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# Patterns of axial and appendicular movements during aquatic walking in the salamander *Siren lacertina*

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## Abstract

Most studies of salamander locomotion have focused either on swimming or terrestrial walking, but some salamanders also use limb-based locomotion while submerged under water (aquatic walking). In this study we used video motion analysis to describe the aquatic walking gait of *Siren lacertina*, an elongate salamander with reduced forelimbs and no hindlimbs. We found that *S. lacertina* uses a bipedal-undulatory gait, which combines alternating use of the forelimbs with a traveling undulatory wave. Each forelimb is in contact with the substrate for about 50% of the stride cycle and forelimbs have little temporal overlap in contact intervals. We quantified the relative timing and frequency of limb and tail movements and found that, unlike the terrestrial gaits of most salamanders, axial and appendicular movements are decoupled during aquatic walking. We found no significant relationship between stride frequency and aquatic walking velocity, but we did find a statistically significant relationship between tailbeat frequency and aquatic walking velocity, which suggests that aquatic walking speed is mainly modulated by axial movements. By comparing axial wavespeed and distance traveled per tailbeat during swimming (forelimbs not used) and aquatic walking (forelimbs used), we found lower wavespeed and greater distance traveled per tailbeat during aquatic walking. These findings suggest that the reduced forelimbs of *S. lacertina* contribute to forward propulsion during aquatic walking.
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## Introduction

Salamander locomotion is commonly characterized by two distinct modes, each associated with one stage of a bi-phasic life-history strategy. Swimming is the primary mode of locomotion in larval life-stages and adults of permanently aquatic species, whereas walking is the primary mode of locomotion in terrestrial forms. Swimming in salamanders consists of traveling waves of lateral undulation, which originate in the anterior portion of the body and generate forward thrust as they travel posteriorly (e.g. Daout and Aerts, 1997; Gillis, 1997). During swimming, all four limbs are held tightly against the body in order to reduce hydrodynamic drag (Delvolve et al., 1997; Bennett et al., 2001). Terrestrial walking in most salamanders consists of synchronous use of diagonal limb pairs, which generate forces against the ground to propel the salamander forward. The appendicular components of terrestrial walking are typically combined with a standing axial wave which functions to increase stride length (Edwards,

1976). Axial bending associated with terrestrial walking generally consists of synchronous muscle activity of one side of the trunk, which generates nodes (areas of zero curvature) at the pelvic and pectoral girdles (Frolich and Biewener, 1992). The current data on the kinematics and muscle activation patterns associated with the two modes of salamander locomotion highlight differences in the relative coordination of the axial and appendicular systems during swimming and terrestrial walking (Carrier, 1993; Bennett et al., 2001).

In addition to swimming and terrestrial walking, some semi-aquatic or fully aquatic species of salamanders also utilize limb-based locomotion against the substrate while submerged underwater (aquatic walking; Brand, 1996; Eubanks et al., 2002; Ashley-Ross and Bechtel, 2003). Interestingly, aquatic walking has recently been proposed as the primitive locomotory function of the tetrapod limb (reviewed in Clack, 2002a). Mounting anatomical evidence now suggests that the earliest tetrapods were primarily aquatic organisms and functional interpretations of the tetrapod limb need to be made within the context of an aquatic environment (Coates and Clack, 1991; Clack, 2002b). However, despite the evolutionary significance of aquatic walking, functional and biomechanical generalities associated with this mode of locomotion remain unclear. In this study, we describe the aquatic walking gait of *Siren lacertina*, an elongate, aquatic salamander with reduced forelimbs and no hindlimbs (Fig. 1). Behavioral observations of this species in the field suggest that aquatic walking is utilized during seasonal migrations and is potentially an important component of the locomotor repertoire of this salamander (Eubanks et al., 2002).

One goal of this study is to quantify the frequency and phase coupling of axial and appendicular movements during aquatic walking in *S. lacertina*. Examination of whether forelimb movements are tightly coupled to axial movements will allow us to test the hypothesis that elongation of the trunk and reduction of the limbs (as observed in *S. lacertina*) lead to greater locomotor reliance on axial movements and ultimately to a decoupling of the axial and appendicular systems (Gans, 1986; Gans and Fusari, 1994; Renous et al., 1995). Based on comparative locomotor studies of lizards with varying amounts of axial elongation and limb reduction, decoupling of locomotor circuits is thought to precede the evolution of limblessness (Gans, 1985).

Another goal of this paper is to assess the validity of the common conception that the reduced forelimbs of *S. lacertina* are ineffectual (Holbrook, 1842). We examine the locomotor contribution of the forelimbs of *S. lacertina* by comparing swimming, in which the forelimbs are held against the body, and aquatic walking, in which the forelimbs are used to push against the substrate. We predict that if the forelimbs are significantly contributing to forward propulsion, the average axial wavespeed used at a given velocity will be lower during aquatic walking. We also predict that if the forelimbs are significantly contributing to forward propulsion, the average distance traveled per tailbeat will be higher during aquatic walking. Finally, we compare the aquatic walking gait of *S. lacertina* to other aquatic walkers in order to develop some functional generalities associated with this mode of locomotion.

