Locomotion in elongate fishes: A contact sport

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ABSTRACT

Despite the physical differences between water and air, a number of fish lineages are known to make terrestrial excursions on land. Many of these fishes exhibit an elongate body plan. Elongation of the body can occur in several ways, the most common of which is increasing the number of vertebrae in one or both regions of the axial skeleton – precaudal and/or caudal. Elongate species are often found in three-dimensionally complex habitats. It has been hypothesized that elongate fishes use this structure to their locomotor advantage. In this study, we consider how elongation and differences in vertebral regionalization correspond with the use of wooden pegs, which are provided as analogs to vertically oriented substrate, structures that protrude above the ground. We compare aquatic and terrestrial locomotor behaviors of *Polypterus senegalus*, *Erpetoichthys calabaricus*, and *Gymnallabes typus* as they move through a peg array. When considering axial elongation we find that the highly elongate species, *E. calabaricus* and *G. typus*, contact more pegs but on average move slower in both environments than *P. senegalus*. When considering axial regionalization, we find that the precaudally elongate species, *P. senegalus* and *E. calabaricus*, differ in the patterns of peg contact between the two environments whereas the caudally elongate species, *G. typus*, exhibits similar peg contact between the two environments. Our study highlights the importance of incorporating body shape and vertebral regionalization to understand how elongate fishes move in water and on land.

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1. Introduction

Several fishes are able to withstand ground reaction forces and apply a counteractive force onto the substrate for forward propulsion to make terrestrial excursions (Johnels, 1957; Blob et al., 2006; Van Wassenbergh et al., 2006; Pace and Gibb, 2009; Hsieh, 2010; Gibb et al., 2011; Cucherousset et al., 2012; Van Wassenbergh, 2013). A recent review of terrestrial locomotor behaviors for amphibious fish species identified three functional categories: axial-based, appendage-based, and axial-appendage based loco-motion (Pace and Gibb, 2014). Of these categories, axial-based, using only the body to propel forward and axial-appendage based, where the body and fins (pectoral, tail, and/or pelvic) are mainly relied upon for forward movement, appear to be more common for extant amphibious species studied to date.

Body shape may also facilitate terrestrial forays as amphibious fishes tend to exhibit an anguilliform or eel-like body plan (reviewed in Pace and Gibb, 2014). Elongation is the result of lengthening of the axial skeleton, decrease of body width or depth,

increases in the cranial skeleton, or a combination of these factors. The axial skeleton is lengthened through either an increase in the overall number of vertebrae or through an increase in the length of individual vertebrae (Ward and Brainerd, 2007; Mehta et al., 2010). While the majority of fishes elongate their bodies through adding vertebrae in the caudal region, numerous lineages add vertebrae to both the precaudal and caudal region. Only one clade, Polypteriformes, is known to add vertebrae strictly to the precaudal region (Ward and Brainerd, 2007).

Few studies have focused on how vertebral regionalization affects locomotory performance (Swain, 1992; Kelley et al., 1997). One compelling study that highlights the interaction between vertebral characteristics and survival performance with stickleback larvae (*Gasterosteus aculeatus*) revealed that selection was acting on the ratio of precaudal to caudal vertebrae and that as larvae increased in length, the optimal vertebral ratio decreased (Swain, 1992). Thus individuals with relatively more caudal vertebrae exhibit increased performance in their aquatic escape response (Swain, 1992). While some species exhibit dramatic anatomical differences between the major regions of the vertebral column, the functional implications of vertebral regionalization on locomotor performance have yet to be realized.

Similar to tetrapods where highly elongate forms tend to exhibit limb reduction and/or loss, elongation of the body in fishes tends to be correlated with a reduction in both the length and width of the pectoral fins (Ward and Mehta, 2010). Pectoral fin reduction has been suggested to constrain highly elongate fishes to using only the axial skeleton to produce movements on land (Pace and Gibb, 2014). Interestingly, while most fishes use axial-based movements for aquatic locomotion, highly elongate amphibious species from disparate clades appear to employ axial-based lateral undulations during terrestrial locomotion although kinematic differences exist between species (Gillis, 1998; Ellerby et al., 2001; Pace and Gibb, 2011).

Depending on the shape and flexibility of the fish, the number of bends along the body can differ during locomotion (Breder, 1926). Having more vertebrae is presumed to increase body flexibility; there are more joints at which the body can bend (Brainerd and Patek, 1998). Vertebral number also tends to be correlated with standard length in fishes of similar shape (Lindsey, 1975). Elongate terrestrial vertebrates, such as snakes, are known to use push points in their environment for forward propulsion (Gray and Lissmann, 1950; Jayne and Bennett, 1990; Kelley et al., 1997). Increased flexibility would therefore allow individuals to reach more distantly spaced push-off points in the environment (Kelley et al., 1997). Similarly, body elongation in actinopterygian fishes may facilitate the use of push points that are staggered throughout the environment. However, much of the study of fish locomotion has focused on movement in an open water context, with one recent exception (Ellerby and Gerry, 2011). Most importantly, the link between vertebral number and terrestrial locomotor movements has yet to be examined.

Polypteriformes provide an excellent group to investigate how differences in body shape affect locomotion. Polypteriformes is considered the most basal lineage of extant actinopterygians (e.g., Alfaro et al., 2009). Extant members of the group are included in two genera: *Erpetoichthys* and *Polypterus*. *Erpetoichthys* is monotypic while *Polypterus* has thirteen species (Froese and Pauly, 2014). These genera differ remarkably in body shape, although both are relatively elongate compared to other fishes. *Erpetoichthys*, commonly known as ropefish, has a serpentine-like body plan whereas all thirteen species of *Polypterus* are more stout-bodied (Brainerd et al., 1989; Ward and Brainerd, 2007; Ward and Kley, 2012). All Polypteriformes are facultative air breathers (Abdel Magid, 1967; Pettit and Beitinger, 1981). Ropefish are known to make terrestrial excursions (Sacca and Burggren, 1982; Pace and Gibb, 2011) and a recent study showed how terrestrially raised *Polypterus senegalus* can use their pectoral fins to effectively locomote on land (Standen et al., 2014). *Erpetoichthys calabaricus* has approximately twice as many precaudal vertebrae (100) as *P. senegalus* (45), while both genera have similar caudal vertebral numbers (Ward and Brainerd, 2007; Ward and Kley, 2012).

