Husbandry and Care of Amphibians (Chapter 34, ZOOKEEPING)

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Introduction

The amphibians are a highly diverse class comprising more than 6,600 species in 45 families. There are three orders in the class amphibia: Gymnophiona (Apoda), the legless amphibians; Caudata (Urodela), the newts and salamanders; and Anura (Salientia), the frogs and toads. Although there are exceptions, most amphibians—as suggested by the Greek root of the name *amphibios*, which means "double life"—have a two-phase life cycle with an aquatic larval stage and a terrestrial adult phase. All families share a number of characteristics. The main shared characteristic is the permeable skin that serves as a major surface for water absorption and gas exchange. Some species, such as the Plethodontid salamanders, rely entirely on their skin for the transfer of oxygen and carbon dioxide and have reduced or eliminated the lungs or gills.

Gymnophiona: These are legless amphibians known as caecilians. Caecilians are adapted primarily for burrowing, and some also have adaptations for an aquatic lifestyle. There are approximately 181 species within the order Gymnophiona. Caecilians have reduced tails, reduced eyes, segmented skin with small dermal scales, strongly built and heavy boned (ossified) skulls for burrowing, and acute olfactory systems. These animals have an elongated body form and lack even the internal elements associated with limbs (a pelvic or pectoral girdle); many look more like giant earthworms than typical amphibians. Caecilians are found in tropical South America, Africa, and Asia. They possess a penis-like intromittent organ (phallodeum) for internal fertilization. Some species of caecilians exhibit a level of parental care in which the young feed inside the female before birth and on the female after birth (e.g., the Kenyan caecilian, *Boulengerula* sp.). Most caecilians have lungs and also use their skin for gas exchange. Caecilians have retractable tentacles, which are sensory organs, on either side of the head between the eye and the nostril.

Caudata: These are tailed amphibians, salamanders, and newts. They resemble the early generalized amphibian body form. There are approximately 584 species within the order Caudata. The body is elongated and divided into head (cephalic), trunk (thoracic), and tail (caudal) regions. Some salamanders can lose their tails (tail autonomy) as a defense mechanism to distract potential predators. Once a tail has been lost, it can be regenerated. Larvae in this order resemble adults but have external gills. Most caudates have nonfunctional auditory and vocal structures, but also have a welldeveloped olfactory communication that uses pheromones from glands in the cloaca and the skin. Most rely on internal fertilization, in which the male deposits a spermatophore that the female picks up with her cloaca.

Anura: These are "tailless" amphibians, the frogs and toads. Anura is the largest order of amphibians, with approximately 5,834 species. Frogs and toads have reduced bone in the cranial (head) region and distal limbs, and as adults most have no ribs present. They are specialized for jumping, and have a short bodies and elongated hind legs. The sacral (pelvic) vertebrae are fused into a unique pelvic skeletal structure, known as the urostyle, that facilitates this mode of locomotion. Most of these animals rely on external fertilization. This is the most successful order of amphibians, with a geographic distribution that includes all the continents except Antarctica.

Nearly one third of the world's amphibian species (more than 1,800 species) are threatened, making amphibians a priority conservation group. Many zoos are expanding or modifying their amphibian collections in response to International Union for Conservation of Nature (IUCN) recommendations to address the worldwide decline in amphibian populations. Further, many zoos actively participate in "rescue" projects, where threatened populations of amphibians are brought into captivity in the face of catastrophic losses in the range country. The hope is that rescued species can be returned to the wild once the threats to their survival have been mitigated. This requires maintaining amphibians in biosecure facilities to ensure that they can be used in future reintroductions.

This chapter provides fundamental amphibian care guidelines and the basic keeper skills required to successfully manage amphibians in a captive setting. After studying this chapter, the reader will understand the anatomical and physiological terms used in describing the three different orders of amphibians.

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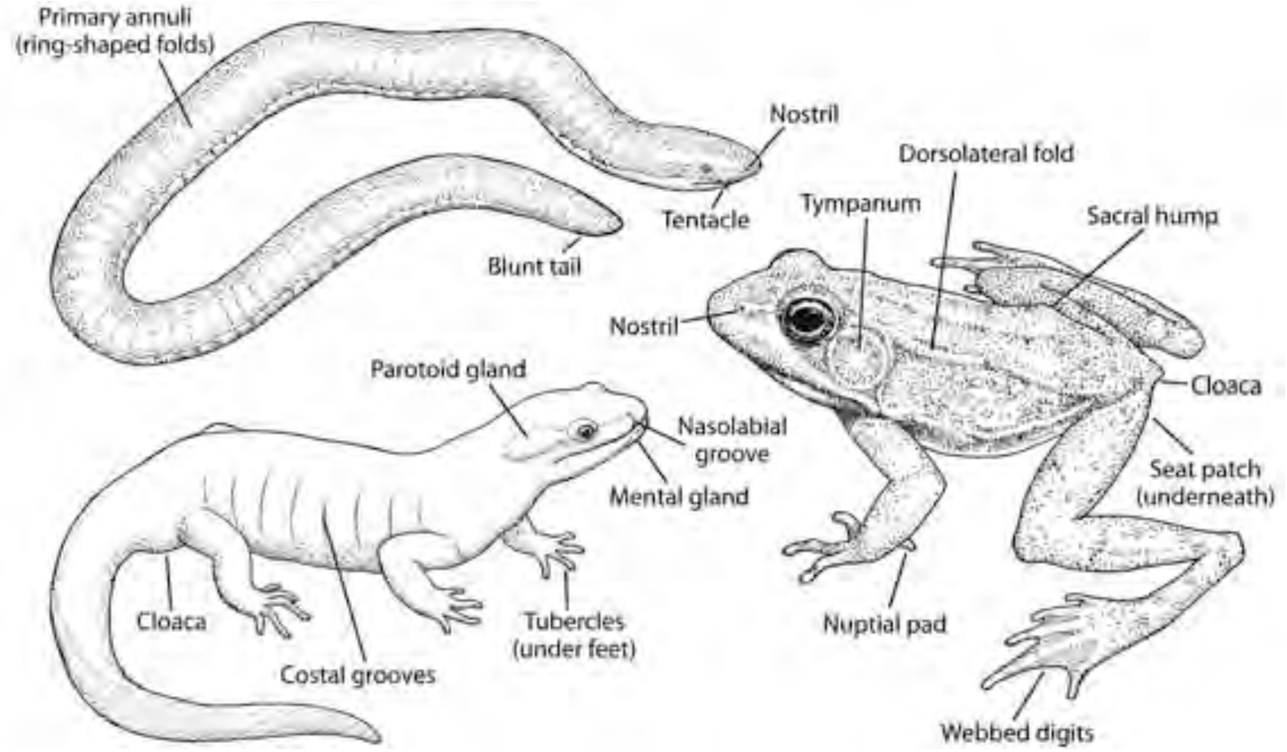

Fig 34.1. Basic anatomical features of amphibians. Clockwise from upper left: Caecilian (legless, order Gymnophiona), frog (tailless, order Anura), salamander (tailed, order Caudata). Illustrations by Kate Woodle, www.katewoodleillustration.com.

