



SYMPOSIUM

Vertebrate Land Invasions—Past, Present, and Future: An Introduction to the Symposium

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Synopsis The transition from aquatic to terrestrial habitats was a seminal event in vertebrate evolution because it precipitated a sudden radiation of species as new land animals diversified in response to novel physical and biological conditions. However, the first stages of this environmental transition presented numerous challenges to ancestrally aquatic organisms, and necessitated changes in the morphological and physiological mechanisms that underlie most life processes, among them movement, feeding, respiration, and reproduction. How did solutions to these functional challenges evolve? One approach to this question is to examine modern vertebrate species that face analogous demands; just as the first tetrapods lived at the margins of bodies of water and likely moved between water and land regularly, many extant fishes and amphibians use their body systems in both aquatic and terrestrial habitats on a daily basis. Thus, studies of amphibious vertebrates elucidate the functional demands of two very different habitats and clarify our understanding of the initial evolutionary challenges of moving onto land. A complementary approach is to use studies of the fossil record and comparative development to gain new perspectives on form and function of modern amphibious and non-amphibious vertebrate taxa. Based on the synthetic approaches presented in the symposium, it is clear that our understanding of aquatic-to-terrestrial transitions is greatly improved by the reciprocal integration of paleontological and neontological perspectives. In addition, common themes and new insights that emerged from this symposium point to the value of innovative approaches, new model species, and cutting-edge research techniques to elucidate the functional challenges and evolutionary changes associated with vertebrates’ invasion of the land.

The transition of early tetrapods from living in water to living on land was a seminal event in vertebrate evolution. These newly terrestrial animals eventually radiated into a diverse array of physical and biological niches. However, the first stages of this evolutionary transition challenged these ancestrally aquatic organisms in myriad fundamental ways. First, the ability to acquire information from the environment was impaired because many vertebrate sensory systems are finely attuned to the surrounding fluid (water versus air). Second, the challenges of the terrestrial environment for internal homeostatic mechanisms are fundamentally different from those of the aquatic environment, particularly with regard to gaseous exchange and water balance. Third, the

musculoskeletal system, one of the body’s primary effectors, is placed under dramatically different physical demands on land when compared with water. Fourth, certain behaviors (e.g., reproduction) are very tightly linked to the aquatic environment and may limit the ability to range widely within the terrestrial realm. Thus, emergent vertebrates experience extraordinary challenges to their ability to sense the environment, respire, osmoregulate, and move about to evade predators, acquire food, and reproduce.

As comparative biologists, we seek to clarify the sequence of evolutionary changes that enabled early tetrapods to overcome the challenges associated with this drastic transition in habitat. In this symposium, we take two complementary approaches: studies of

extant amphibious organisms, which elucidate the functional demands of two very different habitats, and studies rooted in the fossil record, which yield insight into why modern amphibious taxa perform as they do. With these complementary model systems, we endeavor to understand the complex transition to life on land and shed light on the evolutionary mechanisms that facilitated this shift to eventually create the amazing diversity of vertebrate life on earth.

One way to determine how solutions to new challenges have evolved is to examine the fossil record. The recent discoveries of key vertebrate taxa such as *Acanthostega*, *Ichthyostega*, and *Tiktaalik* have yielded new information about the morphological changes that converted a body plan specialized for movement underwater to one capable of withstanding the novel physical conditions of the terrestrial realm. Extrapolation from paleontological studies can also provide insight into the origins of the broad diversity of terrestrial vertebrates found today. For example, convergence in body form among sarcopterygian fishes, extant crocodylians, and the fossil ancestors of modern whales has provided new insights into the likely ecological significance of an elongate and compressed body form for nearshore, sit-and-wait predators (Zimmer 1999). In addition, paleontological studies, in combination with studies of evolutionary development, suggest that the functional solutions employed by modern species to bridge aquatic and terrestrial habitats have deep phylogenetic origins (Shubin et al. 2004), and that alternate morphologies once arose that are not evident in extant taxa. Thus, information from past terrestrial invasions may provide insight into the form and function of modern amphibious taxa—including fish, amphibians, reptilomorphs, and mammals.

