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Activity of trunk muscles during aquatic and terrestrial locomotion in *Ambystoma maculatum*

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SUMMARY

The activity of seven trunk muscles was recorded at two sites along the trunk in adult spotted salamander, *Ambystoma maculatum*, during swimming and during trotting in water and on land. Several muscles showed patterns of activation that are consistent with the muscles producing a traveling wave of lateral bending during swimming and a standing wave of bending during aquatic and terrestrial trotting: the dorsalis trunci, subvertebralis lateralis and medialis, rectus lateralis and obliquus internus. The interspinalis showed a divergent pattern and was active out of phase with the other muscles suggesting that it functions in vertebral stabilization rather than lateral bending. The obliquus internus and rectus abdominis showed bilateral activity indicating that they counteract sagittal extension of the trunk that is produced when the large dorsal muscles are active to produce lateral bending. Of the muscles examined, only the obliquus internus showed a clear shift in function from lateral bending during swimming to resistance of long-axis torsion during trotting. During terrestrial trotting, muscle recruitment was greater in several muscles than during aquatic trotting, despite similar temporal patterns of muscle activation, suggesting that the trunk is stiffened during terrestrial locomotion against greater gravitational forces whereas the basic functions of the trunk muscles in trotting are conserved across environments.

Key words: trotting, swimming, hypaxial, epaxial, electromyography, amphibian, tetrapod.

INTRODUCTION

Salamanders are often chosen for studies of the evolution of tetrapod locomotion because of their generalized body form, range of locomotor modes, and amphibious lifestyle. The prominence of lateral trunk movements during locomotion in salamanders has also made them favorite subjects in investigations of epaxial and hypaxial muscle function in tetrapods (Blight, 1976; Frolich and Biewener, 1992; Carrier, 1993; Delvolve et al., 1997; Bennett et al., 2001; Azizi and Horton, 2004; Brainerd and Azizi, 2005). Despite this attention, the locomotor functions of relatively few trunk muscles have been examined electromyographically, and additional muscles must be investigated to assemble a complete picture of trunk muscle function in salamanders.

Previous research has revealed that both epaxial and hypaxial muscles are involved in lateral bending during terrestrial walking and trotting and during swimming in salamanders (Frolich and Biewener, 1992; Carrier, 1993; Delvolve et al., 1997; Bennett et al., 2001). The most massive trunk muscle, the dorsalis trunci, is activated unilaterally in a craniocaudal sequence during swimming, in accord with a traveling wave of trunk bending, but it is activated synchronously along the trunk during walking and trotting in a pattern that is consistent with the observed standing wave of trunk bending. The oblique hypaxial muscles exhibit activity during both swimming and trotting that fits these patterns (Carrier, 1993; Bennett et al., 2001), but it is not known if they exhibit a traveling wave of activation during swimming.

Some trunk muscles have been found to perform stabilizing functions in addition to lateral bending. The fibers of the oblique hypaxial muscles are oriented such that they can produce long-axis torsion of the trunk or resist torsion that occurs when opposite fore

and hind limbs support the body during trotting. Their activity is timed appropriately to perform this function (Carrier, 1993). No epaxial muscles have been examined for stabilizing functions and these muscles have been predicted to lack such function in amphibians (Ritter, 1995). However, some epaxial muscles, such as the interspinalis (Francis, 1934), are in an ideal position to stabilize the vertebral column and this potential function should be examined.

The locomotor functions of several trunk muscles – the interspinalis, rectus abdominis, subvertebralis, and rectus lateralis – have not been examined electromyographically during trotting and swimming, and their disparate positions within the trunk suggest disparate functions in lateral bending and stabilization against gravitational forces, against locomotor forces, and against forces generated by other trunk muscles.

To advance our knowledge of trunk muscle function in tetrapod locomotion, we examined the electromyographic activity of seven trunk muscles at two sites along the trunk of *Ambystoma maculatum*, during swimming and during trotting in water and on land. By examining changes in activity patterns across two locomotor modes and two environments, we attempted to assess both trunk bending and trunk stabilizing functions of the trunk muscles. The specific aims of this study were to determine (1) which muscles are active during lateral undulation in swimming salamanders; (2) which trunk muscles may play a role in stabilizing the trunk during lateral undulation (e.g. if the rectus abdominis and obliquus muscles are activated inappropriately to produce lateral bending); (3) the possible role of the interspinalis muscle in stabilization of the vertebral column, given its poor mechanical advantage for trunk bending and high percentage of red and intermediate muscle fibers (Schilling and Deban, 2009); and (4) which muscles are active in support of

the trunk against gravitational forces that favor trunk sagging and long-axis torsion.

MATERIALS AND METHODS

Salamanders

Five adult female *Ambystoma maculatum* (Shaw 1802) (82–91 mm snout-vent length; 12–17 g body mass) were purchased from an animal supplier, housed individually in plastic boxes at 22°C on a substrate of moist paper towels, and maintained on a diet of crickets.

Four animals provided data for swimming (nos. 1, 2, 3 and 5) and four provided data for terrestrial and aquatic trotting (nos. 1, 2, 3 and 4). All procedures in this study were approved by the Institutional Animal Care and Use Committee of the University of South Florida.

Anatomy

Activity of seven trunk muscles was recorded during swimming and during terrestrial and aquatic trotting. These muscles have a wide range of orientations, moment arms and cross sectional areas, as evident in the following short descriptions.

The m. interspinalis (IS) is situated dorsally and laterally to the vertebrae (Francis, 1934). The short, monosegmental fiber bundles arise from the posterodorsal edge of the postzygapophysis of one vertebra and insert along the dorsal surfaces of the neural arch of the next caudad vertebra, thus filling the concavity between the zygapophyses and the neural spines (Fig. 1A). Its fibers are also referred to as intervertebral fibers IV-1 and IV-2 (Auffenberg, 1959).

The m. dorsalis trunci (DT) forms the bulk of the epaxial muscle mass and is segmented throughout the trunk by myosepta, which attach to the neural spines and the transverse processes of the vertebrae and follow a peculiar, complex course. Within the muscle and between successive myosepta, the muscle fibers run in a more or less sagittal direction (Francis, 1934). The fibers of the dorsalis trunci muscle are also referred to as intermyoseptal fibers (Auffenberg, 1959).

The subvertebral muscles make up the bulk of the hypaxial muscle mass directly lateral and ventral to the vertebral column (Fig. 1A).

Two to three parts are usually distinguished in salamanders. The pars medialis (Maurer, 1911) (SM) attaches to the lateral aspect of each centrum as well as to the ventral face of the transverse processes. Its fibers run more or less longitudinally [pars subvertebralis according to Francis (Francis, 1934)]. On its lateral edge, it merges, sometimes imperceptibly, with the pars lateralis (Maurer, 1892) (SL), which attaches to the ventral and lateral surfaces of the ribs [pars transversalis according to Francis (Francis, 1934)]. The fiber orientation of pars lateralis is more oblique than that of the pars medialis (Maurer, 1911; Francis, 1934).

The abdominal wall muscles (the muscles ventral to the sulcus lateralis) comprise the rectus and the obliquus groups with fibers running parallel or oblique to the body axis, respectively. Of the rectus group, the m. rectus abdominis trunci (RA) covers the ventral aspect of the abdomen to both sides of the linea alba (Maurer, 1892; Maurer, 1911; Francis, 1934). The two muscle halves extend from the pelvis to the level of the sternal cartilage (pars superficialis) or the hyoid (pars profundus). The superficial part, from which we recorded (Fig. 1A), is organized in metamers along its whole length because of the inscriptions tendinae (septa intermuscular), which attach to the skin. The fibers of the m. rectus abdominis run longitudinally.

The m. rectus lateralis (RL) extends along the whole trunk and is situated ventrally to the sulcus lateralis and superficially to the other hypaxial abdominal wall muscles (Maurer, 1911). As the m. rectus abdominis, the m. rectus lateralis is metameric because of the intermuscular septa that attach to the skin.

The lateral abdominal wall consists of four different layers (superficial to profound: mm. obliquus externus superficialis et profundus, m. obliquus internus, m. transversus) (Fig. 1A). In this study, we recorded from the m. obliquus internus (OI), in which the fibers run in a dorsocaudal direction between the intermuscular septa (Maurer, 1892).