- that the unique physiology of amphibians affects the housing, nutrition, and reproduction of these animals in the zoo and aquarium environment.
- best practices for daily care, handling, and transport of amphibians.
- key habitat and environmental requirements, particularly water quality and temperature.
- principal issues involved in medical management of amphibians.

Orientation to Keeping and Husbandry of Amphibians

In order to manage populations of amphibians for conservation outside of their natural surroundings (*ex situ*) and to monitor the well-being of individuals, keepers must be able to identify the individual animals in their care. Marking and identification techniques for individuals include tags, microchips, and elastomer (a polymer with the elastic properties of natural rubber) dyes that can be injected just below the translucent skin of many amphibians. The least invasive method of individual identification relies on differences in the appearance of individual animals. In many species, each individual has a unique pattern (dorsally, ventrally, or on the limbs) that can be photographed and used to identify it. In some species these patterns can change over time, so the keeper should update identification photographs periodically. Individuals or groups can also be kept isolated from each other and identified by their enclosures.

Amphibians are ectothermic (often referred to as coldblooded) and their body temperature is dependent on the environment. Ectothermic animals obtain the energy they need to raise their body temperature from the sun or from radiant heat, and they regulate their body temperature by exchanging heat with their surroundings. Ectothermic animals require 10 to 14% of the energy used by similarsized endothermic animals (birds and mammals), a point to consider in keeper-controlled diets. This enables them to redirect the energy that endotherms would use to maintain a constant high body temperature to other activities instead, such as growth or reproduction. Ectothermic vertebrates, therefore, can successfully exploit environments of low biological productivity that could not support similarly sized endotherms. Most amphibians are thermoconformers; that is, they exist at the same temperature as their surroundings. Others engage in behavioral thermoregulation to maintain their body temperature within preferred limits. Ectothermy and thermoregulation affect the ecology, behavior, morphology, and physiology of amphibians.

Temperature tolerance: Species-specific temperature adaptations have been well documented. Some temperate species, such as the wood frog (*Rana sylvatica*), are adapted to low temperatures and are actually freeze-tolerant. Their embryonic temperature tolerance ranges from 2 °C to 20 °C (36 °F to 68 °F), and adults can freeze with their body temperature dropping to as low as -7 °C (20 °F). Some tropical amphibian species, such as the waxy monkey tree frogs (*Phyllomedusa sauvagei*), are active at temperatures ranging from 22 °C to 41 °C (72 °F to 106 °F). The salamanders are generally active at lower temperatures than frogs and toads, a fact which is reflected in their predominantly temperate distribution. The "web-toed" salamanders (genus *Bolitoglossa*) are an exception and are found in the New World tropics. Amphibians generally select a microhabitat to achieve a preferred (but variable) body temperature, since they have to balance increasing body temperature with increased evaporative water loss from their permeable skin.

Water Balance: Amphibians face several challenges in maintaining water balance, because of their highly permeable skin. In aquatic environments they have a greater amount of dissolved solutes in their tissues than the surrounding water (hyperosmotic), and therefore they face a loss of ions and an increase in water content (i.e., they absorb water). Amphibians therefore have adaptations that allow them to excrete excess water. In most amphibians the kidney produces large quantities of dilute urine to this end. In a terrestrial environment, amphibians constantly lose water and face the threat of lethal dehydration. Amphibians living in extremely dry (xeric) environments have water-conserving adaptations that allow them to avoid dehydration. Such adaptations include the ability to store and reabsorb water in their bladders, mechanisms for efficient uptake of water from the environment, specialized secretions that reduce skin permeability, the ability to produce insoluble uric acid for efficient nitrogenous waste excretion, and various behaviors to avoid desiccation. The waxy monkey tree frog (*Phyllomedusa sauvagei*) inhabits arid areas of South America and is constantly faced with the threat of desiccation. This species has evolved the ability to secrete a waxy (lipid) substance that it spreads over its entire body by grooming with its limbs. In this way, the frog covers its normally permeable skin with a waterproof coating that reduces evaporative water loss to 5 to 10% of that seen in most other anurans —a rate similar to rates seen in desert lizards.

Most amphibians do not drink. Most of their fresh water intake takes place through their skin. The "seat patch," an area of highly vascularized thin skin on the pelvic region of anurans, facilitates dermal water absorption from free water or damp substrates.

Keeping Amphibians

Amphibians are a highly diverse group that includes both generalists and specialists that pose challenges to ex situ husbandry and conservation. To meet these challenges, keepers need to adopt approaches very different from those required for mammals and birds. The keeper is responsible for creating, monitoring, and maintaining an environment that provides thermal and water (hydrological) features which allow amphibians not only to maintain homeostasis (regulate their internal environment so as to maintain a stable state), but thrive in the captive environment.

Daily Maintenance

Daily exhibit and off-exhibit servicing includes checking lighting, temperature, water quality, and humidity levels, and correcting them if they are outside the desired limits for the species. All fecal material and uneaten food items should be removed daily to reduce the biological load on the system and prevent excessive bacterial contamination. Water features (pools, filters, recirculation pumps, etc.) should be checked daily and maintained per an established schedule. Pools should be free of excessive algae. Water bowls and unaltered pools should be scrubbed daily and refilled with dechlorinated (aged, carbon filtered, or chemically dechlorinated) fresh water. Water should be at the appropriate temperature for the species. Its temperature can be adjusted by mixing hot and cold dechlorinated water, or by maintaining a reservoir of water at the correct temperature. Most amphibians will consume their own shed skins; however, remnants of shed skid will occasionally adhere to the glass of a tank, particularly for some arboreal species, and these should be removed daily. Dead and overgrown plant material should also be removed. The final step in daily maintenance should be a thorough cleaning of the viewing glass. Water droplets on glass are unsightly, and if not cleaned they can leave permanent mineral deposits that will require the replacement of the entire glass. Daily drying of the glass with a lint-free cloth or squeegee after servicing or misting will prevent unsightly mineral buildup. A mild white vinegar solution (one part household vinegar [3-5% acetic acid] and one part water) can be used. One should remove the animals from the enclosure, wipe down the spotted glass with the vinegar solution, and then rinse thoroughly and squeegee the area dry.

Since amphibians have delicate skin, which they use for gas exchange and water balance, care in the use of disinfectants is essential. Keepers must avoid transferring irritants such as oils, soaps, lotions, or acids from their hands to the animals. Disposable gloves (powder-free vinyl or latex) are recommended. Most amphibians secrete toxins through their skin or through the enlarged parotid glands behind their heads. Keepers should always wash and thoroughly rinse their hands before and after handling any amphibian species. Aquatic species can be handled using a soft fish net or simply by scooping them up in a plastic bag to avoid traumatic injury to their delicate skin. If bare hands are used, they should be wet to prevent loss of the protective mucus from the amphibians skin.

The Zoo and Aquarium Environment

Amphibian keepers face the challenge of creating microhabitats that meet the needs of the animals and the visitors. Enclosure design must provide adequate humidity, access to clean water, refugia (shelters and plants), and lighting that meets behavioral and physiological needs. To meet the needs of the captive amphibian, a keeper must first research and understand the animals to be cared for. Researching the geography and microhabitats they exploit in the wild is a good place to start. Climate data from the regions where the animals are found is readily available from credible government or academic internet sites, from libraries, or from colleagues. Knowing the seasonality and temperature range and precipitation that a species experiences in the wild will guide the keeper in designing an *ex situ* environment suitable for that species. Knowing a species' natural history and habits (terrestrial/arboreal, aquatic/fossorial, nocturnal/diurnal, etc.) will also aid a keeper in designing a habitat structure that meets its behavioral and reproductive needs. In the following paragraphs the fundamental principles of amphibian habitat design, as well as more advanced and specialized multihabitat automated systems commonly used to house amphibians, will be discussed. Specialized multihabitat rack units with automated misting systems and false-bottomed flow-through enclosures are commonly used to house amphibians.