## Materials and methods

### Animals

Four adult *S. lacertina* (Linnaeus) were purchased from a commercial herpetological vendor and used for
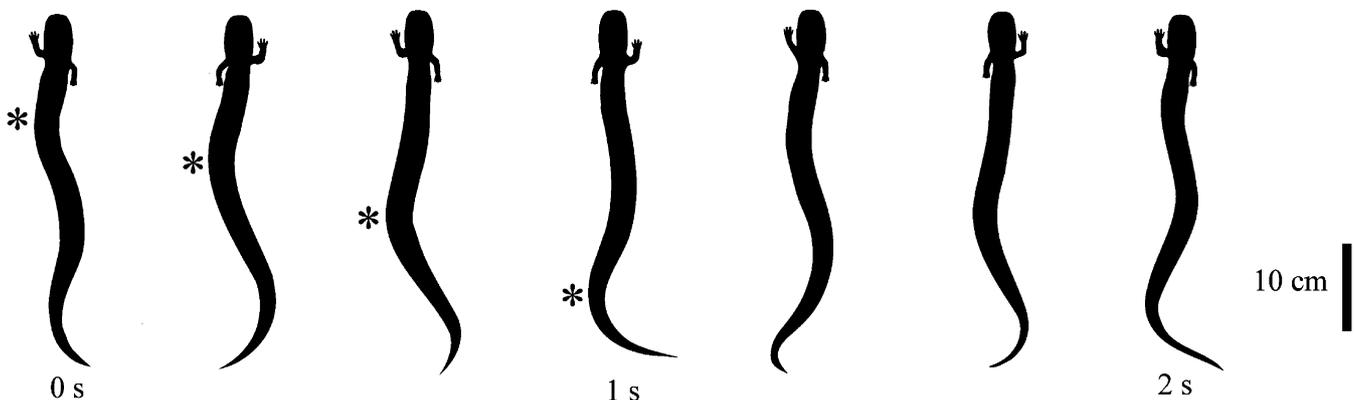


**Fig. 1.** Silhouettes of *S. lacertina* using a bipedal-undulatory gait during aquatic walking. Images are in dorsal view and have been redrawn from a video sequence. Three complete stride cycles are shown. The silhouettes highlight the alternating limb use and axial wave propagation, which are characteristic of a bipedal-undulatory gait. Asterisks highlight the rearward propagation of one undulatory wave. Note: *S. lacertina* lacks hindlimbs.

**Table 1.** Experimental sample sizes and morphometric measurements of salamanders used in the study

| Individual | $n$[a] | Total length (cm) | Snout-vent length (cm) | Limb length (cm) |
|---|---|---|---|---|
| 1 | 18 | 48.5 | 32.4 | 3.8 |
| 2 | 16 | 39.5 | 27.0 | 3.0 |
| 3 | 15 | 42.3 | 31.4 | 3.2 |
| 4 | 16 | 45.5 | 30.0 | 3.6 |

[a] Number of walking sequences analyzed.

this study. All individuals were housed in separate aquatic tanks (51 cm × 26 cm × 32 cm) that were maintained at $22 \pm 1 °C$. The salamanders were fed approximately four earthworms per week. Morphometric measurements were taken while the salamanders were anesthetized with a buffered solution of MS-222 (Tricaine Methanesulfonate) (Table 1). The University of Massachusetts Institutional Animal Care and Use Committee approved all animal care and experimental protocols.

### Data collection

Video was captured in dorsal view as salamanders walked along the bottom of a water-filled trackway (260 cm × 60 cm × 33 cm). This tank was filled to a height of 15 cm with reverse osmosis water which was maintained between 21 °C and 23 °C. Video signals from two Sony Digital Handycam video cameras (models DCR-VX2000 and DCR-VX700) were synchronized using a Panasonic Digital AV Mixer. This process allowed us to capture the total length of the trackway in all video frames. The video output from the mixer was recorded on a Sony digital video deck (model GV-D900). Due to the slow nature of aquatic walking in *S. lacertina*, high-speed video was not necessary; therefore, all video sequences were recorded at 30 frames/s (60 fields/s). Salamanders either walked voluntarily along the bottom of the tank or were stimulated to locomote by touching the tip of the tail.

### Video analysis

Aquatic walking sequences that contained steady bouts of locomotion with a minimum of four complete stride cycles were selected for data analysis. Walking bouts with non-linear paths or potential wall interference were not used. In addition, sequences in which the salamander was not in contact with the substrate were noted during data collection and excluded from our analysis. Between 15 and 18 walking sequences were selected and analyzed for each individual (Table 1). The

selected video clips were uploaded to a Macintosh computer using the application iMovie (v. 3). Selected sequences were then exported to the application Image J (v. 1.29 ×), calibrated spatially using video footage of a ruler and analyzed frame-by-frame to extract kinematic data.

