SYMPOSIUM

Propulsive Forces of Mudskipper Fins and Salamander Limbs during Terrestrial Locomotion: Implications for the Invasion of Land

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Synopsis The invasion of land was a pivotal event in vertebrate evolution that was associated with major appendicular modifications. Although fossils indicate that the evolution of fundamentally limb-like appendages likely occurred in aquatic environments, the functional consequences of using early digited limbs, rather than fins, for terrestrial propulsion have had little empirical investigation. Paleontological and experimental analyses both have led to the proposal of an early origin of "hind limb-driven" locomotion among tetrapods or their ancestors. However, the retention of a pectoral appendage that had already developed terrestrial adaptations has been proposed for some taxa, and few data are available from extant functional models that can provide a foundation for evaluating the relative contributions of pectoral and pelvic appendages to terrestrial support among early stem tetrapods. To examine these aspects of vertebrate locomotor evolution during the invasion of land, we measured three-dimensional ground reaction forces (GRFs) produced by isolated pectoral fins of mudskipper fishes (*Periophthalmus barbarus*) during terrestrial crutching, and compared these to isolated walking footfalls by the forelimbs and hind limbs of tiger salamanders (*Ambystoma tigrinum*), a species with subequally-sized limbs that facilitate comparisons to early tetrapods. Pectoral appendages of salamanders and mudskippers exhibited numerous differences in GRFs. Compared with salamander forelimbs, isolated fins of mudskippers bear lower vertical magnitudes of GRFs (as a proportion of body weight), and had GRFs that were oriented more medially. Comparing the salamanders' forelimbs and hind limbs, although the peak net GRF occurs later in stance for the forelimb, both limbs experience nearly identical mediolateral and vertical components of GRF, suggesting comparable contributions to support. Thus, forelimbs could also have played a significant locomotor role among basal tetrapods that had limbs of sub-equal size. However, the salamander hind limb and mudskipper pectoral fin had a greater acceleratory role than did the salamander forelimb. Together, data from these extant taxa help to clarify how structural change may have influenced locomotor function through the evolutionary invasion of land by vertebrates.

Introduction

The invasion of land was a pivotal event in vertebrate evolution. The penetration of terrestrial habitats, beginning with shallow shores and marginal habitats before culminating in sub-aerial substrates, required major changes in the functional demands faced by fishes and tetrapods due to the dramatic physical differences between aquatic and terrestrial environments (Clack 2002; Coates et al. 2008). One of the functions most dramatically affected by these physical differences is locomotion (Martinez 1996; Gillis and Blob 2001); yet, there are only limited data on the specific impacts of these differing physical conditions on locomotor performance, and how such performance may have influenced morphological and ecological transitions in early tetrapods.

Although living tetrapods often exhibit a fundamental shift from axial-based swimming in water to appendage-based stepping on land (e.g., Gleeson 1981; Frolich and Biewener 1992; Russell and Bels 2001; Ashley-Ross and Bechtel 2004), the fossil record suggests that underwater walking was a likely stage during the water-to-land transition (Gunter 1956; Edwards 1989; Lebedev 1997;

Boisvert 2005; Shubin et al. 2006; Coates et al. 2008; Clack 2009). Thus, one early stage in the evolutionary changes that facilitated the invasion of land must have been the assumption of a new functional role for the appendages, involving contact with the substrate for propulsion and support. Yet, how did the two appendicular systems, pectoral and pelvic, contribute to this transition?

The earliest structural changes toward a tetrapod-like morphology appear in the pectoral appendage (Lebedev 1997; Clack 2009), with enlargement of the endoskeletal girdle and implied increased musculature present among taxa such as the fossil elpistostegalid *Panderichthyes*, outside of crown group tetrapods (Coates et al. 2002; Boisvert 2005). By the emergence of tetrapods such as *Acanthostega*, which likely was still aquatic (Coates and Clack 1991; Coates 1996), character changes in the pelvic appendage have outpaced those in the pectoral appendage, with the pelvic larger than the pectoral one (Coates et al. 2002, 2008). The appendages also underwent morphological changes, including a reduction in the number of axial segments, evolution of digits and distinct wrists and ankles, and the loss of fin rays (Coates 1996; Coates et al. 2008). Behavioral studies of African lungfish (*Protopterus annectens*) suggested an even earlier phylogenetic origin than *Acanthostega* for "hind limb-driven" locomotion (King et al. 2011). When moving along a substrate underwater, *P. annectens* use gaits that resemble bipedal walking, propelling themselves strictly with the pelvic appendages with the anterior body elevated from the buoyant lungs. However, recent studies modeling the range of motion for each limb joint in the early tetrapod *Ichthyostega* have suggested that the hind limbs could not be used for propulsive substrate contact, and that this taxon would have propelled itself with simultaneous "crutching" movements of the forelimbs that resembled the patterns in modern seals and mudskippers (Pierce et al. 2012). Although some aspects of hind limb morphology contributing to such a locomotor style might be specialized features of *Ichthyostega*, Pierce et al. (2012) proposed that similarities to features in other stem tetrapods, such as *Acanthostega* and *Hynerpeton*, suggest that the range of mobility found in *Ichthyostega* could more broadly reflect appendicular function in ancestral stem tetrapods.

