Amphibians as Animal Models for Laboratory Research in Physiology

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Abstract

The concept of animal models is well honored, and amphibians have played a prominent part in the success of using key species to discover new information about all animals. As animal models, amphibians offer several advantages that include a well-understood basic physiology, a taxonomic diversity well suited to comparative studies, tolerance to temperature and oxygen variation, and a greater similarity to humans than many other currently popular animal models. Amphibians now account for ~1/4 to 1/3 of lower vertebrate and invertebrate research, and this proportion is especially true in physiological research, as evident from the high profile of amphibians as animal models in Nobel Prize research. Currently, amphibians play prominent roles in research in the physiology of musculoskeletal, cardiovascular, renal, respiratory, reproductive, and sensory systems. Amphibians are also used extensively in physiological studies aimed at generating new insights in evolutionary biology, especially in the investigation of the evolution of air breathing and terrestriality. Environmental physiology also utilizes amphibians, ranging from studies of cryoprotectants for tissue preservation to physiological reactions to hypergravity and space exploration. Amphibians are also playing a key role in studies of environmental endocrine disruptors that are having disproportionately large effects on amphibian populations and where specific species can serve as sentinel species for environmental pollution. Finally, amphibian genera such as *Xenopus*, a genus relatively well understood metabolically and physiologically, will continue to contribute increasingly in this new era of systems biology and "X-omics."

Key Words: amphibians; animal model; genomics; physiology; physiomics; systems biology

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Why Amphibians as Animal Models?

The Concept of Animal Models

The concept of "animal models" is ancient, dating back to the Greek physician/philosopher Galen (131-201 AD), who transferred his knowledge of the anatomy of pigs and apes to the human condition. William Harvey, generally viewed as the father of modern experimental physiology, used both human and animal dissections to revolutionize physiology in the early 1600s. The use of animal models in physiology was firmly cemented with Claude Bernard's physiological experimentation with animals in the 19th century. Success stories from the use of animal models in physiology abound, perhaps none more fitting than the use of dogs as models for humans in the investigation of diabetes in the early part of the 20th century. These studies led to the isolation of insulin and the saving of millions of human lives (Bliss 1982), and these discoveries are now being used—fittingly—to save the lives of diabetic dogs. Indeed, approximately two thirds of the Nobel prizes in physiology and medicine awarded since 1901 have involved experimentation with laboratory animals, leading to untold numbers of treatments and therapies for humans as well as major advancements in our understanding of the basic principles of genetics, physiology, biochemistry, and behavior (Table 1).

Although the selection, the classification, and ultimately the utility of animal models continue to be debated (Bird and Parlee 2000; Burggren 1999/2000; Burggren and Bemis 1990; Carroll 2005; De Deyn et al. 2000; Feder 2006; Morrison 2002; Tkacs and Thompson 2006; Uvarov 1985), there is no doubt that "the animal model" is one of the major experimental paradigms of current biological and biomedical research. As we explore in this article, amphibians have featured prominently in this animal model paradigm for physiology and will continue to do so in the future. First, however, it is worth reviewing why amphibians occupy this pivotal position in physiological experimentation.

Advantages of Amphibians as Models

Amphibians have many compelling features that make them ideal as animal models. These characteristics are briefly discussed below and include the following: basic physiology, diversity, favorable phylogenies, wide range of habi-

Table 1 Nobel prizes associated with the use of amphibian models

Date	Awardees	Subject	Species
1920	Krogh	Capillary regulation	*Rana esculenta; R. temporaria*
1922	Hill and Meyerhof	Muscle metabolism	*Rana temporaria*
1935	Spemann	Organizer center	*Triton cristatus, T. taeniatus*
1936	Dale and Loewi	Chemical basis of nerve transmission	*Rana esculenta*
1947	Houssay	Hypophyseal regulation of carbohydrate metabolism	*Bufo arenarum; Rana pipiens*
1963	Eccles, Hodgkin, and Huxley	Ionic basis of action potentials	*Rana esculenta; Rana temporia*
1991	Neher and Sakmann	Function of single ion channels	*Rana esculenta*

tats, temperature and oxygen tolerances, sufficient similarities to mammals, and straightforward maintenance.

Basic physiology. The basic physiology of amphibians is relatively well understood. This knowledge results not only from previous research using amphibians as models for mammals but also because they have been extensively investigated in their own right as interesting vertebrates that occupy a key position in the evolution of terrestrial vertebrates. The basic physiology of amphibians has been described in an extensive array of reviews and books, to which we refer the reader (Duellman and Trueb 1986; Feder and Burggren 1992; Lofts 1974, 1976; Moore 1964; Noble 1931).