An alternative approach to improving our understanding of how early tetrapods overcame the demands of new terrestrial habitats is to examine modern species faced with analogous demands. Just as the first sarcopterygian fishes to move onto land likely lived at the margins of bodies of water and moved between water and land, many living species of fishes and amphibians make use both of aquatic and terrestrial habitats. This amphibious habit requires body systems to function in both environments. Numerous researchers have inferred the capabilities of early tetrapods through comparisons with living amphibians (Schaeffer 1941; Gray 1968; Edwards 1977) and sarcopterygian fish (King et al. 2011). Thus, studies of extant amphibious organisms may clarify our understanding of the initial evolutionary transition by vertebrates to terrestrialism by

elucidating the functional demands of two very different habitats.

The synthesis presented by this symposium is particularly timely. In the past decade, there has been a surge in studies of the paleontological record of the water-to-land transition in vertebrates (e.g., Shubin et al. 2004; Laurin 2010) and experimental examinations of the biological and functional demands of the terrestrial habitat faced by amphibious fishes (Blob et al. 2006, Pace and Gibb 2009, Hsieh 2010, Gibb et al. 2011) and other vertebrates (Gillis 1998; Gillis and Blob 2001; Ashley-Ross et al. 2009). The impact of such work has been far-reaching, promoting outreach to public audiences (Clack 2002) and stimulating related work in other disciplines (e.g., robotics; Ijspeert et al. 2007).

Therefore, our intent in organizing this symposium was to bring together researchers who study both past and present systems, thereby generating reciprocal insights, syntheses and approaches to understanding organism–environment interactions, and to facilitate conversations and collaborations that will guide future studies. In the pages that follow, we, the symposium organizers, highlight the findings of the symposium’s participants that provide new insights into key functional problems faced by animals moving across the transition from water to land.

Movement

Emergent aquatic vertebrates may be unable to produce effective overland movement because musculoskeletal systems that evolved in the context of movement in a viscous, low-gravity environment are ill-suited for producing terrestrial locomotion. Indeed, it was long assumed that the evolution of the tetrapod limb was the limiting step in the invasion of land (Raven et al. 2007), and that all early tetrapods moved across land in a manner similar to living salamanders (Gray 1968). Although many small tetrapodal forms likely did move in a salamander-like fashion using lateral bending of the vertebral column coupled with limb movements, Pierce et al. (2013, this issue) suggested that side-to-side bending was not universal. Reconstruction of the joint surfaces in *Ichthyostega* to determine ranges of motion for the pectoral and pelvic appendages reveals that a sprawling, salamander-like posture was unlikely, and that movements of *Ichthyostega* may have been more similar to “crutching” of mudskippers—during which the body is thrust forward through synchronous, cyclic, abduction–adduction of the pectoral appendages. Kawano and Blob (2013, this issue) measured ground reaction forces (GRFs) generated

by mudskippers' pectoral fins while crutching over land, and compared these with GRFs produced by salamander forelimbs and hind limbs during terrestrial walking. The pectoral fins of mudskippers bear comparable proportions of body weight, and have an acceleratory role similar to those of salamanders' hind limbs. By extrapolation from these findings, it can be inferred that *Ichthyostega* generated propulsive forces through mechanics similar to those of mudskippers; thus, somewhat surprisingly, the mudskipper, rather than the salamander, may be a more appropriate postural model for some early tetrapods.

What of the tetrapod foot? The evolutionary modification of fins into limbs with digits has long been considered a unique adaptation of terrestrial vertebrates. However, Davis (2013, this issue) demonstrated the deep evolutionary roots of fin/limb patterning: patterns of *Hox* gene expression that drive development of the manus/pes and digits in mammals and birds are also found in basal actinopterygians such as the paddlefish (*Polyodon*). Davis suggests that the process of changing fins into limbs is more subtle than heretofore appreciated. Knuesel et al. (2013, this issue) supported the observation that radical changes are not required in the water-to-land transition by demonstrating that central pattern generator (CPG) models can successfully output coordination patterns for swimming and walking with only minor modifications, and that these patterns are remarkably similar to CPG models of swimming by lampreys.