Although knowledge of the fossil taxa spanning the fish-to-tetrapod and water-to-land transitions has grown considerably through recent discoveries and analyses (e.g., Boisvert 2005; Daeschler et al. 2006; Shubin et al. 2006; Boisvert et al. 2008; Pierce et al. 2012), data from extant taxa serving as

functional models that provide a foundation for evaluating the relative contributions of pectoral and pelvic appendages to terrestrial support among early tetrapods are much more limited (Fricke and Hissmann 1991; Pridmore 1994; Ashley-Ross and Bechtel 2004; Ijspeert et al. 2007; Macesic and Kajiura 2010; King et al. 2011). Most locomotor studies of terrestrial lineages closest in body plan to early tetrapods, such as amphibians and reptiles, have focused on the hind limb, often with the view that the hind limb is the primary propulsor (Ashley-Ross 1994; Reilly and Delancey 1997; Irschick and Jayne 1999; Blob and Biewener 2001; Gillis and Blob 2001; Sheffield and Blob 2011). Much less is known about forelimb function in such taxa, and empirical data that compare the locomotor roles of forelimbs and hind limbs within the same animal are uncommon for such species.

One study of a taxon using sprawling posture like that of early tetrapods that did compare the locomotor roles of forelimbs and hind limbs was conducted on the gecko *Hemidactylus garnotti*, a lizard with forelimbs and hind limbs subequal in size, in which ground reaction forces (GRFs) were measured from footfalls of individual feet during trotting over level ground (Chen et al. 2006). In contrast to trotting quadrupeds with upright limb posture like mammals, in which each footfall typically shows deceleration followed by acceleration (Lee et al. 1999; Witte et al. 2002), the forelimbs and hind limbs of *H. garnotti* were found to have different roles. Although vertical forces were comparable between forelimbs and hind limbs, medially directed forces were moderately larger for the hind limbs; moreover, the forelimbs produced only deceleratory forces, whereas the hind limbs produced small deceleratory forces, followed by larger acceleratory forces late in the step (Chen et al. 2006). A comparative study of seven additional lizard species not only found similar patterns of forelimb deceleration and hind limb acceleration but also found that as the hind limbs increased in size relative to the forelimbs, medial forces became correspondingly larger for the hind limb relative to the forelimb (McElroy 2009). Data from alligators, in which the hind limbs are considerably larger than the forelimbs, are consistent with these patterns. Moderately larger medial forces and slight deceleration was followed by acceleration primarily of the hind limb; however, the forelimb also showed slight acceleration at the end of the step after a mainly deceleratory force (Willey et al. 2004).

Despite questions about the ancestry of the use of posterior appendages through the invasion of land,

consensus has emerged that limbs evolved from limb-like fins among aquatic animals (Shubin et al. 2006; Boisvert et al. 2008; Coates et al. 2008; Clack 2009). Given that the evolution of digits and the loss of fin rays occurred underwater, what biomechanical factors may have facilitated the use of limbs with digits, or limited the use of fins, during the evolutionary invasion of land? Bowler (2007) suggested potential differences in locomotor performance between fins and limbs, because the fins of the ancestors of stem tetrapods were likely adequate for benthic, underwater locomotion, but a stronger pectoral appendage would have been required for sustained forward propulsion on land. Some structural reinforcement of the pectoral appendage can be observed among amphibious fishes that use their fins to power terrestrial movement. For example, morphological specializations among *Periophthalmus* mudskippers (members of the actinopterygian lineage), such as greater ossification and stiffening of the fin rays (Harris 1960), likely contribute to the capacity of these fish to use simultaneous "crutching" of the pectoral fins to move over terrestrial surfaces (Pace and Gibb 2009). Among basal tetrapodomorphs, the evolution of digits and the loss of fin rays, in addition to enlargement of the endoskeletal bones of the pectoral girdle, probably made the pectoral appendage more robust and efficient at supporting the body off the ground (Bowler 2007). However, direct comparisons of appendicular mechanics that could evaluate the relative functional capabilities of fish fins (with rays) and tetrapod limbs (with digits) during terrestrial locomotion have not been performed.

Extant amphibious fishes and amphibians could provide informative models for understanding the functional challenges faced by vertebrates through the evolutionary transition from water to land (Ashley-Ross et al. 2004; Graham and Lee 2004). To improve the foundation for understanding the changing roles of pectoral and pelvic appendages, and the contrasting capabilities of fins and limbs, during the evolutionary invasion of land by vertebrates, we compared measurements of three-dimensional GRFs produced during terrestrial locomotion by the pectoral fins of a representative amphibious fish, the African mudskipper (*Periophthalmus barbarus*), and a representative amphibious tetrapod, the tiger salamander (*Ambystoma tigrinum*). There are some limitations to the use of both of these taxa as functional models for the stem tetrapods that spanned the water-to-land transition. For example, because mudskippers are actinopterygians rather than sarcopterygians, they are not on the same

evolutionary line that led to tetrapods and do not have homologous limb elements. However, both taxa also have advantages that make them among the best extant models available (Long and Gordon 2004). First, mudskippers and salamanders readily use their appendages for locomotion over ground. Second, the forelimbs and hind limbs of tiger salamanders show limited disparity in size, resembling the limbs of many extinct Paleozoic amphibians. This provides an appropriate comparison for fossil taxa spanning this evolutionary transition, but in a model that, as an amphibian, might be physiologically more similar to early tetrapods than alternative taxa such as lizards. Third, the projection of the mudskipper's pectoral girdle beyond the body wall provides a functional analogue to the tetrapod elbow (Harris 1960; Pace and Gibb 2009), and recent proposals of crutching as a mode of terrestrial locomotion among some early tetrapods (Clack 1997; Ahlberg et al. 2005; Pierce et al. 2012) make comparisons of force production between stepping and crutching relevant for understanding early stages of terrestrial locomotion. Other amphibious fishes are less appropriate models for appendicular GRF production, either because they do not use the pectoral appendages for moving on land, for example, *Anguilla* eels (Gillis and Blob 2001), climbing perch (Sayer 2005), and ropefish (Pace and Gibb 2011), or because they primarily used movements of the axial system to generate thrust while the pectoral fins had less of a locomotor role, for example, *Claris* catfish (C. M. Pace, unpublished Master's thesis), blennies (Hsieh 2010), and stichaeids (S. M. Kawano, personal observation).