Here, we compare terrestrial and aquatic locomotion in Polypteriformes with *Gymnallabes typus*, a clariid catfish. Clariidae contains many members capable of undergoing terrestrial excursions. The most well-known are the "walking catfishes" (*Clarias* spp.) which are propelled with large pectoral fins (Johnels, 1957). However, there are other species, with reduced pectoral fins, that locomote terrestrially via axial undulation. *Channallabes apus* has been observed undulating through grassy vegetation (Adriaens et al., 2002) and uses dorsoventral flexion of the anterior aspect of the vertebral column to bite prey terrestrially (Van Wassenbergh et al., 2006; Van Wassenbergh, 2013). Elongate catfishes tend to have more caudal vertebrae than close relatives (Adriaens et al., 2002; Ward and Brainerd, 2007). *G. typus* has 19 precaudal vertebrae and 73 caudal vertebrae (Ward, pers. obs.). Therefore, anguilliform catfishes such as *G. typus* provide an interesting comparison to the precaudally elongated ropefish.

In the present study, we investigated the effects of environment (aquatic and terrestrial) on peg use in elongate amphibious fishes. We consider the pegs to be analogous to vertical substrate (reeds, rocks, etc.). As terrestrial axial undulation has been shown to generate less force compared to aquatic axial undulation (Ellerby et al., 2001), we designed locomotor trials in two environments where fishes had uniform access to vertical substrate (i.e., wooden pegs). Here, we simply asked, "Do differences in elongation and vertebral regionalization affect peg use in the aquatic and terrestrial environment?"

2. Materials and methods

2.1. Animals and animal care

We obtained the following three freshwater fish species through the pet trade: four individuals of *P. senegalus* (total length (TL) range: 7.7–9.4 cm; maximum body width (BW) range 0.94–1.01 cm), three individuals of *E. calabaricus* (TL range: 23.5–27.4 cm; BW range: 0.93–1.03 cm), and three individuals of *G. typus* (TL range: 16.7–22.0 cm; BW range 0.96–1.36 cm). Each individual was kept in a 38-liter glass aquarium maintained at a temperature range of 24–27 °C with standard aquarium heaters. Individuals were fed either frozen bloodworms (Chironomidae) or live blackworms (*Lumbriculus variegatus*) three times per week. All experiments performed were approved by the Adelphi University Institutional Animal Care and Use Committee.

2.2. Experimental design

A 50.8 cm × 33.0 cm plexiglass pegboard was fitted with 77 wooden pegs (diameter = 4.88 mm, length = 3 cm). Pegboard studies have long been used to examine axial locomotion in terrestrial taxa (Gray and Lissmann, 1950; Jayne, 1988; Jayne and Bennett, 1990). Peg spacing influences the velocity (Gasc and Gans, 1990) and pattern (Gans and Gasc, 1990) of movement in lizards. Peg arrays with narrow spacing, less than 2 times the body width of the animal, constrained the direction of motion in anguid lizards while pegs spaced at distances at least 6 times anguid body widths were so great that they inhibited locomotion. While total lengths for *P. senegalus*, *E. calabaricus*, and *G. typus* varied greatly, body widths were not statistically significantly different between the three species ($F_{2,9} = 3.35$; $p = 0.10$). Therefore, we chose the distance between the centers of adjacent pegs to be 3 cm; this distance was at least two times maximum body width of the individuals included in the study. The pegboard was placed in a plastic container (length × width × depth = 55.9 cm × 39.4 cm × 14.0 cm). During aquatic trials, the water level was 4 cm. During terrestrial trials, enough water was added to the tank to keep the pegboard wet. Filming was performed at 30 fps with a Panasonic HD camcorder.

Individuals were allowed to acclimate and explore the pegboard environment 5 min prior to the onset of filming. Once acclimated, a trial began by encouraging individuals to move by gentle coaxing with a hand net. A trial was defined as the animal crossing the field of pegs with continuous motion. All trials were filmed and we saved only those where individuals were moving continuously. Four to six locomotor bouts were analyzed for each individual in each environmental treatment. We quantified the following six variables: (1) number of pegs touched per trial – defined as the total number of pegs an individual contacted during a single trial standardized by body lengths traveled during that trial, (2) contact duration (s) – defined as the amount of time the body touched an individual peg, (3) "on" position – defined as the point on the body where peg contact first occurred, standardized by body length (BL), (4)

"off" position – the posterior-most point along the body where the body leaves the peg, standardized by BL, (5) body contact length – the total length along the body (or the proportion of the body) that touched an individual peg; body contact length was calculated as the difference between "off" position and "on" position, and (6) average velocity – calculated as the displacement of the snout during a trial divided by the trial duration, standardized by body length (BL/s). Contact duration, body contact length, and "on" and "off" positions were averaged across pegs for each trial.

2.3. Statistical analysis

We used a two-way nested ANOVA to examine the effects of species and environment on number of pegs touched per trial, contact duration, "on" position, "off" position, body contact length, and average velocity. We ran a principal components analysis, without rotation, to reduce the dimensionality of the data set and to examine which variables accounted for the greatest variation between species across the two environmental treatments. Inter-individual variance of the eigenvalues was calculated for each species in each environment for the first three principal components axes. All analyses were run in JMP 8 (SAS Institute, Inc., Cary, NC, USA).

3. Results

In both environments, fish were willing to move through the pegboard. During aquatic treatments, there were a few trials where *P. senegalus* swam through the pegboard without touching a single peg. We analyzed only the trials where *P. senegalus* contacted a peg. Although the body width of each fish was at most half the distance between pegs, *E. calabaricus* and *G. typus* always contacted pegs during aquatic locomotion. For terrestrial locomotion, all species touched pegs when moving through the array. The highly elongate species (*E. calabaricus* and *G. typus*) kept their pectoral fins against the body during locomotion. *P. senegalus* exhibited more movements in the pectoral fins, but still maintained the pectoral fins against the body when moving forward. While only the Polypteriformes, *P. senegalus* and *E. calabaricus*, had distinct anal fins and *P. senegalus* and *G. typus* had pelvic fins, neither of these fin types were used to propel the fish forward; only axial-based propulsion was observed.