Electromyography

Bipolar hook and patch electrodes were constructed from formvar-coated, 0.025 mm diameter nichrome wire (A-M Systems #7615,

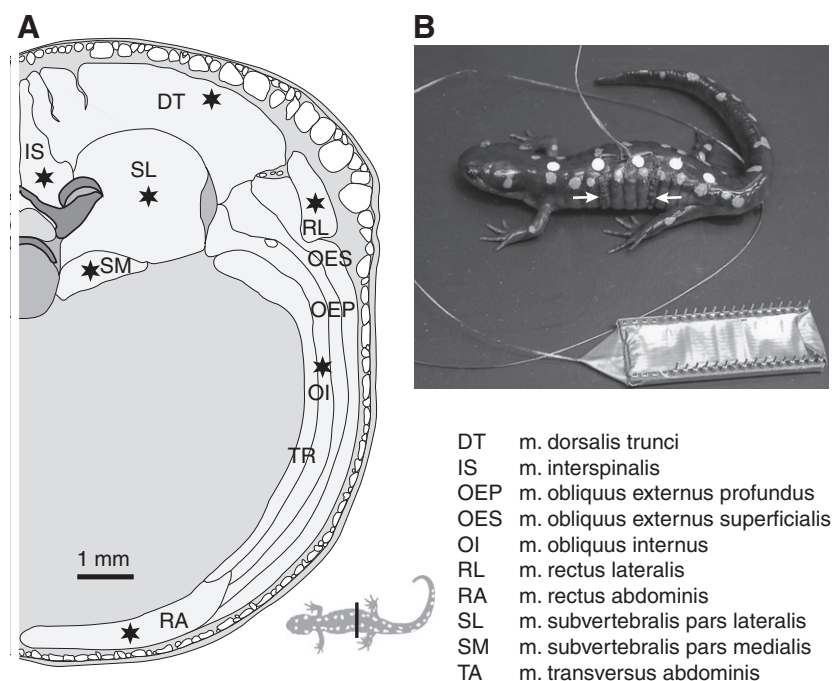


Fig. 1. (A) Topography of trunk muscles of *Ambystoma maculatum* in cross section at the indicated longitudinal position. Note the large cross-sectional area of the dorsalis trunci (DT) and subvertebralis lateralis (SL) compared with the abdominal wall muscles, and the position of the interspinalis (IS). Stars indicate the positions of recording electrodes. Abbreviations are the same as in the text. (B) Experimental animal, showing dorsal markers, positions of trunk segments with electrodes (arrows), and the electrode wires with connector.

Sequim, WA, USA). Electrodes were made of two strands of wire approximately 80 cm long, glued together along their length. Hook electrodes were made by threading the wire through a 30 gauge hypodermic needle, removing the insulation from the tips of the wires and bending the two strands away from each other at the ends. Patch electrodes were made by separating the first few centimeters of wire and threading them individually through a square of silicone rubber sheeting. Insulation was removed from both wires on the inner surface of the electrode. Dipole orientation of the patch electrodes was parallel to the muscle fibers upon implantation, and the silicone provided shielding from neighboring muscles.

Before electrode implantation, salamanders were anesthetized by immersion in a buffered 2% aqueous solution of MS-222 (3-aminobenzoic acid ethyl ester; Sigma, St Louis, MO, USA) for 10–30 min. For surgery each salamander was positioned on its right side on moist paper towels on the stage of a dissecting microscope (Wild Heerbrugg M5 Stereomicroscope, Heerbrugg, Switzerland). Electrodes were implanted through two incisions in the skin of left side of the trunk, at the fourth and eighth trunk segments (i.e. muscle segment between the myosepta corresponding to costal grooves 4 and 5 and grooves 7 and 8; Fig. 1B). Hook electrodes were inserted into the m. interspinalis (IS), m. subvertebralis lateralis (SL) and the m. subvertebralis medialis (SM) with hypodermic needles, and the needles were withdrawn leaving the wire held in place by the hooks of the electrode tip. Patch electrodes were placed against the superficial surface of the m. dorsalis trunci (DT), the m. obliquus internus (OI), the m. rectus lateralis (RL) and the m. rectus abdominis (RA). Patches were held in place by overlying muscles (e.g. the m. obliquus externus in the case of the OI) and skin. Incisions were closed with polypropylene monofilament sutures, and electrode wires exited at the dorsal end of each incision. Electrode wires from both recording sites were bundled together, sutured to the skin at the dorsal midline and glued with modeling cement along their length. The ends of the wires were stripped and soldered to a 40-pin IC plug (Fig. 1B), which fitted into an IC socket on the amplifier probe. White markers (3 mm round disks of DuPont Tyvek) were glued to the dorsal midline with surgical adhesive (Fig. 1B). A marker positioned over each limb girdle and one positioned midway between them (at costal groove seven) were used in measurements of lateral trunk bending.

Electromyographic (EMG) signals were amplified 2000–10,000 times and filtered to remove 60 Hz line noise using a 16-channel differential amplifier (A-M Systems 3500). Amplified signals were sampled at 4 kHz with a PowerLab 16/30 analog to digital converter coupled to Chart software (AD Instruments, New South Wales, Australia) running on an iMac G5 computer (Apple, Cupertino, CA, USA). EMG recordings were synchronized with digital images *via* a trigger shared with the camera. Signals were obtained in at least one individual at all 14 recording sites and in four individuals for most of the sites.

Locomotor experiments

After recovery from surgery (12–24 h), salamanders were imaged (1024 × 1024 pixels, 125 Hz frame rate) in dorsal view with a Photron Fastcam 1024 PCI as they swam or trotted across the bottom of a plastic tray (91 × 62 cm) at approximately 22°C (water and air temperature). Terrestrial trotting was performed on the rough plastic surface of the tray which provided traction and which was kept moist to prevent the salamander from sticking. Aquatic trotting was performed on the same surface in approximately 0.5 cm of water such that the surface of the water was at mid-trunk height. Deeper water was found to elicit swimming instead of trotting.

Manipulating the locomotor environment in which trotting was performed was intended to alter the gravitational forces acting on the trunk directly but also by the limbs *via* the limb girdles. These forces were estimated to be reduced by approximately 50% when the salamanders trot in water *versus* on land because the trunk was half submerged. Specifically, we expected that the trunk experiences smaller ground reaction forces during limb support and hence less long-axis torsion in water than on land, and that the trunk is buoyed in water and therefore experiences less sagittal sagging. In addition to decreasing gravitational forces, the aquatic environment increased drag forces and thereby resisted limb movements and trunk bending movements as well as forward progression of the salamander. EMG recordings of the trunk muscles in both conditions was expected to provide an indication of how the trunk muscles responded to changes in these forces.

Swimming was performed in 2–3 cm of water. Examining swimming enabled us to assess the role of the trunk muscles in producing lateral bending of the trunk in the absence of propulsive forces generated by the limbs, which remain pressed against the sides of the trunk.

Approximately 175 bouts of locomotion were recorded from which locomotor cycles were chosen for analysis. Of the variety of locomotor modes the salamanders employed (walking, swimming, paddling and trotting), only swimming and trotting in a straight line and at a steady speed were analyzed for footfall, trunk-bending and electromyographic patterns. Swimming was identified as undulatory movement with the limbs pressed against the sides of the body, and trotting was identified as a symmetrical gait in which a forelimb and the contralateral hindlimb were moved in phase with one another and out of phase with the other diagonal pair of limbs. During swimming, the trunk exhibited a traveling wave of lateral bending and during trotting it exhibited a standing wave of bending. Locomotor bouts were selected in which trunk bending frequencies for each individual were confined to a narrow range (within 0.2 Hz) for both aquatic trotting and terrestrial trotting (1.4 ± 0.2 Hz overall) to provide a basis for comparison of footfall patterns, trunk-bending amplitudes, and EMG timing and integrated area measurements. Likewise, all swimming bouts analyzed were confined to a trunk bending frequency within a narrow range (4.3 ± 0.3 Hz). All recording of locomotion was performed in 1 day, after which salamanders were killed and electrode placement was confirmed by dissection.

