under water and on land (Clarac et al., 1987; Hui, 1992; Martinez, 1996; Martinez et al., 1998) provide evidence that animals can accommodate drastic shifts in their external environment without altering the structures being used for propulsion. Although both examples noted above involve appendage-based modes of locomotion, axial-based undulatory locomotion can also be used, most notably

by snakes, to move through or across a diverse range of external environments (Gans, 1986; Jayne, 1988).

In addition to snakes, several diverse species of elongate fish are also known to make transitory excursions into the terrestrial environment (Gordon and Olson, 1995). Anguillid eels are a good example, being known to move across land under a variety of circumstances (Gray, 1968; Lindsey, 1978; Tesch, 1977). Yet, unlike snakes, whose axial musculoskeletal system is derived from a terrestrial ancestor, eels possess the serially arranged myotomal musculature common among fishes and designed to produce propulsion in water. How is it that this axial musculoskeletal system which evolved in an aquatic environment and is designed for undulatory propulsion through a buoyant, viscous and dense fluid is also capable of producing propulsive thrust on land, where gravitational forces dominate? Do the same patterns of muscle activity that create the movements responsible for swimming also permit movement across land, or do eels and other fish capable of terrestrial locomotion possess a range of motor output that exceeds that

observed during a variety of aquatic locomotor behaviors? Given that eels can swim at low speeds (up to $0.4Ls^{-1}$, where L is total body length) using relatively low levels of body undulation (i.e. low degrees of muscle strain) (Gillis, 1998a,b), it is likely that the shift to terrestrial locomotion, where the lateral displacement and level of undulation along the whole body are much greater at similar speeds (Gillis, 1998a), should require greater degrees of muscle force and work generation.

In the present study, I use high-speed video and electromyography to investigate the influence of the external physical environment (water/land) on the motor control of lateral undulatory locomotion in the American eel *Anguilla rostrata*. In particular, the main goal of this study is to examine the patterns of muscle activity underlying terrestrial locomotion and to address the extent to which axial muscle activity patterns in eels change (relative to those used during swimming) in order to generate locomotor forces on land.

Materials and methods

Animals

Kinematic and electromyographic data were collected from five eels [*Anguilla rostrata* LeSueur, 35–40 cm total length (L), mean 37.6 cm]. Animals were obtained from a commercial supplier in Pennsylvania, USA, in June 1997. Eels were kept individually in 40 l aquaria, provided with polyvinylchloride tubes for hiding, and maintained on a diet of earthworms (*Lumbricus* sp.). Water temperature within the aquaria was kept at $19.5 \pm 1.5^\circ\text{C}$, and a 12 h:12 h light:dark photoperiod was established. All experiments were performed between November 1997 and July 1998.

Electrode implantation

Electrodes were made and implanted following the basic procedures outlined by Gillis (1998b). Briefly, bipolar electrodes were constructed by threading two insulated stainless-steel wires (0.002 mm diameter bifiler wire; California fine wire Co.) through a 26 gauge 5/8 hypodermic needle. The wire tips were stripped of insulation (0.5 mm), spread 0.5–1.0 mm apart, and bent to form a fishhook-like structure that was pulled back into the opening of the needle's barrel.

During electrode implantation, eels were anesthetized using a buffered tricaine methanesulfonate (MS-222) solution (0.45 g l^{-1}). In early trial experiments, it was clear that red muscle electrodes were prone to being pulled out because of their relatively shallow implantations; therefore, for the present work, only white muscle was consistently implanted and recorded from in six locations (at approximately 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 L ; see Table 1 for exact implant locations in each individual). White muscle electrodes were implanted into the epaxial musculature to a depth approximately midway between the skin and the vertebral column and approximately 5–7 mm dorsal to the lateral line (into the anterior-pointing cone). Such positioning allowed the electrode tips to be medial and typically slightly dorsal to the wedge of superficial red

Table 1. Locations of white muscle implants from which electromyographic activity was recorded

Implant location (L)	Eel 1	Eel 2	Eel 3	Eel 4	Eel 5	Mean
0.2	0.23 (19)	0.23 (19)	0.22 (17)	*	‡	0.23 (18)
0.3	0.30 (26)	0.31 (27)	0.31 (26)	0.29 (26)	0.33 (29)	0.31 (27)
0.4	0.37 (33)	0.38 (34)	0.37 (32)	0.35 (32)	0.38 (34)	0.37 (33)
0.5	‡	0.48 (44)	0.49 (43)	0.49 (45)	0.48 (44)	0.48 (44)
0.6	0.58 (55)	0.57 (53)	0.60 (54)	0.58 (54)	§	0.58 (54)
0.7	0.68 (65)	0.68 (64)	§	§	§	0.68 (65)

Locations are described both as a proportion of total length, L , and as intervertebral joint number (in parentheses).

*No electrode was implanted at 0.2 L in individual 4.

‡Sites from which electrodes were dislodged during an experiment.

§Sites where electrode design and implantation looked good but from which no clearly discernible activity was recorded (for statistical tests, site 0.7 L was not included because of the absence of data from three individuals; site 0.6 L for individual 5 was considered to show zero intensity).

muscle (confirmation that electrode tips were indeed located in the white muscle was obtained by dissection after each experiment). Implanted electrodes were sutured to the body at four locations to help prevent them from being dislodged. All the electrode wires were sutured to the body at the origin of the dorsal fin and glued together into a cable using plastic cement. After the implantation procedure, the animals were allowed to recover for several hours before locomotor trials began.

