RESEARCH ARTICLE

Locomotor behavior across an environmental transition in the ropefish, *Erpetoichthys calabaricus*

Cinnamon M. Pace* and Alice C. Gibb

Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, USA

*Author for correspondence (Cinnamon.Pace@nau.edu)

SUMMARY

Many amphibious organisms undergo repeated aquatic to terrestrial transitions during their lifetime; limbless, elongate organisms that make such transitions must rely on axial-based locomotion in both habitats. How is the same anatomical structure employed to produce an effective behavior across such disparate habitats? Here, we examine an elongate amphibious fish, the ropefish (*Erpetoichthys calabaricus*), and ask: (1) how do locomotor movements change during the transition between aquatic and terrestrial environments and (2) do distantly related amphibious fishes demonstrate similar modes of terrestrial locomotion? Ropefish were examined moving in four experimental treatments (in which the water level was to lowered mimic the transition between environments) that varied from fully aquatic to fully terrestrial. Kinematic parameters (lateral excursion, wavelength, amplitude and frequency) were calculated for points along the midline of the body and compared across treatments. Terrestrial locomotion in the ropefish is characterized by long, slow, large-amplitude undulations down the length of the body; in contrast, aquatic locomotion is characterized by short-wavelength, small-amplitude, high-frequency undulations that gradually increase in an anterior to posterior direction. Experimental treatments with intermediate water levels were more similar to aquatic locomotion in that they demonstrated an anterior to posterior pattern of increasing lateral excursion and wave amplitude, but were more similar to terrestrial locomotion with regard to wavelength, which did not change in an anterior to posterior direction. Finally, the ropefish and another elongate amphibious fish, the eel, consistently exhibit movements characterized by 'path following' when moving on land, which suggests that elongate fishes exhibit functional convergence during terrestrial locomotion.

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Key words: amphibious fish, swimming, terrestrial undulation, kinematics.

INTRODUCTION

Many organisms undergo transitions between aquatic and terrestrial environments – either during their lifetime or as a result of an amphibious habit. Such transitions present a drastic change in physical conditions, as these environments differ in viscosity, density and gravitational effects (Vogel, 1994; Graham, 1997). Some such organisms employ different locomotor structures (or different combinations of structures) during aquatic and terrestrial locomotion. For example, mudskippers (e.g. *Periophthalmus*) and some salamanders (e.g. *Taricha*) primarily use their tail for aquatic locomotion, but rely more on their paired appendages during terrestrial locomotion (Frolich and Biewener, 1992; Ashley-Ross and Bechtel, 2004; Pace and Gibb, 2009). However, elongate limbless vertebrates (or vertebrates with non-weight-bearing appendages) must employ the same propulsive structure, the axial skeleton and musculature, across both habitats. This raises the question: how is the same anatomical structure employed to produce an effective behavior across such disparate habitats? Despite the tractability and simplicity of elongate fish as a model system in which to address this question, few studies have directly compared axial-only locomotion in aquatic *versus* terrestrial environments. In addition, most such studies have considered only undulation frequency and/or overall animal velocity (Jayne, 1986; Shine and Shetty, 2001; Shine et al., 2003), with a much smaller subset encompassing other kinematic variables (Gillis, 1998; Ellerby et al., 2001).

In addition to moving in both aquatic and terrestrial environments, amphibious animals also move through transitional environments, i.e. environments that are neither entirely aquatic nor entirely terrestrial. Such environments can range from steep slopes at the land–water interface to very shallow aquatic environments where some of the body protrudes above the water surface. In fact, animal movements during transitions from one environment to the next likely depend on both the physical forces that the environment exerts on the organism and the organism's response to changing conditions. For example, a different neuromuscular routine may be employed in a transition zone relative to the routine used by the same animal when it is either on land or in the water. Unfortunately, to our knowledge, only one study has examined the movements of an amphibious vertebrate (a salamander; *Taricha torosa*) entering/exiting the water on a slope (Ashley-Ross and Bechtel, 2004), and no studies have examined such movements in limbless organisms that employ the same propulsive organ across environments. Here we ask: when an elongate vertebrate moves from one environment to another, what kinematic changes characterize the transition from one locomotor regime to the next, and under what conditions does this transition occur?

In addition, we seek to determine whether elongate limbless aquatic vertebrates produce functionally convergent movements during terrestrial locomotion. Studies of eel locomotor behavior (Gillis, 1998; Ellerby et al., 2001) suggest that terrestrial locomotion in these elongate

fishes (*Anguilla rostrata* and *A. anguilla*) is similar to the terrestrial lateral undulations that are well documented for many snake species. During snake terrestrial lateral undulation, large-amplitude movements occur in both the posterior and anterior regions of the body and posterior regions exhibit a 'path-following' behavior, wherein points on the body follow each other to traverse a similar path of movement. However, snakes and other functionally limbless tetrapods can also exhibit a variety of other terrestrial locomotor behaviors, including sidewinding, concertina and rectilinear movements (Gans, 1986; Jayne, 1986); each of these behaviors is characterized by a distinct movement pattern. Can elongate amphibious fishes exhibit the same diversity in terrestrial movements, or are they limited in their functional repertoire? Do amphibious fishes consistently move using lateral undulation, or do divergent fish taxa demonstrate distinct locomotor responses to this environmental challenge?

The ropefish, *Erpetoichthys calabaricus*, is an elongate polypterid native to central Africa that inhabits bodies of water ranging from flowing rivers to seasonal flood plains (Welcomme, 1979; Udoidiong and King, 2000). This species makes voluntary terrestrial excursions, both in the wild and in the laboratory. One laboratory study investigating air breathing in *E. calabaricus* recorded an average of six terrestrial excursions in 24 h (per fish), with each excursion lasting an average of 2 min (Sacca and Burggren, 1982). In addition, *E. calabaricus* captures terrestrial insects on land, and can avoid obstacles when moving terrestrially (Pettit and Beitinger, 1985). Based on qualitative observations, it has been suggested that this species uses an 'undulatory' mechanism when on land (Pettit and Beitinger, 1985). However, although the aquatic head retraction response of *E. calabaricus* has recently been quantified (Bierman et al., 2004), the mechanism that generates forward propulsion in water or land remains undescribed.