Amphibian diversity. Amphibians are not just frogs, as Feder (1992) indicates in his discussion of "the myth of the 'typical' amphibian." The three orders of amphibians— Anurans (frogs and toads), Urodeles (salamanders), and Gymnophiona (caecilians or apodans, a group of limbless amphibians)—present a huge range of diversity in terms of morphology, physiology, and life histories. As only one example, amphibians exhibit great variety in terms of developmental processes, which range from direct development (e.g., the coqui frogs, *Eleutherodactylus*) to accelerated development (many xeric species of toads) to paedomorphosis (e.g., the axolotl). Amphibians thus provide a rich source of experimental material for probing all types of physiological questions. As espoused by August Krogh, the great Danish physiologist, for every physiological question there is an animal best suited for its study (Bennett 2003; Burggren 1999/2000; Krebs 1975). A corollary of Krogh's principle could thus be "for many physiological questions there is likely to be an amphibian model well suited for its study."

Favorable phylogenies. Amphibians have phylogenies that are both well understood and complex, which lend themselves to sophisticated experimental designs shaped by evolutionary history. While it is a time-honored tradition to compare two species, one with a trait of interest and one without, the last few decades have been characterized by the emergence of a variety of approaches that emphasize multiple species comparisons with careful attention to phylogenetics (Garland 2001). Garland gives the example of a biologist interested in altitudinal adaptations comparing two

groups of animals—a high-altitude and a low-altitude group—each with three species. Unfortunately, if the high- and low-altitude groups each contain closely related species, then there is a chance for "phylogenetic pseudoreplication." Garland (2001) argues, then, that a better example would be comparing three genera, each of which contains a low- and high-altitude species. Alternatively, one can control for phylogenetic bias by examining multiple species from a single genus. These types of experimental approaches work best when there are many species, many genera, and many traits. The diversity of amphibians will likely enhance their future popularity as animal models in phylogenetically rigorous studies.

Wide range of habitats. Amphibians are found in a wide range of habitats and, accordingly, show a wide range of morphological and physiological adaptations to these habitats. Some, like *Xenopus laevis*, are completely aquatic. Although *X. laevis* do breathe air, they show significant cutaneous respiration in addition to pulmonary respiration. Other amphibians are found in surprisingly xeric conditions (e.g., Couch's spadefoot toad, *Scaphiopus couchi*). Amphibians are also found at altitudes as high as 4000+ meters (e.g., *Rana sauteri* in Taiwan) with the attendant environmental stressors of low temperature and low oxygen. Frogs are also found at surprisingly high latitudes where they survive severe winters by entering a torpor state or, in the most extreme cases, actually survive freezing. In contrast, the frog *Eleutherodactylus coqui*, which dwells in caves in Puerto Rico, experiences almost constant temperature and humidity. Amphibians have even developed an ability to make sojourns into sea water (the crab-eating frog, *Rana cancrivora*), conquering problems of osmoregulation along the way. Evoking yet again the "Krogh principle," there are prominent environmental and evolutionary physiology questions that can be answered best by an amphibian that is uniquely adapted to a specific habitat.

Temperature and oxygen tolerances. Amphibians are poikilotherms (cold-blooded animals) and thus typically survive, if not thrive, over a range of body temperatures (Hutchinson et al. 1992; Rome et al. 1992; Spotilla et al. 1992). Similarly, they are typically tolerant of hypoxia even at quite severe levels. The combination of these char-

acteristics makes them not only hardy animals from an animal husbandry perspective but also interesting subjects for targeted research. As one example, amphibians would present an excellent model for studying the interactions of heat shock proteins and hypoxia-inducing factors because amphibians could easily be subjected to a matrix of body temperature × environmental oxygen.

Sufficient similarities to mammals. As mentioned above, amphibians exhibit physiological traits in common with all vertebrates, including mammals. From the perspective of biomedical research, a criticism of nonprimate, nonmammalian models is that they are taxonomically too distant from humans to be relevant. However, if one considers the tremendous knowledge that has been gained from studies with zebrafish (Ackerman and Paw 2003; Anderson and Ingham 2003) or, even more remotely, the nematode *Caenorhabditis elegans* (Barr 2003; Hoffenberg 2003), then one can hardly dismiss amphibians as suitable models given that they occupy even closer positions to humans than these other popular models.