Locomotor trials

As eels were recovering, electrodes were connected to Grass P511 preamplifiers. During locomotor trials, analog EMG signals amplified 5000 times and filtered (60 Hz notch filter and 100–3000 Hz bandpass) were recorded onto tape using a TEAC XR 5000 cassette data recorder. Eels were lowered carefully via a plastic container into a rectangular arena (0.75 m × 1.0 m) constructed of bricks set inside an empty inflated plastic wading pool (diameter 1.3 m). This arena was filled with wet sand (average grain size approximately 1 mm^3 ; for more details, see Gillis, 1998a) packed by hand to a depth of approximately 1 cm (3:1 sand:water ratio by volume) and was used to restrict the movement of the animals to the limits of the field of view of the video camera. Wet, packed sand was chosen because it seemed to be more similar to the terrestrial substratum that these eels might encounter in the wild than something like a pegboard which provides obvious and stiff

vertical projections to push against. Wet sand was also used because it was light enough in color (unlike mud) to allow the dark skin along the dorsal surface of the animals to be distinguished on the videotapes for digitizing purposes.

After being lowered into the arena, eels were released from the container onto the sand and typically began to move within the arena immediately. If eels remained stationary, light pinches of the tail tip would always elicit locomotion unless the animals were exhausted, at which point the locomotor trials were stopped. EMG data were collected from eels as they undulated across the wet, packed-sand environment. Simultaneous high-speed video recordings of the dorsal view of undulating eels were collected using a NAC HSV 500 video system recording at $250 \text{ fields s}^{-1}$. Electromyographic and kinematic data were synchronized by recording a 100 Hz pulse, whose shape changed predictably over time, simultaneously onto both the TEAC and NAC tapes, thus allowing identical times to be located on the video and EMG recordings.

EMG analysis

Much of the data analysis followed the procedures outlined in more detail by Gillis (1998b). Data were analyzed from four locomotor sequences per individual (except for one individual for which only three sequences were analyzed). Depending upon the trajectory of the animal across the arena, 2–4 full cycles of unimpeded locomotion could usually be recorded in a given sequence before the animal encountered one of the brick walls. Sequences were chosen for analysis on the basis of whether animals moved continuously and in a consistent direction across the substratum. Using a Keithley A/D converter, EMG data from each locomotor sequence were sampled at 8000 Hz and converted to digital signals. Digital data were filtered using a finite impulse response filter and then analyzed using a personal computer.

Using a custom-designed EMG analysis program, EMG bursts were digitized following the procedures of Jayne and Lauder (1993, 1995) and Gillis (1998b). The onset and offset time of each EMG signal were located visually using amplitude differences relative to the baseline for identification. In general, EMG traces were magnified, and bursts were identified as signals whose amplitude was at least three times that of the baseline and lasted at least 200 ms with no gaps in activity longer than 30% of the overall duration of the burst. The duration (in s) and rectified area (in mV s) of all bursts of activity were subsequently calculated by computer. EMG duty cycle was calculated by dividing each burst duration by the undulatory cycle time. The undulatory cycle time (in s) was calculated as the time between sequential onsets of white muscle activity at an anterior site ($0.2L$ or $0.3L$, where L is total body length). The instantaneous intensity (in mV) of each muscle burst was also calculated by dividing the rectified area of the burst by its duration. The velocity of the wave of muscle activity was calculated for all cycles by dividing the distance between electrodes implanted at $0.3L$ and $0.6L$ (or $0.5L$ in one individual) by the difference in the onset times of muscle activity at those sites during each undulatory cycle.

Video analysis and determination of the timing of muscle activity relative to muscle strain

Video recordings of two locomotor sequences (a subset of those sequences used for overall EMG analysis) from each individual were used for determining longitudinal patterns of intervertebral flexion and lateral amplitude and the timing of EMG activity at a given site relative to the estimated muscle strain cycle at the joint closest to that site. Only two sequences for each individual were used because of the time-consuming nature of the video digitization. Sequences for digitizing were chosen on the basis of the quality of the video recording (i.e. the two sequences from each individual in which shadows were minimized due to the trajectory of the eel relative to the external lighting).

As in several recent studies (see, for example, Wardle et al., 1995), the muscle strain cycle was defined as a 360° cycle, where 0° is the time at which a fiber is at its resting length during lengthening, 90° is the time at which it reaches its maximum length, 180° is the time at which the fiber returns to resting length during shortening, and 270° is the time at which it reaches minimum length. White muscle strain cycles were estimated using calculated patterns of intervertebral flexion, which in turn were determined as follows. First, after experiments, eels were X-rayed to determine the longitudinal position of all intervertebral joints and the locations of all electrode tips relative to these joints. The eel outlines from 20 video fields per tailbeat cycle were then digitized from each of the locomotor sequences. A customized kinematic analysis program described by Jayne and Lauder (1993) and Gillis (1997) was then used to fit a series of cubic splines to these eel outlines and to calculate the midline of the fish for each digitized video image. Each midline was then converted into a series of straight-line segments whose positions and lengths were defined by the longitudinal locations of all the axial skeletal joints determined by X-ray photography. Because the outline of the fish bent during undulation, the calculated midline also bent, and the individual joints along the midline differentially flexed according to their position, their spacing and the degree of curvature of the midline.

Patterns of flexural excursion at the intervertebral joints closest to each of the implanted electrodes were then determined. The timing and degree of muscle strain were considered to be equivalent to the timing and degree of joint flexion. In other words, maximal flexion at a joint implied maximal muscle strain on the convex side of the flexed joint and minimal strain on the concave side; zero flexion at a joint implied no muscle strain. Points of flexion closest in time to a phase of 0° were identified and used to define the beginning and end of each undulatory cycle, 0 and 360° , respectively. Then, for every cycle, muscle onset and offset times were determined (relative to the 360° cycle of flexion) for all electrodes from which recordings were obtained.