In addition, although the fin-to-limb transition was undoubtedly important in the permanent colonization of the terrestrial landscape, Gibb et al. (2013, this issue) showed that many extant teleosts can move proficiently over land without the use of limbs at all. In this recently described behavior, phylogenetically-distant taxa display the ability to “jump” via stereotyped flexion-plus-extension movements of the axial musculoskeletal system. We note that postures and abilities used in jumping covary with the likelihood of encountering land, which suggests a continuum of terrestriality for extant fishes and provides a new model system with which to investigate invasions of the land.

Feeding

One of the most significant insights from recent evolutionary and biomechanical studies is that feeding successfully on land may have been far more difficult for the earliest tetrapods than “simply” moving around. Further, capture of prey was not the most difficult step—swallowing was. When feeding in the water, fish can change the ambient pressure in the

buccal cavity and—because the density of prey and surrounding water is approximately equal, the food moves as the water does—both food and water are forced posteriorly, into the esophagus. This same strategy cannot be used on land because in this environment, the density of air is much lower than that of the prey. As most fishes also lack a tongue that might assist in manipulation of food, options for intra-oral transport are limited. Exploitation of new sources of food has been hypothesized as a driving force for the invasion of the terrestrial environment—which implies that modifications of the skull that enable terrestrial feeding should appear early in the evolution of tetrapods. However, Anderson et al. (2013, this issue) challenged this long-held assumption by demonstrating that modifications of the lower jaw associated with the diversification of diet appear considerably after the transition to a terrestrial locomotor system.

How might early tetrapods have fed when on land? Again, comparison with extant systems provides crucial insight. Van Wassenbergh (2013, this issue) showed that fully-aquatic eel-catfish are able to capture prey terrestrially by direct prehension of the jaw; however, to swallow they must return to the water and use hydrodynamic means of intra-oral transport. Thus, extant amphibious fishes are tied to the water in a previously unrecognized way, and this limitation may have also restricted the geographic dispersal of early tetrapods.

Respiration

Although the ancestors of the earliest tetrapods were almost certainly air-breathing fishes, locomotion in the gravity-dominated realm of land likely placed unaccustomed metabolic demands on the earliest tetrapods, which required the evolution of new, or enhanced, mechanisms capable of facilitating acquisition and transport of aerial oxygen. However, it is unlikely that tetrapodomorph fishes had substantial sustained aerobic capacity, and this may have limited their capability for activity on land. However, the atmosphere when the first vertebrates emerged onto land (the late Paleozoic era) was higher in oxygen content (30–35% of total) than at present. Jew et al. (2013, this issue) examined the effects of oxygen concentration on terrestrial performance in mudskippers on a treadmill and showed that activity endurance and the speed of repayment of the oxygen debt both increase with increased atmospheric oxygen concentration. Thus, the vertebrate invasion of land may have been facilitated and even accelerated by the favorable atmospheric conditions of the late Paleozoic.

Reproduction

For the majority of extant amphibians, their single strongest remaining tie to the aquatic environment is reproduction. Like extant amphibians, early tetrapods must have produced delicate anamniotic eggs, which would have been prone to desiccation and predation. Because of this, the amniotic egg, with its relatively water-impermeable shell, is often regarded as a key terrestrial innovation (Kardong 2012). However, Martin and Carter (2013, this issue) considered the reproductive biology of extant fishes and reached a different conclusion. Many extant teleosts that are not otherwise prone to emergence will leave the water to deposit their eggs on land; these fishes have evolved a suite of unusual behavioral and life-history characteristics that accommodate and facilitate this reproductive strategy. Because there are such a large number of fishes from phylogenetically diverse groups that reproduce on land, it appears that the benefits of placing eggs on land must outweigh potential costs. Indeed, the terrestrial landscape during the Paleozoic must have presented many fewer predators, relative to the diversity of aquatic forms in oceans and streams of that time. Accordingly, terrestrial reproduction may have been another force driving tetrapods out of the water.