Straightforward maintenance. Last, but most certainly not least, many amphibians are relatively easy to rear, taking a variety of foods and tolerating a relatively wide range of environmental conditions (Pough 2007). Many amphibians also can be bred in captivity (Browne and Zippel 2007). One of the reasons that *Xenopus* is such a successful animal model is because it can be easily bred in captivity. Indeed, it was this success that led to the widespread use of *Xenopus* for pregnancy testing in the 1930s and 1940s. Finally, with proper husbandry, most amphibian diseases can be successfully managed and eliminated (Densmore and Green 2007; Gentz 2007; Smith 2007).

Amphibians as Animal Models in Physiology Research

An Historical Perspective

Amphibians have held an important place in physiology from the earliest days of the discipline. They were the model of choice for many early physiologists because of their small body size, local availability, and tolerance of surgical procedures. Galvani, for example, used isolated organs from frogs in the 1780s to demonstrate the importance of electrical activity in muscle activation (Bennett 1999). Because this work took place long before the days of perfusion pumps and temperature regulators, the use of poikilotherms ("cold-blooded" animals) made these experiments possible. In retrospect, we now realize that the amphibian tolerance of hypoxia and body temperature variation was the key to success in these early isolated organ studies. Amphibians were also in demand in the early days of cell biology and electrophysiology because their large cell size allowed studies on red blood cells, single cell electrical recordings, and other investigations requiring access to or observation of individual cells.

The well-known ease of embryonic observation and manipulation also made amphibians a popular animal model in descriptive and experimental embryology. A comprehensive survey of the various amphibian species that have contributed substantially to the advancement of developmental biology was recently published by Callery (2006) and is also reviewed elsewhere in this issue (O'Rourke 2007). Amphibia have long interested biologists with a vast diversity of natural histories (e.g., aquatic, sylvan, xeric, marine, arboreal, burrowing). However, amphibians are probably best known for their numerous developmental modalities, including such unexpected features as direct developing species (i.e., no larval stage), parental care, maternal nutrition provision, and metamorphic and nonmetamorphic species. Consequently, amphibians continue to occupy an important position in the discussion of the relationship between ontogeny and phylogeny (Gould 1985; Safi et al. 2006).

Modern physiologists continue to explore physiology with amphibian models and can be divided by approach: those who study amphibians to understand amphibians, and those who use amphibians as general vertebrate models to address questions of evolution, disease, development, and a myriad of related topics. For example, comparative physiologists have made extensive inroads into understanding the physiology of tolerance to hypoxia (West et al. 2006) and even anoxia (Hedrick et al. 2005). While there is an extensive literature on basic physiology, the majority of data relates to Anurans (frogs and toads), with much less available for the Urodeles (salamanders) and almost nothing known of Gymnophiona (caecelians) physiology. Availability is an advantage of those species easily bred in laboratory settings, most notably *X. laevis* and the faster developing *Xenopus tropicalis*. Laboratories with limited resources may consider using local invasive amphibians (collected under permit). Examples of amphibian invaders are the cane toad (*Bufo marinus*) now almost worldwide in tropical areas, bullfrogs (*Rana catesbeiana*) in the western United States and Canada, Central and South America, and elsewhere, and the Puerto Rican coqui (*E. coqui*) in Hawaii. Amphibians are also a speciose order, providing fertile ground for studies of heterochrony (Schlosser 2001) and the application of nonbiased phylogenetic independent contrasts.

For those who study amphibians primarily as general vertebrate models, the ability of amphibian tissue to survive with much less stringent regulation of temperature and gas composition compared with mammals is probably the primary reason for the continuing involvement of amphibians in many areas of physiological investigation. Studies of functional aspects of genetic alterations are increasingly common in *Xenopus* laboratories and promise to contribute even more to the future use of amphibians as models.