Kinematic analyses

To quantify the timing and amplitude of overall lateral trunk bending during locomotion and to provide sampling windows for the EMG signals, the positions (*x*, *y* coordinates) of the mid-dorsal markers were recorded from each image of the locomotor sequences. The angle formed on the left side of the body by the markers, ipsilateral to electrode implantation, was computed trigonometrically from marker positions and used to provide a measure of overall trunk bending. Locomotor cycles were defined from trunk bending, with 0% and 100% representing maximum bending of the trunk to the side of EMG electrodes (i.e. maximum left-side segment shortening) as defined by the minimum value of this angle within the cycle (Fig. 2). The time of maximum extension of the left side of the trunk, as defined by the maximum value of this angle, was calculated for terrestrial and aquatic trotting as a proportion of the cycle (e.g. 47.5%). Amplitude (deg.) of bending was calculated for each cycle by subtracting the minimum angle by the mean of the two maxima (one at 0% of the locomotor cycle and the other at 100%).

Duty factors for each limb were obtained from 10 locomotor cycles from both aquatic and terrestrial trotting for each individual,

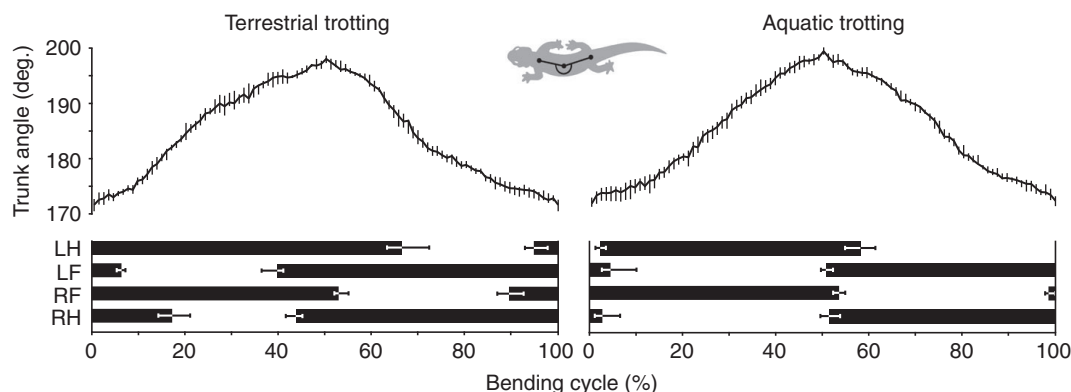


Fig. 2. Representative trunk bending (above) and footfall patterns (below) from 10 strides from a single individual trotting on land and in water. The trunk bending traces show median trunk angle ipsilateral to the electrodes. Note the similar degrees of maximum trunk bending. In order to examine multiple strides of varying length, increasing and decreasing phases of bending were time-normalized separately so that maximum bending occurred at 50% of the locomotor cycle. Quartiles are shown as vertical lines. The footfall patterns show median values of touchdown and liftoff events (ends of thick black bars) for each foot as a percentage of the locomotor cycle. Bars thus represent stance. Upper and lower quartiles of these events are shown as error bars. Note the shorter stance phase in aquatic trotting, which is produced by earlier liftoff and later touchdown than in terrestrial trotting, as well as the shorter overlapping support phases.

sampled from the same trials from which EMG data were sampled. The times of touchdown and liftoff for each limb were recorded for each stride from the image sequences. Touchdown was defined as the first contact of the foot with the substrate at the beginning of stance (hard contact), and liftoff was defined as the last contact of the foot at the end of stance. Footfall timing events (in frames) were converted to a percentage of the locomotor cycle (Fig. 2) and duty factor was calculated as the percentage of the locomotor cycle that the foot was in contact with the substrate.

Average forward velocity of the salamander was calculated for each locomotor bout used in the kinematic and EMG analyses by dividing the total displacement of the anterior marker by the duration of the bout.

Analysis of electromyograms

Relationships between trunk bending and muscle activity were examined by generating average EMGs for each muscle from 16–20 trunk-bending cycles for each animal for each locomotor condition (swimming, terrestrial trotting and aquatic trotting). Using a custom program (LabVIEW, National Instruments, Austin, TX, USA), EMG signals were rectified and averages were generated from a sampling window that extended from 0% to 100% of the trunk-bending cycle. The sampling window was identified as successive maxima in the synchronized trace of trunk angle (Fig. 2) and varied slightly in duration and hence in the number of data points. Therefore each EMG sample was duration-normalized (Carrier et al., 2008) to generate a new sample with 120 bins regardless of original length. All points from the original sample were partitioned into these 120 bins. For example, the first of the 120 bins contained the sum of the point values from the first 120th of the locomotor cycle. Likewise, the second bin contained the sum of the point values from the second 120th of the cycle, and so on. EMG averages were then generated by averaging the value for each of the 120 bins across the 16–20 cycles sampled for a given muscle. The averaging process facilitates comparisons of locomotor cycles of different duration.

To illustrate the effects of environment (aquatic *versus* terrestrial) on the EMG patterns, EMG averages were first amplitude-normalized across individuals. The amplitude of each of the 120 bins of the EMG average from a given muscle were normalized across salamanders. This was accomplished by first computing the mean value of all 120

bins from a given muscle for terrestrial trotting in each salamander. The value of each bin for both terrestrial and aquatic trotting was then divided by this mean value. Hence, the amplitude of each of the 120 bins was normalized to the mean amplitude of that muscle during terrestrial trotting. Once bin amplitudes for each individual were normalized, an average bin amplitude was generated across all individuals. By normalizing amplitudes for each salamander before averaging across salamanders, the pattern from one salamander did not overwhelm the pattern from another should signal strength vary among electrodes.

To examine the effect of environment on muscle recruitment, the integrated area of the calibrated, rectified EMG (not amplitude-normalized) was generated for each muscle by summing the 120 bins for each sample (i.e. for each trunk-bending cycle). A single value for EMG integrated area of a given muscle for each individual was thus obtained for each locomotor cycle.

Statistical analyses

ANOVA was conducted to examine the effects of environment (terrestrial *versus* aquatic) on several variables: EMG integrated area for each muscle, average forward velocity, duty factor for each limb, trunk-bending amplitude and frequency, and time of maximum ipsilateral trunk extension. Four separate one-way ANOVAs were conducted (Table 1), one for each individual. A simultaneous Bonferroni correction for experiment-wide error adjusted the significance level from $\alpha \leq 0.05$ (to values ranging from 0.0022 to 0.0024) because 21–23 comparisons were performed, depending on the number of muscles compared. Two separate two-way ANOVAs were conducted, one on kinematic variables (Table 2) and another on EMG integrated areas, to examine the effects of individual and environment; both included all individuals. Because the ANOVA revealed a significant effect of individual on all EMG areas ($P < 0.0001$), we examined the effect of environment by computing the ratio of EMG integrated area in aquatic trotting to the integrated area in terrestrial trotting for each recording site in each individual. The mean and 95% confidence intervals were then generated for each recording site across individuals and compared with the hypothesized ratio of 1.0 signifying no effect of environment (Table 3). Statistical analyses were performed on an Apple iMac computer using JMP 5.1 software (SAS Institute).