The Enclosure

Enclosure types available to house amphibians are varied. Due to the diversity of species involved, the most appropriate enclosure will depend on the particular species and life stage of the animals being housed. The most readily available and versatile enclosure is the glass aquarium. Aquariums are generally inexpensive and work very well as the foundation of a captive habitat for terrestrial and aquatic amphibians. Most amphibians are very good climbers and can escape through a regular aquarium lid or hood. Therefore, a secure escapeproof lid is essential. Such lids can be made of screen in a metal or plastic frame that fits snugly into the plastic trim of the aquarium. The lid should be secured by latches or hasps; one should not rely on weights to keep a lid in place.

The complexity of the habitat depends on the purpose of the enclosure. An exhibit enclosure with natural substrate and live plants will usually be more complex than a holding enclosure that may be set up in a more sterile manner for improved hygiene or

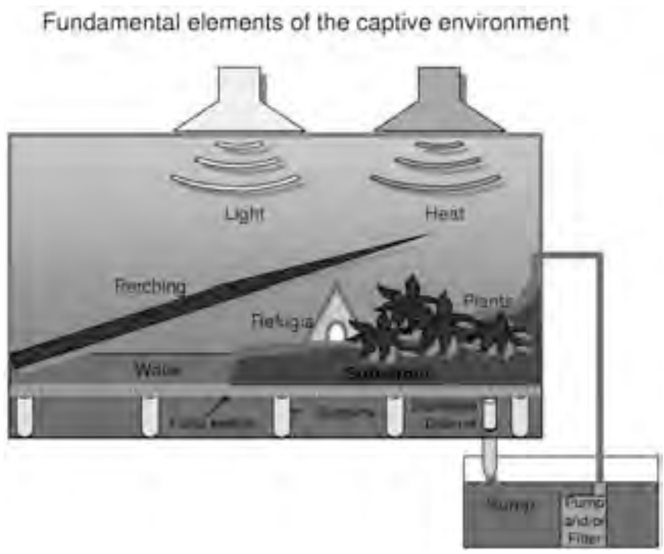

Fig 34.2. The fundamental elements of an amphibian enclosure: water, lighting, temperature control, refugia, and substrate, with appropriate variation or gradients for each. Courtesy of A. Lentini.

treatment of sick animals. The fundamental aspects to include in any enclosure design are water, lighting, temperature control, refugia, and substrate, with appropriate variation or gradients in each (fig. 34.2). Glass aquariums may not be sufficient for many complex modern exhibits, which may instead require custom-designed and built enclosures.

A popular enclosure setup technique for exhibit and holding tanks is the false-bottom design. Using plastic (polystyrene) light diffusers (often called "egg crate diffusers") covered with fiberglass (fine mesh) window screen, a permeable and snugly fitting elevated bottom is created to fit in an aquarium with a drilled drain hole in the tank's glass bottom. This allows for the liberal use of water in the system and also facilitates the use of advanced filtration. Tanks with false bottoms can also be decorated with naturalistic backdrops of expanding foam insulation sealed with silicone sealant (which is inert when cured) and natural long-lasting substrates such as coir (coconut fiber), peat moss, sphagnum, mulch, or gravel. Detailed instruction on enclosure setup (false bottoms, glass drilling, etc.) can be found on the internet (www.amphibiancare.com or the AZA and EAZA Amphibian Husbandry Resources at www.aza.org).

Water Sources

Amphibians are so closely tied to water that water sources, availability, and quality are of utmost importance in the ex situ environment. Amphibian enclosures benefit from the addition of water features such as pools, waterfalls, streams, and drip walls that support live plants. These enhance the appearance of exhibits, help to increase humidity, and often encourage natural breeding behavior. Water features can be open systems that constantly introduce fresh water or closed systems in which water is retained. They may employ some type of recirculation with or without filtration. Closed systems with low biomass, where water circulation is more for aesthetic purposes than for the benefit of the animals, may not require filtration.

Substrates used in terrestrial amphibian enclosures must facilitate drainage that is adequate for the water features used. If a substrate lacks proper drainage it will become stagnant, resulting in accumulation of waste products (ammonia) and bacterial overgrowth which can lead to a toxic environment for amphibians. Stagnant substrates can also result in parasite infestation. Mixes of coco husk chips, haydite (heat expanded shale, slate, or clay) or other lightweight aggregate and activated charcoal provide a safe substrate that provides good drainage, breaks down slowly, provides for good aeration, and retains moisture. Other substrates include peat moss, sheet moss, pebbles, gravel, and potting soil. The use of perlite (a lightweight heat expanded volcanic glass) in a substrate mix is not recommended, since it is somewhat sharp and can also be high in fluoride. Amphibians occasionally ingest some substrate when eating, so keepers should observe the animals in their care to see whether the substrate poses a threat. If potting soil is used, it should not have perlite or added fertilizers. Long-lasting and well-drained substrates meet the needs of the animals and also support plant growth. Water can be obtained from natural sources such as wells, springs, or rainfall; however, natural water sources can be contaminated both chemically and biologically. Deep wells are often oxygen-deprived, and cold water sources can be supersaturated with other gases. Well water is usually high in minerals and metals and can often have some unwanted salinity. Rainwater is naturally soft, but it can have variable pH. Rainwater is also prone to environmental contamination from pollutants and runoff (e.g., from a metal roof). Further, none of these natural water sources meet biosecurity requirements, because they can be contaminated with bacteria, viruses, fungal spores, or parasites.

Bottled water has also been suggested for captive amphibians. The quality of bottled water is uncertain, since monitoring requirements are not as stringent as are those for municipal tap water. In many Western countries, bottled water is seldom of higher quality than tap water. Further, all plastic bottles leach synthetic chemicals and their effects on amphibians are uncertain.

Reverse osmosis (RO) filtration systems yield nearly pure water by forcing water through a semipermeable membrane. Clean water passes through the pores in the membrane and the impurities are left behind. However, RO removes not only harmful components but also the beneficial, naturally occurring minerals in water. Therefore, RO water must be reconstituted (important minerals must be added back) prior to use in amphibian husbandry. Products for reconstituting RO water are commercially available from aquarium supply companies. A recipe for reconstituting RO water is available in the Amphibian Husbandry Resource Guide from the AZ A website (see AZA and EAZA amphibian husbandry resources). However, RO systems are relatively expensive and also waste a large quantity of water. Most systems generate approximately two to three liters of waste water for every liter of RO water they produce. In areas where the only water available is of very poor quality, RO may be the only option available.