The continued importance of amphibian studies over the last 5 years, as revealed by the number of PubMed entries, is illustrated in Figure 1. Currently, the most commonly studied amphibian species include the bullfrog (*R. catesbeiana*), the marine toad or cane toad (*B. marinus*), the African clawed frog (*X. laevis*), and salamanders (*Ambystoma* spp.).
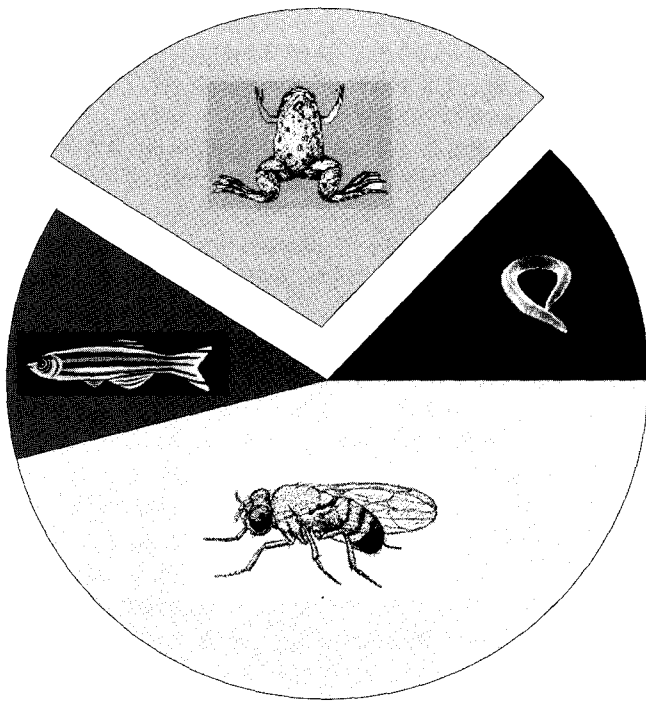
Figure 1 *Danio rerio* (zebrafish), *Caenorhabditis elegans*, and *Drosophila melanogaster* are acknowledged highly successful animal models. However, amphibians (especially from the genera *Xenopus*, *Bufo*, and *Rana*) are used more frequently in biological experiments than either the zebrafish or *C. elegans*, as determined from the number of PubMed entries between 2001 and 2006.

The diploid *X. tropicalis* is rapidly overtaking the pseudo-tetraploid *X. laevis* as a preferred model for genetic studies, but that trend has yet to be noticeably reflected in physiological studies.

Systems Physiology

The field of comparative physiology is rife with studies on all aspects of amphibian physiology, and it is beyond the scope of this paper to provide a complete summary. In the text below, we provide some current examples of ongoing research in selected areas to provide a broad overview of the diversity of topics wherein amphibians are models of choice. One very promising field of amphibian physiological exploration is developmental physiology. While amphibians have been studied from the beginning of physiological investigation, they are also widely acknowledged as a key model in developmental biology. Thus, with the current explosion of interest in evolutionary developmental physiology (Warburton et al. 2006), the breadth and depth of developmental information and techniques available for amphibians makes them the obvious choice with which to study the physiology of developing organs and organ systems.

Xenopus is currently the focus of intense sequencing

efforts (Klein et al. 2006) and in situ expression studies in organogenesis (Blitz et al. 2006). Unfortunately, physiological studies have not similarly focused on *Xenopus*. The benefits of *Xenopus* in the laboratory (e.g., easy breeding, large and rapidly developing larvae, ease of genetic transformation) are offset by their more delicate nature and lesser ability to tolerate surgical procedures relative to their more robust relatives such as *Rana* spp. or *Bufo* spp. There is a great need for better alignment in species usage at the interface of molecular biology and physiology (i.e., "systems biology"), either by exploring the application of *Xenopus*-derived molecular techniques to *Rana* and *Bufo*, or by increasing the use of *Xenopus* as an animal model in physiological studies systems biology. Such alignment is increasingly important with the maturation of functional genomics.

In Gerald Thomsen's (2006) introduction to a volume devoted to exploring amphibian genetics and development, the author notes,

> "An exciting aspect of studying organogenesis in *Xenopus* is the rapid rate at which 'classical' experiments can be done, and *X. tropicalis* genetic screens promise to augment such approaches to uncover the rules for making an organ. Studies of organogenesis in *Xenopus* are also likely to inform the human condition in ways not usually encountered in our basic science driven field" (p. 79).

The obvious sequelae of studying the "making of an organ" is the study of organ function in the making.

Let us now turn to the contributions of amphibian animal models to our understanding of physiology of specific organ systems. What follows is not intended to be a comprehensive review of current amphibian physiological studies but rather is intended to familiarize the reader with some of the fields wherein amphibians are being studied, either as a result of an interest in amphibians per se or in using amphibians as a general vertebrate model.

Skeletal muscle. Many of the early studies on innervation of muscle and muscle dynamics were performed on amphibians, and these protocols are still used currently in only slightly modified fashion in teaching laboratories. The form and function of amphibian skeletal muscle continues to comprise a fertile field spanning the range from calcium sparks (Baylor 2005), cellular metabolism (Walsh et al. 2006), and channel kinetics to studies of the high-speed kinetics of ballistic tongue movements (Lappin et al. 2006). A recent review of calcium spark methodology emphasizes the dominant role played by frog studies (Klein and Schneider 2006). Recent studies on frog nerve-muscle interaction include long-term modulation of synaptic function (Belair et al. 2005). Clearly, amphibians have served as important models in muscle physiology.

