Facility Design and Associated Services for the Study of Amphibians

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Abstract

The role of facilities and associated services for amphibians has recently undergone diversification. Amphibians traditionally used as research models adjust well to captivity and thrive with established husbandry techniques. However, it is now necessary to maintain hundreds of novel amphibian species in captive breeding, conservation research, and biomedical research programs. These diverse species have a very wide range of husbandry requirements, and in many cases the ultimate survival of threatened species will depend on captive populations. Two critical factors have emerged in the maintenance of amphibians, stringent quarantine and high-quality water. Because exotic diseases such as chytridiomycosis have devastated both natural and captive populations of amphibians, facilities must provide stringent quarantine. The provision of high-quality water is also essential to maintain amphibian health and condition due to the intimate physiological relationship of amphibians to their aquatic environment. Fortunately, novel technologies backed by recent advances in the scientific knowledge of amphibian biology and disease management are available to overcome these challenges. For example, automation can increase the reliability of quarantine and maintain water quality, with a corresponding decrease in handling and the associated disease-transfer risk. It is essential to build facilities with appropriate nontoxic waterproof materials and to provide quarantined amphibian rooms for each population. Other spaces and services include live feed rooms, quarantine stations, isolation rooms, laboratory space, technical support systems, reliable energy and water supplies, high-quality feed, and security. Good husbandry techniques must include reliable and species-specific management by trained staff members who receive support from the administration. It is possible to improve husbandry techniques for many species by sharing knowledge through common information systems. Overall, good facility design corresponds to the efficient use of space, personnel, energy, materials, and other resources.

Key Words: amphibian; captive breeding; facilities; husbandry; quarantine; research; UVB; water quality

General Design

Facility layout depends on the number and variety of species to be housed, their environmental requirements, the number and distribution of enclosures, the regional climate, and the purpose of the facility. The environmental range of species and their climatic requirements determine the number and design of temperature-controlled rooms. The distribution of amphibians between enclosures determines the number of enclosures and racks. Within this system, personnel isolate amphibians by single species or species assemblage (an amphibian faunal group that naturally occurs in the range country), optimum temperatures, life stage, or other factors. The regional climate affects facility layout, and climate extremes especially limit the dispersal of structures within the facility. For information about amphibians that is beyond the scope of this article we recommend that readers consult the following literature: for *General Husbandry*: Gresens (2004), Halliday (1999), Mattison (1987), Nace et al. (1974), O'Reilly (1996), Pough (1992), Reed (2005), Schimdt and Henkel (2004), Schultz and Douglas (2003), Wake (1994), Wright and Whitaker (2001), and Zippel (2005); *Biology*: Duellman and Trueb (1994), Feder and Burggren (1992), Frazer (1976), Hofrichter (2000), Stebbins and Cohen (1995); and *Larval Biology and Larval Rearing*: Browne et al. (2003), and McDiarmid and Altig (1999).

Quarantine is critically important to prevent the spread of pathogens from surrounding environments into the facility, within the facility, and from the facility to surrounding environments. When investigators intend to eventually release amphibians, the amphibians' isolation from other populations in the facility is of utmost importance. It is also essential to keep only a single species or species assemblage per room.

The published and web literature in aquaculture is replete with information on the safety of materials and the design of enclosures, water systems, and other physical components of systems for the maintenance of aquatic and amphibious animals. Exemplary publications include Barnett et al. (2001), Lucas and Southgate (2004), Nace et al. (1974), Pough (1992), Pough (1989), and Wheaton (1977).

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In moderate climates, facilities that house local species may have a minimum of insulation. It may be desirable in those climates to have an open design with different rooms placed in a cluster of different buildings. The advantage of clusters of structures is that quarantine is more assured for specific amphibian rooms. By contrast, in extreme climates including northern continental climates, it is desirable to have a more centralized facility. However, it is still necessary for the amphibian facility to accommodate full quarantine, including the water supply, for pedestrian traffic and for materials including substrates for enclosures. Within the facility, it is necessary to be able to quarantine the animals between enclosures, arrays, and amphibian rooms.