Future directions

Common themes and new insights that emerged from this symposium point to future research that may elucidate the ecological challenges and evolutionary changes associated with vertebrates' invasion of the land. One emergent theme is that—even though the shift from aquatic to terrestrial habitats necessitates evolutionary alteration of a wide range of body systems and functions—extinct and extant taxa both often demonstrate a “patchwork” of functional adaptations. Indeed, some vertebrate lineages demonstrate terrestrial adaptation in only one functional system (e.g., reproduction) (Martin and Carter 2013, this issue), whereas others have evolved a suite of adaptations that affect multiple functional systems (e.g., mudskippers). It is also clear that functional changes need not occur simultaneously during the evolution of terrestriality; for example, locomotor and feeding innovations can be decoupled (Anderson *et al.* 2013, this issue). Yet, terrestrial habitats are most effectively exploited when a suite of terrestrial adaptations appear—and this may be the key to adaptive radiation. However, these findings also raise new questions. Why did some vertebrate lineages move beyond marginal habitats onto land, and others remained tied to the water's edge? What

role do phylogenetically diverse intrinsic morphogenic and physiogenic processes, such as development (Davis 2013, this issue), play in generating divergent evolutionary trajectories?

A second theme is that the original invasion of land by tetrapods occurred under environmental conditions that were significantly different from those we see in the present (e.g., Jew *et al.* 2013, this issue). To what extent do specific abiotic and biotic environmental conditions influence or constrain the success of terrestrial radiations? Additional work assessing the functional capacities of modern amphibious taxa in the context of past environmental conditions will provide new opportunities to examine this question. As is clear from the symposium, our understanding of these environmental transitions is greatly improved by reciprocal integration of paleontological and neontological perspectives (e.g., Kawano and Blob 2013; Knuesel *et al.* 2013; Pierce *et al.* 2013, in this issue).

A third emergent theme of the symposium is that our ability to elucidate the evolutionary steps that underlie the invasion of land improves as we expand our model systems. Studying unusual or novel taxa (e.g., Davis 2013; Gibb *et al.* 2013; Van Wassenbergh 2013, in this issue) can both cause us to reconsider our preconceptions and generate new questions and experimental hypotheses. In addition, the use of new technologies and computational/mechanical systems (e.g., Karakasiliotis *et al.* 2012; Pierce *et al.* 2012) allows us to ask and answer questions that were not tractable with traditional experimental techniques.

Finally, we conclude by suggesting that the colonization of the land by vertebrates was not inevitable. Clearly, the vertebrates that first invaded the land possessed a series of pre-adaptations, such as air-breathing and limb-based locomotion, that allowed them to move about effectively on land; however, other behaviors such as reproduction and swallowing likely tied these vertebrates to the water. In addition, the more we survey the ecology of extant osteichthyan fishes, the more we realize that aquatic vertebrates have invaded the nearshore terrestrial environment hundreds of times—and that they may continue to invade the terrestrial realm in the future. The often under-appreciated frequency of vertebrates' terrestrial invasions begs the question of why the particular group of sarcopterygian fishes that moved out of the shallow waters of the Paleozoic and onto land ultimately gave rise to a prolific radiation of diverse terrestrial species—while other invasions by vertebrates have not. Thus, as-yet-to-be-identified approaches, new model species, and innovative

research techniques will be required to elucidate the key evolutionary steps taken by vertebrates in past invasions, to outline the similarities and differences of past invasions to the ongoing incursions onto land by extant osteichthyan fishes, and to predict how amphibious vertebrate species might be affected by future global environmental change.