Cardiovascular studies. Many of the fundamental studies on cardiovascular physiology were based on frog models. Studies include Krogh's (1920) Nobel Prize work

on capillary regulation and Bowditch's (1871) description of "treppe" or staircase. Although there is some disagreement as to whether Otto Frank and Joseph Coates should get credit for formally describing the relationship of preload or cardiac filling to stroke volume (Zimmer 2002), it is clear that both investigators worked with isolated frog hearts. Thus, while the attribution for formal presentation of this keystone relationship may be unclear, the animal model involved in its discovery is certainly clear.

Amphibians continue to receive the attention of cardiovascular physiologists. The axolotl (*Ambystoma mexicanum*) is of particular interest to those studying developmental cardiovascular biology because of a "heartless" mutant strain. This strain is not actually heartless; the heart develops but never begins to beat. Although "silent" hearts are not uncommon in other vertebrates, this mutation is typically lethal very early in development. In the axolotl, however, development continues, surprisingly, for several days, rendering it useful for studies of oxygen transport, vascular development, and transgenics. The myofibrils of the nonbeating ambystomid heart are normally poorly organized, affording a system for investigating myofibril organization (Zhang et al. 2006). This model has been used, for example, to express a human cardiac tropomyosin gene that is implicated in the signaling of myofibrillar organization (Denz et al. 2004).

Interestingly, some amphibians have the ability to regenerate myocytes, seemingly in contrast to the mammalian lack of such abilities. This capability, of course, makes them of intense interest in the study of myocardial infarction recovery. Studies on newts (*Notophthalmus viridescens*) suggest that their ability to regenerate cardiomyocytes may not be very different from the condition in mammals because in the newt, the majority of injured cardiomyocytes do not appear to be capable of regeneration, as in mammals, and only a subset retain the ability to enter mitosis and repopulate the cardiac field (Bettencourt-Dias et al. 2003).

Microvascular studies continue to utilize amphibian models. For example, recent studies have used frog vasculature to evaluate new imaging techniques for studying permeability (Fu et al. 2005). Amphibians possess a highly developed lymphatic circulation including pulsatile lymph hearts (as do reptiles and a few birds). The function of the lymphatic system of adult amphibians is reasonably well studied (Williams et al. 1998), and investigations have recently been extended into the larval stages of *X. laevis* (Ny et al. 2006). Indeed, *Xenopus* spp. have proven valuable in many aspects of delineating heart development, including heart field induction and the manifold aspects of Nkx involvement (reviewed by Lohr and Yost 2000). In addition, amphibian models are used for investigation of cardiac laterality (Ramsdell et al. 2006).

The interaction of behavior and circulatory function can also be uniquely addressed in Anurans, which are distinctive among tetrapods in that terrestrial species do not drink or depend on metabolic water production for hydration. Instead, they absorb water across a specialized patch of pelvic skin—the seat patch—and alter circulatory patterns and posture to regulate water uptake (Burggren and Vitalis 2005; Viborg et al. 2006).

Renal function. The tetrapod kidney develops via a requisite three-step process of formation—pronephros, mesonephros, and metanephros—with each subsequent step dependent on successful completion of the previous one. In mammals, the pronephros is a nonfunctional ephemeral feature of very early development and, as such, is difficult to study in utero. In amphibians, however, the pronephros becomes functional early in development, as a precursor to the mesonephros serving as an organ of blood filtration. Free-swimming larval stages and amenability to explants and genetic manipulation greatly enhance studies on the function, structure, and genetic events in the formation of pronephros (Chan and Asashima 2006; Jones 2005).

In mammals, the kidneys serve both waste disposal and osmoregulatory functions. Given the importance and highly selective nature of ion transporters, it is perhaps not surprising that they are mostly evolutionarily conserved. More surprisingly perhaps is the finding that the hormonal regulation of ion transporters also appears to be conserved. In a recent study on the regulation of sodium transporters by aldosterone, insulin, and vasotocin/vasopressin, remarkable similarities emerged between amphibian skin and bladder versus mammalian renal tubule (Shane et al. 2006).

Bufo marinus has also proven to be a valuable renal research model in that it is a robust species less dependent on water than the highly aquatic *Xenopus* species. Such studies include environmentally induced gene expression of urea transporters (Konno et al. 2006) and as one of a suite of tetrapods in an evolutionary analysis of mitochondrial density in renal tissue (Hulbert et al. 2006).