The maximum extension of the left limb was used to define the beginning and end of each stride cycle and the maximum lateral deflection of the tip of the tail (to the left) was used to define the beginning and end of each tailbeat cycle. Stride frequency and tailbeat frequency were calculated for all walking sequences by counting the number of strides and tailbeats and dividing by the duration of the walking bout. Average velocity of a walking sequence was calculated by measuring the distance a digitized landmark at the tip of the snout moved over the duration of the walking bout. Tailbeat distance (previously called stride length in swimming studies; Gillis, 1997) was defined as the average distance the salamander progressed per tailbeat in a locomotor bout. The average wavespeed for each bout was defined as the mean rate of posterior propagation of all the undulatory waves in a given sequence. Wavespeed was measured by tracking a digitized landmark on the crest of each undulatory wave. All time variables used in this study were initially measured in terms of the number of video frames and were subsequently converted to seconds (each frame = 0.033 s).

The footfall patterns of *S. lacertina* were characterized using the contact interval of each limb during aquatic walking in five sequences from each individual. A contact interval was defined as the percentage of the stride cycle that a given limb was in direct contact with the substrate.

### Statistical analysis

All linear regressions were performed using the application JMP (v. 5.0 SAS institute, Cary, NC, USA). A reduced major axis regression (assuming equal variance) was used to quantify the relationship between tailbeat frequency and stride frequency during aquatic walking. Least-squares linear regressions were used to determine how tailbeat and stride frequency varied with increasing velocity.

An analysis of covariance (ANCOVA) was used to compare wavespeed and tailbeat distance during aquatic walking and swimming across a range of velocities. Since the swimming data used in this comparison represented means for multiple individuals, aquatic walking sequences from all individuals were pooled prior to these tests. Tests were performed in the application SuperAnova (v. 1.11) with wavespeed and tailbeat distance as the dependent variables, velocity as the covariate, and locomotor mode as the factor.

Results from these tests initially included the effect of an interaction term, which includes differences in the slopes of regression lines. The interaction terms were not statistically significant and were removed in order to compare the *y*-intercepts of the regression lines.

A Rayleigh test (Zar, 1996) was performed to determine whether axial tailbeats occurred at specific times throughout the stride cycle. The Rayleigh test describes the probability that cyclical variables occur randomly with respect to each other. For this test, all tailbeats analyzed for a given individual were pooled and a separate test was conducted for each individual.

## Results

### Bipedal-undulatory gait

The aquatic walking gait of *S. lacertina* combines alternating forelimb movements, similar to those observed during terrestrial walking in salamanders, and axial undulatory waves, similar to those observed during swimming (Fig. 1). We characterized the appendicular component of this gait by quantifying the mean contact intervals of each limb in five walking sequences from each individual. The left forelimb of *S. lacertina* was in contact with the substrate for approximately the first half of the stride cycle (Fig. 2b). The right forelimb was in contact with the substrate for approximately the second half of the stride cycle (Fig. 2b). During steady aquatic walking the forelimbs are never used synchronously, show little if any overlap in contact intervals, and often include short periods of suspension within the stride (Fig. 2a). Based on the contact intervals, this

symmetrical bipedal gait can be described as a slow trot according to the terminology of Hildebrand (1985).

The axial component of this gait is characterized by a traveling wave, which begins in the anterior third of the body of the salamander and propagates posteriorly (Fig. 1). All walking sequences contained axial undulations, which presumably generate thrust for forward movement as well as lift, which keeps the posterior portion of the body off the substrate despite the lack of hindlimbs in this species. The magnitude and frequency of axial undulations used during aquatic walking are similar to movements associated with swimming at slow speeds in this salamander. Maximum lateral deflection of the tail tip (a metric of tailbeat amplitude) is approximately 0.1 bodylengths during both swimming and aquatic walking (Fig. 2a; Gillis, 1997). Tailbeat frequency ranges from approximately 0.6 to 2.0 Hz during slow speed swimming and from 0.6 to 1.3 Hz during aquatic walking (Fig. 5b; Gillis, 1997).

### Axial-appendicular coupling

To examine the frequency coupling of axial and appendicular movements during aquatic walking, we quantified the relationship between stride frequency and tailbeat frequency. For this analysis we performed a reduced major axis regression on the stride frequencies and the tailbeat frequencies of all the walking sequences of each individual (Fig. 3). If the frequencies of axial and appendicular movements were coupled, we would expect a statistically significant positive correlation between stride and tailbeat frequency. We found no significant correlation between stride and tailbeat frequency in any individual (Individual 1: $p = 0.33$; Individual 2: $p = 0.13$;
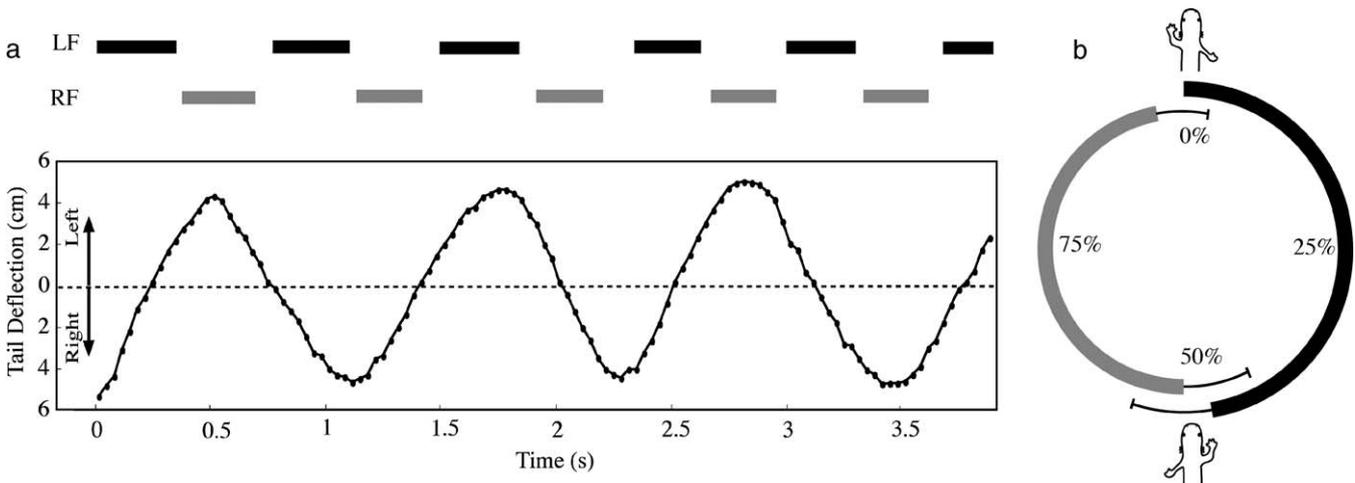


