Amphibians Used in Research and Teaching

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Abstract

Amphibians have long been utilized in scientific research and in education. Historically, investigators have accumulated a wealth of information on the natural history and biology of amphibians, and this body of information is continually expanding as researchers describe new species and study the behaviors of these animals. Amphibians evolved as models for a variety of developmental and physiological processes, largely due to their unique ability to undergo metamorphosis. Scientists have used amphibian embryos to evaluate the effects of toxins, mutagens, and teratogens. Likewise, the animals are invaluable in research due to the ability of some species to regenerate limbs. Certain species of amphibians have short generation times and genetic constructs that make them desirable for transgenic and knockout technology, and there is a current national focus on developing these species for genetic and genomic research. This group of vertebrates is also critically important in the investigation of the inter-relationship of humans and the environment based on their sensitivity to climatic and habitat changes and environmental contamination.

Key Words: amphibians; frogs; research; salamanders

Brief Historical Perspective

Amphibians have long been utilized in scientific research, both as models for the elucidation of physiological and developmental processes and as primary subjects for herpetological investigation. Amphibians derive their name from the Greek "amphibious," which means "double life." Most begin life as aquatic larva and, through the process of metamorphosis, emerge as terrestrial adults. While many species of amphibians do not strictly adhere to this developmental pattern, they remain the only vertebrate class with such a unique adaptation (Zug et al. 2001).

As early as the 18th century, scientists were describing the biology and natural history of frogs (Duellman and Trueb 1994). With the expansion of knowledge regarding amphibian reproduction and development, scientists began experimental manipulation of embryos (Gurdon 2002). In the early 20th century, investigators discovered that injection of urine from pregnant women induced ovulation in African clawed frogs, *Xenopus* (due to chorionic gonadotropin); thereafter, *Xenopus* became an integral component of early pregnancy testing (Bellerby 1934; Callery 2006; Shapiro and Zwarenstein 1934). This ability to reliably induce ovulation year-round with hormone injections made *Xenopus* an ideal choice for developmental studies because it alleviated the constraint of seasonal reproduction. Thus, *Xenopus* emerged as a premier animal model for biological research (Gurdon 2002), and today, *Xenopus* and other amphibians are widely utilized in research and teaching. For additional information on amphibian taxonomy, biology, and natural history, please refer to the following publications: Duellman and Trueb (1994); Petranka (1998); Stebbins and Cohen (1997); and Zug et al. (2001).

Frog Species and Common Research and Teaching Areas

All frogs belong to the order Anura. Examples of frogs include the aquatic African clawed frog, the semiterrestrial bullfrog, and the terrestrial toad. Anurans typically begin life as aquatic tadpoles, with gills and a laterally compressed tail. As development proceeds, limbs appear, the tail is resorbed, and gills are replaced by lungs. Tadpoles and some aquatic adult frogs have a lateral line system, which is a series of pores in the skin that contain mechanoreceptors called neuromasts. Cilia project from the neuromasts, bend in response to current changes and water pressure, and are used to locate food (Zug et al. 2001). Frog skin also contains both mucous and granular glands. Mucous glands are more abundant than granular glands and are located over the entire body. These protective glands secrete slimy mucus, which protects the delicate skin from abrasive trauma, inhibits entry of pathogens, and helps prevent water loss. In aquatic larvae, the mucous layer reduces friction and facilitates movement through the water. Granular glands may be scattered on the body or clustered into parotoid glands, as occurs on the head of toads. Granular glands secrete a variety of compounds, including toxins, pheromones, and antimicrobial substances. Toxins (including neurotoxins, cardiotoxins, and hallucinogens) help defend against preda-

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tors. Antimicrobial compounds protect against bacterial and fungal infection when skin is abraded (Clarke 1997).

Species of frogs commonly used in research include members of the genus *Xenopus* (*Xenopus laevis* and *Xenopus tropicalis)*, *Rana* (*Rana pipiens, Rana catesbeiana*, and others), *Bufo*, and *Hyla. X. laevis* (African clawed frog) and *X. tropicalis* are the most intensively used amphibians today. Taxonomically, *Xenopus* are in the family Pipidae. These totally aquatic species have fusiform bodies and powerful hindlimbs for swimming. The forelimbs are used to push food into the mouth, which lacks a tongue (Zug et al. 2001). *Xenopus laevis* are tetraploid, and females can produce thousands of large, easily manipulated eggs. Oocytes from chorionic gonadotropin-primed females can be surgically harvested or allowed to be deposited. Male and female *X. laevis* can be pair housed to provide naturally fertilized embryos. Embryos will translate foreign mRNA when eggs are injected, a characteristic that has made *X. laevis* indispensable in experimental embryology (Beck and Slack 2001). Jones (2005) has summarized investigations using *X. laevis* embryos to elucidate development of the pronephric kidney. Microsurgical removal of cells from early-stage embryos and subsequent in vitro culture has enabled scientists to study neural crest cell migration (DeSimone et al. 2005). Other uses of *X. laevis* have included investigations into genetic expression in development and regeneration, growth, immune function, and toxicology. The *X. laevis* leptin gene has been isolated and demonstrated to have a role in early limb development as well as in regulation of food intake (Crespi and Denver 2006). Limb regeneration studies have frequently involved this species (King et al. 2003; Satoh et al. 2006; Tassava 2004). Recent investigations into central nervous system axonal regeneration have demonstrated positive capability in *X. laevis* neurons (Gibbs and Szaro 2006). *Xenopus laevis* has also been used to understand evolution of heat shock proteins and the immune system (Robert et al. 2004).