Because of the complex arrangement and orientation of white muscle fibers within myotomes, there has been some question as to whether patterns of midline curvature can accurately predict the timing and degree of white muscle strain.

Two recent reports (Katz et al., 1999; Wakeling and Johnston, 1999) compared white muscle strain histories obtained *via* sonomicrometry and calculated *via* video digitization and spine curvature analysis. Both studies agree that, for superficial white muscle, sonomicrometry and spine curvature analysis lead to similar results, implying that this region of the body bends like a homogeneous beam. However, Wakeling and Johnston (1999) suggest that data from the two different techniques do not necessarily correspond as well for the deeper white muscle, whereas Katz et al. (1999) conclude that the two techniques do indeed lead to similar results for the deep and superficial white muscle. While the method used in the present study to determine muscle strain *via* kinematic analysis is somewhat different from that used by Katz et al. (1999) or Wakeling and Johnston (1999), all use some measure of midline curvature rather than displacement to estimate muscle strain. Because both these studies agree that the pattern of midline curvature can be used to determine accurately the timing and degree of superficial white muscle strain [and Katz et al. (1999) state that this is also true of the deep white muscle], and given that the implants in the present study tend to be just medial and dorsal to the red muscle (i.e. relatively superficial), it is likely that the estimates of the timing of muscle strain used here can be considered reliable. It is important to note that the fiber architecture of white muscle in eels is somewhat different from that in teleosts with more derived musculoskeletal features (Alexander, 1969) such as the carp (*Cyprinus carpio*) and milkfish (*Chanos chanos*) examined by Wakeling and Johnston (1999) and Katz et al. (1999) respectively. However, until similar comparative experiments are performed with the myotomes of anguillid or salmonid fish (teleosts with more primitive features), the influence of such architectural differences on the relationship between midline curvature and white muscle strain will remain unknown.

Statistical analyses

To quantify patterns of electromyographic activity during terrestrial locomotion, the mean and standard error of the mean of EMG duty cycles and intensities were calculated for each longitudinal position across all individuals (by averaging the mean values for each locomotor sequence). In addition, the mean and standard error of the mean of EMG onset and offset times were also calculated for each longitudinal position using the subsample of locomotor sequences for which they were calculated. Two-way mixed-model analyses of variance (ANOVAs) with individual and site as a random and fixed effect, respectively, were used to address individual- and site-related variation in EMG duty cycles, intensities, onsets and offsets. Because muscle activity was recorded from the most posterior site in only two individuals, this position was not included in these ANOVAs. Finally, to determine the effect of the external environment on patterns of muscle activity, one-way ANOVAs were used to compare data from all terrestrial sequences in the present study with white muscle activity during aquatic sequences collected previously from different individuals [data for white muscle activity patterns during

swimming were taken from Gillis (1998b, and unpublished results)]. EMG data from aquatic sequences used for comparison were taken from sites at 0.45, 0.6 and 0.75L during high-speed swimming trials ($1.0 L s^{-1}$) and compared with data taken from sites at 0.4, 0.5 and 0.6L during terrestrial locomotion. High-speed swimming bouts were used for comparison because it is only during these high-intensity activities that white muscle is recruited consistently in water.

Results

In general, eels traverse a terrestrial environment using lateral undulation (*sensu* Gans, 1986), which entails bending the body into waves that pass posteriorly from head to tail. These mechanical traveling waves encounter and push

Table 2. Number, mean duration and average locomotor speed of sequences used for analysis

Individual	Number of cycles	Mean cycle time (s)	Average speed ($L s^{-1}$)
Eel 1			
Sequence 1	4	0.62±0.02	0.41
Sequence 2	2	0.68±0.01	0.55
Sequence 3*	4	0.97±0.04	0.27
Sequence 4*	3	0.93±0.04	0.42
Eel 2			
Sequence 1*	4	1.06±0.04	0.31
Sequence 2	3	1.03±0.11	0.38
Sequence 3*	3	1.24±0.03	0.26
Sequence 4	4	1.19±0.09	0.27
Eel 3			
Sequence 1*	3	1.01±0.04	0.27
Sequence 2*	3	0.84±0.02	0.42
Sequence 3	3	0.89±0.03	0.42
Sequence 4	3	1.34±0.10	0.22
Eel 4			
Sequence 1*	2	1.04±0.06	0.21
Sequence 2*	4	1.36±0.08	0.20
Sequence 3	4	1.52±0.11	0.18
Eel 5			
Sequence 1	3	1.24±0.06	0.22
Sequence 2*	2	1.28±0.02	0.20
Sequence 3	3	1.32±0.06	0.20
Sequence 4*	3	1.05±0.10	0.19
Overall mean	3.16	1.08	0.29
Mean*	3.10	1.08	0.28

Duration is given as mean ± S.E.M.

*Sequences used for analysis of the timing of muscle activity with respect to estimated muscle strain.

Values for average speed were calculated by taking the total linear distance (in cm) moved by a point on the body approximating the center of mass (0.4L) and dividing by the total duration of the sequence (in s). This value was then divided by the length (L) of the animal (in cm) to give speed in $L s^{-1}$.

against sites of resistance between the eel's skin and the substratum, providing the thrust to move the animal forwards. Eels are incapable of moving across land at the high speeds they can achieve while swimming. During the sequences analyzed here, locomotor cycle times ranged from 0.62 to 1.52 s and mean speeds ranged from 0.18 to $0.55 L s^{-1}$ (Table 2). The locomotor cycle times and speeds in those sequences that were digitized and analyzed to determine the relative timing of muscle activity with respect to estimated muscle strain at different body positions ranged from 0.84 to 1.36 s and from 0.19 to $0.42 L s^{-1}$, respectively (Table 2). Maximum flexion at sites of functioning EMG electrodes averaged from 5.2 to 8.5° , while maximum amplitude ranged from 0.08 to $0.135L$.