Our article thus has the following specific objectives. First, we compare GRFs from the forelimbs and hind limbs of salamanders during terrestrial locomotion to evaluate how their roles in force production might differ in a quadrupedal amphibian. Second, we compare GRFs from salamanders' limbs to data from mudskippers' pectoral fins during terrestrial locomotion, to evaluate potential differences in the functional roles and capacities of fins versus limbs on land. Finally, we consider these data in the evolutionary context of the water-to-land transition in tetrapods. Our data show a substantial role of the forelimbs in supporting the body of amphibians on land, although they contribute to propulsion differently than do the hind limbs. In addition, our data provide evidence for a significant evolutionary change in GRF orientation between fins and limbs that might contribute insight into the evolutionary success of limbs as propulsive structures on land.

Materials and methods

Animals

Tiger salamanders, *A. tigrinum* (Green 1825), and African mudskippers, *P. barbarus* (Linneaus 1766), were chosen as model taxa for our analyses because they were the largest available species of salamander and fish that regularly use their appendages to move over land. Salamanders were purchased from Charles D. Sullivan Co. (Nashville, TN, USA) and Underground Reptiles (Deerfield Beach, FL, USA), and mudskippers from Fintastic (Charlotte, NC, USA).

Experimental trials were conducted on five adult salamanders (body mass: 61.72 ± 0.07 g; snout-vent length: 0.100 ± 0.001 m; total length: 0.187 ± 0.005 m) and five adult mudskippers (body mass: 25.10 ± 0.53 g; total length: 0.137 ± 0.001 m). All values represent means ± 1 SE. Animals were housed in individual enclosures, kept on a 12 h:12 h light:dark cycle, and maintained in accordance with procedures approved by the Clemson University IACUC (AUP 2009-071 and AUP2010-066).

Collection of data on 3D GRF

Data for GRFs were obtained from isolated ground contacts of appendages from the right side of the body, using a custom-built multi-axis force platform (K&N Scientific, Guilford, VT, USA) connected to bridge amplifiers. Forces were collected at 5000 Hz using a custom LabVIEW (v.6.1, National Instruments, Austin, TX, USA) routine, with amplifier gains adjusted appropriately for the small body masses of the animals so as to maximize the sensitivity of GRF resolution. Force-plate calibrations were performed daily, and the natural frequency of the plate was 190 Hz in all three directions (vertical, anteroposterior, and mediolateral), sufficiently greater than the step frequencies of our animals, thereby avoiding confounding GRF signals. The force platform was inserted into a wooden trackway with a rubberized surface, providing a flush locomotor path with a 4×9 cm plate area for isolated foot or fin contacts. Animals were encouraged to traverse the plate by gentle tapping and providing a dark hiding location across the plate from their starting location. Animals were allowed to rest in water treated with water conditioner for several minutes between trials to avoid desiccation and were not tested for more than 30 min per day (with at least 1 day of rest between testing sessions). Video was collected simultaneously in dorsal and lateral views (Fig. 1) using two digitally synchronized, high-speed (100 Hz) cameras (Phantom v.4.1, Vision Research Inc., Wayne, NJ, USA) to evaluate aspects of the appendage cycle, such as durations of stance (propulsive phase) and swing (recovery phase). Video data were synchronized with corresponding data on force by coordinating the onset of an LED light on the video with a 1.5 V pulse on the force traces. Full
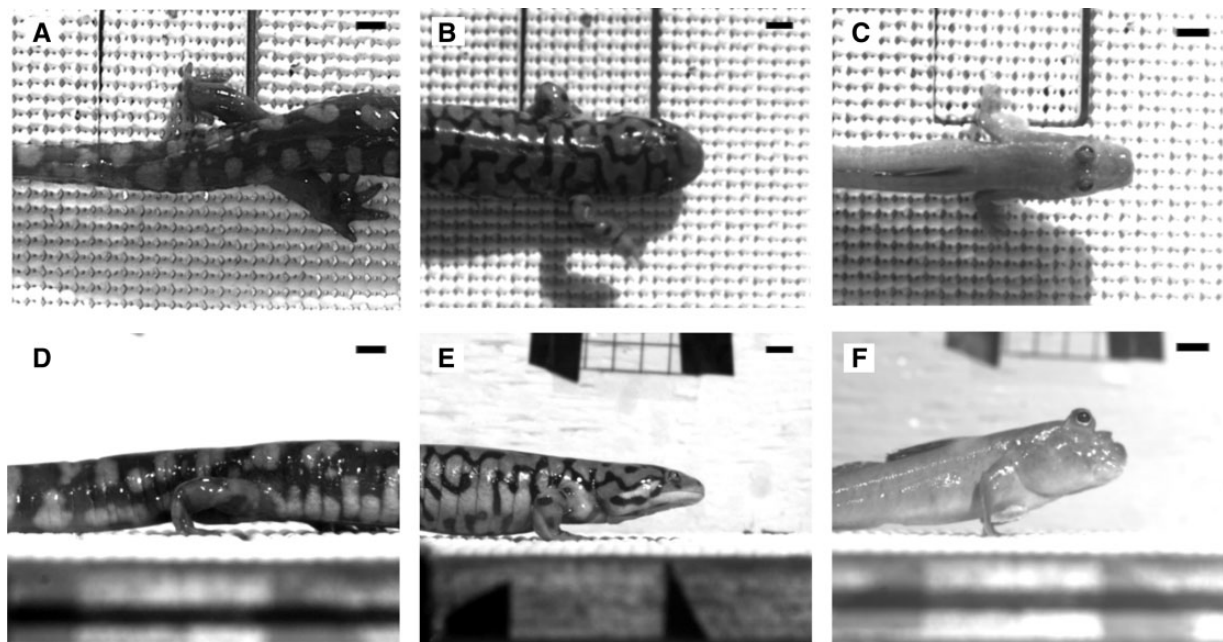

Fig. 1 Dorsal (**A–C**) and lateral (**D–F**) views from high-speed videos of salamanders' hind limbs (**A**, **D**) and forelimbs (**B**, **E**) and mudskippers' pectoral fins (**C**, **F**) at the time of peak net GRF for each of the appendages. Minor adjustments of contrast and sharpness were made to enhance clarity of the image for reproduction. Black lines in upper right corners represent 1-cm scale bars.

details on the experimental set-up and equipment are described by Sheffield and Blob (2011) and Butcher and Blob (2008).