Intuitively, the best captive husbandry of amphibians would provide conditions that simulate their natural habitat. Some small species with complicated behaviors and reproductive requirements (e.g., dendrobatids and mantellas) thrive and reproduce in "natural" systems (Schmidt and Henkel 2004; Zimmerman 1986). Nevertheless, the first choice for the large-scale captive rearing of amenable amphibians is simple, easy-to-maintain, medium- to high-density systems. Descriptions of medium- to high-density aquarium systems for laboratory models include those for *Xenopus* spp. (Reed 2005; Schultz and Douglas 2003) and *Ambystoma mexicanum* (Gresens 2004). Simple medium-density terrestrial systems have been developed for the southern leopard frog (*Rana sphenocephala*)[1] (Nace et al. 1974; Pough 1989), Fowler's toad (*Bufo fowleri*) (Browne et al. 2006a), the cane toad (*Bufo marinus*) (Browne et al. 1998), and the endangered Wyoming toad (*Bufo baxteri*) (Browne et al. 2006b). Medium-density systems have also been developed for the commercial production of amphibians for pets or display (Mattison 1987; Zimmerman 1986). High-density systems are used for commercial ranid species and similar systems should be suitable for the rearing of many other pond species (Figure 1).

The number of threatened amphibians in captive breeding programs is increasing, and research estimates this number will finally include hundreds of diverse species with a wide range of physiological requirements (Hofrichter 2000; Young et al. 2004; Zippel 2005). The maintenance and reproduction of these amphibians with diverse natural histories and from diverse habitats and climates has already proved challenging. These husbandry challenges include the need to meet specific physiological requirements for adults and larvae (Halliday 1999; Stebbins and Cohen 1995), and to house large numbers of adults and larvae needed to produce the numbers of amphibians required for rehabilitation projects (Browne et al. 2003; Culley 1992; Zippel 2005). It is possible to meet the associated challenges in facility design and services by using new technologies. For instance, the control of temperature is a major factor in the growth and sexual maturation of amphibians (Brenner 1966; Horseman et al. 1978; Pancharatna and Patil 1997). With the provision of sophisticated technologies for lighting and heating, many temperate species will bask to select their optimum temperature and to satisfy their ultraviolet (UV[2]) requirement (see Artificial Lighting, Heating, and Humidity below). Reproduction technologies, which also require innovative facility design, include the production of large numbers of larvae by high-density larval rearing and the provision of cryobiology systems (Reed 2005; Browne et al. 1998, 2003; Schultz and Douglas 2003).

Figure 1 Slide-out tanks in racks can provide economical and efficient housing enclosures for amphibians that have limited climbing abilities. It is easy to remove the tanks without fear of breakage for cleaning or inspection.

We recommend considering a modular system of modified shipping containers, which can be adapted to serve as independent units for the maintenance of single species or species groups (ARC 2007). These systems require only external power, water, and waste disposal systems to function. They are well insulated and can efficiently keep amphibians cooler or warmer than the ambient temperature of the surrounding environment. Within these systems, most operations include flushable tanks and automated temperature and lighting. For the maximum storage of tanks, shelves feature a "compactus" design that is similar to those used in archives.

Facility Structure

Natural Lighting and Ventilation

To minimize lighting costs, it is advisable to provide windows in all administration and laboratory spaces if possible.

[1]Frost (2007) has recently described revisions of genus and species names. However, in this article and for consistency with other articles in this issue, we have used the most common names in current use.

[2]Abbreviations used in this article: LC_{50}, median lethal concentration; PCB, polychlorinated biphenyl; UV, ultraviolet; UVB, middle-wavelength ultraviolet light; UVC, short-wave ultraviolet light.

We recommend using UV-penetrable glass in the windows of amphibian holding rooms. The need to control photoperiods in these holding rooms limits the use of windows for lighting. However, windows and skylights can still provide natural light during the light photoperiod. Atriums can provide light to the interior of buildings as well as areas for visitors. For energy efficiency, the size of windows, skylights, and atriums should be minimal to provide adequate lighting and should be double glazed for insulation. It is advisable to attach external shutters or awnings to both windows and skylights to control light and heat. Fixed shutters can both increase light in winter and prevent excessive heat during summer. Alternatively, adjustable shutters operated automatically or manually can provide even greater control of light and heat (see the Wikipedia description of passive solar [http://en.wikipedia.org/wiki/passive_solar]).