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References

- Anderson PSL, Friedman M, Ruta M. 2013. Late to the table: diversification of tetrapod mandibular biomechanics lagged behind the evolution of terrestriality. *Integr Comp Biol* published online (doi: 10.1093/icb/ict006).
- Ashley-Ross MA, Lundin R, Johnson KL. 2009. Kinematics of level terrestrial and underwater walking in the California newt, *Taricha torosa*. *J Exp Zool A* 311:240–57.
- Blob RW, Rai R, Julius ML, Schoenfuss HL. 2006. Functional diversity in extreme environments: effects of locomotor style and substrate texture on the waterfall-climbing performance of Hawaiian gobiid fishes. *J Zool* 268:315–24.
- Clack JA. 2002. *Gaining ground: the origin and evolution of tetrapods*. Bloomington (IN): Indiana University Press.
- Davis MC. 2013. The deep homology of the autopod: insights from *Hox* gene regulation. *Integr Comp Biol* published online (doi: 10.1093/icb/ict029).
- Edwards JL. 1977. The evolution of terrestrial locomotion. In: Hecht MK, Goody PC, Hecht BM, editors. *Major patterns in vertebrate evolution*. New York: Plenum Publishing Corp. p. 553–76.
- Gibb AC, Ashley-Ross MA, Pace CM, Long JH Jr. 2011. Fish out of water: terrestrial jumping by fully aquatic fishes. *J Exp Zool A* 311:240–57.
- Gibb AC, Ashley-Ross MA, Hsieh ST. 2013. Thrash, flip, or jump: the behavioral and functional continuum of terrestrial locomotion in teleost fishes. *Integr Comp Biol* published online (doi: 10.1093/icb/ict052).
- Gillis GB. 1998. Environmental effects on undulatory locomotion in the American eel *Anguilla rostrata*: kinematics in water and on land. *J Exp Biol* 201:949–61.
- Gillis GB, Blob RW. 2001. How muscles accommodate movement in different physical environments: aquatic versus terrestrial locomotion in vertebrates. *Comp Biochem Physiol A* 131:61–75.
- Gray J. 1968. *Animal locomotion*. New York: W.W. Norton & Company.
- Hsieh ST. 2010. A locomotor innovation enables water–land transition in a marine fish. *PLoS One* 5:e11197.
- Ijspeert AJ, Crespi A, Ryczko D, Cabelguen J-M. 2007. From swimming to walking with a salamander robot driven by a spinal cord model. *Science* 315:1416–9.
- Jew CJ, Wegner NC, Yanagitsuru Y, Tresguerres M, Graham JB. 2013. Atmospheric oxygen levels affect mudskipper terrestrial performance: implications for the tetrapod land invasion. *Integr Comp Biol* published online (doi:10.1093/icb/ict034).
- Karakasiliotis K, Schilling N, Cabelguen J-M, Ijspeert AJ. 2012. Where are we in understanding salamander locomotion: biological and robotic perspectives on kinematics. *Biol Cyber* published online (doi 10:1007/s00422-012-0540-4).
- Kardong KV. 2012. *Vertebrates: comparative anatomy, function, evolution*. 6th ed. New York: McGraw-Hill.
- Kawano SM, Blob RW. 2013. Propulsive forces of mudskipper fins and salamander limbs during terrestrial locomotion: implications for the invasion of land. *Integr Comp Biol* published online (doi: 10.1093/icb/ict051).
- King HM, Shubin NH, Coates MI, Hale ME. 2011. Behavioral evidence for the evolution of walking and bounding before terrestriality in sarcopterygian fishes. *Proc Natl Acad Sci USA* 108:21146–51.
- Knuesel J, Bicanski A, Ryczko D, Cabelguen J-M, Ijspeert A. 2013. A salamander's flexible spinal network for locomotion, modeled at two levels of abstraction. *Integr Comp Biol* published online (doi: 10.1093/icb/ict067).
- Laurin M. 2010. *How vertebrates left the water*. Berkeley (CA): University of California Press.
- Martin KL, Carter AL. 2013. Brave new propagules: terrestrial embryos in amniotic eggs. *Integr Comp Biol* published online (doi: 10.1093/icb/ict018).
- Pace CM, Gibb AC. 2009. Mudskipper pectoral fin kinematics in aquatic and terrestrial environments. *J Exp Biol* 212:2279–86.
- Pierce SE, Clack JA, Hutchinson JR. 2012. Three-dimensional limb joint mobility in the early tetrapod *Ichthyostega*. *Nature* 486:523–6.
- Pierce SE, Hutchinson JR, Clack JA. 2013. Historical perspectives on the evolution of tetrapodomorph movement. *Integr Comp Biol* published online (doi: 10.1093/icb/ict022).
- Raven P, Johnson G, Losos J, Mason K, Singer S. 2007. *Biology*. New York: McGraw-Hill.
- Schaeffer B. 1941. The morphological and functional evolution of the tarsus in amphibians and reptiles. *Bull Amer Mus Nat Hist* 78:395–472.
- Shubin NH, Daeschler EB, Coates MI. 2004. The early evolution of the tetrapod humerus. *Science* 304:90–3.
- Van Wassenbergh S. 2013. Kinematics of terrestrial prey capture in the eel-catfish *Channallabes apus*. *Integr Comp Biol* published online (doi: 10.1093/icb/ict036).
- Zimmer C. 1999. *At the water's edge: Fish with fingers, whales with legs, and how life came ashore but then went back to sea*. New York: Touchstone.