In this study, we examined locomotion in *E. calabaricus* in aquatic and terrestrial environments and across two transitional habitats. We quantified the movements produced under these four environmental conditions and addressed the following questions. (1) How do locomotor movements change when an elongate fish moves from one habitat to the next? Based on data for eels (Gillis, 1998), we expect that ropefish aquatic locomotion will be characterized by higher-amplitude undulations of the posterior region of the body, relative to the anterior region, and that terrestrial locomotion will be characterized by high-amplitude undulations of both the anterior and posterior regions. Additionally, we predict that, as water level decreases (i.e. in transitional treatments), lateral excursion will progressively increase in the anterior region of the fish. (2) Do distantly related elongate amphibious fishes demonstrate similar modes of terrestrial locomotion? We predict that terrestrial locomotion in *E. calabaricus* will be similar to that of eels (Gills, 1998), in that it will consist of snake-like, lateral undulations, wherein points on the body will follow each other to traverse a similar overall path of movement. However, the ropefish axial skeleton is almost entirely comprised of abdominal vertebrae, whereas eels have more caudal vertebrae than abdominal vertebrae (Ward and Brainerd, 2007) and there are morphological differences between abdominal and caudal vertebrate (e.g. caudal vertebrae have a haemal arch and spine that abdominal vertebrae lack). In addition, ropefish and eels are very distantly related (Nelson, 1994). Thus, it is possible that these two species employ different movement patterns to produce effective terrestrial locomotion.

MATERIALS AND METHODS
Animals
Erpetoichthys calabaricus (Smith 1865) (hereafter, ropefish) were acquired from commercial vendors and housed in water-filled glass aquaria that contained a floating ramp to provide terrestrial habitat. Ropefish were exposed to a 12 h:12 h light:dark cycle and were maintained on a diet of earthworms and commercial fish food. The five ropefish used in the study were 15.6±2.7 g in mass and 23.6±1.1 cm in total length. All animal care and experiments were conducted in accordance with Northern Arizona University IACUC guidelines under protocol no. 03-091-R1.

Data collection
Locomotor trials were conducted in a 150 l glass (76.2×31.8 cm) aquarium with approximately 3 cm of sand covering the bottom of the tank. Locomotor trials were recorded from a dorsal view using either a Redlake Motionscope digital-imaging system (PCI 1000s; San Diego, CA, USA) or a Vision Research Phantom V5.1 digital-imaging system (Wayne, NJ, USA) at a high sampling rate; however, because voluntary ropefish movements were slow, image sequences were subsequently subsampled to create a frame rate of 20 or 25 frames s^{-1}. When necessary, movement of ropefish was induced by gently prodding their caudal fin.

During locomotor trials, ropefish were presented with four different substrate treatments: a full aquatic immersion treatment (animals were immersed in ~10 cm of water and swam along the bottom of the tank), a full terrestrial emersion treatment (where animals moved across wet sand created using 1.25:3 parts water:sand), and two treatments containing sand and lowered water levels. These four treatments, from aquatic to terrestrial, are considered here as 0, 50, 75 and 100% emersion. As there were slight differences in the size of the ropefish, water levels for the two intermediate treatments were always determined relative to an individual ropefish's body. Thus, in the first intermediate treatment (50% emersion) the water was lowered so that approximately 50% of the dorsal surface of the fish was exposed. At this water level, the ropefish could still fully submerge its head, as the head is not as deep as the rest of the body. In the second intermediate treatment (75% emersion) the water was lowered even further, so that approximately 75% of the fish was exposed; in this treatment, the ropefish could not submerge its head. In the 100% emersion treatment, the sand was wet but the entire body of the fish was exposed to the air.

The velocity of movement during the ropefish locomotor trials was voluntary. Although we attempted to select trials where fish moved at similar velocities, fish did not appear capable of moving at the same velocity across all treatments (Table 1). However, other

Table 1. Mean (±s.e.m.) distance traveled, trial time and velocity of the center of mass for five ropefish, *Erpetoichthys calabaricus*, traveling in each environmental treatment

Environmental treatment	Center of mass		
	Distance (cm)	Velocity (cm s^{-1})	Velocity (% TL s^{-1})
0% emersion (aquatic)	10.3±0.9	8.0±1.7	0.34±0.08
50% emersion	10.6±0.8	10.3±1.9	0.44±0.1
75% emersion	8.3±0.5	6.7±0.9	0.26±0.04
100% emersion (terrestrial)	8.3±0.5	2.7±0.3	0.11±0.01

studies have also found that the very slowest gaits for aquatic locomotion are still faster than terrestrial locomotion for organisms that are primarily aquatic (Shine and Shetty, 2001; Pace and Gibb, 2009). Therefore, this is likely an unavoidable aspect of studies of this nature and may reflect physical and/or biomechanical limitations on movement in these species. Additionally, locomotion in all treatments was slow [<0.5 total length (TL) s^{-1}] (Lauder and Tytell, 2006). For anguilliform swimmers swimming at velocities less than 1.0 TL s^{-1}, anterior body oscillations are minimal (Lauder and Tytell, 2006). Thus, it seems unlikely that our finding that anterior oscillations increase as the water level is lowered is because of the minimal velocity differences between our treatments (see 'Results').