Sensory physiology. Amphibians have been a standard model for vision research for years, and they continue to be used today. The large cells of the amphibian retina make them a model of choice for studies of channel activity and neuronal connection in vision research. Whole cell recordings in *Necturus maculosus* and *Ambystoma tigrinum*, both Urodeles, have been combined with morphological studies to assess function in retinal on-off amacrine cells (Miller et al. 2006). Similarly, spikelet current, which is suspected to be involved with visual integration in the retina, has been studied in *Rana temporaria*, taking advantage of their large cell size and relatively low metabolic rate (Gutmaniene et al. 2006).

The study of other sensory systems also has benefited from some of the nonmammalian characteristics of amphibians. *Xenopus laevis* has proven to be a useful model for studies of inner ear formation and innervation (Quick and Serrano 2005). Amphibians, along with birds, are of special interest for audition researchers because both groups have the ability to regenerate hair cells (Taylor and Forge 2005), an ability lacking in mammals and an important cause of hearing loss in humans. Amphibian feet (e.g., *Bufo punctatus*)

have provided a unique system for studying human taste sensation. As discussed, some amphibians have the ability to osmotically absorb water across a ventral seat patch. Some of these amphibians live in desert environments where patches of hypersaline water exist, and an amphibian in such a pond will actually lose water to the environment. As a protection against such a calamity, these amphibians have salt sensors in their feet to detect the salinity of the water in which they might be standing (Hillyard et al. 2004).

Respiratory physiology. It is in respiratory physiology that amphibians provide a cornucopia of opportunities. Typically adult amphibians exchange gasses with the environment both via lungs and across the skin. There is also a family of lungless salamanders (Plethodontidae) that are completely dependent on cutaneous gas exchange. Gills are typically present during amphibian larval development and may be retained in the adult in rare cases. Thus, there is a multitude of respiratory strategies in the Amphibia, and the study of respiratory control in this group is a challenge. In addition to having multiple sites for gas exchange and mastering the complexities of managing multiple exchange sites (lungs, gills, skin), the amphibious nature of most species means potentially altering gas exchange strategies on a minute-by-minute basis for the transition from air emersion to water immersion or vice versa (Wang et al. 2004). Gas exchange demands must also be balanced against water conservation in these permeable-skinned animals (Burggren and Vitalis 2005).

Finally, metamorphosis presents the ultimate challenge as animals make the transition from one complex set of control issues to another. This complexity provides a wonderful background against which to study respiratory control from the perspective of evolutionary physiology, developmental physiology, and environmental physiology. Regulation of changing respiratory demands and options during metamorphosis involves changing vascular distribution as well as altering neural regulation down to the level of transmitter substances and receptors (Hedrick 2005).

Reproductive physiology. The majority of amphibians reproduce by externally fertilized eggs that develop through larval stages and metamorphose into adults. This pattern is not absolute, however, and many interesting variations on this theme exist. *E. coqui*, the coqui frog endemic to Puerto Rico, is perhaps the most recognized member of the family Leptodactylidae. With only one known exception, all members of this speciose genus share an unusual trait, the compression of larval development to prehatching ("direct development"). Thus, there are no free-living larval forms in this genus.

The somewhat mysterious Gymnophiona (caecilians, apodans) comprise two families for which live birth occurs. In all Gymnophiona, internal fertilization is the norm—the only amphibians to which this characteristic applies. Male caecilians lack the typical mammalian accessory glands, the prostate and seminal vesicles, which are needed to activate sperm. In the caecilians, the müllerian ducts are retained in adult males and function as accessory glands, which secrete the necessary signals to increase sperm motility and supply nutrition (George et al. 2005). The müllerian ducts exist in other vertebrates, but only as an embryological precursor to the adult female reproductive system. Thus, the Gymnophiona provide an example of a potential transition step in the evolution of vertebrate sexuality. Other examples of "evolutionary feasibility studies" abound in the Amphibia. Many of the early arguments of phylogeny versus ontogeny were based on information from invertebrates and amphibians (Gould 1985).

Unlike the diploid *X. tropicalis, X. laevis* is an autotetraploid species. Although the extra genetic material is often considered a hindrance to genetic analysis, this extra genetic material in Leptodactylidae (neotropical frogs) and Hylidae (New World tree frogs) has been used to investigate the evolution of complex genomes by genome duplication. Autopolyploidy is not uncommon in Anurans and is being exploited to investigate gene regulation during periods of recent genome expansion (Beçak and Kobashi 2004).

