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Biomechanics

Limb bone loading in swimming turtles: changes in loading facilitate transitions from tubular to flipper-shaped limbs during aquatic invasions

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Members of several terrestrial vertebrate lineages have returned to nearly exclusive use of aquatic habitats. These transitions were often accompanied by changes in skeletal morphology, such as flattening of limb bone shafts. Such morphological changes might be correlated with the exposure of limb bones to altered loading. Though the environmental forces acting on the skeleton differ substantially between water and land, no empirical data exist to quantify the impact of such differences on the skeleton, either in terms of load magnitude or regime. To test how locomotor loads change between water and land, we compared *in vivo* strains from femora of turtles (*Trachemys scripta*) during swimming and terrestrial walking. As expected, strain magnitudes were much lower (by 67.9%) during swimming than during walking. However, the loading regime of the femur also changed between environments: torsional strains are high during walking, but torsion is largely eliminated during swimming. Changes in loading regime between environments may have enabled evolutionary shifts to hydrodynamically advantageous flattened limb bones in highly aquatic species. Although circular cross sections are optimal for resisting torsional loads, the removal of torsion would reduce the advantage of tubular shapes, facilitating the evolution of flattened limbs.

1. Introduction

Transitions between aquatic and terrestrial habitats represent milestone events in vertebrate history [1–3]. The initial invasion of land by tetrapods was among the most profound of such events [1,3]; however, members of many terrestrial vertebrate lineages have since developed or returned to nearly exclusive use of aquatic habitats (e.g. sea turtles, mosasaurs, penguins and whales). These transitions have often been accompanied by characteristic changes in skeletal morphology, such as shifts from tubular to flattened shafts in the long bones of the limbs [3–5]. Such flattening conveys hydrodynamic advantages to appendages, making them effective propulsors for generating drag- or lift-based thrust during swimming [6]. However, the mechanical environment underlying the structural changes that provide such hydrodynamic advantages is unclear. The shapes of bones can respond to changes in their loading environment, both within generations and over evolutionary time [7–9]. Differences in the forces to which animals are exposed between water and land are also well known [10,11]. Could changes in skeletal loading have facilitated changes in limb bone shape among tetrapod lineages that became primarily aquatic?

For tetrapods that shifted from terrestrial to aquatic habitats, loads imposed on the limbs by both internal (muscular) and external (environmental) propulsive forces are retained; however, the demands of bodily support on the limbs are reduced [11]. Thus, a decrease in overall load magnitudes is expected in aquatic habitats, but the size of this reduction is difficult to predict. Moreover,

overall loading decreases do not clearly correlate with the directional (i.e. flattening) shape change observed in the limbs of primarily aquatic taxa. Such shape changes might, instead, correlate with a change in loading regime. Many terrestrial tetrapods experience significant torsional (twisting) loads on their limb bones [12,13], a regime that tubular bone cross sections are well suited to resist [10]. If such torsion were reduced more than bending loads during aquatic locomotion, the mechanical environment favouring tubular bones might have been released, facilitating the evolution of flattened, asymmetric cross sections.

To test how limb bone loading changes between water and land, we compared *in vivo* strains from the femur of the semi-aquatic slider turtle (*Trachemys scripta*) between swimming and terrestrial walking. Extreme terrestriality is a derived condition among turtles (e.g. tortoises), and sliders are not descended from more terrestrial ancestors [14,15]. However, turtles are particularly appropriate models in the context of understanding changes in limb loading through evolutionary transitions because, with the fusion of the backbone to the shell, they generate all propulsion by the limbs [16]. Thus, comparisons between environments are not confounded by shifts to propulsive structures of the body axis [2]. Moreover, semi-aquatic turtles swim with rowing motions of the limbs [16], which were likely used in the initial stages of evolutionary transitions to highly aquatic lifestyles [2]. Our focus on the femur reflects evidence that the hind limb is the dominant propulsive structure among semi-aquatic turtles [16]. Thus, by quantifying femoral loading differences in turtles between water and land, we could test for the reduction of long bone torsion during limb-propelled swimming compared with walking, potentially facilitating changes in limb bone shape during secondary aquatic invasions.

2. Material and methods

Five adult *T. scripta* (two males, three females; plastron length 19.4 ± 2.5 cm; mass: 1.4 ± 0.6 kg) were collected from Lake Hartwell, Pickens County, SC, USA. Housing and husbandry followed published standards [13].