It is important to find an economical balance between the need for natural ventilation to provide fresh air and the consequent energy costs of heating or cooling. The optimal turnover of air in the facility will depend on the temperatures and humidity of external air. A high turnover of dry air can lower humidity enough to dehydrate amphibians, and low turnover can cause humidity that is high enough to promote the growth of mold. In moderate climates, it is possible to ventilate facilities that house local amphibian species naturally and to climate control only the staff areas.

Floors, Walls, and Ceilings

Because racks or tanks can be heavy, it is necessary to ensure that the floors can support these weights. Floors, walls, and the ceilings of amphibian holding rooms should be waterproof to enable washing or steam cleaning. All construction materials must be able to tolerate high humidity (e.g., drywall [plasterboard] and cellulose drop ceiling panels are inappropriate). Many chemicals used in furniture and coatings may be toxic to amphibians (Sciencesoftware 2007). Floors can be concrete or covered with a waterproof covering. Even in small facilities, it is necessary to designate at least one room as a wet area where personnel can wash and sanitize equipment including tanks and tubs.

It is important to equip wet areas with floor drains that have mesh coverings of sufficient size to prevent the escape of amphibians and the entrance of pests. It is essential to frequently disinfect these areas to prevent disease transmission from occurring within the facility through open floor drains. To prevent the escape of waterborne pathogens, liquid waste should be drained into a holding tank for disinfection before discharge into a municipal system. Facilities should sterilize their waste water on site; reports strongly support a preference for heat and pressure wastewater treatment (http://www.lbl.gov/ehs/biosafety/Biosafety_Manual/html/sterilization.shtml). At a minimum, chlorine treatment of wastewater must take place in an amphibian-safe manner (e.g., in avoidance of chemical fumes from sterilization agents).

Facility Engineering

Fixtures and Electricity Supply

It is necessary to equip each "wet" room with heavy-duty industrial capacity sinks and facilities that meet standards for disposal of amphibian waste (see Quarantine below). In amphibian rooms, the supply of electricity should be through waterproof outlets, preferably through ceiling drops using ground fault circuit-interrupted (GFI) outlets. It is ideal to have outlets for lights wired on timers and to have pumps, heaters, and other electrical equipment directly wired. The amount of electricity supply backup and alarms will depend on essential electricity usage, including lighting, air pumps, water heating, and air conditioning units. A backup generator should be available to provide the essential minimal power requirements to keep filters, air pumps, and other life support systems supplied in case of main failure. An important consideration is a safety system to turn tank lighting and heating off if room temperatures exceed amphibians' stress levels. Ideally, an emergency telephone/pager system that contacts on-duty (or on-call) personnel should notify animal care staff when building or water temperatures are outside the optimal "set" ranges or when there is a power outage in the facility. Personnel who work in wet areas should always wear boots that protect against electric shock and follow other requirements as stipulated in safety standards.

Insulation, Heating and Cooling, and Energy Efficiency

It is important to thoroughly insulate all structures to facilitate temperature control and to save on energy. The amount of insulation will depend on the ambient temperatures and the requirements of temperature-controlled rooms. It is possible to save energy by simple means such as the coupling of heating and cooling of different rooms with air conditioners. A well-insulated room can be used for the maintenance of tank temperatures rather than using immersion heaters.

Compartmentalization

Rooms for Amphibian Facilities

Amphibian holding rooms must provide for the following: quarantine of new arrivals and sick amphibians, reproduction and larval rearing, hibernation and aestivation, and general husbandry. Other designated areas include laboratories, live food culture, general service, storage, administration, and display. Brief descriptions of these rooms and areas appear below. It is critically important to keep groups of amphibians as isolated as possible from disease transmission through the quarantine of all new arrivals, and isolation between and within amphibian holding rooms.

Amphibian Holding Rooms

Amphibian holding rooms often have different temperatures, humidity, and photoperiod because it may be necessary to simulate different climate regimes and their seasonality and be adaptable to a variety of species from different habitats and climates. The provision of this variety of environments is challenging, but it is possible to modify the temperatures and humidity in each enclosure by using heat lamps or aquarium heaters in water bodies and by spraying and manipulating ventilation.

It is necessary to equip amphibian holding rooms with waterproof electrical outlets, hot and cold water, air lines, and resistance against insects. Special baffles on air conditioning vents and door seals will prevent the escape or entrance of insects. Doors should have self-closing devices and be lockable. If possible, to facilitate quarantine and to prevent the escape of insects or amphibians, amphibian holding rooms should be accessible by a short corridor. The control of insect pests should not include the use of chemicals but be through physical methods such as fly papers or traps.