Municipal water is generally highly regulated, monitored, and safe, but variable in composition. The disinfectants used—chlorine and/or chloramines (a combination of chlorine and ammonia), which are both toxic to amphibians— should be removed from municipal water before it is used in amphibian husbandry. Chlorine is volatile and will dissipate if water is vigorously aerated or "aged" (left to stand in an open container for 24 hours or more to allow the chlorine to dissipate). Aeration and aging also de-gas municipal water, which is often supersaturated with gases (N, CO_2, O_2), but they do not remove chloramines. Filtration with activated charcoal and chemical treatment with inorganic reducing agents, such as sodium thiosulfate, are both effective in removing chlorine and chloramines. However, when chloramines are removed with either of these methods, toxic ammonia is released. Thus, thiosulfate or carbon filtration alone is not adequate for eliminating toxicity from chloramines, and ammonia must also be removed using a chemical binding agent, a de-ionizing resin filter, or biological filtration. Treated municipal water appears to be the best option for use in *ex situ* amphibian care, given biosecurity concerns surrounding amphibians in *ex situ* conservation programs.

Lighting

The proper quality and quantity of light will help meet the physiological requirements of animals and will promote natural behavior, promote proper thermoregulation, facilitate plant growth, and improve the aesthetics of exhibits. Proper lighting also provides ultraviolet (UV) radiation that facilitates vitamin D synthesis. Many amphibians benefit from exposure to controlled safe levels of UV light—especially after metamorphosis, when their bone growth and development is increased. UV light can be provided by using commercially available fluorescent blacklight lamps. Blacklight lamps differ from standard fluorescent lamps only in the composition of the phosphor which radiates most of its energy in the near ultraviolet region, peaking at about 350 nanometers. These lamps are safe to use in conjunction with regular lighting over all amphibians. Black lights are effective if placed within 50 cm (20 in.) of the animals. At greater distances, using timed exposure of higher UV output from self-ballasted mercury vapor lamps, such as those manufactured by Westron of Canada Inc. (Dorval, QC, Canada H9P1H2; Westron Lighting Corp., Oceanside, NY 11572-5829) or modified Eiko brand halogen bulbs (Eiko Ltd., Shawnee, KS 66227) is effective (see the AZA Amphibian Husbandry Resource Guide). The use of UV meters is recommended to verify UV levels and required exposure time. Keepers can also refer to Gehrmann et al. (2004) for details on the use of UV light in animal husbandry. See AZA and EAZA amphibian husbandry resources and follow manufacturers' guidelines for instructions and exposure precautions.

Tools and Equipment

Water Quality Parameters

Creating and maintaining a healthy aquatic environment for amphibians requires the keeper to employ the same monitoring and maintenance procedures used in fish husbandry to ensure adequate filtration that provides a safe and stable environment. The equipment, filtration, and life support principles are the same for larval and adult aquatic amphibians as they are for freshwater fish.

A number of water quality parameters should be considered when developing a husbandry plan for amphibian species. Where data exists for water quality in situ, it should be used as a guideline for water quality parameters in captivity. These parameters can be measured regularly, using specialized meters or commercially available color-changing (colorimetric) aquarium test kits that are relatively simple, inexpensive, and effective.

Hardness
Water hardness measures the presence of dissolved minerals, specifically calcium (Ca) and magnesium (Mg), in water. Hardness can be measured with simple inexpensive aquarium testing kits. Adjustments can be made by adding Ca and Mg salts to increase hardness, and by adding RO, distilled or deionized water to lower hardness. Hardness can be quantified as soft (o to less than 60 parts per million (ppm), medium hard (60 to 120 ppm), hard (120 to 180 ppm), or very hard (more than 180 ppm).

PH
Alkalinity and acidity are measured as pH. Pure water has a neutral pH of 7. The acceptable pH range for most amphibians is between 6 and 8. Some amphibian species are found in ponds and in water with high organic content, which lowers pH, and these would likely benefit from slightly acidic water (pH 6-7). Adjusting pH can be accomplished by adding buffers or tannins such as almond leaves (*Terminalia catappa*), aquarium peat, or commercially available extract solutions.

Ammonia
All living creatures give off nitrogenous waste (ammonia) produced by protein metabolism. Ammonia is toxic and must be removed from aquatic systems. Fortunately, a natural process in which biological conversion of ammonia into relatively harmless nitrogen compounds can be used to do this (fig. 34.3). Several species of bacteria convert ammonia (NH_3) to nitrite (NO_2), while others convert nitrite to nitrate (NO_3) which is relatively nontoxic. Since ammonia is highly toxic, concentrations of it should be kept below 0.01 milligrams per liter (mg/L).

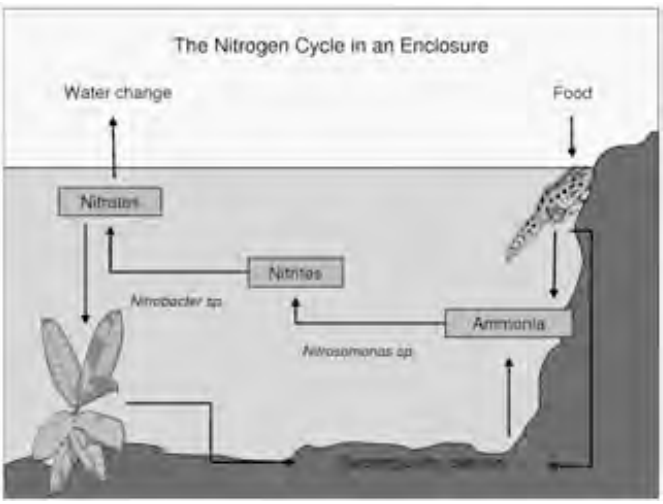

Figure 34.3. The nitrogen cycle in an amphibian enclosure is the bacterial processing of toxic metabolic waste (ammonia) produced by animals into relatively nontoxic compounds (nitrates). Courtesy of A. Lentini.

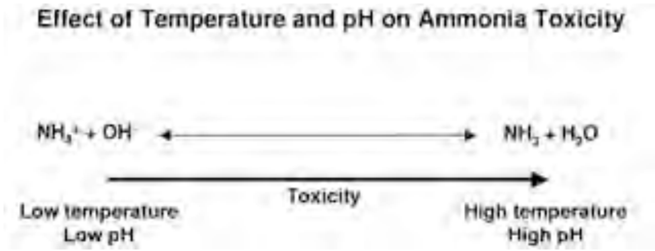

Fig 34.4. An illustration of the relationship between ammonia toxicity, pH, and temperature. Courtesy of A. Lentini. good water quality; the plants use wastes as nutrients and contribute oxygen during photosynthesis.

Ammonia above 0.01 mg/L requires immediate correction. Ammonia should be tested following any suspicious mortality. Nitrites are slightly less toxic; concentrations should be less than 0.1 mg/L. Nitrates are the least toxic, and concentrations below lomg/L are safe. Nitrifying bacteria are present everywhere and once ammonia appears in a system, the desired bacteria will establish a colony in the filter media and substrate; this is called biofiltration.

It is important to be aware of the relationship between ammonia toxicity, pH and temperature (fig. 34.4). Ammonia (NH_3) is much more toxic than the ammonium ion (NH_4+). The relationship between ammonia, ammonium, pH and temperature is described in figure 34.4. At a higher temperature and pH, more of the nitrogen is in the form of toxic ammonia than at lower temperature and pH. It is therefore preferable to maintain pH at or slightly below neutral (pH = 7) for most species.