We observed different patterns of peg contact along the body (Fig. 1). As individuals approached new pegs, contact would occur on the opposite side of the body from the previously contacted peg (Fig. 1A). In more elongate species, oftentimes two consecutive pegs would contact the same side of the body (Fig. 1B). Less frequently, we observed three consecutive pegs contacting the same side of the body (Fig. 1C). In general, once an individual contacted a peg, the rest of the body would slide along that peg (Fig. 2A–D). The two highly elongate species would touch more pegs at any given time than *P. senegalus* during both aquatic and terrestrial trials (Figs. 1 and 2). During a locomotory trial, the highly elongate species contacted more pegs on average than *P. senegalus* ($F_{6,84} = 21.81$, $p < 0.0001$; Fig. 2 and Table 1). *G. typus* contacted more pegs during terrestrial trials than aquatic trials; there was no difference in the number of pegs contacted in the other two species when comparing environments ($F_{6,84} = 2.54$, $p = 0.026$; Fig. 3).

Contact duration exhibited a significant species × environment interaction ($F_{6,84} = 4.52$, $p = 0.001$; Fig. 4 and Table 1). For both *P. senegalus* and *E. calabaricus*, contact duration was significantly greater during terrestrial trials than during aquatic trials. However, in *G. typus*, there was no difference in contact duration between the two environments.

When "on", "off", and body contact length were averaged across pegs within trials, there was no difference between species or
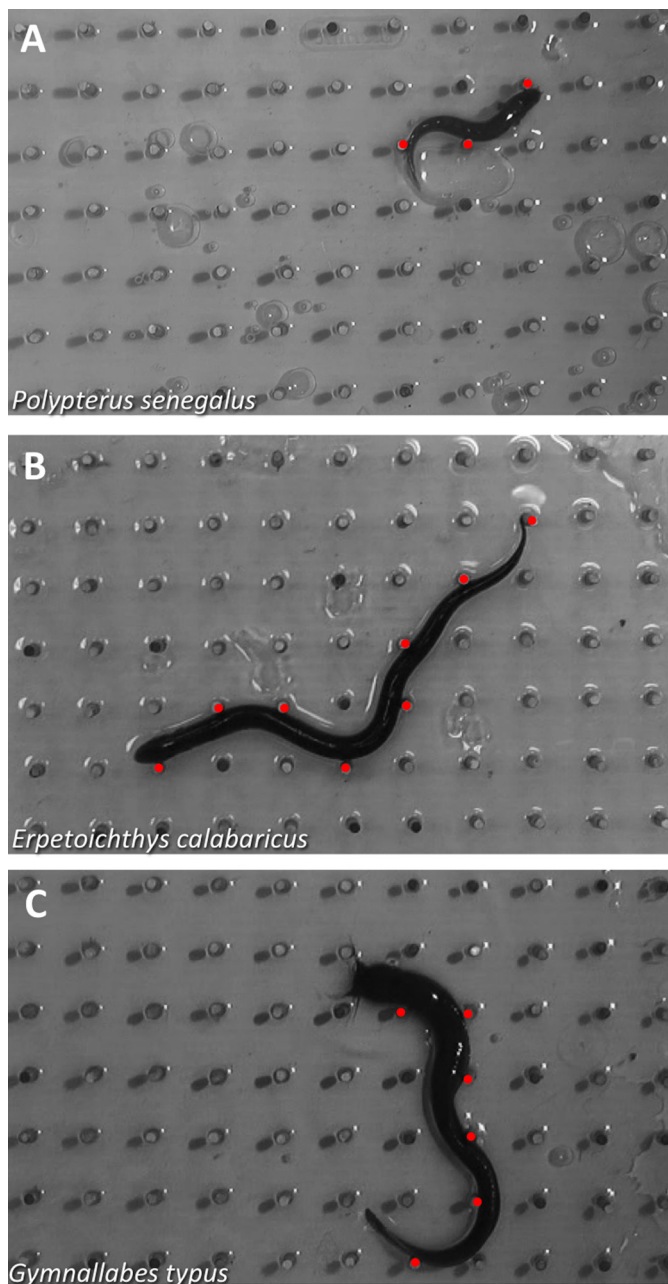

Fig. 1. Representative frames showing the maximum number of pegs contacted at a single point during a terrestrial trial. Pegs that are contacted are red. Notice how pegs are touched on opposing sides of the body.