Data acquisition and variables

Image sequences (sequentially numbered TIFF images) were imported into the program Didge (A. Cullum, Creighton University) for data extraction. Only trials where the center of mass (COM) of the fish was moving continuously for an average linear distance of at least 0.25 TL were digitized. Additionally, only trials where the overall direction of travel for the COM was a straight line were included for analysis (this was assessed by attempting to fit a straight line that bisected the undulations at the COM for each trial). Measurements of body movements during a trial were made using the 'segment' function of Didge, in which the user inputs the TL of the organism and defines a set number of segments for this length; as a point is placed on the midline of the body of the fish by the researcher, Didge restricts the cursor to move a distance equal to the length of the pre-defined segment. In this manner, 21 evenly spaced points were placed along the length of the ropefish by the researcher, such that each segment corresponded to 5% of the total length of the fish. In this manner, the movement of points defining 20 equal segments (0, 5, 10,…,100%TL, where 0%TL is the anterior tip of the head and 100%TL is the posterior tip of the tail) was quantified for each individual in every trial across all treatments.

The two-dimensional (x, y) coordinates were imported into a spreadsheet program to calculate kinematic (movement) variables. Movements of the center of mass (COM), defined here as 40%TL (Bierman et al., 2004), were used to calculate average values of time (s), distance (%TL) and velocity (%TL s^{-1}) for each trial of each individual across all four treatments. In addition, for six points along the length of the body (0, 20, 40, 60, 80 and 100%TL) the distance ratio (DR) was calculated. The DR is the ratio of the average linear distance that a single point traveled *versus* the total distance that same point traveled; this last value includes any lateral excursions by this point that are produced during locomotor movements. Average linear distance was calculated as the linear distance between the position of a given point at the start of the trial and the position of the same point at the end of the trial. Total distance was determined by summing the distance traveled by a specific point from one frame to the next throughout the trial. The DR reveals how much lateral displacement a point on the body undergoes and also indicates the 'efficiency' of movement of a particular region of the body. For example, DR values approaching 1.0 indicate that the point is traveling in approximately a straight line. In contrast, a DR value of 0.5 would indicate that the point moved twice as far in total distance (indicating a great deal of side-to-side displacement) as it did in linear distance. Finally, at three of the body points (0%TL, head; 40%TL, COM; and 100%TL, tail), three additional kinematic parameters were determined: wavelength, wave amplitude and wave frequency. Wavelength was calculated by measuring the distance from the center of one wave to the center of the next across time for the same body point; wave

amplitude was calculated by measuring the maximum height of a single undulation across both sides of the body and dividing by two; and wave frequency was calculated as the average number of complete undulations per second.

Statistical analysis

A three-way ANOVA design was used to compare the four habitat treatments at six points along the body; JMP 7 was used to perform this and all other statistical analyses for this study. Variables for each treatment were calculated using data collected from five individuals (where each individual value for a given variable was the mean of 2–5 trials from that individual; see supplementary material Table S1). For each individual, six points along the body (0, 20, 40, 60, 80 and 100%TL) were used to assess DR, and three points (0, 40 and 100%TL) were used to assess wave frequency, amplitude and length (as described above). For each of the four variables, individual ANOVA models were run in which habitat (i.e. % emersion) and %TL (i.e. position along the body) were considered as fixed factors, with individual included as a random effect. In addition, the three interaction terms (habitat–%TL, habitat–individual and %TL–individual) were also included in these models. Tukey's *post hoc* tests were performed on significant factors for each variable to ascertain relationships between groups within variables. Although all data were normally distributed, they were not homoscedastic. As the DR value is a ratio, it was transformed using the arcsine-square-root transformation; this transformation reduced, but did not entirely alleviate, heteroscedasticity. However, F-tests are robust to deviation in the homogeneity of variance assumption, especially when sample sizes are the same across treatments (Sokal and Rohlf, 1995; Steel et al., 1997; Ott and Longnecker, 2001). In addition, although heteroscedasticity may shift probabilities such that P-values become significant when they should not be, this is most problematic for P-values of borderline significance (i.e. $P \approx 0.05$). Very low P-values (e.g. $P > 0.0001$ for the DR habitat–%TL interaction term; Table 2) are robust to this deviation from the ANOVA assumptions.

RESULTS
General description of aquatic and terrestrial locomotion

Aquatic locomotion (0% emersion) in the ropefish is characterized by cyclical undulation in the posterior regions and little or no undulation in the anterior regions (Fig. 1). Additionally, slow aquatic locomotion can be accompanied by pectoral fin paddling, although this is the only treatment where this behavior was observed. At the anterior of the ropefish, the DR is always greater than 0.9 (Fig. 2), which indicates that this region of the ropefish is traveling in approximately a straight line (Fig. 2). DR values drop successively in an anterior to posterior direction such that at the tail the DR is less than 0.5 (Fig. 2, Table 2), which indicates that the tail is moving more than twice the distance laterally than it is moving in the overall (presumably the 'desired') direction of travel. Wave frequency, wavelength and wave amplitude all differ in the anterior *versus* posterior ends of the ropefish such that that the anterior end has slower wave frequencies, shorter wavelengths and smaller wave amplitudes than the posterior end of the ropefish (Fig. 3).