A keeper's goal is to start with clean water and keep it clean by not overfeeding or overcrowding an enclosure. When animals are kept at high densities, the biomass in the enclosure is often too high for any filtration system to cope with the amount of ammonia present. Water quality should be monitored using appropriate test kits or meters, and regular water changes should be scheduled to maintain water quality by preventing nitrate and organic waste accumulation. Regular cleaning of filters (since they do not eliminate detritus but merely trap it) and use of live plants will also help maintain good water quality; the plants use wastes as nutrients and contribute oxygen during photosynthesis.

Feeding and Nutrition
Nutritional Requirements
As ectotherms, amphibians obtain the energy required to raise body temperature from their environment and therefore require less food energy than similar-sized birds and mammals, which are endothermic. This allows amphibians to exploit environments of low biological productivity, and it means that they require less food in captivity. The daily energy requirements of an amphibian, calculated on the basis of a standardized metabolic rate at 25° C, indicate that a typical 40 g frog requires 1.25 kcal per day. Domestic crickets have 1.9 kcal of energy per gram; therefore, a 40 g frog

needs approximately 4 g of crickets per week (only two large crickets per day). Overfeeding and obesity are common in captive animals, and weights of individuals should be monitored and recorded regularly (at least four times per year) so that intake can be adjusted as necessary. Metabolic rate is temperature-dependent. For every 10 °C drop in temperature, there is a corresponding 50% drop in the standard metabolic rate of an ectothermic animal (Wells 2007). Therefore, amphibians kept at lower temperatures need less food. Bear in mind that if an amphibian is kept too cold, it may not be able to digest properly, resulting in putrification of food in its gastrointestinal tract. The amount of food offered should be controlled, since many amphibians will overeat when food is abundant, resulting in gastric distention, which can be fatal.

Amphibians employ diverse feeding strategies, as both herbivores and carnivores, and their preferred diet varies according to life stage. Aquatic amphibians (adult and larval) will accept prepared diets (gels, pellets, tablets, etc.); however, most terrestrial species require live prey. Amphibians respond to moving prey because their neural processing and brains are "hardwired" to detect motion, which will elicit a feeding response. Some species (tree frogs, aquatic frogs, and salamanders) can be trained to tong feed; however, most will require live prey. Commercially available live prey includes mealworms (*Tenebrio molitor*), gray crickets (*Acheta domesticus*), wax worms (*Galleria mellonella*), cockroaches (usually *Blaberus craniiferus*), wingless fruit flies (*Drosophila melanogaster* and *D. hydeii*), and earthworms (*Lumbricus terrestris*). These and other invertebrate species can be cultured by the amphibian keeper. In order to maintain a reliable supply of appropriately sized (generally no longer than the width of the amphibians head) live foods for the animals in their care, amphibian keepers should be familiar with invertebrate culturing techniques. Amphibians possess a digestive enzyme (chitinase) in the stomach and pancreas that assists in digestion of the chitin found in the exoskeletons of many insects (Oshima *et al.,* 2002). However, care should be taken regarding impaction and perforation when feeding prey with heavy exoskeletons or highly chitinous parts. For example, the large ovipositor of adult female crickets or the spurs on the legs of certain species of cockroach can puncture the gastric wall, and mealworms can cause impaction in certain species that may not commonly feed on such chitinous prey. Also, wax worms are somewhat high in fat and can lead to obesity if fed in excess.

Supplementation

Many invertebrate prey species are generally deficient in vitamins and have a low calcium-to - phosphorous ratio. For this reason, vitamin and mineral supplementation is beneficial. Gut loading (feeding prey species vitaminand mineral-rich foods) and dusting with powdered supplements are methods commonly used to improve the nutritional value of invertebrate prey. However, externally applied supplements wear off (i.e. crickets quickly groom themselves and shed any adhering supplement) and nutrients in the gut are transient. In order to prevent captive amphibians from feeding on nutrient-poor prey, any uneaten food items should be removed from the enclosure the next day. Extending the periodicity of feeding (once or twice a week) ensures that hungry amphibians will quickly consume the supplemented food. Ideally, the food should be consumed within one to two hours of being offered. For amphibians that can be trained to accept food from tongs, this method of feeding also ensures that food items are consumed with supplements. Tongs used for tong feeding should be blunt-ended to prevent injury to the animals, which often will lunge at the presented food item and grasp it and the tongs at the same time.

Behaviour

Amphibians engage in a variety of interesting and often unique behaviors that enable them to go about the daily business of survival. They feed, find mates and reproduce, protect their young, defend territories, and avoid predators. Some of the behavior is geared towards maintaining water balance, thermoregulation, and communication.

Amphibians use behavioral adaptations to maintain their body temperature within acceptable limits (thermoregulate) and to maintain their water balance (hydroregulate). Amphibians are highly susceptible to desiccation because of their thin and highly permeable skin. These animals have been shown to alter their habitat selection and daily movements in response to changes in humidity. Hydroregulation involves moving from areas of different water availability, humidity, and air currents and altering body posture and activity levels, thereby selecting microhabitats in which conditions are more favorable. The keeper must therefore provide an ex situ environment that allows amphibians to display the full array of behaviors associated with homeostasis. Such environments can employ sloped habitats that go from a cool aquatic to a warm terrestrial area within the same enclosure.

Amphibians use chemical, acoustic, tactile, and visual methods of communication. Many of the tailed amphibians possess mental glands (the glandular patches under the chins of some salamanders) and use pheromones and olfaction to communicate. Frogs and toads were the first vertebrates to possess vocal chords, and the anurans rely primarily on vocalization to attract mates, announce territory, or defend themselves when frightened. Visual signaling using the limbs or body (semaphoring behavior) is employed by several amphibians. These use their feet, legs, and bodies as "flags" to signal to other animals; this semaphoring behavior is most common in species found in environments where sound does not carry well. As defense, this behavior is often known as the "unken reflex" and is characterized by reverse flexing of the body to display warning colors of the ventral surface of the limbs and body.

Operant conditioning is not common; however, some tailed amphibians (salamanders and newts) and anurans have been target trained for food rewards. Varying the substrates, basking sites, prey species, feeding stations, and feeding schedules can serve to enrich the *ex situ* environment for amphibians.

Many amphibians will exhibit territoriality in defense of limited resources such as food, shelter, and reproductive (calling and egg laying) sites. Most aggression is associated with courtship, and vocalization by male anurans is a major component of this. Aggression associated with parental care (defense of eggs or larvae) is common in some frogs and salamanders. Male plethodontid salamanders may engage in biting, chasing, and aggressive displays such as open-mouth threats when placed in close quarters. Aggressive territorial behavior can be avoided by not overcrowding and limiting the number of males in a group, and by providing visual barriers and multiple retreat sites within an enclosure.

Transporation

Cooperative management requires animals to move between institutions, and transportation methods for amphibians are designed to reduce stress, reduce time in transit, and reduce exposure to adverse conditions (temperature extremes and desiccation). When arranging a shipment of amphibians, the keeper should anticipate problems that might delay the shipment or expose the animals to unfavorable conditions. Potential problems include weather or traffic delays. When shipping by air, keepers should avoid the last flight of the day, multiple connecting flights, and weekend flights, since problems can result in lengthy delays.

