

Amphibian Care & Handling

Amphibians are scaleless, smooth-skinned, ectothermic (cold-blooded) vertebrates, most of which are closely associated with aquatic or very moist environments. Virtually all amphibians begin their lives in the water as fully aquatic gill-breathing larvae (tadpoles). Although some remain aquatic their entire lives, many amphibians metamorphose into air-breathing adults with lungs and appendages. These animals spend varying amounts of time, depending on species, in both aquatic environments and terrestrial (land) environments.

The types of amphibians commonly represented in laboratories are frogs, toads and salamanders. Certain species of frogs, toads and salamanders adapt well to the laboratory environment and have been studied extensively. Various species have been used for genetic, physiological and neurology/endocrine studies. These include bullfrogs (*Rana catesbeiana*), leopard frogs (*Rana pipiens*), the African clawed frog (*Xenopus laevis*) and the Tiger salamander (*Ambystoma tigrinum*), also called the axolotl.

Handling and Restraint

The slimy skin secretion of amphibians is a protective covering that is similar to that of fishes. Handling these animals with dry hands can cause this skin covering to rub off, allowing the entrance of bacteria. Frogs and salamanders should not be handled unless it is absolutely necessary. Amphibians do not adapt well to handling. When picked up they almost always struggle. This combined with their mucous coating makes it difficult to restrain or hold. If they must be picked up, it should be done with wet gloves or a soft damp net. Frogs are picked up by placing fingers on each side and between the legs. The frog's head will then face the handler's wrist. Axolotls can be injured if restrained in a net; a two-handed lift is the preferred method of restraining.

Sexing and Breeding:

Most amphibians are not bred in laboratories. Rather, they are wild caught or bred and reared by suppliers. There are many variations in sexual characteristics among amphibian species. Among most, the only absolute way to determine sex is to observe the actual courtship, for this is the only time there is a difference in their morphology and behavior. During the breeding season males' vocal sacs become more prominent. In bullfrogs the external tympanic membrane (the ear) which is located caudal to each eye, is approximately the same diameter as the eye in the female, whereas in the males, this membrane is twice as large as the eye. Males also have heavy thumb pads.

Xenopus laevis females have larger ventral flaps and a larger body than males. Male Xenopus have black surfaces on the inner aspect of the fore limbs, and they have larger digits during the breeding season. Adult male and female Xenopus laevis are distinguished by differences in size, presence of cloacal valves and presence of nuptial pads on the forearms during the breeding season. Xenopus should never be released into local ponds or rivers because they are hardy and will compete with native amphibian wildlife.

In Rana pipiens the location of the foramen magnum may be readily identified by a slight depression of the skin on the midline just posterior to the eyes.

Behavior

Amphibian skin is much more porous than that of most other partial or wholly terrestrial vertebrates. Amphibians are therefore particularly sensitive to toxic substances in the water, especially those amphibians that spend little or no time in a terrestrial environment. In planning the water supply for amphibians, care should be taken to ensure its quality with respect to chlorine, fluorides, heavy metals, microorganisms, oxygen, pH, and toxicants. Temperature changes that for most other vertebrates would not be considered extreme, cause behavioral abnormalities such as lethargy and loss of appetite, and can trigger illnesses. Amphibians are also fairly sensitive to sudden temperature changes of more than a few degrees at a time.

Simply providing amphibians with the appropriate food may not be sufficient to ensure feeding. If amphibians become stressed from over handling or too many disturbances, they may stop eating and starve to death. If temperature is sub optimal, frogs will not eat and their metabolism will slow down. Establishment and maintenance of feeding behavior indicates that the amphibian has accepted its environment.

Many amphibians tend to be cannibalistic. Large tadpoles may eat small ones, and large adults may eat tadpoles or smaller adults. Cannibalism among larvae is largely a result of overcrowding. Housing larvae and adult amphibians in a low population density and providing them with adequate food can help reduce or eliminate cannibalism.

Reptile Care & Handling

Reptiles are ectothermic (cold-blooded) vertebrates. They are easily distinguished from other classes of vertebrates by their dry, scaly skin. In addition, turtles have a shell. The class Reptilia includes tortoises and turtles, crocodiles and alligators, and snakes and lizards. Reptiles breathe air by means of lungs at all stages of life. They do not pass through a gill-breathing tadpole stage as do amphibians. The homeostatic abilities of reptiles are far less well developed than birds and mammals. Reptiles are ectothermic and, under natural conditions, select microenvironments in which they can gain or lose heat as required to maintain their body temperatures. Their thick, keratinized skin better protects them from water loss and absorption of noxious substances from their environment than does the moist, permeable skin of amphibians.