environment in the "on" position, the point on the body where a peg was first contacted ($F_{13,84} = 1.78$, $p = 0.059$; Fig. 5, Table 1). The main difference in body contact length between species and environments was driven by the "off" position, the posteriormost point along the body that contacts the peg ($F_{13,84} = 6.928$, $p < 0.0001$; Fig. 5 and Table 1). The "off" position extended closer to the caudal fin rays during terrestrial trials in *P. senegalus* and *E. calabaricus* than during aquatic trials for both species (Fig. 5). There was no difference in the "off" position in *G. typus* when comparing across aquatic and terrestrial trials. Body contact length, defined as the proportion of the body that touched a given peg, also differed between environments and species ($F_{13,84} = 9.87$, $p < 0.0001$; Fig. 5 and Table 1). Ropefish exhibited greater body contact length in terrestrial trials compared to aquatic trials.
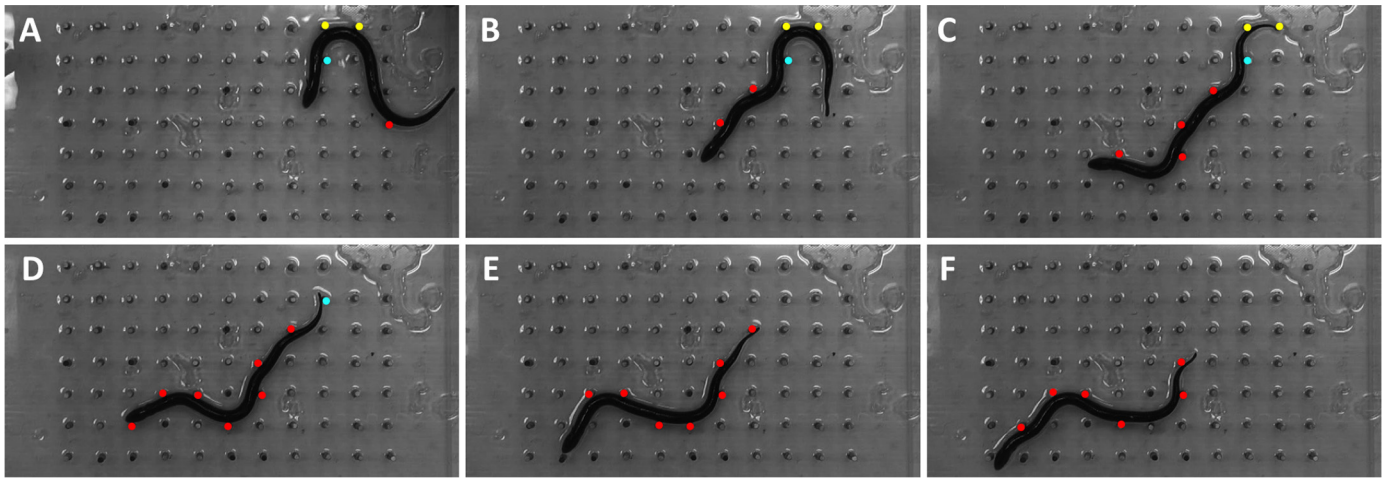
Fig. 2. Successive images during a terrestrial trial of *Erpetoichthys calabaricus*. Images are one second apart. Red dots mark pegs being touched at each time point. Three individual pegs were traced through A–D (marked yellow and turquoise).
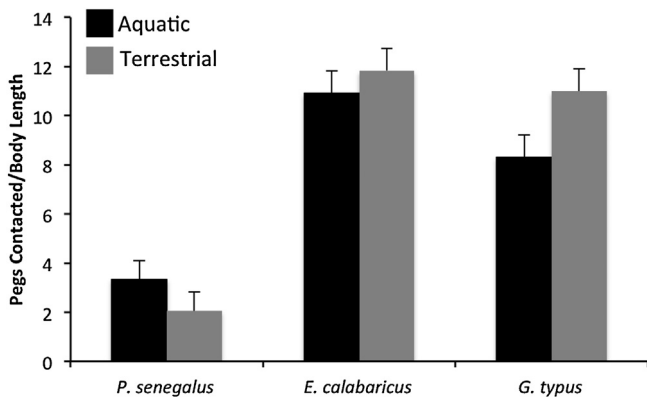

Fig. 3. The average number of pegs (±SE) touched during a single trial for each species standardized by body lengths traveled during the trial. Aquatic trials are black and terrestrial trials are gray. The highly elongate species contacted more pegs on average per body length traveled than *Polypterus senegalus*.
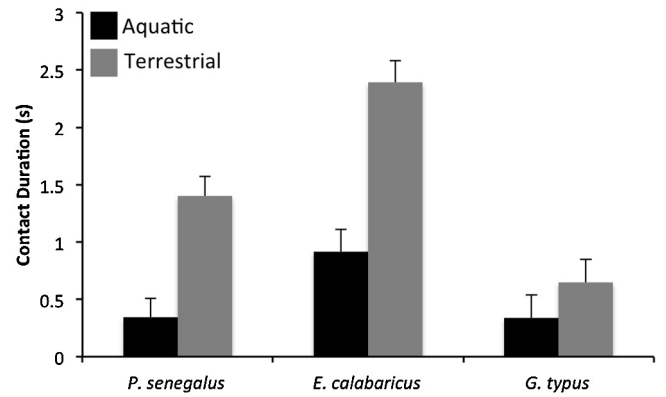
Fig. 4. The average (±SE) contact duration for each species. Aquatic trials are black and terrestrial trials are gray. *Polypterus senegalus* and *Erpetoichthys calabaricus* differed in contact duration between environments while there was no difference between contact duration in the two treatments for *Gymnallabes typus*.
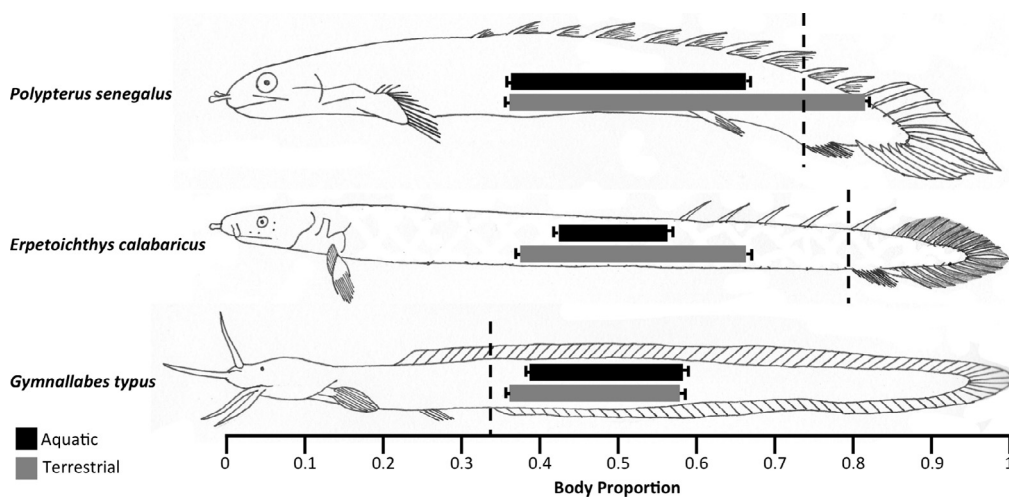
Fig. 5. The average body position that contacts the pegs. The left side of the bar is the mean "on" position ± SE and the right side of the bar is the mean "off" position ± SE. The distance between the "on" and "off" positions is known as the contact length. The dashed line designates the transition between precaudal and caudal regions along the axial skeleton. The range in total length for each of the three species was: *Polypterus senegalus* = 7.7–9.4 cm, *Erpetoichthys calabaricus* = 23.5–27.4 cm, and *Gymnallabes typus* = 16.7–22.0 cm. *P. senegalus* and *E. calabaricus* primarily contact pegs in their precaudal region whereas *G. typus* contacts pegs in the caudal region.
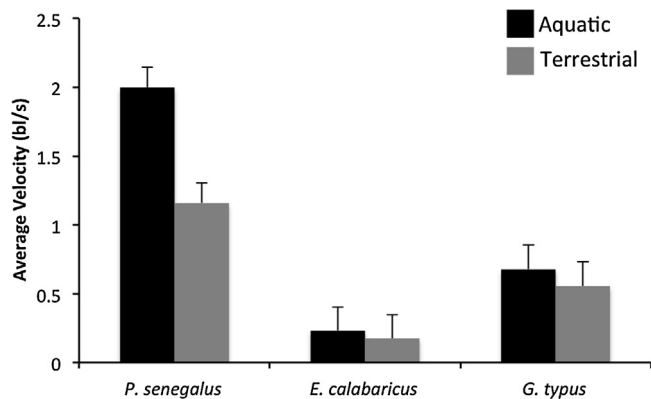
Fig. 6. The average velocity (±SE) for each species. Velocity was standardized by total length. Aquatic trials are black and terrestrial trials are gray. *Polypterus senegalus* was faster in both environments than the highly elongate species.