Physiological Approaches to Evolution and Environment

In addition to the rich history of delineating basic physiological principles, amphibians as animal models have also been used to explore a variety of aspects of evolutionary and environmental questions and their physiological answers. These areas of study are briefly described below.

Evolutionary physiology. Amphibians occupy a transitional state in the evolution of tetrapod vertebrates as well as in the evolution of air breathing and terrestriality. Not surprisingly, then, the physiology of amphibians has been the subject of considerable scrutiny from the perspective of how they can yield insights into the evolution of terrestrial invasion (Feder and Burggren 1992; Little 1983; Randall et al. 1981). An inter-related set of challenges to animals leaving water and exploiting air breathing and terrestriality involves acid-base balance and nitrogenous waste elimination. Because of the low oxygen content of water compared with air, aquatic animals exhibit a high "water convection requirement" for O_2. For example, a fish must breathe 30 to 40 more volumes of water than an equal sized cold-blooded air breather would breathe of atmospheric air. Because of the high solubility of CO_2 and ammonia in water, these waste products are easily lost across the gills, and aquatic vertebrates have little problem with accumulation of either waste product. Acid-base regulation is achieved by secretion of bicarbonate (HCO_3^-) and protons (H^+) because CO_2 cannot be retained in the tissues as a "tool" for acid-base regulation (Boutilier et al. 1992; Branco 1995; Burton 2002; Maina 2002; Randall et al. 1981). With the evolutionary migration onto land and the onset of air breathing, CO_2 elimination is in a sense more difficult, and CO_2 levels increase in body tissues. However, this enables terrestrial

air-breathing animals to use CO_2 modulation to assist with acid-base regulation.

Similarly, elimination of ammonia in strictly water-breathing animals is straightforward because ammonia diffuses out of the gills into the surrounding water before it can increase to toxic levels (Randall and Tsui 2005; Tsui et al. 2004). In terrestrial animals, nitrogenous waste elimination is more problematic because ammonia secretion is ineffective, yet this toxic by-product must be kept at a low concentration in body tissues. Alternative nitrogenous end products (urea and uric acid) exist, but their elimination requires additional energetic input (Bray 1985; Randall et al. 1981; Singer 2003). These intertwined problems and the co-solutions of acid-base balance and nitrogen excretion become especially relevant in studying amphibian physiology. Amphibians represent not only a waypoint in the evolution of terrestriality but also, in most amphibians, a marked *developmental* transition from a strictly aquatic, gill-breathing larva to a more or less terrestrial, air-breathing adult.

Without unduly forcing a metaphor, the "fish-to-mammal" transition that amphibians evidence during their life cycle has provided excellent models for investigating evolutionary transitions in physiology associated with the adoption of air breathing (Burggren 1995; Burggren and Just 1992; Graham and Lee 2004; Remmers et al. 2001; Uchiyama and Konno 2006). Moreover, the great diversity in amphibian life cycles has allowed exquisite testing of physiological hypotheses that relate to an amphibian's mode of respiration. Thus, for example, there have been long-standing hypotheses that the evolutionary transition from water breathing to air breathing involves a general transition from ammonia release to urea/uric acid release, in both amphibians and air-breathing fishes (reviewed by Ip et al. 2004; Shoemaker et al. 1992). It would follow then, considering their developmental transition, that aquatic larval amphibians will be primarily ammonia excretors, while more terrestrial air-breathing adults will eliminate primarily urea, with each developmental stage making corresponding acid-base adjustments.

Several amphibian species with unusual life histories present an opportunity to test this nitrogenous waste/acid-base hypothesis. For example, the Chinese fire-belly newt (*Cynops orientalis*) develops in water, then moves out onto land, but later at sexual maturity returns to water. Yet upon its return to water, this newt retains the capacity for urea synthesis and continues to excrete urea along with ammonia, which moves freely across the skin (Weng et al. 2004). Thus, the mode of nitrogenous waste excretion is tied not only to mode of respiration but also to state of development, with juveniles and adults continuing to synthesize and secrete urea irrespective of mode of breathing. The idea that it is adult, not terrestrial, amphibians that synthesize urea could be tested further in the direct developing anuran genus *Eleutherodactylus* (Jennings and Hanken 1998). In *Eleutherodactylus*, the larvae stay in the egg and develop into a juvenile with the full adult morph, surrounded by perivitelline fluid, before finally hatching (Jennings and Hanken 1998). This mode of development would allow investigators to separate an animal's mode of breathing (water, air) from stage of development (larvae, adult) and thus probe hypotheses in evolutionary physiology.