Table 1. Means \pm standard errors of kinematic variables and electromyographic (EMG) integrated areas for each experimental animal during terrestrial and aquatic trotting, and results of one-way ANOVA testing of all variables for significant effects of environment

	Animal no. 1		Animal no. 2		Animal no. 3		Animal no. 4	
	Terrestrial	Aquatic	Terrestrial	Aquatic	Terrestrial	Aquatic	Terrestrial	Aquatic
Trunk bending								
Frequency (Hz)	1.7 \pm 0.1	1.9 \pm 0.2	1.1 \pm 0.0	1.0 \pm 0.1	1.6 \pm 0.1	1.4 \pm 0.1	1.2 \pm 0.1	1.3 \pm 0.0
Amplitude (deg.)	37.0 \pm 1.7	39.6 \pm 1.9	21.0 \pm 0.8	19.5 \pm 0.8	23.2 \pm 0.9	25.3 \pm 0.8	26.6 \pm 0.6	27.4 \pm 0.8
Max. extension (%)	45.4 \pm 3.1	48.2 \pm 1.0	45.5 \pm 1.8	49.2 \pm 1.0	46.5 \pm 1.6	48.9 \pm 1.5	52.7 \pm 1.4	49.0 \pm 0.7
Footfall timing (%)								
LH duty factor	61.3 \pm 1.9	44.1 \pm 3.0*	57.4 \pm 3.0	65.8 \pm 2.3	64.9 \pm 2.1	55.1 \pm 2.6	71.8 \pm 2.3	56.6 \pm 2.1*
LH liftoff	62.1 \pm 1.9	46.7 \pm 2.9*	71.1 \pm 1.5	67.4 \pm 3.2	59.1 \pm 1.4	56.3 \pm 2.3	67.7 \pm 2.2	59.3 \pm 1.9*
LF duty factor	59.9 \pm 1.9	43.8 \pm 2.0*	61.4 \pm 1.7	67.5 \pm 1.6	55.6 \pm 2.7	52.0 \pm 1.3	64.9 \pm 1.5	55.6 \pm 1.3*
LF liftoff	3.0 \pm 1.5	2.7 \pm 1.6*	15.1 \pm 2.0	13.4 \pm 2.2	1.5 \pm 1.8	0.2 \pm 1.0	6.1 \pm 1.1	5.6 \pm 1.6
RF duty factor	61.3 \pm 2.2	38.6 \pm 1.5*	63.7 \pm 1.5	66.5 \pm 1.4	60.0 \pm 1.8	50.1 \pm 1.3	65.1 \pm 1.8	54.8 \pm 0.9*
RF liftoff	54.0 \pm 2.6	47.0 \pm 1.4*	66.5 \pm 1.1	65.0 \pm 1.5	51.5 \pm 1.4	51.2 \pm 0.8	54.5 \pm 1.3	53.1 \pm 0.6
RH duty factor	63.0 \pm 1.0	43.8 \pm 2.1*	58.2 \pm 1.2	59.2 \pm 1.3	63.2 \pm 6.3	57.9 \pm 1.8*	73.0 \pm 1.1	54.0 \pm 1.8*
RH liftoff	13.4 \pm 1.4	2.4 \pm 1.4*	15.5 \pm 1.9	14.6 \pm 1.8	9.8 \pm 1.5	9.6 \pm 1.6	17.5 \pm 1.4	4.1 \pm 1.5*
EMG area (mV s⁻¹)								
Cranial site								
IS			0.6 \pm 0.1	0.4 \pm 0.1	13.8 \pm 0.4	12.3 \pm 0.4	0.9 \pm 0.0	0.3 \pm 0.0*
DT	0.5 \pm 0.1	0.4 \pm 0.1	10.1 \pm 1.4	3.9 \pm 0.8*	1.9 \pm 0.2	2.0 \pm 0.7	2.3 \pm 0.2	1.4 \pm 0.1*
SL	1.1 \pm 0.2	0.8 \pm 0.1	1.2 \pm 0.1	0.6 \pm 0.1*			0.0 \pm 0.0	0.0 \pm 0.0
SM	1.0 \pm 0.2	0.8 \pm 0.1						
RL			8.8 \pm 0.4	4.7 \pm 0.4*	3.2 \pm 0.2	1.1 \pm 0.1*	4.2 \pm 0.2	2.9 \pm 0.1*
OI	1.0 \pm 0.1	0.1 \pm 0.0*	8.9 \pm 0.5	4.3 \pm 0.3*	4.0 \pm 0.3	2.3 \pm 0.4*	0.1 \pm 0.0	0.0 \pm 0.0
RA	2.0 \pm 0.3	2.6 \pm 0.5	16.0 \pm 1.0	7.8 \pm 0.6*			1.1 \pm 0.1	1.6 \pm 0.1*
Caudal site								
DT	12.0 \pm 0.9	13.3 \pm 1.0	6.2 \pm 0.8	2.0 \pm 0.4*	3.2 \pm 0.3	2.3 \pm 0.2	0.5 \pm 0.0	0.2 \pm 0.0*
SL	1.0 \pm 0.2	1.2 \pm 0.2	0.1 \pm 0.0	0.0 \pm 0.0	4.8 \pm 0.4	1.7 \pm 0.2*	3.0 \pm 0.2	0.9 \pm 0.0*
SM	2.6 \pm 0.6	2.5 \pm 0.4					0.8 \pm 0.1	0.3 \pm 0.0*
RL	23.6 \pm 3.8	18.0 \pm 2.7	24.1 \pm 1.0	9.8 \pm 0.8*	2.5 \pm 0.2	0.6 \pm 0.1*	1.5 \pm 0.2	0.4 \pm 0.0*
OI	0.1 \pm 0.0	0.2 \pm 0.0	4.0 \pm 0.5	1.1 \pm 0.1*	3.9 \pm 0.3	1.9 \pm 0.1*	1.1 \pm 0.1	0.2 \pm 0.0*
RA			6.8 \pm 0.4	3.1 \pm 0.4*	4.1 \pm 0.3	0.9 \pm 0.1*	4.4 \pm 0.2	2.7 \pm 0.1*

*Significant difference between terrestrial and aquatic trotting for this animal in one-way ANOVA at Bonferroni-corrected α (0.0022–0.0024) indicating significant effect of environment.

Maximum extension is the percentage of the locomotor cycle at which maximum extension of the ipsilateral (left) side of the trunk occurs, about midway through the locomotor cycle. Similarly, duty factor is the duration of stance phase as a percentage of the locomotor cycle, and liftoff is the time of the beginning of swing phase as a percentage of the locomotor cycle. Muscle abbreviations are provided in Fig. 1.

RESULTS

Swimming

Salamanders swam using lateral undulation of the trunk in a posteriorly traveling wave that continued onto the tail. Forward velocity of the salamanders was $20.5 \pm 1.0 \text{ cm s}^{-1}$ (mean \pm s.e.m.) and undulatory frequency was $4.3 \pm 0.3 \text{ Hz}$.

All recorded muscles showed a mean activation pattern with one primary burst. The primary burst of each muscle, except the IS, was approximately one third of the locomotor cycle in duration, and began at approximately one quarter of the locomotor cycle, during lengthening of the ipsilateral muscle segments (Fig. 3). The IS showed a longer burst of over one half of the locomotor cycle and was activated out of phase with the other muscles, beginning earlier in the cycle near the time of minimum segment length (Fig. 3).

EMG bursts at the cranial recording site occurred earlier in the locomotor cycle than those at the caudal site for the respective muscles. The IS, OI and RA also showed a much smaller secondary burst occurring one-half cycle out of phase with the primary burst.

Trotting

Salamanders trotted using lateral undulation of the trunk in a standing wave. Forward velocity was significantly higher ($P < 0.0001$) during aquatic trotting than during terrestrial trotting ($12.8 \pm 1.9 \text{ cm s}^{-1}$ versus $7.6 \pm 0.8 \text{ cm s}^{-1}$) despite the similar bending frequency

($1.4 \pm 0.2 \text{ Hz}$), similar bending amplitude (27.9 ± 1.3 versus 26.9 ± 1.1 deg.) and similar time in the bending cycle of maximum trunk bending (48.8 ± 0.5 versus $47.5 \pm 1.1\%$). Duty factor (averaged for all four limbs) in aquatic trotting was significantly lower ($P < 0.0001$) than during terrestrial trotting (54.1 ± 1.6 versus $62.8 \pm 1.1\%$), and the same pattern held for duty factor of individual limbs (Fig. 2, Tables 1 and 2). Individual salamanders showed significant differences in bending amplitude and in duty factors of individual limbs ($P < 0.0045$), but trunk bending frequency, time of maximum trunk bending, and limb liftoff times showed no significant effect of individual in the ANOVA (Table 2).

During terrestrial trotting, trunk muscles were active for most of the locomotor cycle, typically showing reduced activity near the time of minimum segment length. Activation was more spread out and showed more diffuse bursts than during swimming and did not show periods of silence as during swimming (Fig. 4).