White muscle activity travels in a wave-like pattern from anterior to posterior as eels undulate across the terrestrial environment (Fig. 1). The velocity of the wave of muscle activity was typically 2–4 (mean 3.2, $N=19$ sequences) times greater than that of the mean forward velocity of the animal. Some individuals used a much narrower range of velocities (both locomotor and of the muscle wave itself) than others. However, increases in the velocity of the wave of muscle

activity generally led to an increase in locomotor speed (Fig. 2).

Longitudinal position had a significant effect on the duty cycle of EMG bursts ($P<0.05$; d.f.=2,13) but, in general, bursts occupied 0.4–0.5 cycles (Fig. 3A). Longitudinal position also had a significant effect on EMG burst intensity ($P<0.02$; d.f.=2,13), which tended to be lowest at the most anterior and posterior sites (Fig. 3B). In addition, obvious bursts were not always present in the posterior-most locations during terrestrial locomotion (Fig. 1). Both duty cycle and burst intensity also showed significant individual variation ($P<0.0001$ for both variables).

Longitudinal position had a significant effect on both the onset ($P<0.02$; d.f.=2,13) and offset ($P<0.001$; d.f.=2,13) of white muscle activity. In general, the relative timing of muscle bursts changed along the body such that muscle from more posterior sites was activated and deactivated earlier in its strain cycle than muscle located more anteriorly (Fig. 4). For example, the average onset and offset times of white muscle located at $0.2L$ are 138 and 296° , respectively, whereas at $0.6L$, the onset and offset are at 91 and 243° respectively. The shift in the timing of the onset of activity was not as consistent from

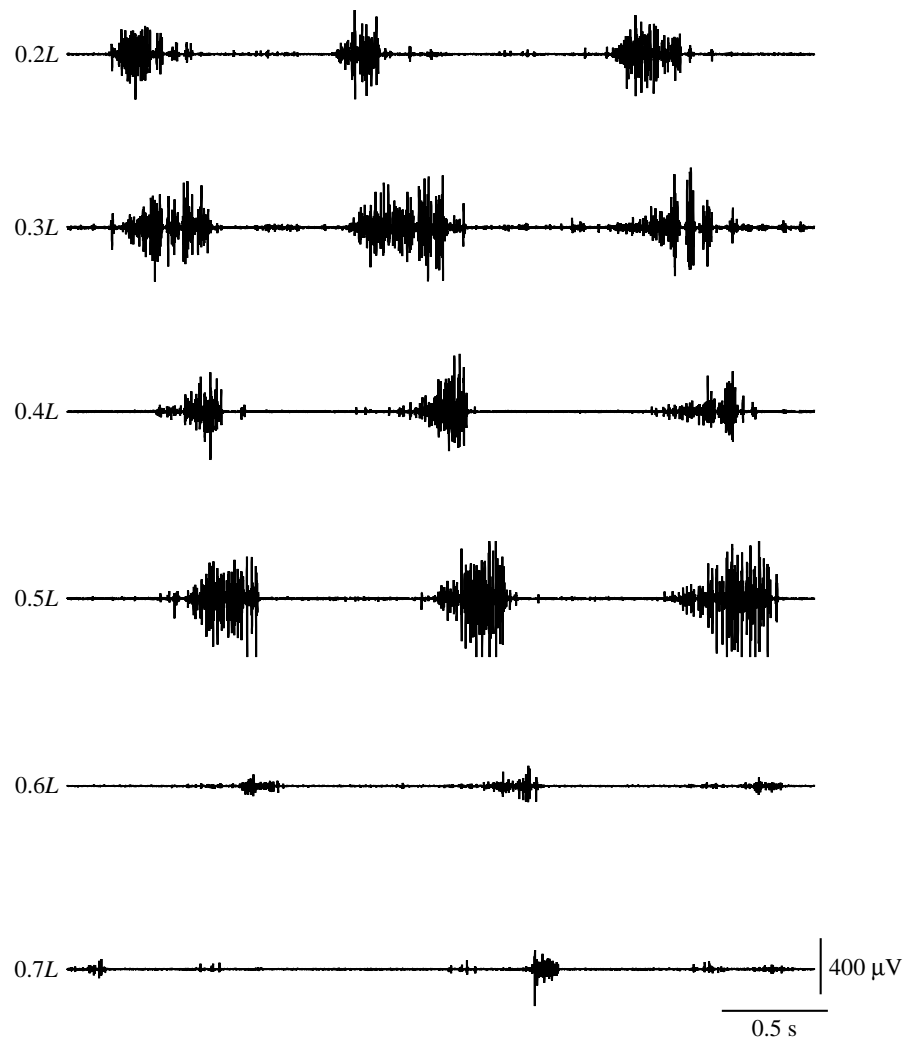


Fig. 1. Series of recordings showing left-side electromyographic (EMG) activity from white muscle at various longitudinal positions during two cycles of terrestrial locomotion. Note the temporal lag between the onsets and offsets of activity at the different sites, showing that EMG activity travels in a wave-like fashion from anterior to posterior along the body during undulatory locomotion on land. Also note the decrease in EMG intensity posteriorly, where some bursts are not even clearly discernible. In several eels, there was a similar absence of discrete EMG bursts at the most posterior sites (0.6 and/or 0.7L). Traces are from an eel moving at $0.27 L s^{-1}$. L is total body length.