In contrast, terrestrial locomotion (100% emersion) is typically slow, halting and characterized by pronounced lateral excursions of both the anterior and posterior regions of the fish (Fig. 1). In fact, all regions of the fish exhibit a DR of less than 0.6, with the head (0%TL) and the tail (100%TL) undergoing the greatest lateral excursions relative to the other regions of the fish, producing DR values consistently near 0.4 (Fig. 2, Table 2). During terrestrial
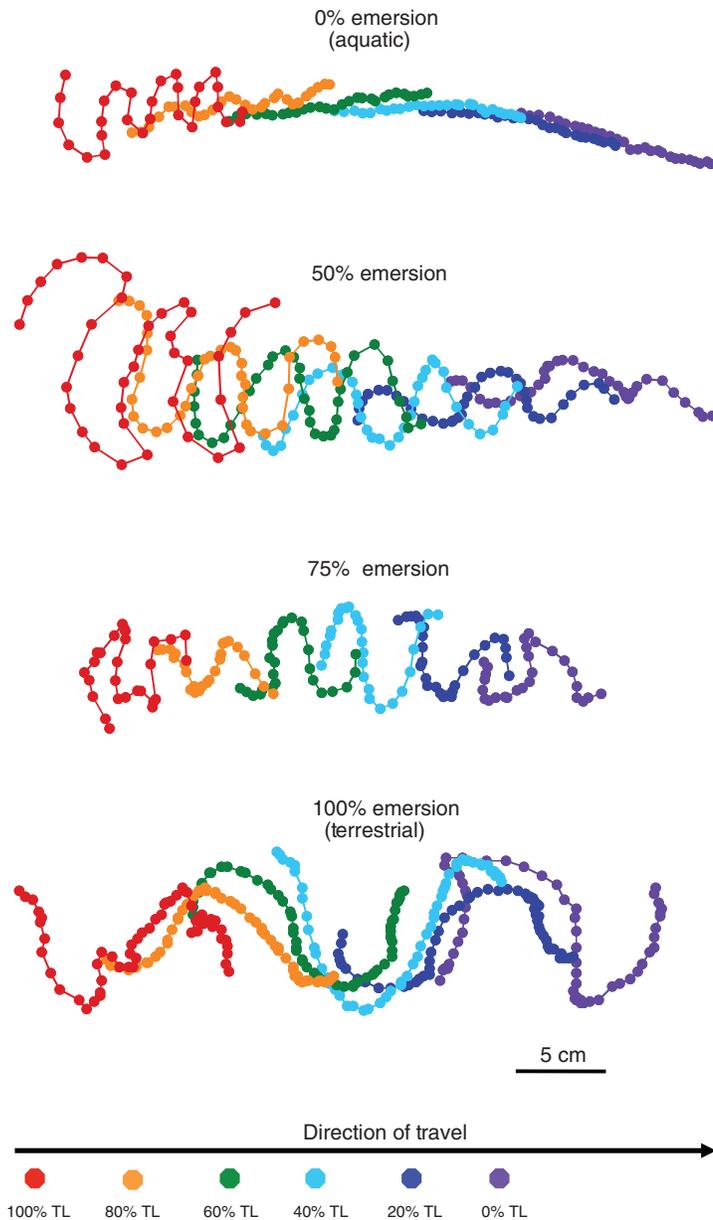
Fig. 1. Representative midline traces of points along the body [0, 20, 40, 60, 80 and 100% total length (TL)] of a single trial of the same individual ropefish moving in each of four environmental treatments: 0 (aquatic), 50, 75 and 100% (terrestrial) emersion. Each body point is designated a specific color and the arrow indicates the direction of movement.

locomotion, there is no significant difference in wave frequency, wave amplitude or wavelength at the head (0% TL), COM (40% TL), or tail (100% TL). In general, wave frequency is low whereas wave amplitude and wavelength are large (Fig. 3).

Transition from aquatic to terrestrial locomotion

Velocities of ropefish in all of the treatments with standing water (0, 50 and 75% emersion) were at least twice as fast as in the 100% emersion treatment. However, ropefish in all treatments moved slowly (Table 1). The ratios of lateral excursion to forward movement (e.g. DR values) varied significantly with habitat, % TL and the habitat–% TL interaction term; Tukey's *post hoc* analysis revealed that the only treatments that didn't differ from one another were the 75% emersion treatment and the terrestrial treatment. For location along the body (% TL), there was a general relationship across treatments wherein points on the body close to one another were more similar to one another than to points farther away, with

the overall result that the anterior and posterior regions of the body differed from one another.

A significant habitat–% TL interaction revealed differences in the degree of lateral excursion between treatments at different points along the body. In general, for all treatments with standing water (0, 50 and 75% emersion), ropefish lateral excursions increased (i.e. DR values were smaller) from the anterior to the posterior region; in contrast, there was little variation along the body in the terrestrial (100% emersion) treatment (Figs 1, 2). In 0% (aquatic) and 50% emersion treatments, the anterior end of the ropefish traveled in approximately a straight line (DR values close to 1). Ropefish moving in the 75 and 100% emersion treatments produced large lateral excursions (DR<0.6) at these same anterior regions. By 60% TL, the 50% emersion treatment was also characterized by large lateral excursions. At the tail (100% TL), all treatments showed large lateral excursions relative to the distance traveled, with the 75% emersion treatment showing the smallest DR values, indicating the
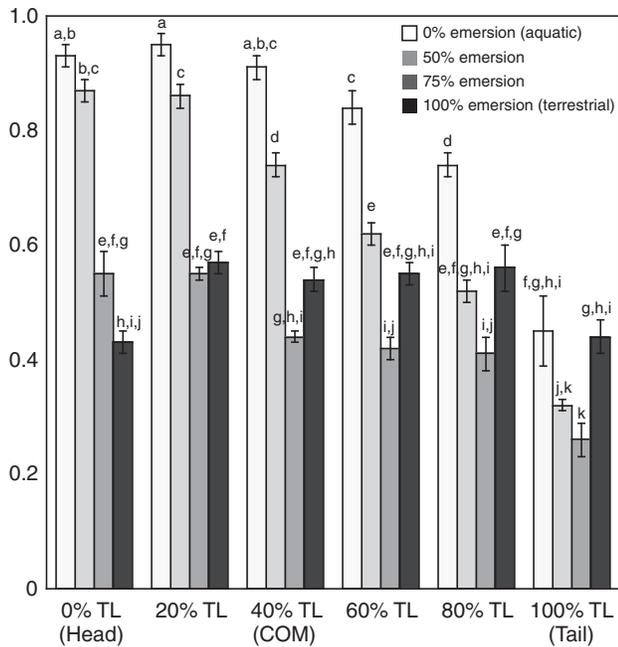
Fig. 2. Distance ratio (the average linear distance traveled by a body point divided by the total distance traveled by that same point) in each treatment at several body points (0, 20, 40, 60, 80 and 100% TL). Values are the means ± s.e.m. of five ropefish. The letters superimposed on the graph represent groupings found *via* a Tukey's *post hoc* test. Body segments and treatments that share a letter are not significantly different from one another; body segments and treatments that do not share any letters are significantly different.