All traces of force (analyzed only during the propulsive stance phase) were processed and filtered in R (v.2.15.2, Vienna, Austria). Magnitudes of force were standardized to units of body weight (BW) to facilitate comparisons across individuals of different sizes. Relative magnitudes of the vertical, anteroposterior, and mediolateral components of force were used to calculate the magnitude and orientation of the net GRF vector. Angular orientations were analyzed with respect to vertical ($0°$): positive values corresponded to the anterior or lateral directions, whereas negative values corresponded to posterior or medial directions. Prior to filtering, the beginning and end of raw force data were padded to avoid edge effects (Smith 1989). A custom second-order, zero-lag, low-pass Butterworth filter was applied to all raw force using the *signal* package in R (available at http://www.r-project.org). Frequency values were normalized to Nyquist frequency to avoid aliasing (Smith 1997). Data filtered during stance were then interpolated to 101 points using a cubic spline to represent 1% increments, from 0% to 100%, of the stance phase.

Several criteria were used to determine whether a trial was valid for inclusion in our analyses. First, the entire right foot/fin was required to contact the force plate. If the pelvic appendage overlapped the pectoral appendage during its contact with the ground (i.e., stance), then those frames of overlap were not included in analyses for either appendage. Animals also must have completed a full appendage cycle in a straight line (i.e., no turning). Trials were not used if the peak net GRF was found to occur at 0% or 100% of stance, or if it occurred during a time of overlap with another body part. Although locomotion at steady speed can be rare among sprawling taxa (e.g., Farley and Ko 1997), effort was made to select trials with locomotor cycles before and after the cycle of interest that were comparable in speed, with preliminary data for speeds evaluated for each trial by digitizing the movement of a point near the center of mass of the animal. Linear mixed-effects models fit by restricted maximum likelihood (REML) with individual as a random effect were conducted using the *lme4* package in R (see "Analyzed variables and statistical comparisons" for details). Speeds of trials for the salamander forelimb (9.9 ± 0.3 cm/s) and hind limb (10.4 ± 0.5 cm/s) were not significantly different ($P = 0.811$). Speeds of the trials for the pectoral fin of the mudskipper (7.6 ± 0.3 cm/s) also did not differ from those for the forelimb ($P = 0.391$) and hind limb ($P = 0.444$). All trials represented typical behaviors of the animals.

Analyzed variables and statistical comparisons

Pair-wise comparisons of force between forelimbs and hind limbs, and between fins and limbs, were conducted in R and Microsoft Excel. These comparisons were approached from two perspectives. First, pairwise linear mixed-effects models fit by REML with appendage type (forelimb, hind limb, or pectoral fin) as a fixed effect, and individual as a random effect (*lme4* package in R), were used to compare response variables. *P* values were generated using Markov–Chain Monte–Carlo methods using 10,000 iterations and were adjusted through sequential Bonferroni correction (Holm 1979) with the *languageR* package. These models were used to compare values of several variables at the time of peak net GRF, providing information about how forces were applied when the weight supported by the appendage was the greatest (Sheffield and Blob 2011). These variables included the timing of the peak net GRF, magnitudes of the components of GRF, and angles of GRF orientation. Second, vector analysis (Hankison et al. 2006; Cullen et al. 2013; Rivera et al. 2013) was used to qualitatively assess the overall similarity of GRF patterns between pairs of appendicular systems. For each trace of force values through stance, 21 mean values of the variable (calculated for each 5% increment through stance, from 0% to 100%) were used to generate vectors with 21 dimensions. The angle between pairs of these vectors could then be calculated using standard equations (Hamilton 1989). Angles near $0°$ indicate nearly identical vectors (i.e., two nearly identical force profiles), whereas angles near $90°$ indicate vectors with perpendicular trajectories, reflecting strong differences between force profiles. In addition to these comparisons of forces, duty factors (i.e., the proportion of an appendicular cycle spent in contact with the ground) were also compared between systems using linear mixed-effects models, as previously described. These were evaluated from the videos of each trial, and were viewed as a possible factor contributing to differences in magnitudes of GRFs between systems (e.g., higher duty factors corresponding to lower peak forces) (Biewener 2003).

Results

Comparison of GRFs between salamander forelimbs and hind limbs

Comparisons of GRFs between salamander forelimbs and hind limbs showed several similarities. For both appendicular systems, net GRF magnitudes were slightly less than 0.5 BW with similar magnitudes of the vertical and mediolateral components when evaluated at peak net GRF (Table 1 and Fig. 2). The GRF also showed a similar medial orientation

Table 1 Comparison of mean GRF parameters between the forelimb and hind limb of *Ambystoma tigrinum* and pectoral fin of *Periophthalmus barbarus* at the time of peak net GRF