Following IATA (International Air Transport Association) guidelines, clearly labeled, rigid-sided, insulated shipping boxes should be used. A polystyrene foam inner box placed in a cardboard outer box or insulated wooden crate can be used. Crumpled newspaper or polystyrene foam packing

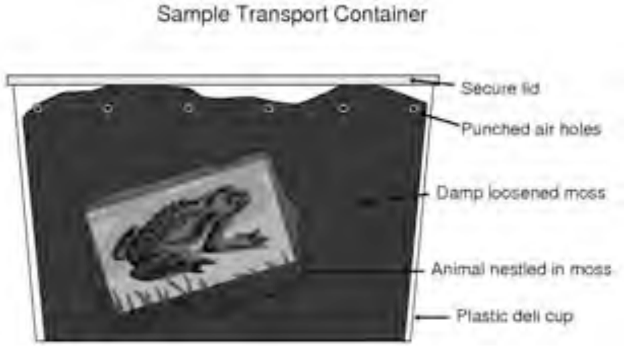

Fig 34.5. An example of an amphibian transit container that provides adequate moisture and a secure shock-absorbing environment for the animal. Courtesy of A. Lentini.

chips should be used around the containers housing the animals. This will prevent jarring and support the containers in the box during transit. Most species should not be shipped if they will be exposed to extremely hot or cold temperatures (above 30 °C or below 8 °C). To protect animals from extremes in temperature, heat or cold packs may be useful.

Containers used for housing amphibians during transit should provide adequate moisture and a secure shockabsorbing environment for the animals. Clear plastic containers (such as Glad-ware or Rubbermaid) work very well since they allow for easy inspection. New or thoroughly washed and rinsed delicatessen or margarine containers also work well (fig. 34.5). Ensure that lids are secure by tying them down with cable ties (encircling the entire container) or closing them with strong and durable adhesive tape (e.g., duct tape or electrical tape). All containers should be clearly labeled with species name and number of animals. Ventilation holes (1/8 to 1/4 in. in diameter) should be punched or drilled in the sides and tops of the plastic container. Be sure to drill from the inside out to prevent rough edges inside the container that could injure the animal.

Each animal container should be filled with an "airy" and damp substrate in which the animal can nestle comfortably. Acceptable packing substrates include slightly dampened sphagnum or sheet moss that has been teased or pulled apart to create air spaces, dampened paper towel, dampened sponge pieces, and chips (aquarium filter sponges work very well and are known to be safe and nontoxic). Whichever substrate is used, it should not be saturated with water, since the weight of saturated substrate can crush, trap, or drown small animals.

Aquatic species can be packed in the same manner as fish. Keepers can use standard plastic fish bags. To prevent small animals from getting trapped in the bottom corners, one can use square-bottomed bags with their corners taped down, or bags with rounded corners. Each bag should be filled one-third to one-half full with clean dechlorinated water. For trips longer than 18 hours, oxygen should be added to the bag before sealing. Products that absorb ammonia, like Poly-filter, (Poly-Bio-Marine, Reading, PA) can also be added to the water for long trips. A keeper should always double-bag an animal, since a leak can result in its death. Further, since airlines will refuse to carry any container that is wet, a shipping container should be lined with a plastic bag.

Reproduction

Some of the more fascinating aspects of amphibian biology are the variations on the typical life history model of egg to-tadpole-to-metamorphosis. Some species have evolved life histories that tend towards parental care and direct development without an aquatic tadpole stage. These include terrestrial nesting species such as the eyelash leaf frog (*Ceratobatrachus guenther*i), in which the tadpole develops within the egg and the metamorphosed adult form emerges from it without ever having gone through a free-swimming larval stage. Other variations include species in which either the male or female carries the eggs for a period of time and either deposits them when they are ready to hatch (e.g., the midwife toad, *Alytes obstetricans*) or carries them until the larvae have fully metamorphosed and emerge as the adult form (Surinam toad, *Pipa pipa*).

Amphibians are sexually dimorphic to varying extents. Keepers can determine the sex of an individual by examining its size and color, and looking for the presence of secondary sex characteristics. A key secondary sexual characteristic in anurans and some newts and salamanders (caudates) is the nuptial pads. These are raised mucus glands located at the base of the thumb and forearm on males. Nuptial pads are used to grasp females during amplexus. Amplexus (from the Latin "embrace") is the copulatory embrace of amphibians in which a male grasps a female with his front legs as part of the mating process. In the anurans throat color can differ between the sexes, with males having a darker and looser skin around the throat. This reflects the fact that generally only males produce mating and release calls. The external ear drum (tympanum) is also larger in the males of some species of anurans. In the caudates, males will often have much larger crests and more glandular or enlarged cloacae. Female amphibians generally appear to be larger and rounder as a result of the eggs they carry, particularly when they are carrying eggs (gravid).

Prior to breeding, some form of environmental cycling may be essential for gamete (egg/ova) maturation. Seasonal changes for most amphibians involve either brumation or aestivation. Brumation, a state of torpor in response to cooler environmental conditions, is similar to hibernation, in which endothermic vertebrates lower their body temperature and pass the winter in a dormant or torpid state. Brumation in amphibians involves a period of reduced metabolism and food intake in response to temperatures that are too cold to allow for normal activity. Keepers can use incubators, refrigerators, or wine refrigerators to replicate the cold temperatures that temperate amphibians would experience in the wild. It is important to ensure that sufficient water or moisture is available to prevent desiccation in a cooler environment. Temperate species can be cooled to 6 °C to 8 °C (43 °F to 46 °F) for 4 to 12 weeks. Although tropical species do not normally brumate, a brief two to three-week cooling period with temperatures ranging from 16 °C to 20 °C (61 °F to 68 °F) may be beneficial for successful breeding. Aestivation is a similar period of inactivity, seen in some species from xeric environments, that allows animals to conserve energy and moisture when their supply of food and water is low.

The danger of fatal desiccation can be mitigated by having a water dish always available while allowing the substrate to dry out. In order to reduce these

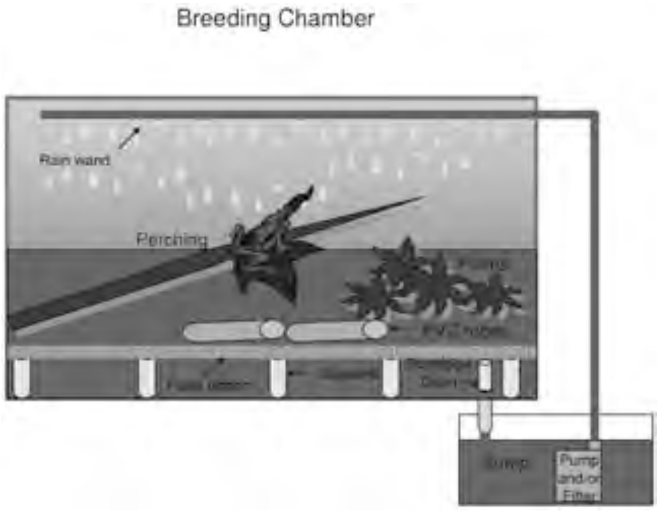

Fig 34.6. A commonly used breeding enclosure, the "rain chamber," uses a pump to recirculate water through an enclosure to simulate a rainy environment. Courtesy of A. Lentini.

animals' metabolic rates, temperatures should be kept 4 °C to 6 °C (39 °F to 43 °F) cooler than during the active season. In captivity, brumation and aestivation may be triggered by reduced lighting, temperature, humidity, and food availability. Prior to inducing brumation or aestivation, keepers should stop feeding two weeks in advance and perform a health and weight check to confirm that animals are of breeding age and in good condition. A breeding plan that specifies the desired temperature profile, photoperiod, humidity, and duration should also be prepared. Changes in temperature and humidity should be made slowly over a period of several days or weeks.