Fig. 3. Wave parameters at 0, 40 and 100% TL: (A) wave frequency (undulations s⁻¹), (B) wave amplitude (% TL) and (C) wavelength (% TL). Values are the means ± s.e.m. of five ropefish. The letters superimposed on the graph represent groupings found *via* Tukey's *post hoc* tests. Body segments and treatments that share a letter are not significantly different from one another; body segments and treatments that do not share any letters are significantly different.

most lateral travel. In general, the DR reveals that, as water level decreases, ropefish experience more lateral movement relative to their desired direction of travel. However, the terrestrial treatment presents a distinct pattern of lateral excursion, wherein all the central body segments have lateral excursions (DR values) of the same magnitude, and the head and the tail have lateral excursions of a slightly greater magnitude.

The other variables (wave frequency, wave amplitude and wavelength) were all significant sources of variation for habitat, %TL and the habitat–%TL interaction (with the exception that wavelength wasn't significant for habitat) (Table 2). For both the 0 and 50% emersion treatments, wave frequency increased in an anterior to posterior direction, whereas the other two treatments showed no difference in wave frequency along the body. The treatments with water showed more variability in wave frequency than the terrestrial treatment and, in general, had greater values and larger standard errors (Fig. 3). The aquatic treatment showed the greatest change in wave frequency along the body, as the head had a very low wave frequency and the tail had a very large wave frequency; however, tail movements in the aquatic habitat (0% emersion) were indistinguishable from the other two treatments with water (50 and 75% emersion) (Fig. 3). Ropefish in the terrestrial treatment moved with low frequencies along the entire length of their body.

In all the treatments with standing water (0, 50 and 75% emersion), ropefish wave amplitude increased in an anterior to posterior direction, whereas ropefish in the terrestrial treatment showed no change in wave amplitude down the length of the body. Ropefish in the terrestrial treatment also had the largest wave amplitudes in the anterior region of the body (Fig. 3). In all
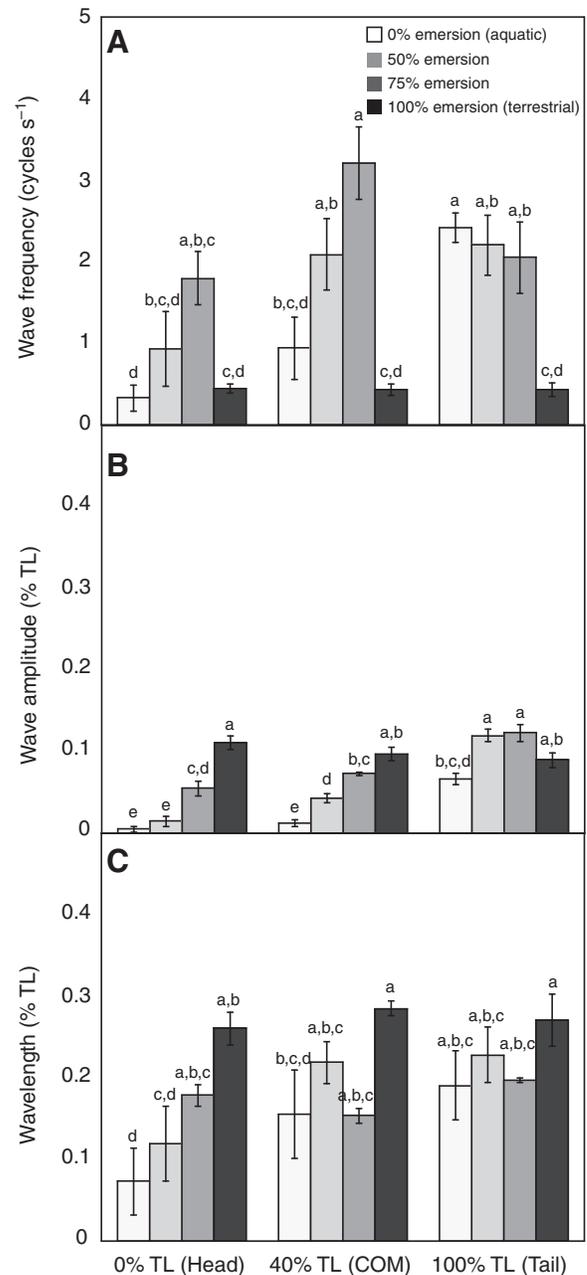
treatments with standing water, wave amplitude was consistently greatest at the tail. Additionally, wave amplitude at the tail was approximately the same in ropefish across treatments, with the exception of the aquatic treatment, which had a slightly lower maximum wave amplitude relative to the other treatments.

At the head, the COM and the tail, ropefish in the terrestrial treatment (100% emersion) had the greatest (longest) wavelengths, and wavelength did not change down the ropefish's body. Ropefish in the 50 and 75% emersion treatments demonstrated a similar

Table 2. *F*-ratios and *P*-values for all factors

Factor	Distance ratio		Wave frequency		Wave amplitude		Wavelength	
	$F_{d.f.}$	P	$F_{d.f.}$	P	$F_{d.f.}$	P	$F_{d.f.}$	P
Overall	44.330_{59}	**<0.0001**	4.8766_{35}	**<0.0001**	27.8009_{35}	**<0.0001**	5.1313_{35}	**<0.0001**
Habitat	87.3244_{3}	**<0.0001**	14.9763_{3}	**0.0002**	27.4220_{3}	**<0.0001**	3.2279_{3}	0.0612
% TL	92.5690	**<0.0001**	14.0174_{2}	**0.0024**	61.9579_{2}	**<0.0001**	13.1913_{2}	**0.0029**
Individual	8.3543_{4}	**<0.0001**	3.2278_{4}	**0.0297**	4.3564_{4}	0.0086	3.5537_{4}	**0.0206**
Habitat–% TL	25.2173_{15}	**<0.0001**	5.2432_{6}	**0.0014**	29.2148_{6}	**<0.0001**	3.2280_{6}	**0.0181**
Habitat–individual	4.7713_{12}	**<0.0001**	1.6685_{12}	0.1381	5.1422_{12}	**0.0003**	5.4632_{12}	**0.0002**
% TL–individual	1.8572_{20}	**0.0341**	0.8693_{8}	0.555	2.2407_{8}	0.0605	0.8037_{8}	0.6056