Fig. 2. (a) Footfall patterns and tail kinematics of a representative aquatic walking sequence. Bars indicate the contact interval of each limb with the bottom of the water trackway. Lateral deflection of the tip of the tail is shown at 0.03 s intervals. Dotted line (0 cm deflection) represents the direction of forward travel. (b) Average footfall pattern for five aquatic walking bouts from each individual. Contact intervals are shown on a polar plot with the radial axis representing the relative stride cycle. Error bars represent the standard error of the mean. LF: left forelimb (black); RF: right forelimb (gray).
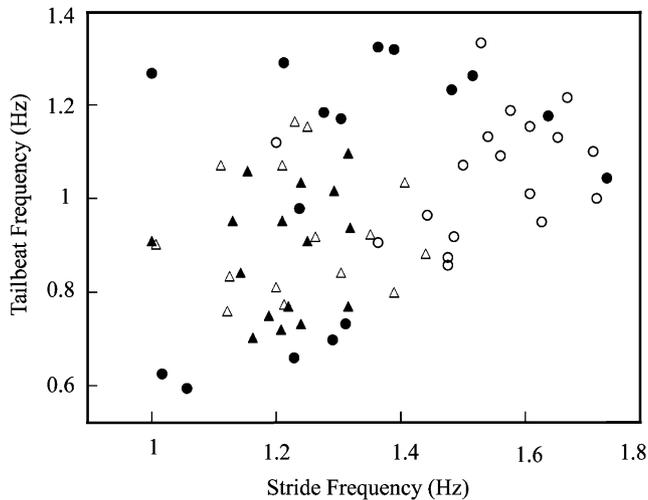
**Fig. 3.** Plot of stride frequency versus tailbeat frequency during aquatic walking in *S. lacertina*. Reduced major axis regressions performed on each individual were not significant. ($\bigcirc$) Individual 1 ($n = 18$, $p = 0.329$); ($\bullet$) individual 2 ($n = 16$, $p = 0.131$); ($\triangle$) individual 3 ($n = 15$, $p = 0.930$); ($\blacktriangle$) individual 4 ($n = 16$, $p = 0.635$); and $n$ = number of aquatic walking sequences analyzed for each individual.

Individual 3: $p = 0.93$; Individual 4: $p = 0.64$). Based on non-significant correlations for all four individuals we conclude that the frequencies of tail and limb movements are not tightly coupled during aquatic walking.

To examine the phase coupling of axial and appendicular movements, we performed a polar analysis on the timing of tail movements relative to the stride cycle. For each individual, the occurrence of maximum lateral tail deflection (to the left) was plotted as a percentage of the stride cycle (Fig. 4). If limb and axial movements were tightly coupled, we would expect lateral deflections of the tail to occur within a specific portion of the stride cycle. A Rayleigh test was used to determine whether maximum lateral deflections of the tail were randomly distributed throughout the stride cycle. For this test, all tailbeats analyzed for a given individual were pooled and separate tests were conducted for each individual. We found that in all four individuals the occurrence of maximum lateral tail deflections within a stride cycle did not differ significantly from a random distribution (Individual 1: $p = 0.69$; Individual 2: $p = 0.15$; Individual 3: $p = 0.07$; Individual 4: $p = 0.98$). These results indicate that the relative phase of axial and appendicular movements of *S. lacertina* is not coupled during aquatic walking.