	Hind limb (HL)	Forelimb (FL)	Pectoral fin (PF)	HL vs. FL (P value[a])	FL vs. PF (P value[a])
Time of peak net GRF (%)	32.80 ± 1.60	61.08 ± 1.01	57.16 ± 1.84	<0.001*	0.297
Net GRF (BW)	0.47 ± 0.01	0.46 ± 0.01	0.42 ± 0.01	0.616	0.118
Vertical GRF (BW)	0.43 ± 0.02	0.45 ± 0.01	0.39 ± 0.01	0.679	0.014*
Mediolateral GRF (BW)	− 0.07 ± 0.01	−0.07 ± 0.004	−0.12 ± 0.01	0.679	0.011*
Anteroposterior GRF (BW)	0.15 ± 0.01	−0.03 ± 0.01	0.05 ± 0.01	<0.001*	<0.001*
Mediolateral angle (deg)	−11.04 ± 1.73	−8.67 ± 0.53	−17.14 ± 0.90	0.679	0.001*
Anteroposterior angle (deg)	21.69 ± 1.98	−3.21 ± 0.10	7.65 ± 0.83	<0.001*	0.002*

Values are means ± SE ($n = 50$ steps across five individuals for each group). For mediolateral GRF and angle, negative values indicate a medial direction; for anteroposterior GRF and angle, negative values indicate a posterior (deceleratory) direction, whereas positive values indicate an anterior (acceleratory) direction.
[a]P values were generated using Markov Chain Monte Carlo methods (10,000 iterations) and adjusted using sequential Bonferroni corrections.
*$P < 0.05$.

between both limbs ($P = 0.679$), inclined $−8.7°$ for the forelimb and $−11.0°$ for the hind limb at peak GRF (Table 1). Frequency of the locomotor cycle did not differ significantly between the forelimb (FL) and hind limb (HL; $P = 0.641$), at $1.45 ± 0.03$ and $1.42 ± 0.05$ Hz, respectively. Duration of swing (FL: $0.19 ± 0.01$ s; HL: $0.16 ± 0.02$ s) and total duration of the appendage cycle (FL: $0.71 ± 0.02$ s; HL: $0.76 ± 0.03$ s) also did not differ (duration of swing: $P = 0.424$; duration of cycle: $P = 0.544$).

However, salamander forelimbs and hind limbs also showed several significant differences in the values of GRF parameters at the time of peak net GRF. Prominent among these was the time of peak GRF itself, which occurred approximately one-third of the way through the step for the hind limb, but nearly two-thirds of the way through the step for the forelimb (Table 1 and Fig. 2). Also, at the time of peak net GRF, the anteroposterior component was large and positive for the hind limb, but small and negative for the forelimb (Table 1 and Fig. 2). These values corresponded to a substantial anterior (acceleratory) inclination of over $20°$ for the hind limb, but a slight posterior (deceleratory) inclination averaging just over $−3°$ for the forelimb (Table 1). Although duty factor was significantly larger for the hind limb than the forelimb ($P < 0.001$), for both limbs it was very high with only a 6% difference between them ($0.80 ± 0.01$ for the hindlimb and $0.74 ± 0.01$ for the forelimb).

Comparison of GRFs between salamander forelimbs and mudskipper pectoral fins

Some similarities in GRF were also identified between the salamander forelimb and the mudskipper pectoral fin (Table 1 and Fig. 2). The timing of peak net GRF

did not differ significantly ($P = 0.297$), occurring at approximately 57% and 60% into stance phase for the pectoral fin and forelimb, respectively. The overall magnitude of the GRF at these points was similar between these pectoral appendages ($P = 0.118$), with values just under 0.5 BW (Table 1 and Fig. 2). In addition, the time spent during the swing phase was not significantly different ($P = 0.706$), at $0.19 ± 0.01$ s (forelimb) and $0.20 ± 0.01$ s (pectoral fin). Duration of stance (FL: $0.53 ± 0.02$ s; PF: $0.39 ± 0.01$ s; $P = 0.358$), duration of the total cycle (FL: $0.71 ± 0.02$ s; PF: $0.59 ± 0.02$ s; $P = 0.422$), duty factor (FL: $0.74 ± 0.01$; PF: $0.66 ± 0.01$; $P = 0.303$), and appendage frequency (FL: $1.45 ± 0.03$ Hz; PF: $1.78 ± 0.06$ Hz; $P = 0.400$) were also not different.

However, salamanders' forelimbs and mudskippers' pectoral fins also showed a number of significant differences in GRF parameters. Differences in all three components of the GRF were observed (Table 1). At the time of peak net GRF, the vertical component was greater for the forelimb but the medial component was greater for the pectoral fin (Table 1 and Fig. 2). As a result, the medial angle of inclination of the GRF for the pectoral fin ($−17.1° ± 0.9$) was almost twice as large as that for the forelimb ($−8.7° ± 0.5$). In a further contrast between these appendages, mudskippers' pectoral fins showed a slight anterior (acceleratory) orientation of the GRF, rather than the slight posterior (deceleratory) orientation found in salamanders' forelimbs (Table 1 and Fig. 2).