Each variable was analyzed using a three-way ANOVA design with habitat (environmental treatment) and % TL (position on the body) as fixed effects, and individual as a random effect. Values in bold are statistically significant (*P*<0.05).

pattern: no significant variation in wavelength along the body. However, ropefish in the aquatic treatment did differ in wavelength down the length of the body, such that the head had the shortest wavelength and the tail had the longest. The greatest difference between treatments occurred at the head, with wavelength increasing as the water level was lowered.

When all of these variables are considered, the 50% emersion treatment is more similar to the aquatic treatment in the pattern of movement and the timing and magnitude of movements than it is to the terrestrial treatment. In contrast, the 75% emersion treatment shows similarities with the aquatic treatment for some variables and similarities with the terrestrial treatment for others. In all treatments with standing water, including 75% emersion, DR values decrease in an anterior to posterior direction. However, during 75% emersion, DR values at the head are very low, and there is no difference in wave frequency and wavelength across different locations on the ropefish; this is similar to the pattern produced in the terrestrial treatment.

DISCUSSION

Surprisingly few studies have examined locomotor behavior across the water–land transition. Here, we have quantified and compared locomotion of the ropefish across four environmental conditions in which the water level varied from a fully aquatic to a fully terrestrial treatment, with two intermediate conditions (created *via* lowered water levels). In the ropefish, aquatic and terrestrial locomotion are distinct from one another and kinematic patterns change during the transition from the fully aquatic to the fully terrestrial environment. However, no intermediate water level was associated with a clear gait transition to a 'terrestrial-like' movement. Instead, locomotion at intermediate water levels possessed characteristics of both aquatic and terrestrial locomotion.

How does the same structure produce movement in aquatic and terrestrial environments?

As in other vertebrate taxa (Gillis and Blob, 2001), ropefish demonstrate distinct kinematics during terrestrial *versus* aquatic locomotion. Ropefish terrestrial locmotion is characterized by several distinct kinematic patterns. First, during terrestrial locomotion, the points on the body follow one another, such that all points travel in approximately the same direction. Second, when moving on land, the DR values and the wave parameters of amplitude, length and frequency do not change along the length of the ropefish's body. This is in marked contrast to aquatic locomotion, in which the anterior regions of the ropefish travel a different path relative to the posterior regions and wave amplitude increases along the body in an anterior to posterior direction. At higher swimming

velocities it is likely that wave frequency and amplitude will increase in the more anterior regions of the ropefish; a trend that was observed in eels (Gillis, 1998) swimming at different velocities and lungfish (Horner and Jayne, 2008) swimming in different viscosities. However, with both eels and lungfish, statistically significant differences are retained between the anterior and posterior regions of the fish at faster locomotor velocities.

Comparisons of muscle activation and strain in aquatic *versus* terrestrial locomotion in other organisms (Gillis and Blob, 2001) suggest that differences in locomotion are controlled actively by the organism and not generated passively through environmental effects. In the ropefish, movement patterns for the anterior region of the fish suggest that the caudal region of the organism plays a very different role during aquatic *versus* terrestrial locomotion. During aquatic locomotion, the caudal end of the body (the tail) likely produces most of the propulsive force, as evidenced by the fact that it undulates when the other body regions do not. During terrestrial locomotion, however, the tail does not appear to play the same role. Because it is laterally compressed, the tail presents little ventrally oriented surface area for potential contact with the ground; in this study, we observed that the tail appeared to lose purchase and slip on the surface more than the other parts of the ropefish. It is even possible that the tail is 'dragged' behind the body during terrestrial locomotion. A similar trend was noted in eels, where muscle activation was greatly reduced in the posterior regions of the animal during terrestrial locomotion compared with aquatic locomotion (Gillis, 1998; Gillis, 2000). It is also interesting to note that, in most fishes, undulatory swimming velocity is primarily controlled by changes in tail-beat frequency (Shadwick and Gemballa, 2006) – this is likely to be true for the aquatic locomotion of the ropefish as well. However, because the tail has a reduced role in propulsion on land, it is unclear what mechanism determines changes in animal velocity during terrestrial locomotion.

How do locomotor movements change during the transition between aquatic and terrestrial environments?

Both of the intermediate treatments (50 and 75% emersion) shared certain characteristics with the aquatic treatment and other characteristics with the terrestrial treatment. Lateral excursions and wave amplitude increased in an anterior to posterior direction in the aquatic, 50% emersion and 75% emersion treatments, but not in the terrestrial treatments. In addition, as the water level was lowered, the magnitude of the lateral excursions and wave amplitude in the anterior regions of the ropefish increased. Similarly, lungfish swimming in fluids of increased viscosity showed an increase in wave amplitude anteriorly, with the posterior wave amplitude changing little and always remaining greater than

the anterior wave amplitude (Horner and Jayne, 2008). Although the increase in viscosity experienced by lungfish is not identical to the change in physical regime experienced by ropefish during transitional treatments (see below), the similarities in movement patterns may indicate the increased effort required for both ropefish and lungfish to move under these circumstances. However, intermediate treatments also shared certain characteristics with terrestrial locomotion. For example, in all treatments where the ropefish was partially or completely exposed, there was no difference in wave frequency from head to tail (although wave frequency differed across treatments). In addition, for ropefish moving in the 75% emersion treatment, wavelength did not increase along the body (a pattern similar to the terrestrial treatment), whereas for ropefish moving in the 50% emersion treatment, wavelength did increase along the body (a pattern similar to that observed in the fully aquatic treatment).

