Introduction

The Art of Amphibian Science

Stephen A. Smith and Michael K. Stoskopf

"I don't see no p'ints about that frog that's any better'n any other frog" (Samuel Langhorne Clemens, "The Celebrated Jumping Frog of Calaveras County," 1865).

"Jeremiah was a bullfrog, was a good friend of mine. Never understood a single word he said, but I helped him drink his wine" (Three Dog Night, "Joy to the World," 1971).

The contributions of amphibians to our understanding of ourselves and our world dates back to the very beginning of science. The selected reproductive strategies and the mysteries of amphibian metamorphosis have intrigued the earliest human cultures. Frogs seem to have received more attention than salamanders throughout time, having been adopted as fertility fetishes of southwestern Indian tribes and associated both with Aphrodite by the ancient Romans and with the shape-shifting goddess Heket by the Egyptians. The impact of frogs on ancient Greek science as models for understanding the biology of humans is evident in Aristotle's "Inquiry Concerning Animals" from the 4th century BC, when he uses frogs to illustrate his thoughts on the anatomical requirements for sound production and speech. Centuries later, in the first century BC, Pliny the Elder ponders the reproductive capacity of anurans in "Natural History" and furthers the misconception that they return to mud as winter approaches. It is apparent that understanding basic husbandry issues was a challenge even then.

From the resurgence of science in the Renaissance to the modern science of today, the ability to study amphibians has greatly facilitated our understanding of physiology and basic cellular processes. In 1798, studies of the reactions of frog muscles to the application of electricity by Luigi Galvani, a physician of Bologna, led to his theory that frog electricity differed from the ordinary kind, sparking a controversy with Allessandro Volta that formed the foundation for our understanding of action potentials in muscle tissue. For centuries, salamanders have served as the primary model for early vertebrate development (Srikrishna et al. 2004). Beginning mainly in Germany in the late 1800s, investigators used salamanders (primarily) and frogs (less frequently) (Beetschen 1996) to identify organizer and inducer regions in developing embryos, which in turn laid the groundwork for Hans Spemann's Nobel Prize work with newt embryos. From the 1920s through the 1950s, urodele embryos were the foundation of experimental embryology. The advances made using amphibian models long predated efforts in mammalian embryology, including the successful cloning of a frog by somatic cell nuclear transfer by Robert Briggs and Thomas King in 1952.

Spemann's Nobel Prize in Physiology or Medicine 1935 was only one of many Nobel prizes awarded through the careful study of amphibians (see Table 1 in Burggren and Warburton 2007). Spemann used newts for his discovery of the organizer effect in embryonic development, although the frog seems to have played a larger role in post-1920s investigations. Even before Spemann's award, the Danish biologist Schack August Steenberg Krogh won the 1920 prize in Physiology or Medicine for his studies of frogs and the capillary motor regulating mechanism. Shortly thereafter, the German Otto Fritz Meyerhof shared the prize in 1922 for his work with frogs, which established the fixed relationship between the consumption of oxygen and the metabolism of lactic acid in muscle. This work was followed in 1936 by the award of a share of the prize for Otto Loewi, using frogs, along with Sir Henry Hallett Dale for their discoveries of chemical transmission of nerve impulses. Elucidation of the role of anterior pituitary hormones in sugar metabolism using frogs earned Bernardo Alberto Houssay a share of the 1947 prize with Carl Ferdinand Cori and Gerty Theresa Cori, née Radnitz. The 1963 prize for discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane was awarded to Sir John Carew Eccles, Alan Lloyd Hodgkin, and Andrew

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Fielding Huxley who again utilized the stalwart frog, as did Erwin Neher and Bert Sakmann, who were awarded the 1991 Prize for their discoveries concerning the function of single ion channels in cells. Importantly, amphibians have not only supported the research for Nobel quality work in Physiology or Medicine but have also played a role in other basic research. For example, the 1943 Nobel Prize in Chemistry went to George de Hevesy for his work on the use of isotopes as tracers in the study of chemical processes using amphibians.