Historically, *X. laevis* gained popularity as the subject of the frog embryo teratogenesis assay: *Xenopus* (FETAX) method. This method utilizes documented normal *Xenopus* development (Nieuwkoop and Faber 1994) to determine effects of putative toxins and teratogens on developing embryos (Dumont et al. 1983; Papis et al. 2006). Recent environmental concerns have focused on effects of endocrine disrupters as well as other environmental contaminants. Due to its carefully defined developmental stages and metamorphosis, *X. laevis* has become a common model for assessing the effects of these compounds (Balch et al. 2006; Degitz et al. 2005; Katbamna et al. 2006; Kloas 2002; Loeffler et al. 2001).

Xenopus tropicalis, another member of the family Pipidae, has recently begun to replace *X. laevis* in many types of genetics research. Although members of the same family, *X. tropicalis* is classified in a separate subgenus from *X. laevis* (Kobel et al. 1996); in fact, it was once assigned to a separate genus, *Silurana*. *Xenopus tropicalis* is smaller than *X. laevis* and has more fastidious temperature requirements. *Xenopus tropicalis* also has a shorter generation time and is diploid (Beck and Slack 2001), factors that make it extremely desirable for transgenic and knockout technology. Laboratories are focusing on breeding and identifying mutants of *X. tropicalis* (Grammer et al. 2005), and the National Institutes of Health have an initiative focused on developing genetic and genomic tools for research involving both *X. tropicalis* and *X. laevis* (Klein et al. 2002).

The second major group of frogs used in research belongs to the genus *Rana* in the family Ranidae. They are typically semiaquatic animals, with powerful legs for jumping great distances. Species commonly seen in laboratories include *R. catesbeiana*, the bullfrog, and *R. pipiens*, the northern leopard frog. *Rana* species were used early in developmental studies (Briggs and King 1952); today they play a key role in investigating endocrine disrupters and environmental toxins (Cassano et al. 2006; Hogan et al. 2006; Mikkelsen and Jenssen 2006). *Rana* are used extensively in physiology research, both as animal models (King 1998; Zayas et al. 2004) and as subjects in their own right (Feder and Burggren 1992; Pryor and Bjorndal 2005). The genus is frequently studied from an ecological standpoint (Halverson et al. 2006; Laurila et al. 2006). Antimicrobial peptides secreted by the granular glands of *Rana* skin have been investigated intensively (Marenah et al. 2006; Xu et al. 2006). Mangoni (2006) recently described antiviral, antibacterial, antifungal, and anti-*Leishmania* properties in temporins from *Rana*. A unique and significant research application for *R. pipiens* has been use of the acetic acid wiping response. Frogs respond to serial dilutions of acetic acid by wiping with the hind limb when the concentration becomes irritating. This well-defined nociceptive response behavior has been used to elucidate the effects of opioids and opioid receptors in this pain research model (Stevens 2004). *Rana* has also been the frog of choice for comparative anatomy dissections and for teaching musculoskeletal physiology, cardiac physiology, and neurophysiology.

Toads of the genus *Bufo* are terrestrial frogs that have been used for a variety of studies, including limb regeneration (Abdel-Karim 1989; Everett and Brown 1996; Michael et al. 1993). They have been subjects of brain lateralization ("handedness") research (Vallortigara et al. 1999). *Hyla* species, the treefrogs, are arboreal frogs that along with *Bufo* are often the subjects of environmental toxin and endocrine disrupter research.

Salamander Species and Common Research and Teaching Areas

Salamanders are in the order Caudata, with approximately 400 living species represented (Petranka 1998). Like frogs, salamanders may begin life as aquatic larva; however, salamander larvae more typically resemble the metamorphosed adult form than do tadpoles. Adult salamanders may breathe by lungs, gills, or buccopharyngeal or cutaneous respiration.