## Velocity effects

Another goal of this study was to quantify and describe the mechanism by which *S. lacertina* increases
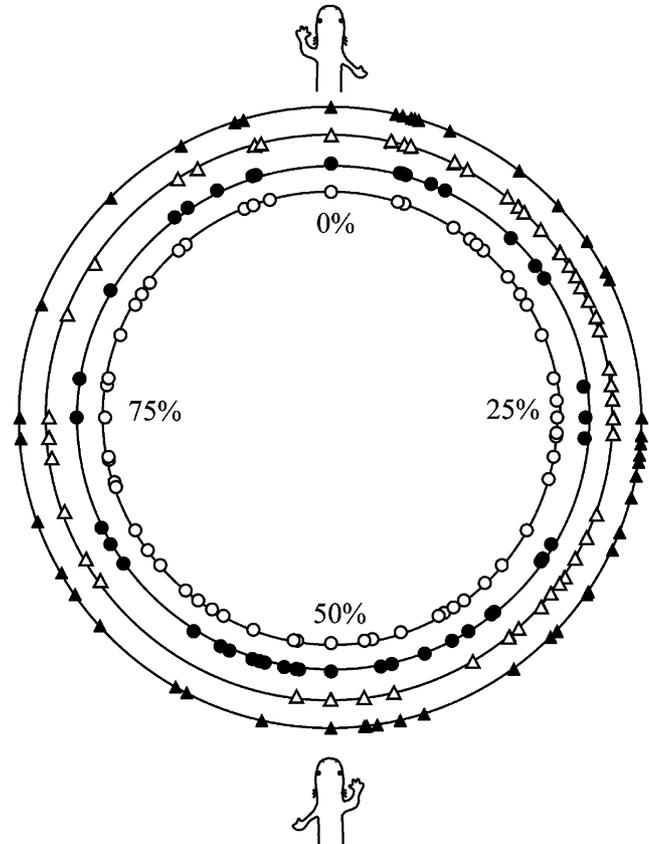


**Fig. 4.** Polar plot of the phase relationship between limb and axial movement in *S. lacertina* during aquatic walking. The radial axis represents the normalized stride cycle. The stride cycle begins when the left forelimb is maximally extended (0%), proceeds clockwise and terminates when the left limb returns back to the 0% position. Each point indicates the relative time of maximum tail deflection (to the left) within the stride cycle. Each ring of points represents all the tailbeats from one individual. Results of a Rayleigh test (a test of circular uniformity) show that tail deflections are randomly distributed throughout the stride cycle in all individuals. ($\bigcirc$) Individual 1 ($p = 0.686$, $n = 84$); ($\bullet$) individual 2 ($p = 0.150$, $n = 58$); ($\triangle$) individual 3 ($p = 0.069$, $n = 59$); ($\blacktriangle$) individual 4 ($p = 0.98$, $n = 52$); and $n$ = number of tailbeats analyzed for each individual.

forward velocity during aquatic walking. We used a linear regression (least-squares) analysis to quantify how stride frequency and tailbeat frequency vary with increasing speed (Fig. 5).

In all four individuals we found a statistically significant linear relationship between tailbeat frequency and velocity (Fig. 5b). Similar to most previous work on swimming, our data suggest that varying tailbeat frequency is one mechanism used to modulate speed during aquatic walking. In addition to tailbeat frequency, tailbeat amplitude has been shown to increase with increasing speed during swimming in *Siren* and is another likely mechanism to modulate speed during
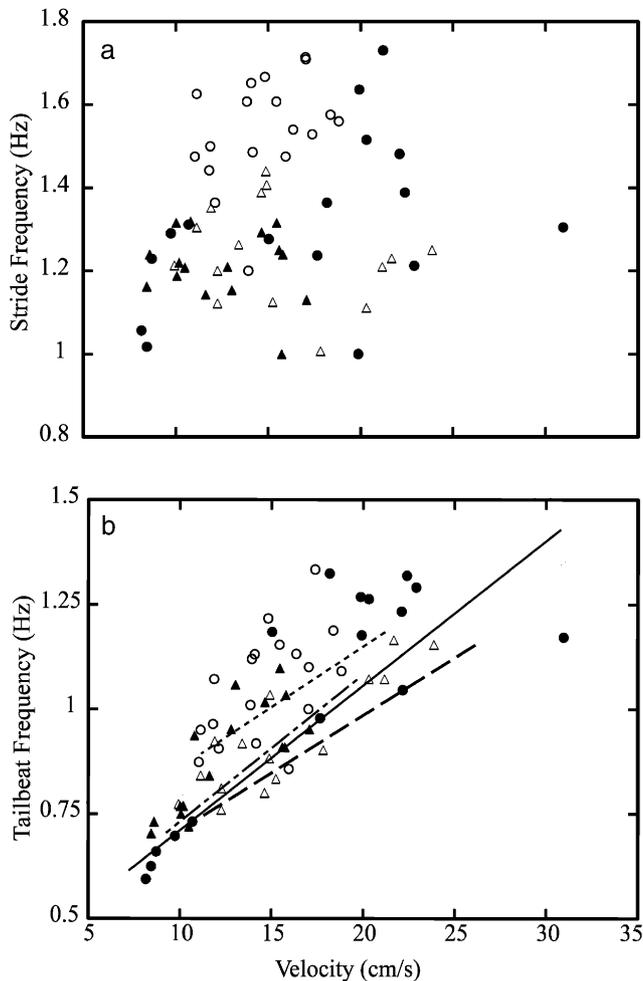
**Fig. 5.** The relationship between stride frequency, tailbeat frequency and walking velocity. (a) Plot of stride frequency versus velocity. A linear regression analysis indicates that no significant relationship exists between stride frequency and velocity for any of the individuals in this study. ($\bigcirc$) Individual 1 ($p = 0.146$, $n = 18$); ($\bullet$) individual 2 ($p = 0.087$, $n = 16$); ($\triangle$) individual 3 ($p = 0.405$, $n = 15$); ($\blacktriangle$) individual 4 ($p = 0.511$, $n = 16$). (b) Plot of tailbeat frequency versus velocity. A linear regression analysis indicates a significant relationship between tailbeat frequency and velocity for all four individuals. ($\bigcirc$) Individual 1 (- - -), $y = 0.029x + 0.632$ ($r^2 = 0.31$, $p = 0.019$, $n = 18$); ($\bullet$) individual 2 (—), $y = 0.035x + 0.431$ ($r^2 = 0.69$, $p < 0.0001$, $n = 16$); ($\triangle$) individual 3 (- - -), $y = 0.028x + 0.498$ ($r^2 = 0.75$, $p < 0.0001$, $n = 18$); ($\blacktriangle$) individual 4 (- - - -), $y = 0.037x + 0.426$ ($r^2 = 0.63$, $p = 0.0002$, $n = 16$); and $n =$ number of walking sequences for each individual.