Although the photoperiod requirements for reproductive maturation in most amphibians are unknown, many studies have shown that photoperiod (even the phase of the moon) affects reproductive maturation in many species (Brenner 1966; Duellman and Trueb 1994; Fraile et al. 1989). For this reason, in captive breeding programs the photoperiod in amphibian holding rooms should reflect that found at the geographical location of the species. Recent advances in lighting have enabled the construction of cheap efficient lighting systems, which can also provide basking sites (see Artificial Lighting, Heating, and Humidity below).

Principles of Quarantine

An effective approach to quarantine includes the following essential steps:

- Quarantine all incoming amphibians and if necessary test them for disease.
- Sanitize all incoming materials.
- Isolate quarantine groups of amphibians within the facility as much as possible.
- Provide sanitization floor mats.
- Prevent the movement of animal pests within the facility.
- Frequently clean and sanitize all floors, surfaces, and drains.
- Use disposable gloves when handling animals.
- Change disposable gloves between different amphibian individuals and groups.
- Practice good general hygiene.
- Sterilize all materials leaving the facility.
- Sterilize all equipment between uses.
- Isolate all sick animals and perform necropsies on dead animals.
- Use disease-free food sources.
- Always work from the most reliably disease-free amphibians to the least reliable.
- Establish appropriate schedules for management.
- Properly train staff.
- Work in a standardized routine to reduce the risk of disease spread.

Currently, one of the main threats to the survival of amphibians is the probably historic introduction of chytridiomycosis to naive amphibian populations from the laboratory model genus *Xenopus* as a consequence of poor quarantine (Weldon et al. 2005). Chytridiomycosis is devastating to many species of amphibians, especially when kept at low temperatures (Berger et al. 2004) and at high densities (Mazzoni et al. 2003). Real-time TaqMan polymerase chain reaction assays can provide an assay of the presence and prevalence of chytridiomycosis (Boyle et al. 2004). If detected the preferred compounds that could be used for treatment include itraconozole (Nichols and Lamirande 2000; http://www.thebdg.org/library/illnesses/chytrid_fungus.htm) or benzalkonium chloride (NSW 2001).

It is possible to disinfect from chytridiomycosis with exposure to 70% ethanol, 1 mg Virkon mL(-1), or 1 mg benzalkonium chloride mL^{-1}, which results in death after 20 seconds. The most effective products are Path-X and the quaternary ammonium compound 128, which can be used at dilutions containing low levels (e.g., 0.012 or 0.008%, respectively) of the active compound didecyl dimethyl ammonium chloride. Bleach that contains the active ingredient sodium hypochlorite is effective at concentrations of 1% sodium hypochlorite and greater. It is important to keep chlorine and other disinfectants from contact with amphibians, including contact through air conditioning. Chytridiomycosis will not survive complete drying after 4 hours at 24ºC, 3 hours at 37ºC, or 30 minutes at 47ºC (Johnson et al. 2003).

This example of the virulence of pathogens, and their devastating effect especially on naive populations, shows that the diverse range of amphibian species now being kept in research or captive breeding programs requires the implementation of the strictest quarantine measures, particularly with species kept outside their natural range. There is a particular risk from interspecies disease transmission in zoos that house a range of amphibians as well as other vertebrates including birds, reptiles, and fish. These other vertebrates are often from locations remote from those of adjacent amphibians and can carry potential pathogens to which many amphibians are naive. Especially in captive breeding programs, there is often a range of amphibians from different geographical regions, and prevention of disease transmission between these species is essential. For a discussion of some factors that affect interspecific disease transmission, see Caley and Hone (2004).

To achieve zero risk of pathogen release from amphibian facilities to the environment, quarantine stations, and quarantine rooms, investigators and all personnel must supplement traditional methods of quarantine with improved management practices and facility designs. These practices include the sterilization of water, substrates, and other "waste" to and from the facility. It is important to treat waste water to minimize the risk of introducing foreign pathogens out of the facility and into the surrounding area. Treatment before release is best accomplished by storing wastewater in a tank where chlorination/dechlorination is used for sterilization, or for small quantities heat and pressure sterilization. It is possible to accomplish chlorination/dechlorination using sodium hypochlorite to chlorinate and then sodium thiosulphate to neutralize the chlorine before disposal (Wedemeyer 2000). It is necessary to sterilize solid waste from the facility by incineration or by having a medical waste hauler remove it.