One rosette (FLK-1-11) and two single element (FRA-1-11) strain gauges (Tokyo Sokki Kenkyujo Co., Ltd., Japan) were surgically implanted onto the midshaft of each turtle's right femur following published methods [13]. After 24 h recovery, individuals were prompted to swim in a flowtank [16] and walk on a motorized treadmill [13] while *in vivo* bone strains were collected (see [13] for details). Trials were conducted at the highest speed that individuals could maintain for several seconds (flowtank, $0.44\text{--}0.86$ m s⁻¹; treadmill $0.04\text{--}0.20$ m s⁻¹). Such speeds may not be strictly dynamically equivalent, but do provide comparable ecological relevance for understanding selection pressures on skeletal design. During aquatic trials, microconnectors between the animal's strain gauge wires and the shielded amplifier cable were sealed with plumber's epoxy to prevent water leakage into contacts. Strain trials were simultaneously filmed from lateral and dorsal (walking) or ventral (swimming) views (100 Hz; Phantom V5.1, Vision Research Inc., Wayne, NJ, USA). Turtles were euthanized following recordings (Euthasol® pentobarbital sodium solution; Delmarva Laboratories Inc., Midlothian, VA, USA; 200 mg kg⁻¹ intraperitoneal injection). For each gauge location in each turtle, strains were compared between the thrust and recovery phases of swimming (determined from video records), and between walking and swimming, using Mann-Whitney *U*-tests, which were conducted in SAS® (SAS v. 9.3, SAS Institute Inc. 2010, Cary, NC, USA).

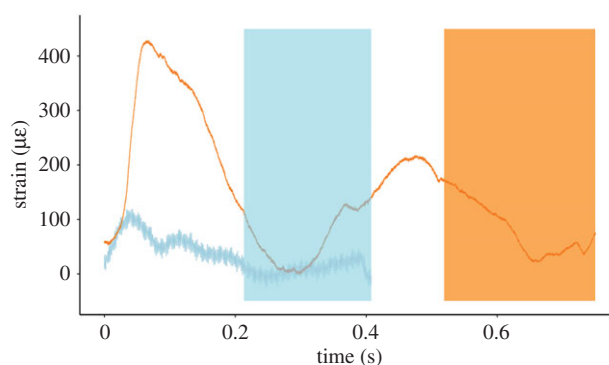


Figure 1. Femoral strain traces from single, representative limb cycles during swimming and walking in *T. scripta*. Shaded regions indicate recovery phase during swimming (blue, shorter trace, left shaded area) and swing phase during walking (orange, longer trace, right shaded area). (Online version in colour.)

3. Results

During swimming, longitudinal strains at each gauge location typically maintained the same orientation (i.e. tensile or compressive) during both thrust and recovery (figure 1 and table 1). Thus, the direction of femoral bending did not change as the direction of limb oscillation reversed between retraction and protraction. However, absolute magnitudes of peak strain (longitudinal, principal and shear) were greater during thrust (retraction) than during recovery (protraction) for 18 of 19 comparisons, and significantly greater for 15 of 19 comparisons (table 1). The orientation of peak principal tensile strain to the long axis of the femur (φ_T) showed small differences between thrust and recovery (averaging 3.7° across animals); however, φ_T averaged under 10° throughout the limb cycle (table 1), showing close alignment of strains with the femoral long axis (i.e. limited torsion; table 1).

Longitudinal strains at each gauge location typically maintained the same orientation between swimming and walking (figure 1 and table 2). Peak strain magnitudes during swimming were significantly lower than during walking (figures 1 and 2, and table 2) except at the ventral gauge location owing to its proximity to the femoral neutral axis [13]. Focusing on the phases of the limb cycle when strains are highest in each behaviour (thrust and stance), peak swimming strains from non-ventral gauges are roughly 33.1% of peak walking strains. This difference in strain magnitude between environments was even more prominent for shear, for which swimming magnitudes were only approximately 10% of those during walking (table 2 and figure 2). Though owing partly to an overall reduction in femoral loading while in water, the difference in shear between swimming and walking also appears to reflect a difference in load orientation. Whereas φ_T for swimming shows a nearly longitudinal average orientation of -6.1° , during walking φ_T averages -19.8° (figure 2b), nearer to an absolute value of 45° that signifies maximal torsion [8,12,13].