Amphibians continue to fuel our understanding of physiology and cell biology, yet one can argue that their most important role in science today is in helping us understand the ecology of our changing environment. Although the ability of salamanders to regenerate their limbs, tail, lens, retina, spinal cord, and even heart musculature continues to intrigue physiologists and cell biologists, the massive declines in amphibian populations around the globe, whether real or simply the result of more careful observations, are now inspiring scientists to examine issues of direct import to the survival of amphibians themselves (Linder et al. 2003; Ross and Richards 1999). This work is providing a better understanding of the complex relationships between environmental conditions and amphibian population viability and, at the same time, is identifying important emerging diseases of amphibians. Chytrid fungal infections and tiger salamander virus are only two examples of diseases virtually unknown only a decade ago that are now known to have major impacts on important amphibian taxa (Bollinger et al. 1999; Jancovich et al. 1997; Weldon et al. 2004). The unique position of amphibians at the aquatic/terrestrial interface makes their population health an important marker of ecosystem health. The challenge of understanding the translational impacts of their physiology and ecology, such as their unusual and diverse skin structures that affect absorption kinetics of xenobiotics, is critical to our understanding of human health (Willens et al. 2006). The more we learn about the impacts of ecological factors on amphibian health, the better we understand the necessity of managing amphibians in laboratories with utmost care.

The proper management and husbandry of amphibians are as important to good science as is the appropriate, species-specific care when using terrestrial animal models such as rodents. The same issues and challenges face the investigator. Investigators have long known that many issues of nutrition beyond the vagaries of feed composition (e.g., timing of feeding and environmental factors such as temperature, light intensity, and light cycling) can have profound impacts on amphibian physiology and have the potential of affecting experimental results (Paniagua et al. 1990). The complexities of the interactions of these factors and their wide impact on amphibian physiology are still being discovered. The impacts of water quality on amphibian physiology, also well documented historically (Whitford and Hutchison 1965), are being found to be even more complex than first recognized. Novel issues with amphibian husbandry, which include appropriate seasonal habitat shifts in land:water ratios, have huge implications in laboratory management of even well-established amphibian research models. Thus, although our knowledge of the proper husbandry of amphibians used in laboratory research has taken some important steps toward refining the art of repeatable science using these complex animals in recent years, there is still much to be learned. Considering the diversity of amphibian species, there is great opportunity for innovation and refinement of both husbandry and experimental design as we continue to explore the questions amphibians are able to help us answer.

This issue of *ILAR Journal* brings together the historical and current knowledge of contemporary amphibian issues. The initial article by O'Rourke (2007) describes commonly maintained amphibian species used for research and teaching, including the commonly used *Xenopus* frog and the axolotl salamander. In this article, the author illustrates the importance of amphibians to reproductive, developmental, genetic, and physiological research, and why certain species have come to the forefront as models for human processes and environmental indicators. This article is followed by a treatise by Browne et al. (2007) on the basic facility requirements for accommodating small numbers of individuals and for housing large populations of multiple species. The authors discuss environmental issues that range from water quality to ventilation and lighting (visible and ultraviolet), as well as issues related to the fulfillment of specific physiological requirements for adult and larval stages. The advantages of compartmentalized system design and various construction materials are examined. The authors also stress the importance of biosecurity (quarantine and isolation protocols) and staff management.

The next two articles extensively cover the biology, reproduction, and husbandry of amphibian species. The first article by Pough (2007) explores the enormous biological and life history variation that exists in the taxanomically diverse amphibian species. Arising from a larval aquatic life stage and metamorphosing to an aquatic, terrestrial, or arboreal adult life form can obviously present challenges for the captive maintenance of many of these species. In addition, changes in dietary preferences from herbivore to carnivore as the amphibian matures may require use of a variety of food items from commercially prepared diets to live prey. The author also underscores the subtle albeit important social behavior of amphibians (e.g., complex territorial and dominance hierarchies) that may influence feeding and reproductive behaviors. In the second of these two articles, Browne and Zippel (2007) discuss the current state of reproductive technologies (ovulation, spermiation, oocyte and sperm collection, fertilization, embryonic development, and metamorphosis) for commonly kept amphibians. The authors cover both natural reproductive patterns and artificial systems for the captive maintenance of amphibians while emphasizing the genetic diversity and broodstock management steps required to have successful captive breeding programs or laboratory colonies. Amphibians also exhibit distinct reproductive strategies that commonly require environmental cues (photoperiod, temperature, hibernation, aestivation, and rain events) for successful completion. An in-depth discussion of oocyte induction, spermiation, in vitro fertilization, and sperm cryopreservation are also included. Finally, the authors discuss larval rearing in terms of rearing densities and dietary requirements.