They have an elongated shape, and most have four limbs and a tail (McDiarmid 1994). Like frogs, salamanders possess both mucous and granular glands. In newts and other salamander species, granular glands may secrete pheromones, which are integral components of courtship behavior (Clarke 1997). Many salamanders, such as *Ambystoma mexicanum* (the axolotl), retain some larval features while otherwise developing into reproductively active adults. This failure to develop adult morphological characteristics is referred to as paedomorphosis (the process formerly called neoteny). Paedomorphosis is widespread in salamanders and has been documented in at least 57 species representing nine of ten families (Denoel et al. 2005). Similarly, other salamander species can exhibit variation in timing and rate of growth (heterochrony).

Among the salamander genera commonly represented in research are the aforementioned axolotl, *Notophthalmus* (newt), and *Necturus* (mudpuppy). To the laboratory research community, the axolotl is likely the most familiar of salamanders (Gresens 2004). Axolotls are totally aquatic and breathe through large, feathery external gills. The developmental stages of the axolotl have been carefully characterized (Bordzilovskaya et al. 1989), therefore these animals have been extensively used in developmental research. The presence of nephrostomes on the axolotl kidney has resulted in the use of this species to study tubulointerstitial activation and subsequent fibrosis caused by protein loading (Gross et al. 2002). Limb regeneration has been a subject of investigation for many years; axolotls figure prominently in this area along with other salamander species such as newts (Brockes 1994; Morrison et al. 2006; Tassava and Olsen-Winner 2003; Vinarsky et al. 2005). Studies of sequences from the *Ambystoma* genetic map have contributed to greater understanding of structure and evolution of amniotic genomes (Smith and Voss 2006). *Necturus* has been used to study germ cell progression in spermatogenesis (Pierantoni et al. 2002). This species has also been utilized extensively in a variety of physiological studies, including investigations into olfaction and locomotion (Delay and Dionne 2003; Fok and Stein 2002) and as an instructional model for anatomy laboratories. The tiger salamander, *Ambystoma tigrinum*, is a long-standing animal model for research involving the olfactory system (Kauer 2002).

Both salamanders and frogs are widely studied as research subjects in their own right. An extensive body of information exists on the natural history of amphibians (Petranka 1998; Stebbins and Cohen 1997). Amphibians exhibit a wide diversity in behavior and reproductive strategies, including social monogamy, multiple paternity, clutch piracy, egg brooding, and feeding of young (Gillette et al. 2000; Liebgold et al. 2006; Vleltes et al. 2004; Wise and Jaeger 1998; Zug et al. 2001). Advances in molecular techniques have enhanced and broadened the scope of ecological research. Studies on feeding ecology of poison dart frogs in the wild have demonstrated a correlation between food type and type of skin toxin produced (Darst et al. 2005). Blending traditional field techniques, semicaptive controlled environmental studies, and laboratory methods allows for greater depth of understanding of amphibians and their inter-relationships with the environment (Rissler et al. 2004; Wilczynski et al. 2005).

Amphibian Sources, Population Concerns, and Health Issues

A major area of concern with amphibian use involves acquisition of research and teaching subjects. For many years, animals were collected from the wild, with no thought given to population impact or health issues. Recent investigations have demonstrated that amphibian populations are declining, likely due to multiple factors (Linder et al. 2003). These stressors could include introduction of toxins and other agents into the environment, habitat destruction or alteration, emergence of pathogens, and climatic changes. Studies have shown that exposure to chemicals with hormonal activities may alter behavior and affect survivability of amphibian species (Eroschenko et al. 2002). Likewise, human introduction of non-native predatory species can cause decline of amphibian populations. In some cases, predator removal can reverse this trend (Vredenburg 2004). Emerging infectious diseases of amphibians, such as chytridiomycosis, are likely exacerbated by human introduction of infected animals into previously unaffected areas (Daszak et al. 2001). Clearly, society should not tolerate the irresponsible removal of animals from the wild in light of these circumstances.

Coupled with population depletion concerns is an equally important consideration—the health status of wild-caught animals. Amphibians collected at certain times of the year may be predisposed to developing serious disease problems as a result of collection and shipping stressors. Methods for holding captured animals can be suboptimal, and conditions of crowding, lack of food, and inappropriate temperature can lead to stress and subsequent disease. This situation is worsened by pathogens such as chytridiomycosis (Mazzoni et al. 2003). Therefore, whenever possible, those who use amphibians in research or in teaching should make every effort to acquire captive-bred amphibians from reputable breeders and suppliers, and to ensure that only healthy animals are obtained and used. Some examples of breeders include Rana Ranch Bullfrog Farm (Twin Falls, ID, bullfrog@safelink.net), the Ambystoma Genetic Stock Center at the University of Kentucky (http://bigapple.uky.edu/~axolotl/), Xenopus I (Dexter, MI, www.xenopusone.com), Xenopus Express (www.xenopus.com), and Nasco (http://www.enasco.com/xenopus/).

Whether they are used as animal models, environmental indicators, or primary research subjects, amphibians remain critically important to scientific investigation and the advancement of knowledge. Their use should be thoughtful, judicious, and respectful.

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