4. Discussion

We found single peaks of loading during the thrust phase of swimming that were consistently higher than the variable strains experienced during limb recovery. This difference in femoral strain magnitudes between limb cycle phases parallels that found in walking turtles [13], and highlights the impacts of external (environmental) versus internal (muscular) forces acting on the limb. During thrust, the paddle of

Table 1. Mann–Whitney U results for comparisons of peak femoral strains during thrust versus recovery phases of swimming in *T. scripta*. Values are mean \pm s.e. pT, principal tensile strain; pC, principal compressive strain; φ_T , angle of principal tensile strain to femoral long axis.

animal	gauge location	strain type	N	thrust ($\mu\epsilon$)	recovery ($\mu\epsilon$)	$ Z $	p
TS01	anterior	longitudinal	52	-169.8 ± 100.2	-119.6 ± 84.7	2.75	0.0060*
	ventral	longitudinal	11	-176.6 ± 72.9	-128.9 ± 60.9	2.63	0.0086*
	posterior	longitudinal	52	-118.5 ± 55.3	-126.4 ± 83.6	0.17	0.8670
TS03	anterior	longitudinal	129	98.2 ± 74.6	48.2 ± 32.6	5.69	<0.0001*
	posterior	longitudinal	129	102.9 ± 53.5	48.8 ± 36.0	8.83	<0.0001*
TS04	anterior	longitudinal	71	124.7 ± 120.6	51.2 ± 45.3	4.90	<0.0001*
	posterior	longitudinal	71	135.7 ± 85.8	76.5 ± 42.8	8.83	<0.0001*
TS05	anterior	longitudinal	47	-22.7 ± 9.4	-13.9 ± 10.7	0.45	0.6528
	ventral	longitudinal	47	-105.2 ± 39.0	-83.9 ± 39.7	2.19	0.0280*
	posterior	longitudinal	47	84.1 ± 15.4	33.1 ± 11.3	3.10	0.0019*
	posterior	pT	47	136.1 ± 19.9	76.9 ± 5.9	3.87	0.0001*
	posterior	pC	47	-84.4 ± 7.3	-60.4 ± 4.1	2.61	0.0091*
	posterior	φ_T^a	47	-3.1 ± 1.2	-1.7 ± 0.8	0.94	0.3484
	posterior	shear	47	53.7 ± 8.4	25.7 ± 4.4	2.58	0.0099*
TS07	anterior	longitudinal	54	-101.5 ± 32.8	-21.9 ± 35.0	1.34	0.1794
	ventral	longitudinal	54	-49.3 ± 15.2	2.5 ± 19.1	2.02	0.0429*
	posterior	longitudinal	54	58.5 ± 4.6	15.1 ± 6.1	5.51	<0.0001*
	posterior	pT	54	71.4 ± 3.7	46.8 ± 3.1	4.97	<0.0001*
	posterior	pC	54	-38.9 ± 2.9	-36.5 ± 2.2	0.49	0.6252
	posterior	φ_T^a	54	-9.1 ± 1.9	-3.2 ± 1.9	2.64	0.0083*
	posterior	shear	54	50.5 ± 4.7	26.9 ± 3.6	3.91	0.0001*

^aUnits for φ_T in degrees ($^\circ$).* $p \leq 0.05$.**Table 2.** Mann–Whitney U results for comparisons of peak femoral strains during swimming versus walking for the thrust/stance phase of limb cycle by *T. scripta*. Values are mean \pm s.e. Abbreviations follow table 1.

ID	location	N (swim; walk)	swim ($\mu\epsilon$)	walk ($\mu\epsilon$)	$ Z $	p
TS03	anterior	129; 49	98.2 ± 74.6	188.4 ± 122.8	4.71	<0.0001*
	posterior	129; 49	102.9 ± 53.5	185.1 ± 84.7	6.12	<0.0001*
TS05	anterior	47; 27	-22.7 ± 9.4	—	—	—
	ventral	47; 27	-105.2 ± 39.0	-42.2 ± 15.5	0.62	0.5323
	posterior	47; 27	84.1 ± 15.4	804.5 ± 37.1	7.12	<0.0001*
	pT	47; 27	136.1 ± 19.9	1099.6 ± 46.1	7.12	<0.0001*
	pC	47; 27	-84.4 ± 7.3	-984.4 ± 41.5	7.12	<0.0001*
	φ_T^a	47; 27	-3.1 ± 1.2	-22.1 ± 0.4	7.03	<0.0001*
	shear	47; 27	53.7 ± 8.4	1446.4 ± 63.4	7.12	<0.0001*
TS07	anterior	54; 29	-101.5 ± 32.7	-601.2 ± 157.8	3.41	0.0007*
	ventral	54; 29	-49.3 ± 15.2	-83.4 ± 12.7	1.19	0.2344
	posterior	54; 29	58.5 ± 4.6	231.5 ± 46.2	4.89	<0.0001*
	pT	54; 29	71.5 ± 3.7	462.2 ± 36.9	7.17	<0.0001*
	pC	54; 29	-38.9 ± 2.9	-286.1 ± 45.7	7.45	<0.0001*
	φ_T^a	54; 29	-9.1 ± 1.9	-17.6 ± 4.4	1.52	0.1277
	shear	54; 29	-50.5 ± 4.7	546.9 ± 93.0	6.38	<0.0001*

^aUnits for φ_T in degrees ($^\circ$).* $p \leq 0.05$.