In aquatic trotting the position of peak activation within the locomotor cycle was similar to that in terrestrial trotting (Figs 5 and 6). However, bursts were more concentrated in periods of segment shortening and were more distinct from periods of low or no activity. Several muscles showed inactivity near the time of minimum segment length (DT, SL, SM, RL and RA). Eight of the 13 recording sites showed significantly lower integrated EMG area in aquatic trotting compared with terrestrial trotting (Tables 1 and 3). Individual

Table 2. Means \pm standard errors of kinematic variables during terrestrial and aquatic trotting with results of ANOVA testing for effect of environment and individual

	Terrestrial	Aquatic
Trunk bending		
Frequency (Hz)	1.4 \pm 0.2	1.4 \pm 0.2
Amplitude (deg.)	26.9 \pm 1.1	27.9 \pm 1.28 [†]
Max. extension (%)	47.5 \pm 1.1	48.8 \pm 0.53
Footfall timing (%)		
LH duty factor	63.8 \pm 1.4	55.4 \pm 1.7* [†]
LH liftoff	65.0 \pm 1.3	57.4 \pm 1.7*
LF duty factor	60.5 \pm 1.1	54.7 \pm 1.6* [†]
LF liftoff	6.4 \pm 1.2	4.1 \pm 1.3
RF duty factor	62.5 \pm 0.9	52.7 \pm 1.7* [†]
RF liftoff	56.6 \pm 1.2	54.1 \pm 1.2
RH duty factor	64.4 \pm 1.0	53.7 \pm 1.3* [†]
RH liftoff	14.1 \pm 0.9	7.7 \pm 1.1*

*Significant difference in ANOVA at Bonferroni-corrected $\alpha=0.0045$, indicating significant effect of environment.

[†]Significant effect of individual at $\alpha=0.0045$.

N=4 salamanders. Explanation of variables is given in Table 1.

Table 3. Mean integrated EMG area for aquatic trotting *versus* terrestrial trotting, with upper and lower 95% confidence intervals of each mean

	N	Mean	Upper CI	Lower CI
Cranial recording site				
IS	3	0.62*	0.96	0.28
DT	4	0.73	1.02	0.44
SVL	3	0.54*	0.78	0.31
SVV	1	0.85		
RL	3	0.52*	0.72	0.32
OI	4	0.38*	0.60	0.16
RA	3	1.10	1.71	0.49
Caudal recording site				
DT	4	0.63*	1.00	0.26
SVL	4	0.51*	0.95	0.06
SVV	2	0.66	1.23	0.09
RL	4	0.41*	0.65	0.18
OI	4	0.66	1.36	0.04
RA	3	0.43*	0.66	0.20

*Significantly different than hypothesized mean of 1.0, indicating effect of environment.

N, number of individuals for each comparison.

salamanders showed significant differences in all EMG areas ($P<0.0001$) and varied in which muscles showed a significant difference; however, all but one of the 26 cases of significant differences in EMG area were in the same direction – declining in aquatic trotting compared with terrestrial trotting.

DISCUSSION

Swimming

The observed activation patterns are consistent with the production of a traveling wave of trunk bending during swimming, which has been observed previously in *Ambystoma* and in other salamanders (Frolich and Biewener, 1992; Carrier, 1993; Delvolve et al., 1997; D'Août and Aerts, 1997; Gillis, 1997; O'Reilly et al., 2000; Bennett et al., 2001; Azizi et al., 2002; Azizi and Horton, 2004; Brainerd and Azizi, 2005). Our results reveal that all trunk muscles examined with the exception of the IS are activated nearly simultaneously, peaking in phase with one another just before maximum segment

length (i.e. at the end of segment lengthening), in a pattern that can produce lateral bending.

The activation of the more posterior trunk segment later in the locomotor cycle than the anterior segment is consistent with the trunk muscles contributing to a traveling wave of bending (Frolich and Biewener, 1992; Carrier, 1993). At the average 4.3 Hz undulation frequency of the swimming trials, the locomotor cycle lasts 233 ms. The trunk muscles become activated approximately one-quarter cycle before the start of ipsilateral bending (Fig. 3), which corresponds to approximately 58 ms between the start of muscle activity and the start of trunk bending. This pattern of activation before muscle segment shortening has previously been found in *Ambystoma* and several species of fish during undulatory swimming (Frolich and Biewener, 1992; Johnson et al., 1994; Jayne and Lauder, 1995; Coughlin and Rome, 1999). The duration of offset in timing that we found in *Ambystoma maculatum* is within the range found in the dorsalis trunci of *Ambystoma tigrinum* [45–135 ms (Frolich and Biewener, 1992)] and within the range of times to achieve peak tension of 56–66 ms found in *in vitro* preparations of a limb muscle of *Ambystoma tigrinum* (Else and Bennett, 1987); this offset duration is also similar to the time to peak tension of 43 ms found for red muscle in *Micropterus* (Johnson et al., 1994). Given similar time to develop tension in the trunk muscles examined here, these muscles are likely to be generating force mainly during shortening and would thus be doing positive work at these segments.

The DT has the largest anatomical cross-sectional area (a-csa) of the trunk muscles (Fig. 1A) and its anatomical position, lateral to the vertebral column, together with its activation pattern can produce lateral bending during swimming, as has been shown previously in salamanders (Frolich and Biewener, 1992; Delvolve et al., 1997). Because it is located dorsal to the spine, activity of the DT would also cause sagittal extension. Therefore, when activated, the DT is expected to extend the trunk dorsolaterally. The SL also has a relatively large a-csa and is in a position to produce nearly pure lateral bending with almost no sagittal component. The SM has a much smaller a-csa and poorer mechanical advantage than the DT and SL, attaching to the ventral aspect of the vertebral bodies, but when activated it can be expected to produce lateral bending combined with sagittal flexion. Its sagittal component could counteract the extensor moment of the DT. The combined action of these three muscles (DT, SM and SL), based on their anatomical positions and their complete coactivation during swimming, would be mainly lateral bending with perhaps some sagittal extension if they were fully recruited.

The RA has relatively poor leverage for producing or resisting lateral bending given its position near the ventral midline, but this location gives it good leverage to flex the trunk in the sagittal plane. This muscle, activated in phase with the major trunk muscles, may provide a trunk flexor moment that could counteract a combined extensor moment of the DT and SL. Its smaller secondary burst may counterbalance the extensor moment produced by the synchronous activity of the contralateral dorsal muscle mass. The RL has a small a-csa compared to the other trunk muscles but has an excellent moment arm for producing lateral bending. Its coactivity with the above muscles, during the last two thirds of the bending cycle, is consistent with this function.

The OI is a thin sheet of muscle fibers with an oblique orientation that can produce lateral bending and long-axis torsion of the trunk (Carrier, 1993; Bennett et al., 2001; Brainerd and Azizi, 2005) as well as some sagittal flexion. The main burst of activity of the OI is in phase with the above muscles and is likely