P. senegalus moved faster during aquatic trials than during terrestrial trials ($F_{6,84} = 2.54$, $p < 0.026$; Fig. 6, Tables 1 and 2) and exhibited faster locomotor velocities compared to the two highly elongate species. *G. typus* and *E. calabaricus* did not vary their locomotor velocity across environments (Fig. 6 and Table 2).

A principal components analysis enabled us to examine the variables driving inter-individual trial differences between species and environmental treatments (Fig. 7). Principal components axis (PC) 1 accounted for 42.62% of the variation, 24.85% was described by PC 2, and 18.89% was described by PC 3. The variables that loaded strongly and positively on PC 1 were body contact length and "off" position. The variable "number of pegs contacted" loaded strongly and negatively on PC 1. Contact time loaded strongly and positively while average velocity had a strong negative loading on PC 2. "On"

Table 1
Results of two-way nested ANOVA for the six variables considered in this study.

Variable	DF	*F*-ratio	*P*-value	Eta-squared
Pegs touched/body length	13, 84	12.87	**<0.0001**	
Species (individual)	6	26.27	**<0.0001**	0.63
Environment	1	0.61	0.439	0.00
Species × environment (individual)	6	1.42	0.216	0.03
Contact duration (s)	13, 84	10.58	**<0.0001**	
Species (individual)	6	10.18	**<0.0001**	0.27
Environment	1	50.06	**<0.0001**	0.23
Species × environment (individual)	6	4.52	**0.001**	0.12
"On" position (BL)	13, 84	1.78	0.059	
Species (individual)	6	2.05	0.068	0.11
Environment	1	1.65	0.203	0.02
Species × environment (individual)	6	1.59	0.160	0.09
"Off" position (BL)	13, 84	6.93	**<0.0001**	
Species (individual)	6	10.80	**<0.0001**	0.37
Environment	1	13.46	**0.0004**	0.08
Species × environment (individual)	6	1.85	0.099	0.06
Body contact length (BL)	13, 84	9.87	**<0.0001**	
Species (individual)	6	15.38	**<0.0001**	0.44
Environment	1	21.14	**<0.0001**	0.10
Species × environment (individual)	6	2.18	0.053	0.12
Average velocity (BL/s)	13, 84	4.88	**<0.0001**	
Species (individual)	6	7.47	**<0.0001**	0.30
Environment	1	7.15	**0.009**	0.05
Species × environment (individual)	6	1.94	0.084	0.08

Bolded *p*-values indicate significance <0.05.

Table 2
Least-square mean and standard error of average velocity.

Species (individual)	Environment	Mean	SE	Post hoc grouping
Polypterus senegalus				
1	Aquatic	2.49	0.29	A
2	Aquatic	1.24	0.30	C, D, E
3	Aquatic	2.11	0.30	A, B
4	Aquatic	1.09	0.11	C, D
1	Terrestrial	0.97	0.29	C, D, E, F
2	Terrestrial	1.29	0.29	B, C, D
3	Terrestrial	1.23	0.30	C, D, E
4	Terrestrial	0.69	0.11	E, F, G
Erpetoichthys calabaricus				
1	Aquatic	0.16	0.29	F, G
2	Aquatic	0.59	0.30	C, D, E, F, G
3	Aquatic	0.50	0.30	E, F, G
1	Terrestrial	0.36	0.29	F, G
2	Terrestrial	0.16	0.29	G
3	Terrestrial	0.25	0.30	F, G
Gymnallabes typus				
1	Aquatic	0.63	0.27	D, E, F, G
2	Aquatic	1.44	0.32	B, C
3	Aquatic	0.67	0.32	C, D, E, F, G
1	Terrestrial	0.73	0.29	C, D, E, F, G
2	Terrestrial	0.61	0.29	C, D, E, F, G
3	Terrestrial	0.58	0.32	C, D, E, F, G

Significance is based on a post hoc Student's *T*-test. Individuals not connected by the same letter are significantly different from one another. A is the fastest velocity and G designates the slowest average velocity.

Table 3
Loadings for the six locomotor variables on the first three principal components axes.

Species	PC 1 (42.62%)	PC 2 (24.85%)	PC 3 (18.89%)
Pegs touched/body length	−0.531	0.161	−0.022
Contact duration (s)	0.085	0.732	−0.076
Body contact length (BL)	0.574	0.147	−0.217
"On" position (BL)	−0.121	0.148	0.897
"Off" position (BL)	0.507	0.283	0.301
Average velocity (BL/s)	0.331	−0.561	−0.022

position was the variable that loaded strongly and positively on PC 3 (Table 3).

The trials of all species separate along PC 1 with *P. senegalus* occupying the area defined by longer body contact length and more posterior "off" position (Fig. 7). Trials for the highly elongate species are characterized by greater peg contact. PC 2 separates trials by environment for the Polypteriformes with terrestrial trials defined as having longer contact duration and slower average velocities. For PC 1, *P. senegalus* has the greatest variance during aquatic trials while *E. calabaricus* has the least. For terrestrial trials, *E. calabaricus* has the greatest variance in PC 1 scores and *G. typus* has the least. Both aquatic and terrestrial trials of *G. typus* occupy a narrow region of PC 2 as exemplified by the lower variances (Table 4). Both PC 2 and PC 3 show similar trends with *P. senegalus* having the greater variance (larger inter-individual variation) and *G. typus* having the least variance across all trials.

Table 4
Total inter-individual variance calculated by species and environment for the first three principal components.