Following a period of inactivity, external environmental cues such as increased temperature and extended day length stimulate hormone secretion by the hypothalamus, pituitary, and gonads in amphibians. In females this triggers gonad maturation and spawning behavior. In males it results in activation of the testes and release of sperm into the urine, and stimulates both calling and amplexus. In captivity, the use of recorded calls, rain chambers, and increased humidity and temperature can provide the cues to initiate reproduction. However, cycling captive amphibians at lower temperatures does carry some risk. As mentioned earlier, desiccation can be fatal. Further, at low temperatures the immune system can be compromised, resulting in infections and parasite infestations. Artificial hormone techniques can also be used to stimulate reproductive behavior. Assisted reproduction using hormones is an effective method of inducing or coordinating reproduction without the need to cycle some animals. The use of hormones will not be effective if the animals are not in breeding condition. If animals are in poor condition—for example, if females do not have sufficient energy stores to produce eggs—hormone injections will not be effective.

Animals selected for breeding can be left in an existing enclosure if that environment is suitable for mating and egg laying. Often, however, it is more effective to move the selected animals to a special breeding enclosure. A commonly used breeding enclosure is the "rain chamber" (fig. 34.6). This setup involves the use of a misting or watering system that replicates a rainy environment. Misting and watering systems generally recycle water through filtration in order to ensure that the water is of a suitable quality and temperature. For terrestrial and semiaquatic species, the water level in a rain chamber can be 10 to 20 cm deep. Keepers should provide small "islands" of floating vegetation and perching that extends above the water line so that the animals can rest. Other cage furniture, such as plastic plants or PVC tubes that serve as egg deposition sites, should also be provided. To augment the environmental stimuli provided to anurans, keepers should also provide auditory cues. This can be accomplished by playing a recording of males calling in order to simulate the breeding choruses that are heard in the wild.

Mating

Amphibian species employ a variety of strategies to accomplish successful mating and fertilization. Most anurans typically use a method of external fertilization in which the male clasps the female with the male dorsal to the female, aligns his cloaca with hers, and simultaneously releases sperm as the female deposits her eggs. This clasping posture and behavior is known as amplexus. Referred to as "amplectant positions," a variety of amplexus postures are used by different species. The typical amplectant position is the axillary clasp (e.g., in the American toad, *Anaxyrus* [*Bufo*] *americanus*), in which the male places his forelimbs under the female's so that their cloacae are next to each other (in juxtaposition). Another amplectant position is the inguinal clasp (e.g., in the Surinam toad, *Pipa pipa*), in which the male places his forelimbs around the female's hind legs and groin. Yet another amplectant position is the cephalic clasp, in which the male grasps the female about the head. In the caudates, internal fertilization occurs when the male attaches a spermatophore (a packet of sperm) to substrate, twigs, branches, or stones in the water or on land, and the female then pulls them into her cloaca. Courtship leading up to fertilization can take the form of amplexus or "liebeespiel" (love play), in which both sexes engage in nudging and bumping, guided by olfactory and visual cues. Caecilian breeding has not been described in detail, but it likely includes olfactory and tactile communication. Caecilians are the only order of amphibians to rely entirely on internal fertilization. The male inserts the penis-like phallodeum into the cloaca of the female for several minutes or hours to inseminate the female.

Eggs are usually deposited singly or in masses, and can be laid in water, in ground nests, or attached to vegetation. Eggs laid in water are often free-floating or attached to submerged vegetation, and are protected by several mucus layers. The eggs vary in size and color with different species. They can be dark or pale. They can be laid in long stands (toads' eggs look like a bead necklace) or in a jellylike mass (some frogs lay eggs in a floating mass, and some salamander eggs form firm gelatinous balls). Structures such as plastic aquarium plants, live plants, branches, or stones provide egg deposition sites in the captive environment. When eggs are present, it is important not to disturb them. Some frog and caecilian species protect their eggs and will aggressively defend them. Filters and return water should be moved away from any eggs, and filter intakes should be covered with fine netting (panty hose work well) or sponges to avoid damaging the eggs. Covering the filters in this way also protects tadpoles from being trapped inside them. Once the tadpoles have hatched, keepers should delay feeding until the yolk has been consumed (usually takes 24 hours after hatching) which coincides with free swimming behavior; prior to this the tadpoles typically rest motionless, clinging to either the sides or the bottom of the tank Larval rearing involves close attention to water quality parameters and correct feeding. Tadpoles engage in a variety of feeding strategies. They can be opportunistic omnivores, herbivores, carnivores (in the case of most of the caudates), cannibals, and even filter feeders.

For herbivorous tadpoles, tank algae that consist mostly of blue-green algae (*Leptolyngbya cf. boryana*) and diatoms (green-brown algae) have been found to provide good nutrition. Algae should be cultured for several weeks prior to a planned breeding to provide an adequate source of food for herbivorous tadpoles. Tank algae can also be harvested and stored frozen until needed. The use of algae from ponds or other non-biosecure sources should be avoided, in order to avoid possible pathogen introduction. Alternatively, fresh high-quality commercial fish foods such as Sera-micron,® Tetramin,® Sera-san Color and Growth Enhancing flakes,® Tetra 4 in 1FD menu,® aquaria herbivore diet, and spirulina flakes can also be used. For grazing tadpoles, mixed flakes can be moistened with a few drops of water to form a firm paste that can be dropped into the water for the tadpoles to feed on. Alternatively, by adding more water to a flake mixture, keepers can create a slurry that can be "painted" onto clean glass microscope slides and allowed to dry. The coated slides can then be placed in the water for tadpoles to graze on. Tablet fish foods can also be used. Frozen or heated (either microwaved or blanched, to rupture the plant cells) spinach and lettuce can be fed to supplement the diet. For filter feeders, flake foods can be finely ground and sprinkled on the water for the tadpoles.

Some anuran tadpoles and all larval salamanders and caecilians are carnivorous and will benefit from live foods such as microworms, daphnia, brine shrimp (hatchlings and adults), whiteworms, and tubifex worms. "Survival of the fittest" applies to most carnivorous tadpoles and larval amphibians since they become cannibalistic, consuming any smaller siblings they can overpower. Dependent on program goals, in cases were only a few individual animals are to be raised from a large clutch, cannibalism can be allowed to proceed and the more robust animals will survive. Where the goal is to raise as many of the young as possible, larval amphibians should be reared individually to prevent cannibalism.