Environmental physiology. Amphibians have long been used as indicators of environmental quality (Boyer and Grue 1995; Venturino et al. 2003). This subject is extensively reviewed in a companion article in this issue (Hopkins 2007) to which we refer readers. Many of the studies assessing water quality with amphibians have relied on mortality data to form bioassays (Demichelis et al. 2001). However, as a worldwide decline of amphibian populations is being documented, environmental biologists are increasingly hypothesizing that this phenomenon has physiological roots. More specifically, the industrial and urban release of endocrine disruptors is now well established as having major deleterious effects on amphibian reproduction and development (Hutchison et al. 2000; Iguchi et al. 2001; Kloas 2002; Tattersfield and Egmond 2000). However, physiologists are researching amphibian endocrinology not only from an ecological perspective regarding amphibian populations but also from the human health perspective, and amphibians are regarded as sentinel organisms for pollution dangers to humans.

Amphibians have also been used to model physiological processes subjected to extreme environments, again often relating back to learning about environmental physiological effects on humans. Perhaps one of the highest profile examples is that of studies on amphibians in hypogravity, induced by either parabolic airplane flights (Wassersug et al. 2005) or actual deployment into low space orbits using the Space Shuttle or the International Space Station (Dournon 2003; Horn 2004). Not surprisingly, due to technical limitations (laboratory space and equipment), current experiments have had more of a morphological or behavioral perspective, with modifications of embryonic and larval development as a popular theme. However, the launch of private, commercial space vehicles with an abundance of room for research and development (e.g., Bigelow Aerospace's Genesis 1 module launched in 2006) bodes well for future physiological studies on amphibians and other animals in hypogravity. At the other end of the gravity spectrum, amphibians have also been the focus of *hyper*gravity experiments, including physiological investigations of the effects of both acute and chronic exposure to elevated gravity (Boser and Horn 2006).

Amphibians have additionally been the focus of cryobiological experiments. Many frogs have natural cryoprotectants (antifreezes) that prevent their freezing (Voituron et al. 2005; Woods and Storey 2006). This characteristic not only affords fascinating study of an evolutionary adaptation to an extreme environment but also has broader cellular and molecular implications, including the study of more efficient methods of tissue storage (e.g., the recent study of preserving immunoreactivity in the pituitary of *X. laevis* [Wang et al. 2005]).

Systems Biology, Physiology, and X-Omics

Undeniably, the most rapid and far-reaching advances in amphibian biology are currently under way in the delineation of the *Xenopus* genome. Early efforts were focused on *X. laevis*, but the tetraploid genome adds substantial complexity to genetic manipulations in this species. For that reason, many investigators have switched to *X. tropicalis*, slightly smaller in body size but haploid. For translational genomics to be applied at levels higher than biochemical pathways, it will be necessary to find a convergence species, (i.e., one amenable to both genetic analysis and physiological measurements).

As *Xenopus* genomics spreads to *Xenopus* proteomics and on to metabolomics, it will ultimately arrive at the *Xenopus* physiome. However, many physiologists would prefer to study species such as *Rana* spp. and *Bufo* spp., both of which are more hardy in terms of surgical processes and for which there is already a substantial body of physiological information. Unfortunately, the extent to which the genetic information and approaches (e.g., gene chips) in *Xenopus* are transferable to distantly related genera such as *Rana* and *Bufo* has not been demonstrated. Moreover, the extent to which *Rana* and *Bufo* will be targets of efforts in the near future is uncertain, although we speculate that there will not be a wholesale switch to ranids and bufonids away from *Xenopus*, given the abundance of existing data for *Xenopus*.

The progression of research described above is a good example of the "first model effect," where the first model eventually becomes the best model because so much is known about it, as opposed to it having intrinsically superior characteristics as a model (Burggren 1999/2000). Unfortunately, a large gap is left between the species favored by molecular biologists and the species favored by physiologists. Of course, the most straightforward solution would be agreement on a single "model species." However, both molecular biologists and physiologists have well-founded reasons beyond tradition for studying particular groups of amphibians. It remains to be seen how widely transferable *Xenopus* gene chips will be to other amphibians, and how widely transferable physiological data (e.g., cardiovascular data from *Bufo*) will be to other amphibians. In any event, the continued reliance by biologists on numerous amphibian models appears to be destined and will certainly prosper if more traditional models can be fused with novel evolutionary and genetic approaches.

Acknowledgments

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