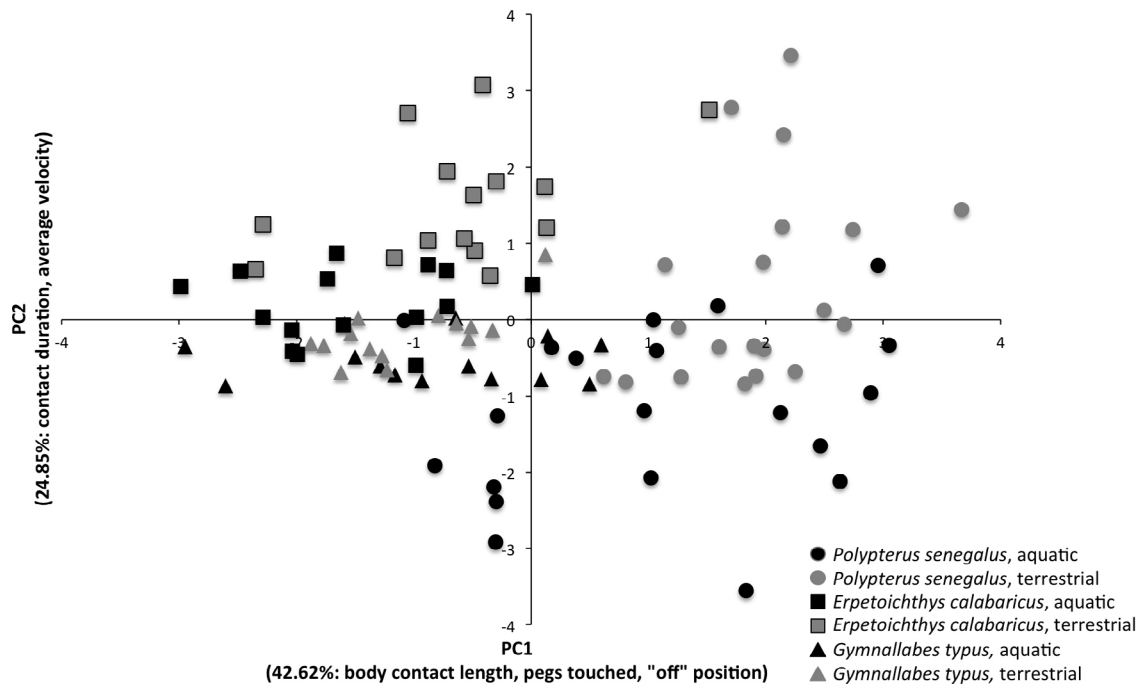| Species	Environment	PC 1	PC 2	PC 3
Polypterus senegalus	Aquatic	1.798	1.273	2.989
Polypterus senegalus	Terrestrial	0.499	1.692	1.220
Erpetoichthys calabaricus	Aquatic	0.650	0.216	0.517
Erpetoichthys calabaricus	Terrestrial	0.876	0.628	0.555
Gymnallabus typus	Aquatic	1.147	0.090	0.427
Gymnallabus typus	Terrestrial	0.370	0.143	0.379

Fig. 7. Plot of principal components (PC) axis 1 versus principal components axis 2 for the six variables included in this study. Variable loadings are noted in parentheses. The trials of *Polypterus senegalus* separate from the trials of the highly elongate species along PC 1. For PC 2, the trials of *Erpetoichthys calabaricus* and *P. senegalus* separate between environmental treatments.

4. Discussion

In the present study, we examined peg use during both aquatic and terrestrial locomotion in three fish species that vary in axial elongation. While *P. senegalus*, *E. calabaricus*, and *G. typus* are considered elongate with an increased number of vertebrae compared to close relatives, the proportions of the precaudal and caudal regions differ dramatically. *P. senegalus* and *E. calabaricus* exhibit precaudal elongation whereas *G. typus* has increased body length by caudal elongation.

During locomotion trials, fishes contacted the 3 cm spaced pegs during both aquatic and terrestrial locomotion (Fig. 3). In a few aquatic trials (that were not included in our data set), *P. senegalus* swam the array without contacting any of the pegs. The highly elongate species consistently contacted pegs during both aquatic and terrestrial trials. In fact, from our preliminary trials, we observed that *E. calabaricus* and *G. typus* were struggling to move forward on plain plexiglass without the pegs. This was not surprising because the animals did not have anything to utilize for propulsion; Gillis (1998) noted in his studies on terrestrial locomotion of American eels (*Anguilla rostrata*) that the eels would depress the sand forming a shallow trench. The eels could then propel forward by pushing against the sides of the trench (Gillis, 1998). In the present study, the substrate (plexiglass) was manipulated by the addition of vertical substrate, pegs, to allow for forward propulsion. The highly elongate fishes contacted more pegs during a locomotory trial per body length traveled than *P. senegalus* (Fig. 3). There were no differences in the number of pegs touched per body length between environmental treatments (Table 1). From these results we speculate that elongate fishes are pushing against the pegs for forward propulsion as described in limbless tetrapods (Gray and Lissmann, 1950; Jayne, 1988; Gans and Gasc, 1990; Gasc and Gans, 1990; Kelley et al., 1997). In some instances, the highly elongate species appeared to suddenly propel themselves forward, advancing a considerable distance by pushing off of the pegs. During these events, we observed the pegs moving in the opposite direction from where an individual made contact. Future studies incorporating force measurements

with peg use, our proxy for vertical substrate use, will enable us to understand the magnitude of forces highly elongate fishes need to propel themselves forward as well as which regions of the axial skeleton are producing force.

4.1. Vertebral regionalization and locomotor performance

The significant differences in velocity, measured in BL/s, between species ($p < 0.0001$; Fig. 6) were remarkable. *P. senegalus* could move up to 2 BL/s in the aquatic environment, which was reduced to just over 1 BL/s in the terrestrial environment. This was achieved by touching one-third to a half as many pegs per body length traveled as the highly elongate species. Despite the dramatic difference in vertebral regionalization, there was no difference in velocity between highly elongate species (Fig. 6 and Table 2). However, the highly elongate species moved significantly slower than *P. senegalus*. This was, in part, due to differences in behavior. The highly elongate species tended to meander through the peg array whereas *P. senegalus* individuals would swim straight through the center of the array in aquatic treatments. While their velocities were not significantly different, *G. typus* possibly moved more body lengths compared to ropefish because it not only contacted more pegs but the time it spent on any one peg was shorter (Figs. 3 and 4).