Two articles review the diseases and veterinary care of amphibians. Densmore and Green (2007) review the infectious (viral, bacterial, parasitic, fungal) and noninfectious diseases (neoplasia, nutritional) of amphibians. Although their coauthored article focuses mostly on common diseases of captive amphibians, much of the information is also relevant for free-ranging amphibian health management. The authors point out that many of the health problems observed in captive individuals are directly or indirectly related to husbandry and management issues. This article is followed by a brief review by Gentz (2007) of the general veterinary techniques used in the medicine and surgery of amphibians. After a short overview of some of the unique anatomical and physiological characteristics of these animals, the author describes several useful nonlethal diagnostic assays. Common surgical procedures and pre- and postoperative considerations are then discussed, as well as techniques for the sedation and anesthesia used for these procedures. The article concludes with a comment on the humane euthanasia of amphibians.

The next series of articles provides a comprehensive coverage of amphibians used as laboratory models for physiological and environmental research. Burggren and Warburton's (2007) article describes the use of amphibians as experimental animals for musculoskeletal, cardiovascular, renal, respiratory, reproductive, and sensory physiology. Their discussion is replete with examples of various amphibian species used for specific research questions. They also provide a synopsis of how amphibians have been used in investigations to study the evolution of air breathing and terrestrial life styles. In the subsequent article, Hopkins (2007) illustrates how amphibians have been used as models for ecological studies. The author notes the numerous characteristics that render amphibians particularly sensitive to environmental disturbances, including their permeable skin and reliance on both aquatic and terrestrial habitats. The author then explores the current global decline of amphibians (i.e., "the greatest mass extinction of land vertebrates since the dinosaurs") and cites possible explanations of critical habitat loss, environmental contamination, diseases, and climate change.

The final articles directly support the important mission of ILAR by providing information pertinent to the humane and appropriate care and use of these animals in a research and teaching setting. The information provided by Alworth and Harvey (2007) is essential reading for all institutional animal care and use committees (IACUCs[1]) that face the many novel issues concerning the use of amphibians in laboratory and field situations. As vertebrates, amphibians are encompassed by Public Health Service Policy and are subject to IACUC review and oversight. However, some issues are indeed distinct to amphibians, and Alworth and Harvey address the need for committee, investigator, and staff education in the complexities of amphibian use. In addition, they discuss potential zoonotic and other occupational health hazards. The authors close with a discussion of indicators of stress and disease in amphibians, which complements the Densmore and Green (2007) article on amphibian diseases. Nolan and Smith (2007) then provide a guide to readily available Internet resources relating to amphibians. The authors present these web sites, list servers, and databases in a format ordered by general discipline, with a brief description of what can be found at the site. The issue then concludes with an Appendix, by Smith (2007), which provides information about the drugs and compounds commonly used to treat or anesthetize amphibians. This referenced compendium categorizes the drugs and compounds by use (e.g., antibacterial, antifungal, antiparasitic, hormones, anesthetics) and provides suggested dosage ranges.

Taken together, the articles in this issue of *ILAR Journal* provide the most extensive update of amphibian management in science in more than a decade. The techniques and methods described in the issue are intended not only to update the ILAR (NRC 1974) publication *Amphibians: Guidelines for the Breeding, Care, and Management of Laboratory Animals* but also to help refine the work of scientists around the world who study these incredible animals. It is our two-fold hope that readers of this issue will benefit from the wealth of information, and that both accomplished and new scientists will be inspired to increase their understanding of the amphibian's needs in captivity and to create even better approaches to their husbandry.