aquatic walking (Gillis, 1997). Conversely, we found no significant relationship in any individual between stride frequency and velocity (Fig. 5a). These data suggest that increasing stride frequency is not a mechanism used to increase velocity during aquatic walking. However, the role of stride length in modulating speed remains unclear because measures of stride length during a

bipedal-undulatory gait are confounded by the contribution of the axial system to forward propulsion.

## Contribution of limbs

To examine the contribution of the limbs to aquatic walking, we compare the axial wave patterns of *S. lacertina* during aquatic walking and swimming. Previously published data on the swimming kinematics of *Siren* (Gillis, 1997) provide an opportunity to compare slow-speed swimming in which the limbs are tucked against the body and aquatic walking in which limbs are used. This comparison is possible because the data being compared are collected from salamanders of similar size; salamanders in this study have a mean total length of 38 cm and salamanders in the swimming study have a mean total length of 36.3 cm (Gillis, 1997). In addition, the two locomotor behaviors being compared have similar axial kinematics and have a substantial overlap in velocities.

We compared the axial wavespeed (speed of rearward propagation of an undulatory wave) at varying velocities during aquatic walking and swimming (Fig. 6a). We predict that if the limbs significantly contribute to forward propulsion, axial wavespeed will be significantly lower during aquatic walking when compared to swimming at similar speeds. We found that length-specific wavespeed (BL/s) during aquatic walking increases linearly with velocity. Data pooled from all individuals can be described by the line $y = 0.84x + 0.21$ ($r^2 = 0.71$, $p < 0.0001$) (Fig. 6a). Similarly, length-specific wavespeed (BL/s) during swimming has been shown to increase linearly with velocity (Gillis, 1997). Mean swimming data pooled from four individuals at four distinct speeds (0.3, 0.45, 0.6, and 0.9 BL/s) can be described by the line $y = 1.08x + 0.37$ ($r^2 = 0.98$, $p < 0.0001$) (Fig. 6a; Gillis, 1997). These lines are found to have indistinguishable slopes and significantly different $y$-intercepts ($p < 0.0001$; ANCOVA). Our results suggest that at any given velocity aquatic walking requires a lower wavespeed than swimming.