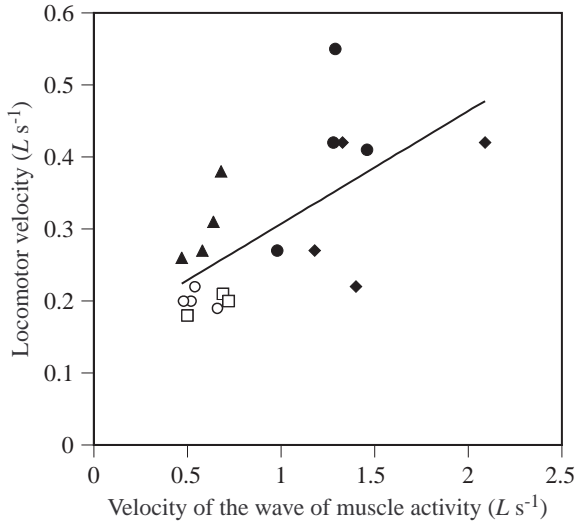


Fig. 2. Plot of locomotor velocity as a function of the velocity of the wave of underlying white muscle activity. Different symbols represent different individuals, and each point represents the mean value for one locomotor sequence (consisting of 2–4 undulatory cycles). Note how some individuals (open squares and circles and filled triangles) tended to generate waves of muscle activity of similar velocities across sequences. Also note that some individuals (filled circles and filled diamonds) always generated waves of muscle activity of higher velocities than those of other individuals. The equation for the line fitting all the data is $y=0.16x+0.15$ ($r^2=0.44$, $P=0.002$). L is total body length.

anterior to posterior as was the shift in the timing of the offset of activity (Fig. 4). Muscle at all sites is not activated until after it has begun to shorten (i.e. onset time $>90^\circ$) and, for some sites, muscle activity did not end until after the muscle has begun to re-lengthen (i.e. offset time $>270^\circ$). Individual variation was significant for EMG onset ($P<0.0001$), but not for EMG offset ($P=0.14$).

Discussion

Neuromuscular control of terrestrial versus aquatic locomotion

During both aquatic and terrestrial undulatory locomotion, waves of muscle activity travel posteriorly along the body creating waves of curvature that also travel posteriorly down the animal, propelling it forwards. In this study, only white muscle activity was addressed as electrodes implanted in the superficial red muscle were prone to becoming dislodged by the exaggerated undulatory movements eels use on land. The speed at which eels pass waves of white muscle activity along their body tends to be much lower during the terrestrial locomotion trials studied here (overall mean 35 cm s^{-1}) than during high-speed (1.0 L s^{-1}) aquatic locomotor trials (overall mean 74 cm s^{-1}). In addition, the ratio of the speed of the wave of muscle activity to the forward speed of the animal is much greater on land (mean 3.2) than during swimming (mean 1.8), suggesting a lower locomotor efficiency for terrestrial

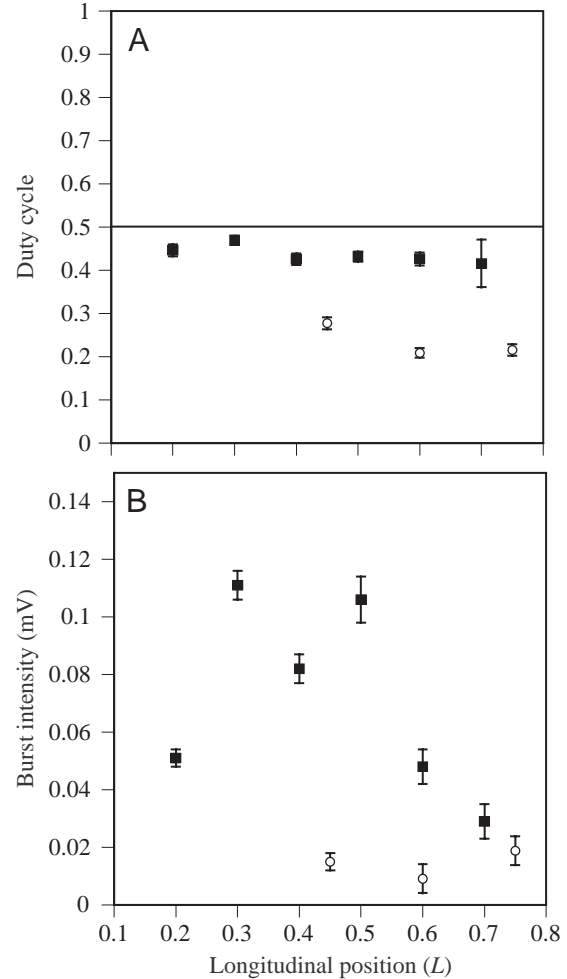


Fig. 3. Graphs showing the mean duty cycle (A) and mean burst intensity (B) of white muscle electromyographic (EMG) bursts during terrestrial locomotion (filled squares) and during swimming at 1.0 L s^{-1} (open circles). Values are means \pm S.E.M. (for values of N , refer to Tables 1 and 2). The horizontal line in A represents a duty cycle of 0.5. During terrestrial locomotion, there is a significant difference among body positions in terms of the mean duration of EMG bursts, measured as a proportion of undulatory cycle time (i.e. duty cycle) although, in general, all sites are typically active for 0.4–0.5 cycles. EMG burst intensity shifts dramatically along the body and is much lower in the most anterior ($0.2L$) and posterior (0.6 and $0.7L$) sites. During swimming, duty cycles and burst intensities tend to be much lower than on land. Values for burst intensity only take into account sequences for which bursts were present (i.e. no values of zero were used to indicate the absence of activity at a given site). Taking into account sequences in which no bursts were present despite a good electrode implantation would reduce the mean intensity at the most posterior site during terrestrial locomotion severely because three of the five individuals showed no obviously discernible EMG bursts at this site. L is total body length.

locomotion (i.e. a slower rate of forward progression with respect to a given rate of muscle activation along the body).