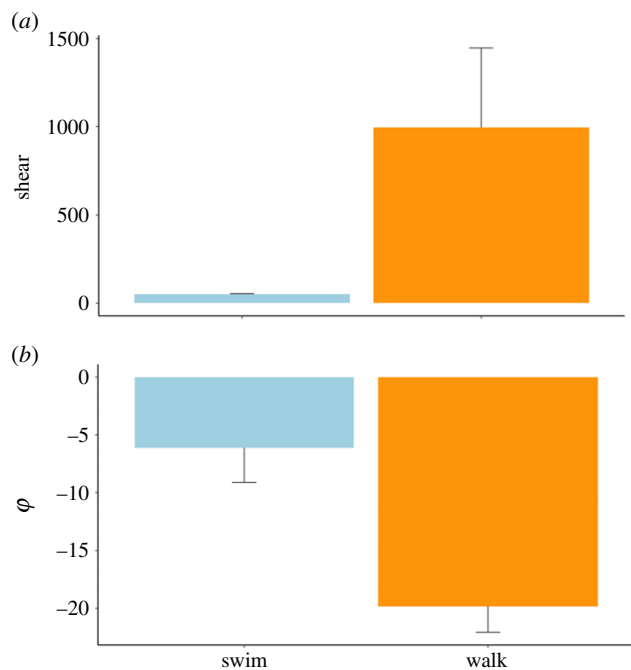


Figure 2. Comparison of femoral shear strain (a) magnitudes and (b) orientation (ϕ_T) between swimming and walking for *T. scripta* ($N = 2$ individuals, 101 swimming limb cycles, 56 walking limb cycles). Shear is significantly lower during swimming, owing at least in part to lower ϕ_T that reflects a decrease in femoral twisting in water. (Online version in colour.)

the foot is oriented perpendicular to the flow of water [16], maximizing drag for the production of thrust [2,6]. Such external forces compound the internal forces applied to the femur by active limb muscles [17], elevating strains. By contrast, the paddle is parallel to oncoming flow during recovery [16], minimizing drag that could impede forward swimming [2,6]. Such drag reduction appears to greatly decrease environmental forces on the femur, significantly reducing peak strains during recovery.

Femoral loads of rowing turtles also differ substantially between water and land. Peak longitudinal strains are reduced by two thirds during swimming, and torsional (shear) strains decreased by a factor of 19. Some reduction in femoral shear strains during swimming reflects the lower overall magnitudes of loading in water. However, reorientation of femoral loading also plays a considerable role in reducing shear strains, as ϕ_T shifts from values on land that indicate considerable twisting,

to values in water that indicate close alignment of strains with the femoral long axis (table 2 and figure 2). Given the prominence of foot rotation during the aquatic limb cycle of swimming turtles [16], the limitation of longitudinal twisting of the femur is puzzling. However, just as humans can pronate and supinate the hand at the wrist independent of oscillations at the shoulder, rotation of the foot in rowing turtles may be achieved largely through the action of distal limb components that have limited impact on femoral loading during fore–aft oscillations.

That femoral torsion is reduced during swimming in rowing turtles suggests a mechanism that may have facilitated the evolution of hydrodynamically advantageous limb bone flattening among tetrapods that shifted to primarily aquatic habitats. Although tubular shapes are advantageous for resisting torsional loads [8,10], the reduction of torsional loads during rowing could have released aquatic tetrapods from a mechanical environment favouring tubular limb bones, opening opportunities for diversification into hydrodynamically specialized limb morphologies. How broadly such patterns might apply across the multiple secondary invasions of water by tetrapods [2,3,5,11] is uncertain, particularly for lineages in which limb bone torsion was already limited [9]. However, at least for amphibian, reptilian [12,13] and, potentially, avian [18] lineages in which torsion during non-aquatic locomotion is high, the combination of changes in both the magnitude and regime of limb bone loads during aquatic propulsion may have provided conditions for the eventual morphological specialization of these taxa as swimmers.

Ethics. Animals were collected under South Carolina Department of Natural Resources Permits (29-2012, 43-2013). Experiments were conducted under Clemson University IACUC guidelines (protocol 2012-056).

Data accessibility. Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.0jd3t>. See also [19].

Authors' contributions. Both authors designed the study, collected, analysed and interpreted data, and contributed to and approved the final manuscript.

Competing interests. Authors declare no competing interests.

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