Comparisons of GRF patterns throughout the duration of stance

Based on comparisons at peak net GRF, salamanders' forelimbs appeared to demonstrate more similarities
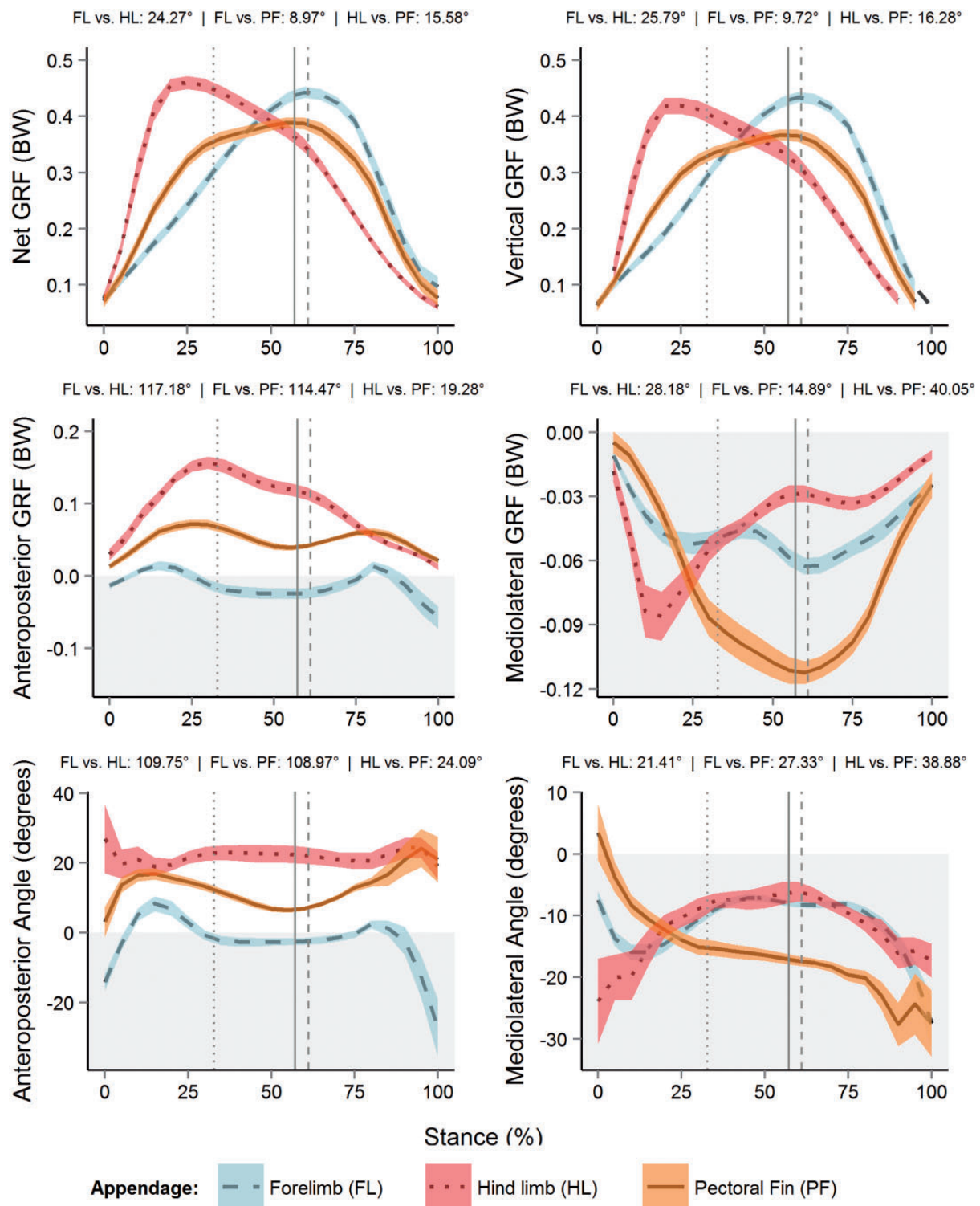
FL vs. HL: 24.27° | FL vs. PF: 8.97° | HL vs. PF: 15.58°　　FL vs. HL: 25.79° | FL vs. PF: 9.72° | HL vs. PF: 16.28°

FL vs. HL: 117.18° | FL vs. PF: 114.47° | HL vs. PF: 19.28°　　FL vs. HL: 28.18° | FL vs. PF: 14.89° | HL vs. PF: 40.05°

FL vs. HL: 109.75° | FL vs. PF: 108.97° | HL vs. PF: 24.09°　　FL vs. HL: 21.41° | FL vs. PF: 27.33° | HL vs. PF: 38.88°

Stance (%)

Appendage: - - Forelimb (FL)   ··· Hind limb (HL)   — Pectoral Fin (PF)

Fig. 2 Dynamics of GRF parameters during stance. Lines represent means from pooled trials for each appendage ($N = 50$ averaged across five individuals for each appendage), and shading surrounding each line represents its standard error. Salamanders' forelimb (FL) traces are represented by light blue dashed lines, and hind limb traces (HL) by dark red-dotted lines; mudskippers' pectoral fin traces (PF) are in orange solid lines. The gray background in the bottom four plots represents negative values (e.g., medial and posterior in the mediolateral and anteroposterior plots). Vertical lines are coded according to of appendage type, identifying the timing of the peak net GRF for each appendicular system. Divergence angles between pairs of appendicular systems are reported above each plot; values close to 0° indicate similarity between pairs of plots whereas values close to 90° indicate strong differences. Darker areas of shading for force traces indicate areas of overlap between standard errors of traces.

to salamanders' hind limbs than to mudskippers' pectoral fins. However, comparisons of overall force profiles throughout stance for these appendages complicate this perspective (Fig. 2). Vector analyses showed that overall profiles for the medial inclination of the GRF were still most similar (i.e., had the smallest divergence angle) between salamanders' forelimbs and hind limbs. However, the net GRF and the vertical component of the GRF were most similar between the salamanders' forelimbs and mudskippers' pectoral fins, with divergence angles under $10°$ versus approximately $25°$ between salamanders' forelimbs and hind limbs. Moreover, with regard to anteroposterior forces and angles, overall profiles were much more similar between the mudskippers' pectoral fins and the salamanders' hind limbs, with divergence angles under $25°$, than either was to the salamanders' forelimbs, which showed divergence angles of over $100°$ compared to the other two appendicular systems.