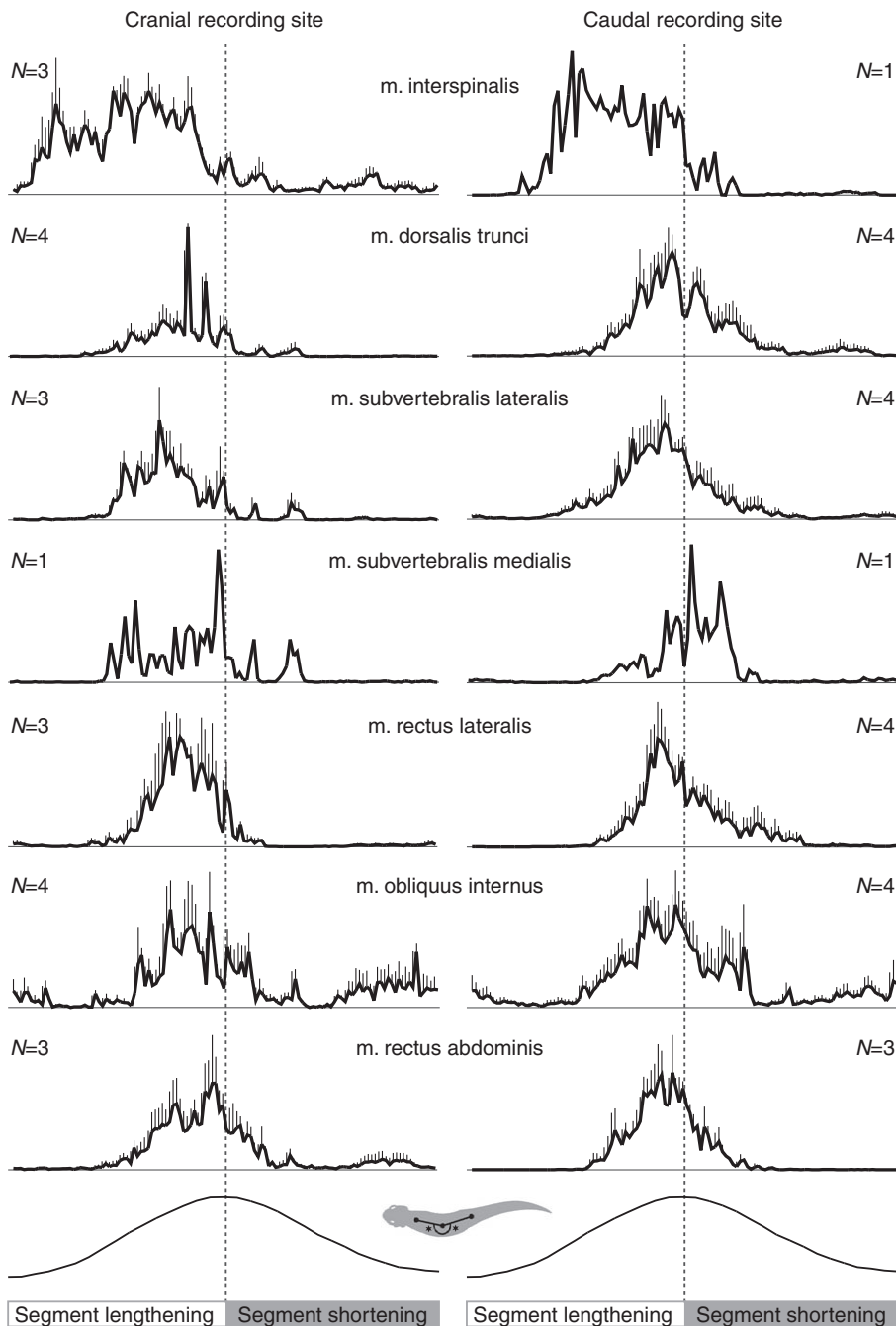


Fig. 3. Average electromyographic signals (EMGs) obtained during swimming in *Ambystoma maculatum* from seven trunk muscles at two recording sites. Note the nearly synchronous bursts from all muscles except the interspinalis, and the secondary bursts in the obliquus internus and rectus abdominis. Error bars are standard errors of means (means of 16–20 locomotor cycles from each individual, in turn averaged across five individuals; see Materials and methods for details). Amplitude is normalized to the maximum amplitude for each electrode; therefore, each trace has a different vertical scale. Numbers of individual animals are shown for each average. Bending at mid-trunk is shown at the bottom, with minimum values representing maximum shortening of muscle segments on the side ipsilateral to the electrodes.

to be contributing to lateral bending. The second smaller burst is out of phase with the main burst and may produce sagittal flexion forces that balance the extensor moment of the contralateral DT and SL. The bilateral activity of the OI, which has shallow muscle fiber angles (Simons and Brainerd, 1999), may also hydrostatically shorten the abdominal region of the trunk, causing sagittal flexion (Kier and Smith, 1985). Activity of the OI on one side of the body could also resist or produce long-axis torsion of the trunk, and the latter could be resisted by the activation of the obliquus externus, which has nearly orthogonal fiber orientation (Carrier, 1993).

The IS is positioned dorsal to and near the vertebral column (Fig. 1B) and is thus in a poor position mechanically to produce or resist trunk bending during swimming, but its monosegmental

topography is well suited to ensure the integrity of the spine and thus resist vertebral dislocation that could result from contralateral trunk bending muscles. Its extended activity during segment lengthening and reduced activity during segment shortening (i.e. absorbing work) is in accord with a local stabilizing role of this kind, as is its fiber insertion directly on the vertebrae (Francis, 1934; Auffenberg, 1959). Our results contrast with previous studies (Frolich and Biewener, 1992) which found no activity in the IS during swimming or trotting.

Trotting

The increase in forward speed during aquatic trotting compared with terrestrial trotting at the same undulation and stride frequency suggests that the salamanders were generating thrust with lateral

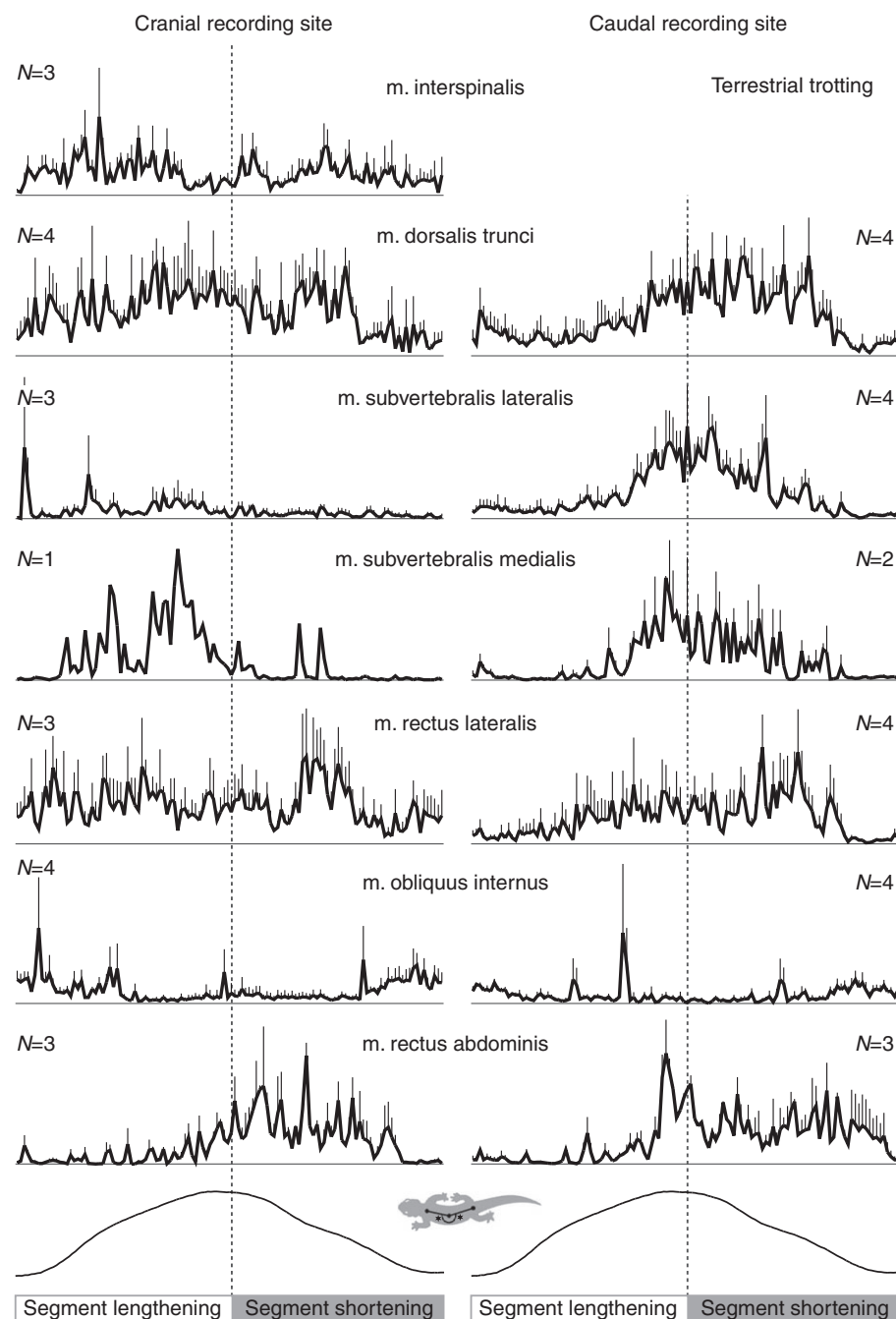


Fig. 4. Average electromyographic signals (EMGs) obtained during terrestrial trotting in *Ambystoma maculatum* from seven trunk muscles at two recording sites. Note the prolonged activity of the interspinalis and dorsalis trunci and the activity of the obliquus internus that is out of phase with the other muscles. Details are the same as in Fig. 3.