[1]Abbreviation used in this Introduction: IACUC, institutional animal care and use committee.

References

Alford RA, Richards SJ. 1999. Global amphibian declines: A problem in applied ecology. Ann Rev Ecol System 30:133-165.

Alworth LC, Harvey SB. 2007. IACUC issues associated with amphibian research. ILAR J 48:278-289.

Beetschen JC. 1996. How did urodele embryos come into prominence as a model system? Int J Dev Biol 40:629-636.

Bollinger TK, Mao J, Schock D, Brigham RM, Chinchar VG. 1999. Pathology, isolation, and preliminary molecular characterization of a novel iridovirus from tiger salamanders in Saskatchewan. J Wildl Dis 35:413-429.

Browne RK, Odum RA, Herman T, Zippel K. 2007. Facility design and associated services for the study of amphibians. ILAR J 48:188-202.

Browne RK, Zippel K. 2007. Reproduction and larval rearing of amphibians. ILAR J 48:214-234.

Burggren WW, Warburton S. 2007. Amphibians as animal models for laboratory research in physiology. ILAR J 48:260-269.

Clemens SL. 1865. "The Celebrated Jumping Frog of Calaveras County." N Y Saturday Press, November 18, 1865.

Densmore CL, Green DE. 2007. Diseases of amphibians. ILAR J 48:235-254.

Gentz EJ. 2007. Medicine and surgery of amphibians. ILAR J 48:255-259.

Hopkins WA. 2007. Amphibians as models for studying environmental change. ILAR J 48:270-277.

Jancovich JK, Davidson EW, Morado JF, Jacobs BL, Collins JP. 1997. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. Dis Aquat Org 31:161-167.

Linder G, Krest SK, Sparkling DW, eds. 2003. Amphibian Decline: An Integrated Analysis of Multiple Stressor Effects. Pensacola: Society of Environmental Toxicology and Chemistry.

Nolan MW, Smith SA. 2007. Amphibian resources on the Internet. ILAR J 48:290-296.

NRC [National Research Council]. 1974. Amphibians: Guidelines for the Breeding, Care, and Management of Laboratory Animals. Washington DC: National Academy Press.

O'Rourke DP. 2007. Amphibians used in research and teaching. ILAR J 48:183-187.

Paniagua R, Fraile B, Saez FJ. 1990. Effects of photoperiod and temperature on testicular function in amphibians. Hist Histopathol 5:365-378.

Pough FH. 2007. Amphibians biology and husbandry. ILAR J 48:203-213.

Smith SA. 2007. Compendium of drugs and compounds used in amphibians. ILAR J 48:297-300.

Srikrishna P, Smith JJ, Walker JA, Rondet M, Weisrock DW, Monaghan J, Samuels AK, Kump K, King DV, Maness NJ, Habermann B, Tanaka E, Bryant SV, Gardiner DM, Parichy DM, Voss SR. 2004. From biomedicine to natural history research: EST resources for ambystomatid salamanders. BMC Genomics 5:54.

Three Dog Night. Joy to the World. Available online (http://www.last.fm/music/Three+Dog+Night/_/Joy+To+The+World+(Jeremiah+Was+A+Bullfrog), accessed March 30, 2007.

Weldon C, du Preez LH, Hyatt AD, Muller R, Speare R. 2004. Origin of the amphibian chytrid fungus. Emerg Inf Dis 10:2100-2105.

Whitford WG, Hutchison VH. 1965. Effect of photoperiod on pulmonary and cutaneous respiration in the spotted salamander, *Ambystoma maculatum*. Copeia 1965:53-58.

Willens S, Stoskopf MK, Baynes RE, Lewbart GA, Taylor SK, Kennedy-Stoskopf S. 2006. Percutaneous malathion absorption by anuran skin in flow-through diffusion cells. Environ Toxicol Pharmacol 22:255–262.