White muscle duty cycles and burst intensities are significantly greater during terrestrial locomotion than during aquatic locomotion ($P<0.001$; d.f.=1,261 for terrestrial

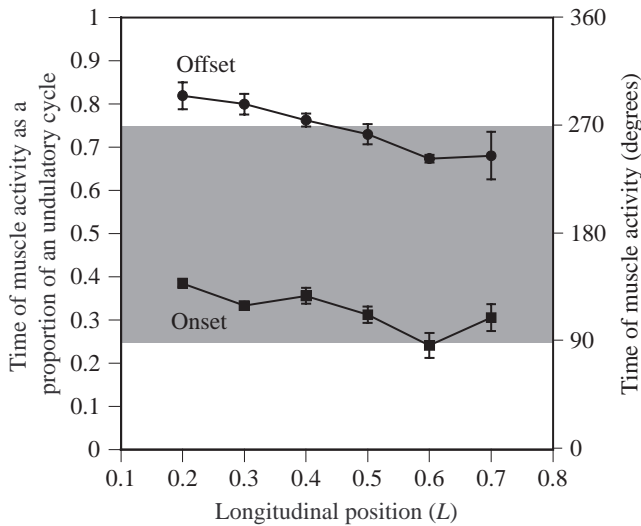


Fig. 4. Graphs of white muscle onset and offset during terrestrial undulatory locomotion. Values are means \pm s.d. (for values of N , refer to Tables 1 and 2). Timing data are indicated both as a proportion of a tailbeat cycle (left axis) and as phase of the muscle strain cycle in degrees (right axis). The shaded area represents the period during which the muscle is shortening. The timing of muscle activity shifts to being relatively earlier in the posterior regions of the fish. However, on average, muscle activity always begins after the fibers themselves have already begun to shorten.

locomotion; d.f.=1,267 for aquatic locomotion). Increased EMG burst intensities and durations are also seen in crustacean limb muscles (Clarac et al., 1987; Grote, 1981) and snake epaxial muscles (Moon and Gans, 1998) when loads carried during locomotion are increased either artificially or *via* a shift from aquatic to terrestrial locomotion. It is likely that increased load (and thus the need for increased force production) to some degree underlies the shifts in muscle burst duration and intensity observed in eels moving on land as well.

Undulatory amplitude and intervertebral flexion are much greater on land than during swimming (Gillis, 1998a), and it is likely that this enhanced degree of bending and lateral displacement along the body also underlie the longer duration and higher intensity of muscle bursts observed during terrestrial locomotion. The fact that terrestrial muscle bursts tend to be twice as long as and, at some sites, five times the intensity of those used during swimming, and yet produce much lower overall locomotor speeds, also suggests low locomotor efficiency on land as well as high costs of transport during terrestrial locomotion relative to swimming.

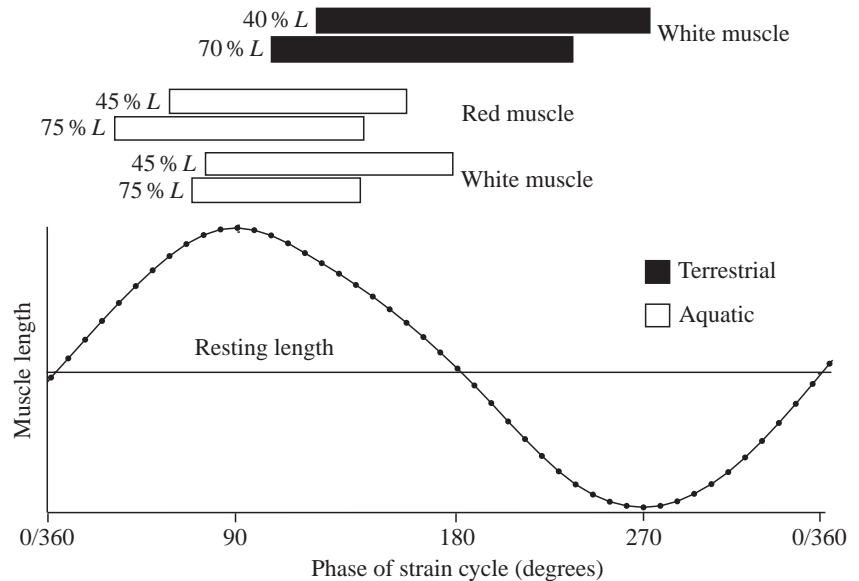
Interestingly, the intensity of white muscle activity is lowest at the most posterior site (0.7L). In three of five individuals, no clearly discernible rhythmic activity was recorded from this site despite good electrode design and implants (e.g. Fig. 1; Table 1). This minimal (or lack of) activity posteriorly is in marked contrast to the situation during swimming, where the highest absolute intensities of red muscle activity tend to be recorded in the most posterior regions (G. B. Gillis, unpublished results). In addition, it is white muscle in the tail

region that is recruited before more anteriorly located white muscle to increase speed during swimming. This difference between aquatic and terrestrial locomotion in the pattern of muscle recruitment and intensity between more anterior and intermediate sites (0.3–0.5L) and more posterior locations (0.6–0.7L) makes sense given the difference in the site of the bulk of propulsive thrust generation across environments. In water, it is the laterally flattened, dorso-ventrally expanded tail whose lateral surfaces produce the majority of the propulsive thrust (because the anterior regions of the body undergo little to no lateral undulation) (Gillis, 1998a). On land, however, it is largely the ventral and ventro-lateral surfaces of the eel that contact the ground and are capable of providing propulsive force. The most posterior regions of the animal have very little white muscle volume and almost no ventral surface area because of the large degree of lateral compression and rarely, therefore, gain any purchase or 'bite' on the sand. Instead, much of the tail tends to flop onto its lateral surface and 'slip' from side to side across the terrestrial substratum with relatively high amplitude rather than providing useful thrust. So, although the posterior regions of the body are probably important for generating thrust during steady swimming, it is probably the more central regions of the fish that generate most of the forces responsible for propelling them on land.