Discussion

The physical properties of the terrestrial environment are drastically different from those of the aquatic realm, in which vertebrates originated and lived for millions of years. To facilitate the penetration of terrestrial habitats, a wide range of morphological, physiological, and life-history adaptations were ultimately required (e.g., Anderson et al. 2013, this issue; Gibb et al. 2013, this issue; Jew et al. 2013, this issue; Martin and Carter 2013, this issue; Pierce et al. 2013, this issue; Van Wassenbergh 2013, this issue). Among the suites of features that experienced such changes were the appendages; these anatomical structures encountered new demands for supporting body weight to allow locomotion on land. How did the functional differences between fins versus limbs with digits influence the conquest of land by tetrapod vertebrates? To address this broad question, we focused on two more specific questions. First, what were the likely contributions of the front and rear appendages to locomotion in early tetrapods? Second, how do the function of fins and limbs differ for locomotion on land? This study helps to answer these questions using GRFs collected from the forelimbs and hind limbs of salamanders and the pectoral fins of mudskippers, providing a framework for comparing how these structures contribute to locomotion on land.

Functional roles of fore and hind appendages across the fin-to-limb transition

Salamanders present a useful model for gaining insight into the potential capacities for terrestrial locomotion

by early tetrapods for several reasons, including their use of a sprawled limb posture with forelimbs and hind limbs of similar size. Our data on GRF patterns from salamanders' hind limbs are largely concordant with those reported in a previous study (Sheffield and Blob 2011), indicating net magnitudes of GRF just under 0.5 BW with medial inclinations of approximately $-10°$, and a strong anteriorly directed component. Our new data show how the locomotor role for the forelimb follows these general trends. Forelimb function shows a number of similarities to hind limb function in salamanders, including having similar total durations and frequencies of limb cycles, similar magnitudes of GRF (e.g., vertical, mediolateral, and net), and similar medial inclinations of GRF (Fig. 2 and Table 1). These results indicate that the forelimbs and hind limbs of salamanders have a similar weight-bearing capacity, much like the gecko *H. garnotti*, which also uses a sprawling posture with similarly sized limbs (Chen et al. 2006). However, the forelimb differed markedly from the hind limb in its anteroposterior GRF, with the hind limb exhibiting a strong acceleratory component at peak net GRF, but the forelimb showing a small deceleratory component. It is possible that drag produced by the tail contributes additional deceleration, which together with the forelimbs would balance the acceleration generated by the hind limbs. In broader comparisons, however, this pattern of deceleration of the forelimb and acceleration of the hind limb also matches that observed in geckos (Chen et al. 2006) and alligators (Willey et al. 2004), suggesting this may be a general pattern for sprawling quadrupeds, with an ancestry deep in the use of stepping locomotion.

These results also call attention to distinct aspects of what has been categorized as "hind limb-driven" locomotion: (1) weight support and (2) the provision of acceleration versus deceleration during an appendage's contact with the ground. As might be expected, similarly sized limbs bear similar responsibilities for weight support. Thus, even if the hind limbs provided the primary acceleration for early tetrapods, the forelimbs still would have been expected to bear a major responsibility for support of weight, based on the size of these structures (e.g., Coates 1996). Early experiments on salamanders by Evans (1946) suggested that forelimbs played major roles in support of body weight and in forward propulsion. For instance, vertically suspended salamanders could pull themselves back up from the edge of a shelf using only their forelimbs (Evans 1946). However, the extent to which the hind limbs were the primary source of acceleration in a taxon might depend on the size of its tail. In geckos, with rather

short tails (∼40% snout-vent length based on measurements of published figures) for which dragging was not documented, forelimb GRFs were deceleratory for the entire step (Chen et al. 2006). In contrast, in salamanders with larger tails (87% snout-vent length) that dragged on the ground (dragging of the tail is visible in Fig. 1D), forelimb GRFs were initially acceleratory at the beginning of the step (Fig. 2), and became only slightly deceleratory by peak GRF (Table 1). Such a model may be more appropriate than geckos for comparison with early tetrapods with heavy tails (Coates 1996), and suggests that with a particularly massive tail the forelimb may have had an even more substantial role in providing acceleration. For instance, *Alligator mississippiensis* has a relatively large tail that accounts for about 8% of its total body weight, and although the forelimb has a net deceleratory role, it plays a slight acceleratory role later in stance when the acceleratory role of the hind limb has decreased (Willey et al. 2004). A similar late acceleratory peak for forelimbs can be seen in our data on salamanders after acceleration of the hind limb declines sharply near the end of the step (Fig. 2). Empirical data on tail dragging are currently unavailable for mudskippers, but Harris (1960) estimated that the tail supported about 10% of the body weight of the mudskipper, which is comparable with values for *A. mississippiensis* (Willey et al. 2004). Thus, some acceleration contributed by the pectoral fins of mudskippers might serve to offset the frictional forces produced by dragging of the tail in addition to contributing toward forward propulsion.

Viewing the GRFs of mudskippers' pectoral fins in this context, a striking point of comparison is that the pectoral fins show an anterior component of GRF that was acceleratory throughout the entire duration of stance (Fig. 2). In this way, the role of these fins appears to more closely resemble that of salamanders' hind limbs than of salamanders' forelimbs, a conclusion further suggested by our vector analysis that showed the smallest divergence angle between the anteroposterior force traces of the hind limb and the pectoral fin (Fig. 2). This comparison underscores the dramatic change in functional role between pectoral appendages that drag the body via crutching versus those that contribute to propulsion via stepping.