Despite similarities in velocity between the elongate species, the use of the pegs differed between the two types of vertebral regionalization (Figs. 3 and 4). Peg contact duration corresponded more with axial regionalization whereas velocity varied in relation to extent of elongation (elongate to highly elongate). For the species with greater precaudal vertebral numbers (*P. senegalus* and *E. calabaricus*), both peg contact duration and proportion of the body in contact with a peg increased during terrestrial locomotion. Velocity did not differ between environments in *G. typus*, the caudally elongate species (Fig. 6). The differences in peg contact between Polypteriformes and *G. typus* are likely due to the fact that forward propulsion in a highly elongate fish during terrestrial locomotion requires greater muscular force than during aquatic locomotion

(Gillis, 2000); *P. senegalus* and *E. calabaricus* are pushing against the pegs with the precaudal region that presumably would produce less force than the caudal region (Fig. 5). Ellerby et al. (2001) demonstrated that undulation in a terrestrial environment produces less force than during aquatic locomotion. *G. typus* only contacted the peg with the caudal region, but in doing so, was able to produce enough force to propel the individual (Fig. 5).

4.2. Use of substrate during locomotion

A previous study of aquatic locomotion in *E. calabaricus* showed that the major axial movement occurred in the caudal region whereas terrestrial locomotion occurred through large amplitude undulations across the entirety of the body which is more similar to the manner of locomotion that we observed in the present study (Pace and Gibb, 2011; Fig. 2). The differences in locomotory style between free swimming and peg-based movements during aquatic trials indicate that *E. calabaricus* modify their movement patterns when presented with complex environments.

The peg use by elongate, limbless tetrapods has been described in several ectothermic vertebrate groups including snakes and anguid lizards. In laboratory studies using snakes and lizards, forward movement of the body occurred when animals took advantage of peg arrays, pushing off of individual pegs (Gray and Lissmann, 1950; Gans and Gasc, 1990; Gasc and Gans, 1990). For the limbless lizards (*Anguis* and *Ophisaurus*), as peg spacing increased, it became more challenging for the lizards to maintain undulatory locomotion (Gasc and Gans, 1990). *Anguis* was able to contact pegs spaced at greater distances compared to *Ophisaurus*, which is likely due to the fact that *Anguis* have more precaudal vertebrae than *Ophisaurus* (Gasc and Gans, 1990). However, in the garter snake (*Thamnophis elegans*), vertebral number was not correlated with the ability to move through low-density peg arrays (Kelley et al., 1997). In all but the lowest density peg array, individual garter snakes with more caudal vertebrae exhibited greater locomotor speeds than those individuals with fewer (Kelley et al., 1997).

Although elongate fishes are known to make terrestrial forays, we hypothesized that the manner of forward propulsion using vertical substrate would differ between species with divergent axial regionalization. Here we observe that the precaudally elongate ropefish do not use a greater portion of their body, compared to the caudally elongate *G. typus* in the aquatic or terrestrial environments (Fig. 5). This suggests that the primary locomotor region in elongate fishes is the region with the greatest number of vertebrae. It would be interesting to look at peg contact in *Anguilla* sp. where total vertebral number is more evenly distributed across the precaudal and caudal regions. In eel-like fishes, increases in vertebral number to a particular body region may not only enhance flexibility in that region but could provide more lateral area off which an animal can push. Species that elongate the precaudal region over the caudal region may have somewhat of a disadvantage as the viscera are located anterior to the tail and do not provide propulsive forces. Increasing contact duration with vertical substrate, as observed in ropefish especially in terrestrial trials, may aid in providing thrust (Fig. 4).

A recent study examining an early sarcopterygian, *Protopterus annectens*, found that axial movements resulted from trunk muscles with little to no tail activity in the terrestrial environment (Horner and Jayne, 2014). Interestingly, movement onto land shifted lungfish from tail-driven to trunk-driven propulsion. *Protopterus* and other members of Dipnoi have relatively equal numbers of vertebrae in the precaudal and caudal region (Arratia et al., 2001; Horner and Jayne, 2008). Perhaps increases in precaudal vertebral number may enable greater reliance on the axial skeleton when traveling on land in sarcopterygians that have reduced or completely lost their limbs as observed in snakes, a clade that exhibits the greatest

number of precaudal vertebrae in vertebrates (Ward and Mehta, 2014).

It is unknown how density of vertical substrate spacing would affect locomotion although Kelley et al. (1997) suggested that having more vertebrae could lead to longer body sizes resulting in individuals being able to take advantage of push-off points at further distances. While this hypothesis is worthy of exploration, one could also consider how vertebral variation in a specific region of the axial skeleton affects the ability to use substrate for forward propulsion.

4.3. Conclusions

Elongate fishes are often found in highly structured environments such as sand burrows, rock crevices or dense vegetation (Ward and Azizi, 2004; Mehta et al., 2010). Elongation of the body in fishes has been proposed to have opened up the potential for the evolution of complex prey restraint behaviors such as rotational feeding and knotting (Mehta et al., 2010). Oftentimes, these fishes are also known to make terrestrial excursions (Pace and Gibb, 2014). When swimming, highly elongate fishes typically use anguilliform locomotion for forward propulsion; however, these studies tend to observe fishes swimming in open spaces (Gillis, 1998; Ellerby et al., 2001). The anguilliform movement pattern is modified when these fishes cross from an aquatic to a terrestrial environment; for example, wave amplitude is greater during terrestrial locomotion than during aquatic locomotion (Gillis, 1998; Ellerby et al., 2001; Pace and Gibb, 2011). Gillis (1998) noted that during terrestrial locomotion, *A. rostrata* (American eel) would push into the sand producing slight indentations. Our study reveals that when provided vertical substrate in the form of wooden pegs, elongate fishes contact pegs presumably to make forward movements. We also show that differences in axial elongation can affect peg use in the aquatic and terrestrial environment. Future studies integrating body shape metrics with vertebral number and substrate density will further our understanding of how some fish lineages can move on land.

References

Abdel Magid, A., 1967. Respiration of air by the primitive fish *Polypterus senegalus*. Nature 215, 1096–1097.