Virtually all major groups of reptiles contain some endangered species. This and applicable national and international conservation regulations must be considered when choosing reptiles for study in captivity. Reptiles are drastically different from most standard laboratory animals. In research setting there are as yet few defined "laboratory reptiles." The species used varies with the research needs of investigators. Reptiles are commonly used for anatomical and comparative physiological studies.

Handling and Restraint

Reptiles should be moved gently, confidently and quickly. A hesitant or jerky approach often provokes a bite. If repeatedly and gently handled, most reptiles become less aggressive. In time some even become placid. Many reptiles can simply be scooped up by the body and moved quickly. When restraint of the head is necessary, for treatment or experimental purposes, it is important that the reptile's body and legs be supported comfortably. This prevents the body and tail from thrashing about and causing injury. The animal's head can be restrained with the other hand of the person administering the treatment, or by an assistant. The hook is an effective tool for lifting a snake to a holding container or to a clean cage. It is slid under the snake one-third to midway down the body. The snake is then lifted quickly to a meter or so about any surface.

Highly venomous snakes should be handled as little as possible. Only experienced personnel are permitted to handle venomous snakes and only with special training and approval from the Institutional Animal Care and Use Committee.

Small lizards should not be enclosed by the hand for more than a few seconds, since this procedure may injure or kill them by restricting their breathing. Handling a lizard by the tail alone should be avoided whenever possible, due to the potential of tail damage. Most reptiles used in laboratories are wild caught. They have a limited capacity to adapt, and their success in captivity depends on the ability of their keepers to create an acceptable simulation of their natural habitat.

It is generally best to maintain species separately and to keep the number of each species within a primary enclosure to a minimum. This helps reduce competition for food and hiding places.

All reptiles periodically shed their skin. How completely and intact as well as how often a reptile sheds is a health indicator. More frequent shedding usually indicates a healthy animal that is eating and growing. Lizards, turtles and crocodilians shed in many pieces. Snakes normally shed in one piece, from nose to tail, which is peeled off like a sock. Sickness or inadequate humidity may cause a reptile that normally sheds an intact skin to shed in pieces or to be unable to shed. In the latter case, placing a snake in a bowl of warm water aids shedding.

Any deviation from a reptile's natural environment, such as excess handling, inadequate temperature or humidity, improper lighting, overcrowding, damp or unsanitary conditions and lack of a hiding place can cause stress. Stressed animals may refuse to eat and will

die of starvation. The primary goal of husbandry is to establish and maintain feeding behaviors. In general a reptile that eats in captivity is not unduly stressed and has successfully adapted.

Water

A vessel of water placed at ground level or some other accessible level must be provided, although it cannot be assumed that reptiles drink until this is observed. Many reptiles simply soak in these vessels if these containers are large enough. Lizards typically lap water from wetted leaves or from wetted cage surfaces. Many chelonians and snakes can drink from freestanding water bowls.

Aquatic Reptiles

Aquatic housing is a requirement for several reptilian species including sea turtles, crocodylians, venomous sea snakes and a few lizard species. These species are encountered rarely in laboratories so their specialized individual requirements will not be discussed here.

Legal Aspects

It is incumbent upon both dealers and users of amphibians and reptiles to be aware of and abide by the laws established to regulate their use. At the federal level the Endangered Species Conservation Act provides for the conservation, protection and propagation of any wild mammal, fish, wild bird, amphibian, reptile, mollusk or crustacean threatened with extinction or likely within the future to be come threatened with extinction. Reputable users and dealers do not undertake the use or supply of endangered species. Permits for scientific purposes for enhancing the propagation for survival, or for the incidental taking of endangered wildlife must be made on a federal level to the U.S. Fish and Wildlife Service, Federal Wildlife Permit Office and on the state level to the Arizona Game and Fish Department (2221 W. Greenway Rd., Phoenix, AZ 85023). Arizona has numerous rules governing live wildlife possessions under laboratory (research) conditions which must be met before a permit to capture wildlife is issued.

Anesthesia and Euthanasia

Considerations of humane treatment and the research requirement that animals in both acute and chronic protocols be subject to a minimum of stress, introduce the need to apply the best available procedures for anesthesia, analgesia and euthanasia.

Recommendations for anesthesia, analgesia and euthanasia can be found in Appendix D of the Institutional Animal Care and Use Committee's Policy and Procedures Manual on the ASU VPR Web site and from the IACUC Animal Records office by calling 480.965.4387.

Succinylcholine chloride without anesthesia is not approved for use in any animal at ASU because alone it does not render the animal unconscious.

In working with any animals, adequate and frequent handwashing is a key to preventing transmission of diseases from animals to humans and from humans to animals. Prophylactic tetanus immunizations are required before working with animals at Arizona State University.

Any procedures which differ from those listed in the animal use protocol and approved by the Institutional Animal Care and Use Committee must be presented as an amendment to the protocol and approved by the IACUC before being carried out on an animal.