We also compared the forward progress of the salamander per tailbeat (tailbeat distance) during aquatic walking with previously published data on swimming (Fig. 6b; Gillis, 1997). We predict that if the limbs contribute to forward propulsion, tailbeat distance will be significantly higher during aquatic walking when compared to swimming at similar speeds. We found that the average tailbeat distance (BL/tailbeat) during aquatic walking increases linearly with velocity. Data pooled from all individuals can be described by the line $y = 0.45x + 0.20$ ($r^2 = 0.71$, $p < 0.0001$) (Fig. 6b). Similarly, tailbeat distance (called stride length in Gillis, 1997) during swimming has been shown to increase linearly with velocity. Mean swimming data pooled
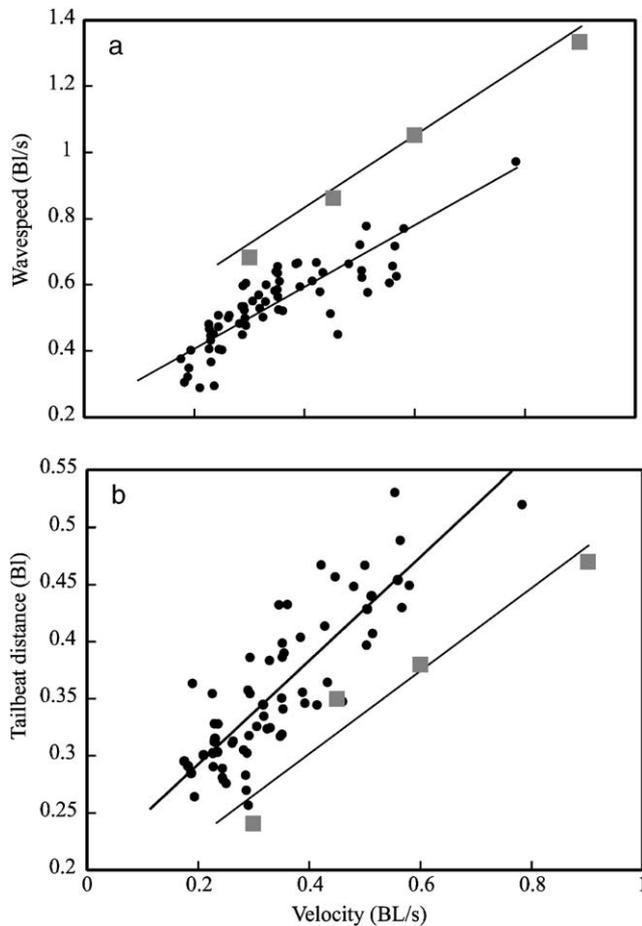
**Fig. 6.** The contribution of the forelimbs during aquatic walking. (a) A comparison of axial wavespeed at varying velocities during aquatic walking and swimming. (●) Average values of axial wavespeed and walking velocity for each aquatic walking sequence analyzed. (■) Average values of axial wavespeed and velocity during swimming (data from Gillis, 1997). For aquatic walking, the data are described by a linear regression line with the equation $y = 0.84x + 0.21$ ($r^2 = 0.71$, $p < 0.0001$, $n = 66$). For swimming, the data are best described by a linear regression line with the equation $y = 1.08x + 0.37$ ($r^2 = 0.98$, $p < 0.0001$, $n = 4$). Wavespeed is found to differ significantly during aquatic walking and swimming ($p < 0.0001$, ANCOVA). (b) A comparison of tailbeat distance at varying velocities during aquatic walking and swimming. (●) Average tailbeat distance and walking velocity for each aquatic walking sequence analyzed. (■) Average tailbeat distance and velocity during swimming (data from Gillis, 1997). For aquatic walking, the data are described by a linear regression line with the equation $y = 0.45x + 0.20$ ($r^2 = 0.71$, $p < 0.0001$, $n = 66$). For swimming, the data are best described by a linear regression line with the equation $y = 0.36x + 0.16$ ($r^2 = 0.94$, $p < 0.0001$, $n = 4$). Tailbeat distance is found to differ significantly during aquatic walking and swimming ($p < 0.0001$, ANCOVA).

from four individuals at four distinct speeds (0.3, 0.45, 0.6, and 0.9 BL/s) can be described by the line $y = 0.36x + 0.16$ ($r^2 = 0.94$, $p < 0.0001$). These lines are found to have indistinguishable slopes and significantly different y-intercepts ($p < 0.0001$; ANCOVA). Our results suggest that each tailbeat results in greater forward progression during aquatic walking than during swimming.

## Discussion

### Aquatic walking gaits

Organisms using limb-based locomotion face significantly different mechanical environments in aquatic versus terrestrial habitats (Martinez et al., 1998). A major difference is that the greater viscosity of water provides greater hydrodynamic resistance, which can mechanically stabilize aquatic organisms. In addition, an organism's natural buoyancy reduces the required support needed to resist the forces of gravity by reducing an organism's effective weight. Therefore, aquatic walking gaits are less constrained to provide stability and support while generating forward thrust and may significantly vary from locomotor patterns observed on land.

Previous studies of aquatic walking in antennariid fishes (Edwards, 1989; Pietch and Grobecker, 1987), the epaulette shark (Pridmore, 1994), and an intertidal crab (Martinez et al., 1998) have highlighted the differences in stability requirements for aquatic versus terrestrial gaits. *Antennarius sp.* uses a slow transverse gallop where both pectoral and pelvic fins are used with little overlap in the contact intervals of the fins (Edwards, 1989). Antennariids also use a "modified walk" where only the pectoral fins are used asynchronously with no overlap in contact intervals (Pietch and Grobecker, 1987). The epaulette shark (*Hemiscyllium ocellatum*) has been shown to use a diagonal walking-trot where diagonal fin pairs have little overlap in contact intervals (Pridmore, 1994). It has also been shown that the intertidal crab (*Grastus tenuicrustatus*) uses significantly smaller contact intervals and a narrower base of support during aquatic versus terrestrial walking (Martinez et al., 1998). Similarly, the California newt (*Taricha torosa*) has been shown to use strides with significantly shorter contact intervals while submerged underwater when compared to terrestrial walking bouts (Ashley-Ross and Bechtel, 2003). The repertoire of gaits used by aquatic walkers shares locomotor patterns that are considered unstable on land, particularly at slow speeds. Many such gaits also include a short period of suspension where no limbs are in contact with the substrate.

Similar to the aquatic gaits described above, *S. lacertina* shows little overlap in the contact intervals of the forelimbs (Fig. 2). This finding is in contrast to previously published data on the terrestrial gaits of most

salamanders, which can have an overlap in the contact interval of the contralateral limbs that corresponds to about 15–30% of the stride duration (Edwards, 1976; Ashley-Ross, 1994; Bennett et al., 2001). Overlap in the contact intervals of contralateral limbs creates a stable base of support throughout the stride and can help increase the stability of the gait at slow speeds (Hildebrand, 1985). Relatively shorter contact intervals of limbs and short periods of suspension within the stride cycle during aquatic walking may be characteristic of most aquatic walkers, which are partially supported by their own natural buoyancy.