Body support on land: consequences of using fins versus limbs

In addition to differing in anteroposterior components of GRF, mudskippers' pectoral fins also differed from both fore and hind appendages of salamanders in vertical and medial components of GRF (Table 1). With lower vertical but higher medial forces, mudskippers' pectoral fins experienced a much more medially inclined GRF at peak force ($-17.1°$) than either the forelimb ($-8.7°$) or hind limb ($-11.0°$). Although differences in speed can influence the magnitudes of the components of the GRF (McLaughlin et al. 1996), such an explanation does not seem likely for the higher medial force of mudskippers (Table 1 and Fig. 2), given the similar speeds between mudskippers and salamanders (see "Materials and methods" section). The presence of such a difference in orientation of the GRF across these taxa is striking, because comparisons of GRFs across a broad range of species (amphibians to mammals) and limb postures (sprawling to parasagittal), including turtles (Jayes and Alexander 1980; Butcher and Blob 2008), iguanian (Blob and Biewener 2001) and scleroglossan (Sheffield et al. 2011) lizards, crocodilians (Blob and Biewener 2001; Willey et al. 2004), and a variety of mammals (Biewener 1983; Biewener et al. 1983; Gosnell et al. 2011) have all found remarkably consistent medial inclinations of the GRF, typically $-10°$ or less. *Hemidactylus* geckos represent an exception to this general pattern, with medial inclinations averaging just over $30°$ (Chen et al. 2006). This difference may be related to locomotor speed, as GRFs were measured in geckos running at an average of 7.8 SVL/s (Chen et al. 2006), but speeds for other sprawling taxa were typically 1 BL/s or slower (Willey et al. 2004; Butcher and Blob 2008; this study). However, iguanas from which GRFs were measured also ran at speeds approaching 8 SVL/s, and still showed medial GRF inclinations of only $-8°$ at the time of peak bone stress (Blob and Biewener 1999, 2001). It is possible that some differences in the orientation of the GRF in mudskippers versus most other sprawling and parasagittal taxa are inherent to their different modes of locomotion (i.e., crutching versus stepping). However, it is also possible that despite the wide range of variation in the shape and proportions of limbs, and in posture among tetrapods, it is the fin-to-limb transition that produces some of the most dramatic consequences for orientation of GRF during terrestrial locomotion (Fig. 3). This change in orientation might be related to the presence of the elbow joint in limbs, which would cause the distal segment of the limb to be directed more vertically compared with the pectoral fin of the mudskipper. As a result, the mudskipper could provide a better functional model for appendicular function in stem tetrapods, such as elpistostegalids, than limbed tetrapods with digits. The posture of the pectoral appendage reconstructed for the elpistostegalid *Tiktaalik*, in which the entire appendage is held at an angle from
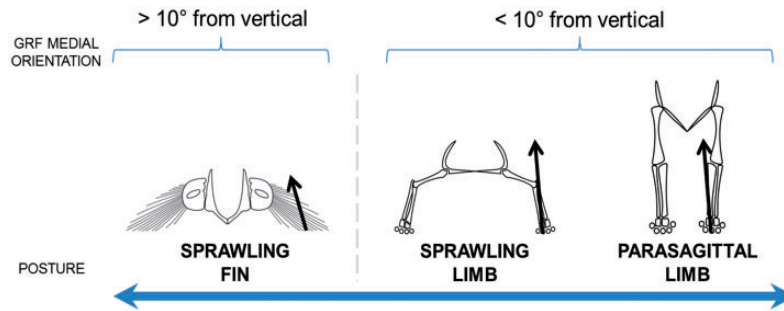
Fig. 3 Data on mudskippers' GRF from this study provide insight into the evolution of the orientation of GRF in vertebrates (indicated by black arrows). Although tetrapods exhibit a wide diversity of postures of the limb and foot, the medial inclination of the GRF is relatively similar across taxa at about −10° or less from vertical. The mudskipper has a sprawling fin posture, and has a GRF oriented more medially than all tetrapods. Inclusion of the mudskippers' GRF data demonstrates how the fin-to-limb transition may have marked a major change in the orientation of the GRF, which can impact the weight-bearing capacities of the appendicular system. Images of the mudskipper from Harris (1960) were used as a guide for illustrating this figure.

the axis of the body (Shubin et al. 2006), strongly resembles that of the mudskippers' pectoral fins, potentially correlating with similarities in force production as well.

What functional consequences might such large medial inclinations in GRF have for the use of fins as locomotor structures on land? One potential impact could be on how the skeletal structures of the appendages are loaded. With a nearly vertical GRF at its peak net magnitude, both sprawling (Blob and Biewener 2001; Sheffield and Blob 2011) and more upright tetrapods (Biewener 1989, 1990) are able to minimize moments of the GRF at the joints of the elbow and knee, reducing the muscular forces required to maintain joint equilibrium and thereby limiting exposure of the limb to bending stresses. Although mudskippers' fins do not have a joint homologous to the elbow, the joint between the radials and the fin rays serves a functionally analogous role. In this context, the greater medial inclination experienced by fins moving over land could increase joint moments of the GRF and potentially elevate bending. In addition, such medial inclination could also increase the distance of the GRF vector from the long axis of the radials, increasing its moment arm for axial rotation and potentially elevating the importance of torsion as a loading regime. Consistent with this possibility, in the late 1800s, Huxley wrote that fins and limbs rotated in different directions from the body and that these rotations would have created an unrealistic amount of torsion in the humeri of fishes with tetrapod-like appendages (Bowler 2007). Because bone performs poorly both in bending and torsion compared with axial compression (Wainwright et al. 1976), the orientation of loads placed on fins could require substantial structural reinforcement to avoid an excessive risk

of failure. Measurement of stresses and safety factors of fins during terrestrial locomotion could give insight into this question, and could ultimately provide a basis for modeling the stresses experienced by the appendages of early tetrapods (e.g., Blob 2001), using a variety of models of their locomotor patterns (e.g., Pierce et al. 2012). Such models could, in turn, provide insight into the transformation of skeletal morphology between aquatic fins and terrestrial limbs, particularly between the robust morphology of appendicular elements exhibited by early tetrapodomorphs to the long, tubular bones found in early tetrapods that were more terrestrial.

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