undulation, as in swimming, and that their propulsive forces more than compensated for the increase in drag. During aquatic trotting, the trunk exhibits a standing wave, but the tail exhibits a traveling wave that can impart rearward momentum to the water. Another possibility is that in water the salamanders were able to redirect ground reaction forces to aid in propulsion because they required lower perpendicular forces to support their body weight. Either interpretation is consistent with observations of the salamander *Siren*, which propels itself aquatically by a combination of lateral undulation and bipedal stepping (Azizi and Horton, 2004). The decreased duty factor of the limbs in aquatic trotting implies less need for support against gravity and agrees with previous reports in which duty factor decreases during submerged aquatic walking in the newt *Taricha* (Ashley-Ross and Bechtel, 2004; Ashley-Ross

et al., 2009) and in other taxa (Azizi and Horton, 2004). However, without recordings of ground reaction forces we are unable to determine the magnitude of the force impulse that contributes to propulsion.

The observed activation patterns of the trunk muscles during terrestrial and aquatic trotting reveal additional functions for many of the trunk muscles beyond lateral bending. Temporal patterns of activity are similar across environments, but most muscles show a decline in amplitude in aquatic trotting despite no change in bending kinematics. This decline suggests a stabilizing role for these muscles against gravitational forces that produce sagittal sagging and long-axis torsion during diagonal limb support, forces that are higher while trotting on land. Some muscles, such as the DT, are not in a position to work against these gravitational forces but nonetheless change

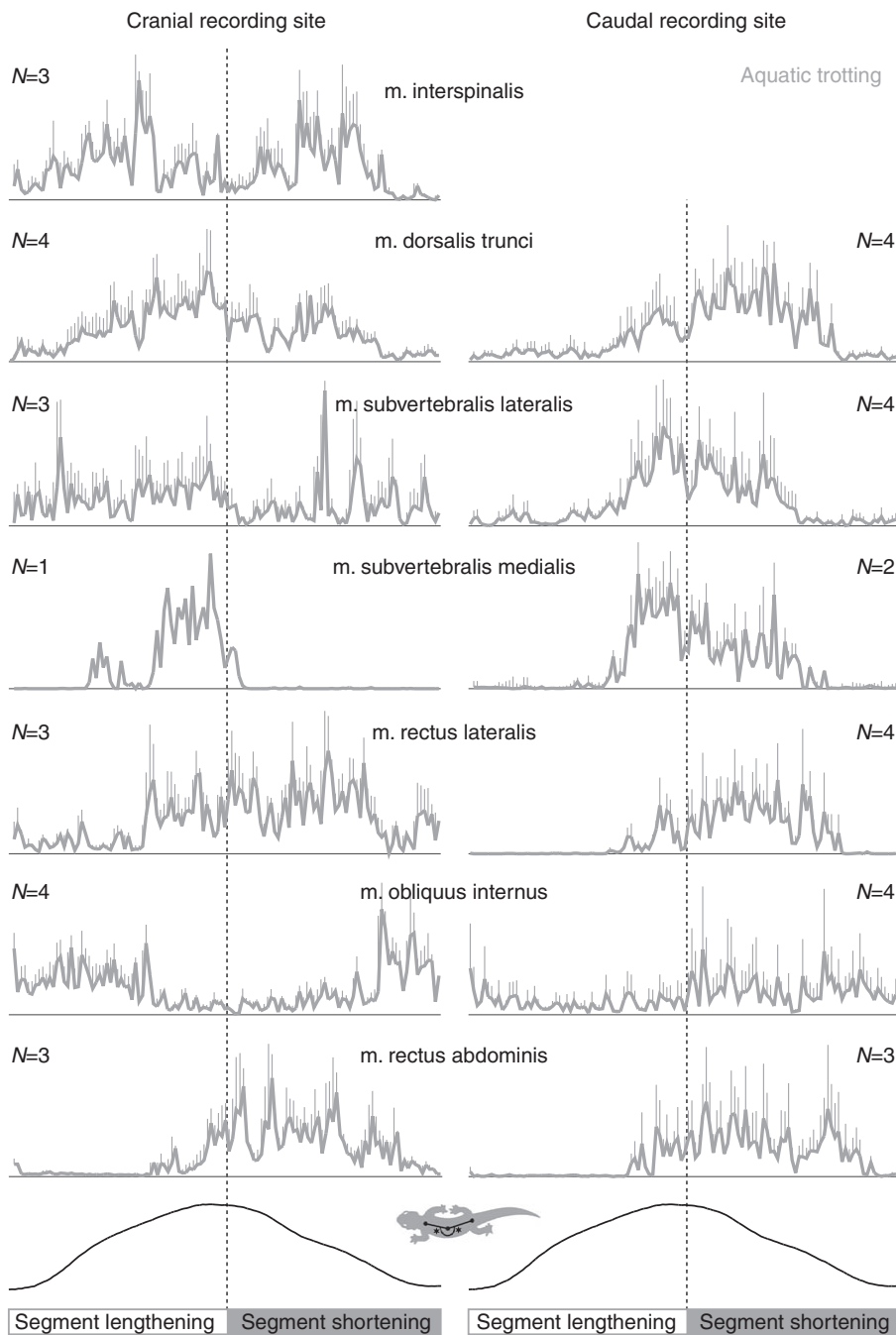


Fig. 5. Average electromyographic signals (EMGs) obtained during aquatic trotting in *Ambystoma maculatum* from seven trunk muscles at two recording sites. As in terrestrial trotting, the prolonged activity of the interspinalis and dorsalis trunci and the activity of the obliquus internus that is out of phase with the other muscles. Details are the same as in Fig. 3.

amplitude of activity across environments, suggesting that they increase activity on land to counteract contralateral antagonists that are activated to stabilize the trunk. This coactivation of antagonists may be required to bend the trunk laterally and at the same time prevent sagging and torsion, and would also increase trunk stiffness.

The OI is active out of phase with all the other trunk muscles examined. This pattern is consistent with its role in counteracting long-axis torsion of the trunk that occurs during the diagonal limb support of the trotting gait, which has been shown in terrestrial trotting in *Dicamptodon* (Carrier, 1993) and *Ambystoma* (Bennett et al., 2001). The dorsocaudal orientation of the OI muscle fibers requires that they be active on the side of the trunk that is lengthening to resist torsion, and therefore their longitudinal force component will also counteract lateral bending (Carrier, 1993). In

terrestrial trotting, long-axis torsion is probably greater than in aquatic trotting and the increase in OI activity we measured should act to resist this.