The relative timing of muscle activity shifts consistently from anterior to posterior during both swimming and terrestrial locomotion in eels (Figs 4, 5). Posteriorly located musculature is activated earlier in its muscle strain cycle than is more anteriorly located muscle. This is indicative of a wave of muscle activity traveling posteriorly at a fairly constant velocity and leading to a mechanical wave of bending that moves more slowly and lags behind the wave of motor activity. Currently, all fish whose muscle activity patterns have been recorded during steady swimming (excluding tuna, *Katsuwonus pelamis*, whose specialized internal red muscle shows an interesting counter-example; see Shadwick et al., 1999), also show some degree of phase shift in the timing of red muscle activity relative to muscle strain between anterior and posterior sites (Hammond et al., 1998; Jayne and Lauder, 1995; Rome et al., 1993; Shadwick et al., 1998; van Leeuwen et al., 1990; Wardle and Videler, 1993; Williams et al., 1989). However, eels also show such a phase shift while undulating across land.

Jayne (1988) found that, during swimming in snakes, a similar phase shift exists in the timing of axial muscle activity between anterior and posterior regions, suggesting that they still possess this fish-like mode of wave generation, despite being derived from terrestrial ancestors. However, during terrestrial locomotion through regularly spaced pegs, the relative timing of muscle activity does not shift along the length of the snake's body. Muscles in anterior and posterior locations become activated and deactivated at similar relative times in their respective muscle strain cycles, thus eliminating the phase shift present during swimming. While it is unclear exactly what the functional ramifications or underlying mechanistic bases of this phase shift elimination are (perhaps

Fig. 5. Bar diagram showing the mean onset and offset of muscle activity at two different longitudinal positions relative to the muscle strain cycle during aquatic and terrestrial locomotion in the American eel. Data for swimming are taken from Gillis (1998b). Note that burst durations are greater during terrestrial locomotion (black bars) and that, for similar body sites, activity begins much later in the muscle strain cycle during terrestrial locomotion. Phase lags exist between muscle onsets as well as muscle offsets between the anterior and posterior sites regardless of environment. More posterior sites on average become activated and deactivated relatively earlier than more anterior sites. L is total body length.



it is simply a function of the artificial pegboard environment), these data suggest that the motor control of terrestrial locomotion in snakes differs from that used by many different animals during undulatory swimming.

Gans (1985) has suggested that the coordination of terrestrial undulatory locomotion (where the environment is heterogeneous and sites of external resistance are unpredictable) is different from and more complex than that required to move through the relatively homogeneous fluid environment of water. In particular, because sites of external resistance on land are unpredictable, a more refined system of feedback is required to detect and act upon localized regions of external force application as the wave of bending passes posteriorly along the body. The fact that the phase shift in the timing of muscle activity disappears during terrestrial locomotion in snakes suggests that they are actively controlling the timing of their bursts of muscle activity in such a way that muscles are always active during the same portion of their muscle strain cycle. Regardless, the data from eels show that this seemingly more active control of muscle timing (i.e. the elimination of the timing phase shift displayed by terrestrially undulating snakes) is not required to generate effective propulsive thrust *via* axial undulations while on land, although it may be more energetically economical to do so. Examining muscle activity during snake locomotion across a more heterogeneous terrestrial environment would allow a better understanding of whether this interesting pattern of muscle timing in snakes is a function of the terrestrial environment in general or just a byproduct of the artificial peg-board environment.

Timing of muscle activity during terrestrial locomotion

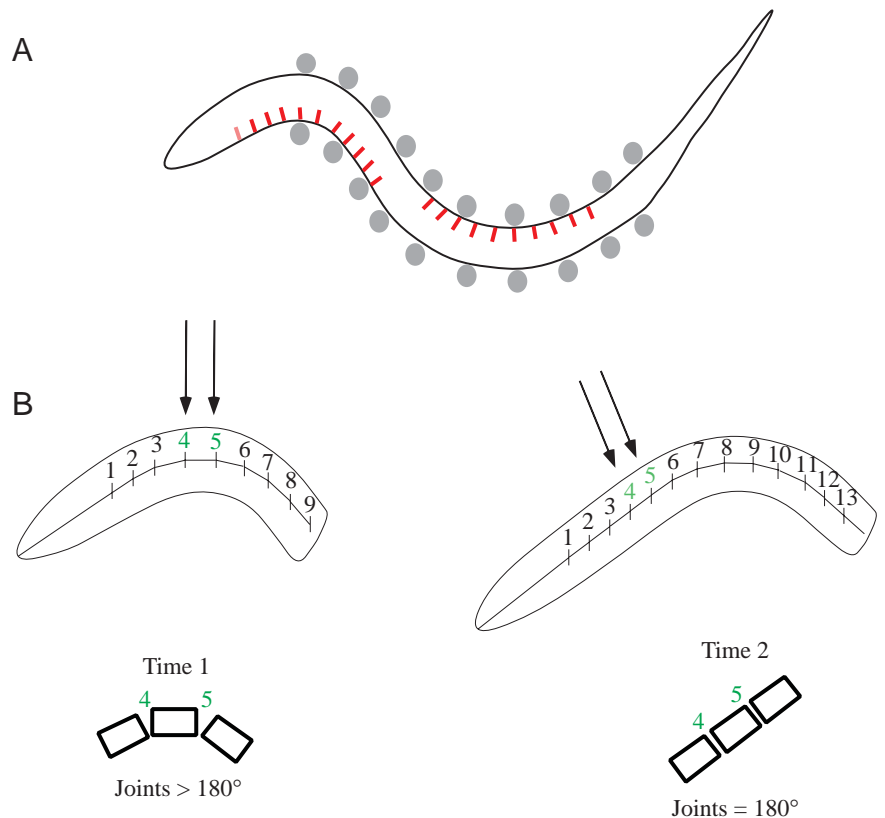
In most studies of the muscle activity patterns underlying cyclic locomotor behavior (where muscles controlling the anatomical structures responsible for producing propulsive thrust cyclically shorten and lengthen repeatedly), it has been