These intermediate treatments, which simulated the transition from an aquatic to a terrestrial locomotor regime, presented the ropefish with changes in fluid viscosity, gravitational acceleration, and friction with the surrounding medium and the substrate. Indeed, both intermediate treatments presented a complex configuration of these various physical forces. For example, when part of the ropefish is exposed, the dorsal region of the body experiences reduced viscosity, but the ventral surface of the ropefish begins to interact with the substrate, generating a new source of friction. The relative contribution of the force produced by gravitational acceleration is difficult to predict in intermediate treatments, as the water may continue to abate the full effects of gravity.

As the ropefish experiences different physical regimes across environmental transitions, it may generate a specific neuromuscular response to each regime. However, it is difficult to determine which components of each movement pattern are due to the effects of the physical regime and which are a result of the behavioral response of the organism. For example, although the values for wavelength and wave amplitude show a consistent gradation from the 0% emersion treatment to the 100% emersion treatment, the values for wave frequency and DR do not. In particular, the 75% emersion treatment demonstrates lower DR values than all other treatments for the posterior half of the ropefish and a higher waver frequency than all other treatments for the COM. It is possible that this unusual pattern occurs because of the unique suite of physical forces present in this treatment. In the 75% emersion treatment, the ropefish does interact with the ground; however, at the same time, the volume of water still present likely generates buoyancy and decreases the friction between the body and the substrate. The combination of these effects may allow the COM of the ropefish to move with a high frequency, whereas the posterior half of the fish continues to demonstrate low DR values relative to terrestrial trials.

Ropefish were also highly variable when moving in the 75% emersion treatment. During 75% emersion, each individual produced trials with characteristics that were more similar to terrestrial trials, and trials with characteristics that were more similar to aquatic trials. Although every effort was made to standardize the substrate both within a single trial and across trials that occurred on different days, it is possible that microperturbations in the substrate influenced the observed locomotor pattern. However, there is another intriguing possibility: in the 75% emersion treatment, where the water level is extremely low, the organism vacillates between two different muscle activation patterns. Other organisms have been shown to vacillate between motor patterns when performing behaviors such as prey capture (Anderson and Nishikawa, 1993), and this may prove an interesting area of future research.

Do distantly related amphibious fishes demonstrate similar modes of terrestrial locomotion?

Terrestrial locomotion appears broadly similar in ropefish and eels (Gillis, 1998). When both organisms move on land, the body is bent into a series of high-amplitude, sinusoidal waves. In both species, absolutely larger undulations occur during terrestrial locomotion (waves are ~0.12% TL for eels and ~0.10% TL for ropefish) relative to aquatic locomotion (~0.07% TL for both eels and ropefish). In addition, both species produce a characteristic path-following behavior, wherein one point on the body directly follows (that is, travels in the same path as) the point immediately anterior to it. However, we note that path following appears less precise in eels and ropefish relative what has been observed for terrestrial lateral undulations of snakes and other organisms. In these elongate fish species, points do not always follow the exact same path, body regions sometimes appear to slide unexpectedly and movements occasionally appear uncoordinated. Some previous studies have used pegboards to elicit lateral undulation (Bennet et al., 1974; Jayne, 1986; Gans and Gasc, 1990; Summers and O'Reilly, 1997), and this structured environment may constrain study organisms to move in a more predictable fashion. However, some of these differences in movement between fish and snakes are probably due, at least in part, to differences in musculature and morphology. For example, snake scales have been shown to be important in terrestrial snake locomotion (Hu et al., 2009) and their presence has clear ramifications for locomotor behavior.

How common is this terrestrial locomotor pattern among amphibious fishes? We posit that the terrestrial movements of the two completely unrelated elongate fishes studied to date are functionally convergent because elongate fishes are limited in their potential mechanisms of terrestrial locomotion. Support for this hypothesis is provided by the Hawaiian climbing gobies; the juveniles of two of these species (*Awaous guamensis* and *Lentipes concolor*) generate lateral undulations of high amplitude for all parts of the body, including the head, while scaling near-vertical surfaces (Schoenfuss and Blob, 2003). It is possible that, regardless of how elongate a fish is, axial locomotion in the terrestrial environment may always be characterized by large lateral undulations at both the anterior and posterior ends of the animal. Although adult *A. guamensis* do not climb at all and adult *L. concolor* do not climb using axial undulations (instead they use their pectoral fins), this pattern may stem from the allometric effects of larger body size (Blob et al., 2007). Scattered across the ray-finned fishes are several other species of amphibious (or potentially amphibious) elongate fish; we predict the synbranchid eels (*Monopterus albus*, *Synbranchus marmoratus*), other anguillids and the muraenid *Gymnothorax pictus* will eventually be categorized as terrestrial undulators. Lateral undulation may be the only way to co-opt a morphology that must continue to function in an aquatic environment for use in a terrestrial environment. However, additional research examining terrestrial movements in these fishes and others that undergo voluntary terrestrial excursions will be necessary to determine whether elongate fishes are constrained in their terrestrial movements to produce functionally convergent locomotor behaviors.

REFERENCES

Anderson, C. W. and Nishikawa, K. C. (1993). A prey-type dependent hypoglossal feedback system in the frog *Rana pipiens*. *Brain Behav. Evol.* **42**, 189-196.

Ashley-Ross, M. A. and Bechtel, B. F. (2004). Kinematics of the transition between aquatic and terrestrial locomotion in the newt *Taricha torosa*. *J. Exp. Biol.* **207**, 461-474.

Bennet, S., McConnell, T. and Trubatch, S. L. (1974). Quantitative analysis of the speed of snakes as a function of peg spacing. *J. Exp. Biol.* **60**, 161-165.