As tadpoles of terrestrial species approach metamorphosis (when one or both of the front limbs have appeared) they should be moved to a rearing tank to prevent drowning. Rearing tanks are set up so that they are sloped with shallow water at one end and easy access to a nonslip land area at the other. There should be plenty of cover at the water-land interface to allow metamorphs to comfortably rest at this point until they are ready to move to land. Metamorphs are quite susceptible to desiccation because of their small size. Even when water is available, they are driven by instinct to leave the natal pond. Therefore, tanks should be securely covered and ventilation should be controlled to maintain high humidity (80-90%).

Veterinary Care

New animal quarantine for amphibians is vital in light of new emerging diseases (e.g., chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis*) in this group of animals, which require high biosecurity standards. By adhering to sound quarantine procedures, keepers can decrease the risk of introducing disease to resident animals and also protect newly arrived animals from acquiring disease from resident animals. This is of particular importance when zoo animals may be used for reintroduction programs. The quarantine period also allows for adaptive management of individuals and diet adjustments, thus allowing the keepers and the animals to get used to each other. Keepers should maintain a quarantine area, with tools dedicated to each enclosure within that area. Proper disinfection procedures for tools and cages, hand washing, and the use of disposable gloves between quarantine enclosures are all essential and should be mandatory. Disinfection using a 1% sodium hypochlorite solution with a contact time of 10 minutes is sufficient to disinfect hard surfaces. Household chlorine bleach ranges from 3% to 5% sodium hypochlorite, and commercially available bulk solutions can be of much higher concentration (up to 12%). It is therefore vital to confirm the concentration on the container label or with the manufacturer or supplier before preparing a dilute working solution. After disinfection, rinse everything thoroughly with hot tap water and leave to air-dry. Porous materials such as bark or driftwood are best replaced if excessively soiled. Iodine toxicity has been seen in amphibians from iodine reversibly bound to plastic holding containers. Because of this potential binding with plastics, it is recommended that iodine not be used to disinfect any material that will come in contact with the amphibians. Other disinfectants are not recommended for use on amphibian tanks or furniture. Care should be taken not to expose animals to chlorine vapors present when concentrated solutions are used for cleaning and disinfection.

The quarantine period should last at least 30 days. However, based on the incubation period of certain diseases, a longer period (60 days) is preferable. Ideally, an "all in/all out" system, where no new animals are introduced until the quarantine period for the first group has ended, is best. During quarantine, temperatures in the range of 18 to 25 °C are safe for most species. Temperatures should be at the cooler end of this range for temperate or montane species, and at the higher end for tropical species. Appetite is one of best indicators of health, and keepers should weigh all animals upon arrival and periodically throughout the quarantine period in order to ascertain whether they are feeding and maintaining their weight. Keepers should also be observing the animals daily and taking note of any obvious changes in their physical appearance or in activity level or posture that may suggest illness. Fecal samples should be submitted to a veterinary laboratory for parasite checks. Animals should not be allowed to clear quarantine until three negative fecals or two negative post-treatment fecals have been produced.

Keepers should always look for the common signs of illness in amphibians under their care. These include loss of appetite, changes in skin texture or color, changes in behavior or posture, reduced righting reflexes, bloating, and change in eye clarity. Diseases of special concern include viral, bacterial, and fungal infections. New emerging diseases (ranaviruses, chytridiomycosis) have been associated with population declines and have been diagnosed in zoo collections. Some disease-causing parasites, such as nematodes, have life cycles that are completed in the soil or water. Regular substrate changes and air drying of the exhibit and props after regular disinfection is often all that is required to prevent reinfestation or transmission of parasites from one animal to another. A schedule for periodic substrate changes and enclosure disinfection should be established and followed. When animals are being treated, it is beneficial to have an identical clean and simple tank already set up, so that the animals can be transferred to a fresh tank once a week. This prevents the buildup of parasite loads and reduces the chance of reinfestation following treatment. Wild-caught amphibians often have heavy loads of parasites (e.g., nematodes such as *Rhabdias ranae*). Antiparasitic drug therapy (e.g., Fenbendazole and Ivermectin) accompanied by tank changes can take several weeks to successfully eliminate the parasite infestation. Due to the potential toxicity of some antiparasitic drugs, dosing should be prescribed by a veterinarian familiar with their use in amphibians.

Conservation and Research
Amphibian declines and extinctions are well documented. The International Union for Conservation of Nature (IUCN) Global Amphibian Assessment of 2004 has found that 42 % of all amphibian species are declining in population, and 32 % of the world's amphibian species are threatened with extinction. These declines are a result of emerging infectious diseases such as chytridiomycosis, toxins, climate change, land use change, unsustainable collection and trade in amphibians, and the introduction of exotic species. Professionally managed zoological institutions play a role in conservation through public education, scientific research, professional training and support of in situ conservation projects (Hutchins *et al.* 1995). Furthermore, zoos are in a position to preserve threatened species in captivity over long periods, thus providing a reservoir of genetic and demographic material that can be used periodically to reinforce, revitalize, or reestablish populations in the wild.

Keepers can participate in amphibian research utilizing the species they care for. Research involving ex situ populations can be used to guide and develop recovery actions and adaptive management strategies. Ex situ populations can also aid in the development of technologies and research techniques that will benefit wild populations. Moreover, databases from ex situ populations can complement research conducted on wild populations. We know little of the detailed biology of some of the many amphibians in zoos. Keepers can contribute to our knowledge by conducting research into the life history, behavior, and biology of the animals they care for. By sharing their experiences and findings with zoo visitors, colleagues, and the scientific community, keepers are able to fulfill their roles as educators as well.

Many zoos actively participate in "rescue" projects in which threatened populations of amphibians are brought into captivity in the face of a catastrophic loss in the range country. Habitat loss and fragmentation remains the primary cause of amphibian declines. The hope is that these rescued species can be returned to the wild once the threats to their survival have been mitigated. This requires maintaining them in biosecure facilities to ensure that they can be used in future reintroductions. Ex situ conservation and management of threatened amphibian species in zoos is one component of a worldwide coordinated amphibian conservation response to the global decline of amphibians. The recommendation for an ex situ population of certain threatened amphibian species can come from a number of recognized sources such as the Global Amphibian Assessment (www.globalamphibians .org), the IUCN Red List (the IUCN Technical Guidelines for the Management of Ex Situ Populations recommend ex situ populations for all critically endangered species), local, regional or national government requests. A successful amphibian conservation program requires institutional support and an advocate to speak on behalf of the species. Keepers can fill this role and champion the species they work with.

In situ conservation activities, with the participation of in-country local partners, are also essential for an effective amphibian conservation program. With local involvement, zoos can contribute to in situ capacity building, so that rescued population can be maintained in country. Keepers can participate in in situ conservation by traveling to rescue centers and sharing their expertise with local conservationists. Keepers have also been instrumental in fund-raising to direct needed resources to in-country projects. This type of support is extremely beneficial to the long-term success of amphibian conservation.

Summary
Amphibians are a group of animals whose life history is intricately associated with water. These fascinating animals come in a variety of forms, from completely aquatic to terrestrial. Some amphibians are among the most vibrantly colored of animals and make beautiful exhibit species. The largest can weigh over 40 kg, and the smallest just a few grams. Amphibians are of high conservation value, since almost one-third of species are threatened with extinction. The specialized needs of this diverse group of increasingly popular animals makes keeping them a challenge, requiring knowledgeable keepers who can create the often complex captive environments that will ensure their well-being.

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