## Decoupling of axial and appendicular systems

Previous studies have hypothesized that elongation of the trunk may result in a number of changes in the motor coordination of locomotion (Gans, 1985; Renous et al., 1995; Renous et al., 1999). One such change associated with trunk elongation may be the loss of inter-girdle coordination between the forelimbs and the hindlimbs (Gans, 1985). A highly elongated trunk may also lead to a loss of the tight coupling relationship between axial and appendicular systems during quadrupedal locomotion (Renous et al., 1995; Renous et al., 1999). The dissociation of axial and appendicular systems may be a result of greater locomotor reliance on the trunk and a subsequent shift from a standing axial wave (characteristic of quadrupedal tetrapods) to a traveling axial wave (characteristic of limbless tetrapods) during terrestrial locomotion (Ritter, 1992; Renous et al., 1995; Renous et al., 1999).

The patterns of axial and appendicular movements during rhythmic locomotor behaviors are thought to be controlled by central pattern generators (CPGs), which function at the level of the spinal cord (Ijspeert and Kodjabachian, 1999; Ijspeert, 2001; Bem et al., 2003). Whole-body neuromechanical models have described the locomotor circuitry of salamanders as consisting of one CPG that controls axial movements and a second CPG that controls limb movements (Ijspeert, 2001; Bem et al., 2003). The accuracy of such models in describing biological patterns of locomotion depends strongly on the phase relationship between the segments (oscillators) which make up a CPG as well as the phase relationship between the CPGs used during a given locomotor behavior (Grillner, 1999).

In this study, we find strong evidence that the axial and appendicular CPGs of S. lacertina are decoupled during aquatic walking. It has previously been hypothesized that decoupling of locomotor CPGs serves as a potential mechanism for the evolution of novel behaviors (Dubbeldam, 2001). This hypothesis is based on the presence and similarity of locomotor CPGs across diverse taxa and the flexibility of such neural circuits in response to both short-term functional demands as well as long-term shifts in external conditions (Pearson, 2000). Our results suggest that the neural control of aquatic walking in S. lacertina differs from both terrestrial walking and swimming in other salamanders. We find support for the hypothesis that the decoupling of CPGs has led to the evolution of a novel locomotor behavior (bipedal-undulatory gait). In addition, our results provide strong support for the hypothesis that axial elongation and limb reduction lead to a decoupling of locomotor systems. The novel aquatic gait of S. lacertina provides an interesting system for linking comparative morphological patterns with shifts in the neural control of locomotion.

## Contribution of limbs

Our findings indicate that the relatively small limbs of S. lacertina, previously described as ineffectual (Holbrook, 1842), do in fact serve a locomotor function (Fig. 6). Our data indicate that at any given velocity the wavespeed used during aquatic walking is significantly lower than the wavespeed used during swimming. It is our interpretation that the contribution of the limbs may increase forward velocity and compensate for the lower axial wavespeed used during aquatic walking. Regression lines, which describe the relationship between wavespeed and velocity, have similar slopes and different y-intercepts (Fig. 6a). This pattern indicates that the contribution of the limbs is constant across speed and is consistent with our previous finding that stride frequency does not vary with velocity (Fig. 5a).

Our results also suggest that each tailbeat results in greater forward progression during aquatic walking than during swimming. Given that the axial kinematics associated with aquatic walking and swimming are similar, the differences observed are likely to be a result of the contribution of the forelimbs. Similar to results described in our wavespeed analysis, the regression lines, which describe the relationship between tailbeat distance and velocity, have similar slopes and different y-intercepts (Fig. 6b). This result is also consistent with our previous finding that stride frequency does not vary with velocity and that the contribution of the limbs is constant across speeds (Fig. 5a).

Although we find that the reduced forelimbs of S. lacertina are contributing to forward propulsion during aquatic walking, these relatively small limbs (<10% of total length; Table 1) would likely be ineffectual on land where limbs need to support, stabilize and propel a locomoting organism. It is likely that the functionality of these limbs is due in large part to the stability and support inherent in an aquatic environment. Similar results have shown that adult sea robins (Trigloporus lastoviza) walk along the substrate using individual fin

rays (Renous et al., 2000). These elements would hardly meet the structural demands of supporting an organism on land. In addition, the relatively small pelvic fin elements (crura) of the little skate (*Raja erinacea*) are considered the primary locomotor structures during aquatic walking (Koester and Spirito, 2003). Due to an organism's natural buoyancy and reduced effective weight in the aquatic environment, the size, structural properties, and muscular support of functional locomotor structures (limbs and fins) differ significantly from terrestrial walkers.

The functionality of relatively small and unimpressive locomotor structures used during aquatic walking highlights important differences in the mechanical demands on limbs and fins in aquatic versus terrestrial environment. These results also bring up an interesting question regarding early tetrapod locomotion and the evolution of tetrapod limbs. If in fact aquatic walking was the primitive mode of locomotion in the earliest tetrapods, then why are the limb elements and girdles of *Acanthostega* and *Ichthyostega* so robust? The robust limbs and girdles of these early tetrapods seem capable of bearing mechanical loads which far exceed those associated with aquatic walking. Based on the anatomy of early tetrapods and the functional demands associated with aquatic walking, it is unclear whether the locomotor repertoire of early tetrapods was in fact limited to aquatic walking.

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