Bierman, H. S., Schriefer, J. E., Zottoli, S. J. and Hale, M. E. (2004). The effects of head and tail stimulation on the withdrawal startle response of the rope fish (*Erpetoichthys calabaricus*). *J. Exp. Biol.* **207**, 3985-3997.

Blob, R. W., Wright, M. K., Becker, M., Maie, T., Iverson, T. J., Julius, M. L. and Shoenfuss, H. L. (2007). Ontogenetic change in novel functions: waterfall climbing in adult hawaiian gobiid fishes. *J. Zool.* **273**, 200-209.

Ellerby, D. J., Spierts, I. L. Y. and Altringham, J. D. (2001). Fast muscle function in the european eel (*Anguilla anguilla* L.) during aquatic and terrestrial locomotion. *J. Exp. Biol.* **204**, 2231-2238.

Frolich, L. M. and Biewener, A. A. (1992). Kinematic and electromyographic analysis of the functional role of the body axis during terrestrial and aquatic locomotion in the salamander *Ambystoma tigrinum*. *J. Exp. Biol.* **162**, 107-130.

Gans, C. (1986). Locomotion of limbless vertebrates: pattern and evolution. *Herpetologica* **42**, 33-46.

Gans, C. and Gasc, J. P. (1990). Tests on the locomotion of the elongate and limbless reptile *Ophisaurus apodus* (Sauria: Anguida). *J. Zool.* **220**, 517-536.

Gillis, G. B. (1998). Environmental effects on undulatory locomotion in the American eel *Anguilla rostrata*: kinematics in water and on land. *J. Exp. Biol.* **201**, 949-961.

Gillis, G. B. (2000). Patterns of white muscle activity during terrestrial locomotion in the American eel (*Anguilla rostrata*). *J. Exp. Biol.* **203**, 471-480.

Gillis, G. B. and Blob, R. W. (2001). How muscles accommodate movement in different physical environments: aquatic *vs.* terrestrial locomotion in vertebrates. *Comp Biochem. Physiol.* **131A**, 61-75.

Graham, J. B. (1997). Air-breathing fishes: evolution, diversity, and adaptation. San Diego, CA: Academic Press.

Horner, A. M. and Jayne, B. C. (2008). The effects of viscosity on the axial motor pattern and kinematics of the African lungfish (*Protopterus annectens*) during lateral undulatory swimming. *J. Exp. Biol.* **211**, 1612-1622.

Hu, D. L., Nirody, J., Scott, T. and Shelley, M. J. (2009). The mechanics of slithering locomotion. *Proc. Nat. Acad. Sci. USA* **106**, 10081-10085.

Jayne, B. C. (1986). Kinematics of terrestrial snake locomotion. *Copeia* **1986**, 915-927.

Lauder, G. V. and Tytell, E. D. (2006). Hydrodynamics of undulatory propulsion. In *Fish Biomechanics* (ed. R. E. Shadwick and G. V. Lauder), pp. 425-468. San Diego, CA: Elsevier Academic Press.

Nelson, J. S. (1994). Fishes of the world. New York: Wiley.

Ott, R. L. and Longnecker, M. (2001). An introduction to statistical methods and data analysis. Pacific Grove: Duxbury.

Pace, C. M. and Gibb, A. C. (2009). Mudskipper pectoral fin kinematics in aquatic and terrestrial environments. *J. Exp. Biol.* **212**, 2279-2286.

Pettit, M. J. and Beitinger, T. L. (1985). Oxygen acquisition of the reedfish, *Erpetoichthys calabaricus*. *J. Exp. Biol.* **114**, 289-306.

Sacca, R. and Burggren, W. (1982). Oxygen uptake in air and water in the air-breathing reedfish *Calamoichthys calabaricus*: role of skin, gills, and lungs. *J. Exp. Biol.* **97**, 179-186.

Schoenfuss, H. L. and Blob, R. W. (2003). Kinematics of waterfall climbing in Hawaiian freshwater fishes (Gobiidae): vertical propulsion at the aquatic-terrestrial interface. *J. Zool.* **261**, 191-205.

Shadwick, R. E. and Gemballa, S. (2006). Structure, kinematics, and muscle dynamics in undulatory swimming. In *Fish Biomechanics* (ed. R. E. Shadwick and G. V. Lauder), pp. 241-280. San Diego, CA: Elsevier Academic Press.

Shine, R. and Shetty, S. (2001). Moving in two worlds: aquatic and terrestrial locomotion in sea snakes (*Laticauda colubrina*, Laticaudidae). *J. Evol. Biol.* **14**, 338-346.

Shine, R., Cogger, H. G., Reed, R. R., Shetty, S. and Bonnet, X. (2003). Aquatic and terrestrial locomotion speeds of amphibious sea snakes (Serpentes, Laticaudidae). *J. Zool.* **259**, 261-268.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*. New York: W. H. Freeman and Company.

Steel, R. G. D., Torrie, J. H. and Dickey, D. A. (1997). Principles and procedures of statistics a biometrical approach. Boston, MA: WCG McGraw-Hill.

Summers, A. P. and O'Reilly, J. C. (1997). A comparative study of locomotion in the caecilians *Dermophus mexicanu*s and *Typhlonectes natans* (Amphibia: Gymnophiona). *Zool. J. Linn. Soc.* **121**, 65-76.

Udoidiong, O. M. and King, R. P. (2000). Ichthyofaunal assemblages of some Nigerian rainforest streams. *J. Aquat. Sci.* **15**, 1-8.

Vogel, S. (1994). Life in moving fluids: the physical biology of flow. Princeton, NJ: Princeton University Press.

Ward, A. B. and Brainerd, E. L. (2007). Evolution of axial patterning in elongate fishes. *Biol. J. Linn. Soc.* **90**, 97-116.

Welcomme, R. L. (1979). Fisheries ecology of floodplain rivers. London and New York: Longman.