found that muscles typically become activated during their lengthening phase and become deactivated before the end of muscle shortening. For example, in swimming fish and salamanders, axial muscles become activated prior to the point at which lengthening muscles begin to shorten, and the offset of EMG activity occurs before the shortening muscles begin to re-lengthen (e.g. Frolich and Biewener, 1992; Gillis, 1998b; Shadwick et al., 1998). Similarly, in flying birds, the pectoralis muscle (responsible for the downstroke) becomes activated during the upstroke (as it is still lengthening) and becomes deactivated during the downstroke (as it is still shortening) (e.g. Biewener et al., 1998; Dial, 1992; Tobalske, 1995). Because the development and decrement of force within a muscle are not instantaneous relative to electrical activation and deactivation, respectively, this pattern allows high active force generation to occur during shortening, with minimal force required to re-lengthen the muscle for the next cycle (as it has already begun to relax) and has been regarded as a means of allowing for the maximization of work output from the muscle (Josephson, 1993).

However, in eels undulating across wet packed sand, the muscle fibers are activated relatively later in their strain cycle than during swimming (Fig. 5). In fact, on land, muscles actually become activated after the joints they span have begun to flex (Figs 4, 5), implying that the initiation of bending at these sites cannot be due to active fiber contraction. Instead, bending must be caused by non-local contraction, perhaps *via* the transmission of forces generated by active muscle contraction at a non-local site, as suggested by Covell et al. (1991) for bending in trout (*Oncorhynchus mykiss*) during fast starts, in combination with external resistance.

When eels move across the packed sand environment used in the present study, the mass of the body tends to make shallow grooves in the sand as it passes over the substratum. These grooves then seem to provide sites of external resistance for the body to push against to propel itself forwards. The

Fig. 6. Schematic diagram demonstrating the pattern of bending prior to active muscle contraction. (A) A schematic diagram of a dorsal view of a terrestrially undulating eel with superimposed sites of muscle activity designated by the red hatching. Gray circles surrounding the eel represent the presence of a groove in the sand caused by the weight of the animal. (B) Enlargements of the anterior region of the eel at two times (100 ms apart) during a locomotor sequence. Intervertebral joints are marked in these lower drawings with thin vertically oriented numbered lines. At time 1 (equivalent to the point in time represented in A), intervertebral joints 4 and 5 (in green) are both flexed concave to the animal's left, as the head swings in that direction. However, as the head continues to move forward and to the animal's left (time 2), joints 4 and 5 straighten out (necessitating that they have begun to flex to the animal's right). Note that, in A, no axial muscle activity was present on the right side of the animal's body near intervertebral joints 4 and 5 prior to this flexion to the right. Thus, the onset of local bending back to the right at these sites was not the result of active local contraction. Instead, many body segments following the path created before them probably move through such a curve *via* the creation of non-local bending forces exerted against an external resistance created by the presence of a groove and can thus undergo passive flexion at such times.



groove left by more anterior portions of the body provides a track through which much of the rest of the body can travel. This track can then constrain the path of movement of more posterior parts of the body to that already traversed by more anterior regions. If a portion of the body follows the path and bends around a curve, it will go from being straight (before it enters the curve) to being bent (as it passes through the curve) to being straight again (as it comes out of the curve; Fig. 6). If activity at non-local sites is generating the forces responsible for moving that portion of the body through the curve, and external structures (potentially the sides of the groove) provide the resistance that is responsible for constraining the moving body to a particular path into, through and out of the curve, then that portion of the body will have undergone passive deformation and bending without the need for locally active contracting muscle (Fig. 6).

Whether this hypothetical scenario accurately explains why portions of the body begin to bend prior to local active contraction is not clear. It is the case, for example, that the amplitude of lateral displacement does vary somewhat along the body [amplitude between sites at $0.2L$ and $0.7L$ range between $0.08L$ and $0.135L$, with the highest amplitudes occurring at sites between 0.4 and $0.5L$ in this study and in Gillis (1998a)] and hence all sites cannot be traveling along exactly the same path. But it does represent a possible and testable mechanistic basis for this intriguing pattern.

Examining axial bending and muscle activity during locomotion along a non-compliant terrestrial environment which prevents the formation of grooves (for example undulation along wetted Plexiglas) would allow one to refute this hypothetical scenario if a similar pattern of intervertebral flexion and muscle activity was still found. Regardless, this pattern of local bending prior to local muscle activation represents an unusual usage of axial muscle among fishes that merits further exploration.

In conclusion, while eels use grossly similar waves of axial muscle activity to produce propulsive thrust during both aquatic and terrestrial undulatory locomotion, specific patterns of timing, duration and intensity are dependent upon the external environment in which the animal is moving. In fact, the magnitudes of timing differences observed between swimming and terrestrial locomotion in this single taxon appear to be as substantial as the timing differences present among the muscle activity patterns used by fish spanning the undulatory locomotor spectrum (see Fig. 8 in Gillis, 1998b). This suggests that physical differences in an animal's external environment can play an important role in affecting not only the mechanics but also the neuromuscular control of locomotion.

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