The activation pattern of the DT suggests a role in lateral bending, given its decreased activity at the time of minimum segment length and its muscle fiber orientation parallel to the long axis of the body (Francis, 1934). Decreased integrated area in aquatic *versus* in terrestrial trotting suggests a role in stabilization against gravitational forces. Furthermore, its fiber orientation is inappropriate for resisting long-axis torsion and its position is poor for resisting sagittal sagging. One explanation for its change in activity across environments is that the DT may act as an antagonist to the contralateral OI, which is coactive. OI activity would counteract long-axis torsion (Carrier, 1993) but also resist lateral bending. In aquatic trotting, torsion and

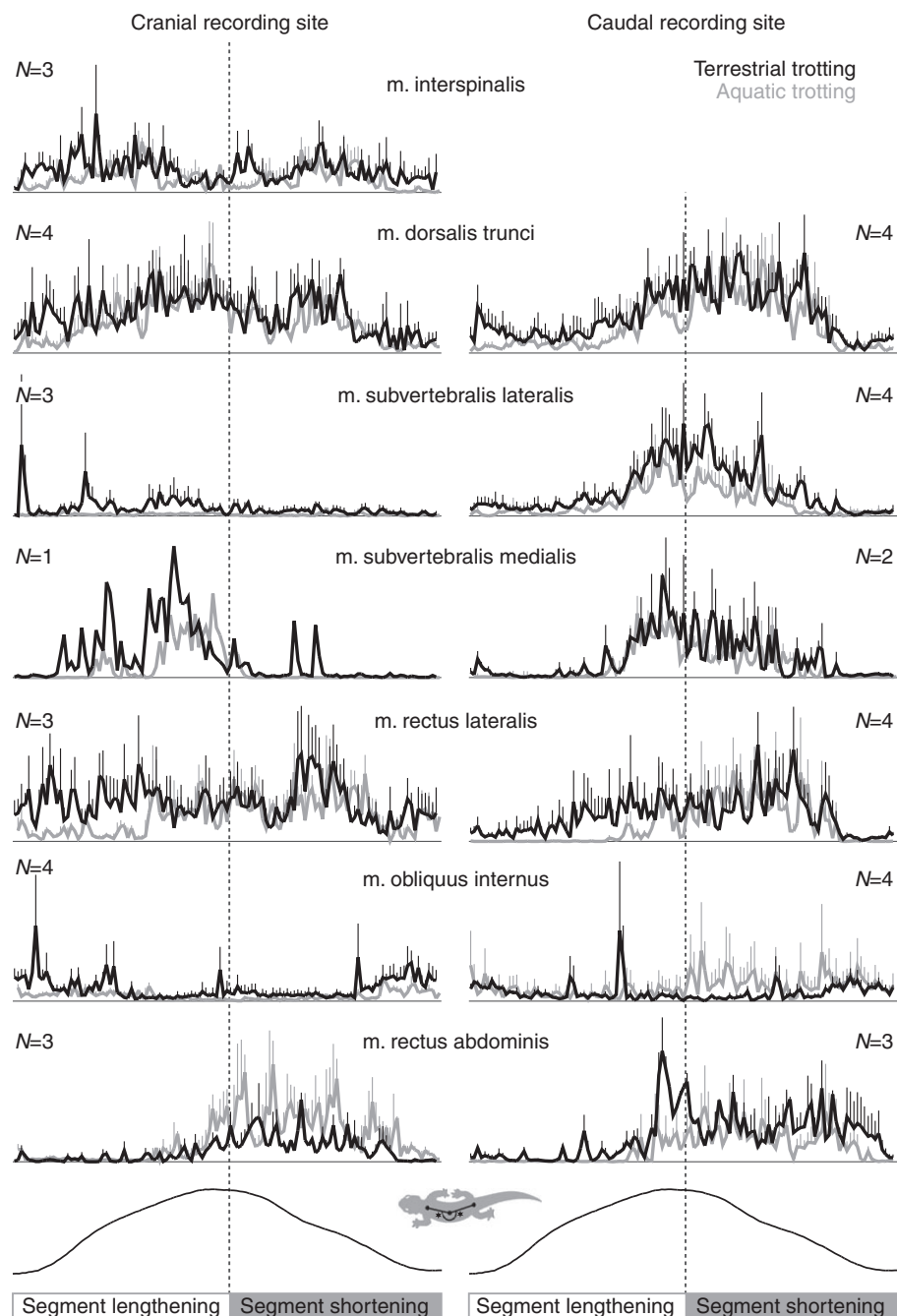


Fig. 6. Average electromyographic signals (EMGs) obtained during both aquatic trotting (gray lines) and terrestrial trotting (black lines) in *Ambystoma maculatum* from seven trunk muscles at two recording sites. Note the decrease in amplitude in aquatic trotting at most sites, and that the temporal patterns of activation are similar in aquatic and terrestrial trotting. Trace amplitudes are normalized to the maximum amplitude for each recording site, separately for aquatic and terrestrial trotting. Details are the same as in Fig. 3.

thus contralateral OI activity are reduced and therefore the force counteracting lateral bending is reduced, and the DT activity that produces a net lateral bending moment can thus be reduced. The activation pattern of the OI is consistent with this proposed functional interaction.

The activity of the SL in trotting indicates that it produces lateral bending and the increase of SL activity in terrestrial trotting compared with aquatic trotting is consistent with its role as an antagonist to the contralateral OI. Similarly, the decreased activity of the laterally positioned RL during aquatic trotting suggests that forces required to bend the trunk laterally are decreased in water, perhaps because of reduced activity of antagonists (and despite an assumed increase in drag). The SM can also serve this lateral bending function, and is also in a position to directly counteract trunk sagging.

Its decline in aquatic trotting in the one individual from which we obtained recordings may indicate this function.

Activity of the RA in aquatic trotting is decreased compared with its activity in terrestrial trotting at the caudal recording site, but the cranial recording site shows the opposite change across environments. These results fail to provide strong support for the hypothesis that this most ventral muscle counteracts sagittal sagging of the trunk, as does the absence of bilateral activity during trotting. Its activity during segment shortening indicates it has roles in both lateral bending despite its poor mechanical advantage for this function, and counteracting the sagittal extension moment of the dorsal muscle mass for which it has good leverage.

As during swimming, the activity of the IS during trotting is distinct. Its activity is tonic or biphasic, suggesting force generation

on both the shortening and lengthening sides of the trunk that stabilizes the intervertebral joints and ensures the structural linking of the vertebrae. Its significant decline in amplitude during aquatic trotting compared with terrestrial trotting (Table 3) is consistent with these muscles playing a stabilizing role against muscular and gravitational forces that diminish in water. A stabilizing function for the epaxial muscles of amphibians has been proposed (O'Reilly et al., 2000) but not previously demonstrated.

Individuals showed significant variation in both kinematic and EMG parameters, suggesting that individuals have different motor control strategies for trotting in a given environment and also differ in their responses to changes in environment. Animal no. 1, for example, showed significant differences in footfall patterns across environments and little change in trunk muscle recruitment, whereas animal no. 2 displayed the reverse pattern, and no. 4 showed changes in both. This variation makes sense when one considers that limb and trunk movements are integrated during trotting and propulsive output can be modulated by limb movements, trunk movements, or both. Trotting locomotion may thus provide more versatility in accommodating changes in environment than purely undulatory locomotion.

Swimming versus trotting

The EMG pattern of most of the trunk muscles is similar in swimming and aquatic trotting, indicating that lateral undulation is well integrated with limb movements and that trunk bending forces are transmitted to the ground through the limbs to propel the salamander. The longer activation period in trotting compared with swimming is probably related to the lower undulation frequency of trotting (1–2 Hz) than of swimming (3–5 Hz). Unlike the other trunk muscles, the OI shifts its activation period 180 deg., a change that is consistent with a change in its role from one of undulating the trunk during swimming to one of resisting long-axis torsion during trotting.

Conclusions

The EMG patterns of *Ambystoma maculatum* presented here reveal that many trunk muscles, both epaxials and hypaxials (DT, SL, SM, RL and OI), contribute to lateral bending during both swimming and trotting. During swimming, both groups of muscles are activated in a traveling wave in discrete bursts that precede the wave of trunk bending, bursts that are separated by periods of electrical silence. The exception to this pattern is the IS, which has a longer period of eccentric activity that indicates stabilization of the intervertebral joints against forces produced by trunk bending. The OI and RA may counteract sagittal extension of the trunk that is produced by the contralateral DT and SL, as revealed by a secondary burst of activity out of phase with the primary burst. The RA may be active solely to prevent sagittal extension.

During trotting, the trunk muscles are activated for a greater proportion of the locomotor cycle and show fewer periods of electrical silence than during swimming, indicating a stabilizing function for many muscles in addition to their function in lateral bending. Activity of the OI out of phase with the other trunk muscles during trotting lends support to previous findings that oblique muscles counteract long-axis torsion of the trunk during trotting (Carrier, 1993; Bennett et al., 2001), as does the increased activity of the OI during terrestrial trotting than during aquatic trotting. This

increase in recruitment was found for several trunk muscles despite similar amplitudes and frequencies of trunk bending, suggesting that the trunk in these salamanders is stiffened during terrestrial trotting and both epaxial and hypaxial muscles assume greater stabilizing function on land, despite conservation of their basic activation pattern during trotting in different environments.

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