Adriaens, D., Devaere, S., Teugels, G.G., Dekegel, B., Verraes, W., 2002. Intraspecific variation in limblessness in vertebrates: a unique example of microevolution. Biol. J. Linn. Soc. 75, 367–377.

Alfaro, M.E., Santini, F., Brock, C., Alamillo, H., Dornburg, A., Rabosky, D.L., Carnevale, G., Harmon, L.J., 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc. Natl. Acad. Sci. U. S. A. 106, 13410–13414.

Arratia, G., Schultze, H.-P., Casciotta, J., 2001. Vertebral column and associated elements in dipnoans and comparison with other fishes: development and homology. J. Morphol. 250, 101–172.

Blob, R.W., Rai, R., Julius, M.L., Schoenfuss, H.L., 2006. Functional diversity in extreme environments: effects of locomotor style and substrate texture on the waterfall-climbing performance of Hawaiian gobiid fishes. J. Zool. 268, 315–324.

Brainerd, E.L., Patek, S.N., 1998. Vertebral column morphology, C-start curvature, and the evolution of mechanical defenses in tetraodontiform fishes. Copeia 1998, 971–984.

Brainerd, E.L., Liem, K.F., Samper, C.T., 1989. Air ventilation by recoil aspiration in polypterid fishes. Science 246, 1593–1595.

Breder, C., 1926. The locomotion of fishes. Zoologica 4, 159–296.

Cucherousset, J., Boulêtreau, S., Azémar, F., Compin, A., Guillaume, M., Santoul, F., 2012. "Freshwater killer whales": beaching behavior of an alien fish to hunt land birds. PLoS ONE 7 (12), e50840.

Ellerby, D.J., Gerry, S.P., 2011. Sympatric divergence and performance trade-offs of bluegill ecomorphs. Evol. Biol. 38, 422–433.

Ellerby, D.J., Spiers, I.L.Y., 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.

Froese, R., Pauly, D. (Eds.), 2014. FishBase. , www.fishbase.org (electronic publication).

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

Gasc, J.-P., Gans, C., 1990. Tests on locomotion of the elongate and limbless lizard *Anguis fragilis* (Squamata, Anguidae). Copeia 1990, 1055–1067.

Gibb, A.C., Ashley-Ross, M.A., Pace, C.M., Long, J.H., 2011. Fish out of water: terrestrial jumping by fully aquatic fishes. J. Exp. Zool. A: Ecol. Genet. Physiol. 315A, 649–653.

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.

Gray, J., Lissmann, H.W., 1950. The kinetics of the locomotion of the grass-snake. J. Exp. Biol. 26, 354–367.

Horner, A.M., 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.

Horner, A.M., Jayne, B.C., 2014. Lungfish axial muscle function and the vertebrate water to land transition. PLoS ONE 9 (5), e96516.

Hsieh, S.-T.T., 2010. A locomotor innovation enables water–land transition in a marine fish. PLoS ONE 5 (6), e11197.

Jayne, B.C., 1988. Muscular mechanisms of snake locomotion: an electromyographic study of undulatory locomotion the Florida banded water snake (*Nerodia fasciata*) and the yellow rat snake (*Elaphe obsolete*). J. Morphol. 197, 159–181.

Jayne, B.C., Bennett, A.F., 1990. Scaling of speed and endurance in garter snakes: a comparison of cross-sectional and longitudinal allometries. J. Zool. 220, 257–277.

Johnels, A.G., 1957. The mode of terrestrial locomotion in *Clarias*. Oikos 8, 122–129.

Kelley, K.C., Arnold, S.J., Gladstone, J., 1997. The effects of substrate and vertebral number on locomotion in the garter snake *Thamnophis elegans*. Funct. Ecol. 11, 189–198.

Lindsey, C.C., 1975. Pleomerism, the widespread tendency among related fish species for vertebral number to be correlated with maximum body length. J. Fish. Res. Board Can. 32, 2453–2469.

Mehta, R.S., Ward, A.B., Alfaro, M.E., Wainwright, P.C., 2010. Elongation of the body in eels. Integr. Comp. Biol. 50, 1091–1105.

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

Pace, C.M., Gibb, A.C., 2011. Locomotor behavior across an environmental transition in the ropefish, *Erpetoichthys calabaricus*. J. Exp. Biol. 214, 530–537.

Pace, C.M., Gibb, A.C., 2014. Sustained periodic terrestrial locomotion in air-breathing fishes. J. Fish Biol. 84, 639–660.

Pettit, M.J., Beitinger, T.L., 1981. Aerial respiration of the brachiopterygian fish, *Calamoichthys calabaricus*. Comp. Biochem. Physiol. A: Comp. Physiol. 68A, 507–509.

Sacca, R., 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.

Standen, E., Du, T., Larsson, H., 2014. Developmental plasticity and the origin of tetrapods. Nature 513, 54–58.

Swain, D.P., 1992. Selective predation for vertebral phenotype in *Gasterosteus aculeatus*: reversal in the direction of selection at different larval sizes. Evolution 46, 998–1013.

Van Wassenbergh, S., 2013. Kinematics of terrestrial capture of prey by the eel-catfish *Channallabes apus*. Integr. Comp. Biol. 53, 258–268, http://dx.doi.org/10.1093/icb/ict036

Van Wassenbergh, S., Herrel, A., Adriaens, D., Huysentruyt, F., Devaere, S., Aerts, P., 2006. Evolution: a catfish that can strike its prey on land. Nature 440 (7086), 881.

Ward, A., Azizi, E., 2004. Convergent evolution of the head retraction escape response in elongate fishes and amphibians. Zoology 107, 205–217.

Ward, A.B., Brainerd, E.L., 2007. Evolution of axial patterning in elongate fishes. Biol. J. Linn. Soc. 90, 97–116.

Ward, A.B., Kley, N.J., 2012. Effects of precaudal elongation on visceral topography in a basal clade of ray-finned fishes. Anat. Rec. 295, 289–297.

Ward, A.B., Mehta, R.S., 2010. Axial elongation in fishes: using morphological approaches to elucidate developmental mechanisms in studying body shape. Integr. Comp. Biol. 50, 1106–1119.

Ward, A.B., Mehta, R.S., 2014. Differential use of axial morphospace in vertebrates. Zoology 117, 70–76.