It is essential to assign the highest priority to the quarantine of threatened amphibians in captive breeding programs when they or their progeny will be released. Facilities for individual species or species assemblages should provide housing in self-contained units such as modified shipping containers or portable buildings (ARC 2007). Threatened amphibians must be kept isolated in individual rooms or in a separate area with other amphibians sampled from the same location. With threatened amphibians, it is important to take every precautionary measure to prevent infection, which includes changing disposable gloves between quarantine groups, using quarantine stations, using disposable protective clothing, performing husbandry of these animals daily before other animals, and showering between working with groups (Figure 2).

Figure 2 Plastic boxes are very inexpensive and ideal for keeping groups of small amphibians or individuals. However, this example shows some poor practices. The storage of other material on the racks encourages pests and disease. In addition, because the tanks are faced in reverse, the caregivers are limited in their observation of the amphibians' condition.

Quarantine stations. Facilities that house amphibians should have a quarantine station for those who enter or exit a compartment or isolated structure. Each quarantine station should have a means to remove loose material from footwear and then sanitize both the material and the footwear. The optimum method for sanitization is a disinfection entrance mat with 25 mm of sponge matting soaked in an antiseptic or foot bath. It is advisable to place the disinfection entrance mat on a dry mat of suitable size for drying footwear and preventing unsafe wet flooring. Alternative methods to use before entering isolated amphibian houses are to wear disposable plastic footwear covers or to utilize spray-on disinfectants.

Quarantine room. When amphibians arrive at a facility, it is necessary to quarantine them to enable the treatment of diseased animals and to prevent the transmission of pathogens to established animals in the collection. It is essential to minimize the handling of amphibians during quarantine to reduce the chance of disease transmission and stress. The quarantine room and its management should prevent transmission of pathogens between amphibians held in the quarantine room to other amphibian holding areas, or to the external environment. It is possible to accomplish this objective by having a good enclosure design and layout in addition to utilizing effective sanitary disposal of waste, water, and air. It is important to seal the quarantine room to prevent the transmission of pathogens through mesh covers on all openings and door seals. Personnel should separate the amphibian enclosures that house each group or individual arrival. Water should drain into a sanitary treatment system. The duration of quarantine will be determined by the results of fecal analyses and physical evaluations. However, the quarantine period should be approximately 30 days for apparently healthy amphibians from other facilities, and 90 days for wild-caught or suspect amphibians.

The treatment of amphibians upon arrival depends on their provenance and means of shipping. However, with all arrivals, it is essential to collect fecal samples and test them for parasites, and to assess the amphibians for their condition through visual inspection and by weighing and measuring. If the animals were shipped individually, it is advisable if possible to take the fecal samples from the shipping containers. If not, we recommend individually housing the animals, covering the enclosure bottoms with wet paper toweling, and supplying a water bowl. It is customary and we believe important to provide artificial furnishings that include a shelter for terrestrial species and plastic vegetation for arboreal or aquatic species.

Other quarantine procedures will depend on the source of the amphibians, but all suspect amphibians should be tested for chytridiomycosis (Boyle et al. 2004). For quarantine of most species and those raised in captivity, simple enclosures are optimal. Simple enclosures facilitate easy monitoring of the amphibians, their feed intake, the collection of samples, and personnel maintenance. Some species of wild-caught amphibians are particularly susceptible to stress. After the stress of capture, handling, travel, and habitat change, it is important to provide these amphibians with substrates similar to those found in their natural environment. These substrates should be sanitized on entering the facility by heating to > 70°C for 20 minutes before use and may include leaf litter, deep sand/loam, or furnishings such as leafy braches and layers of cork bark (Nace et al. 1974). It is possible to rapidly sanitize small quantities of material by placing them in a plastic bag and microwaving them until steam escapes, then agitating them and re-microwaving several times. Always leave heated material spread out for some time to cool and for harmful vapors to escape.

The precise factors that induce stress in amphibians and the consequences of stress are not clear. Nevertheless, we do know that stress suppresses immunity, reduces disease resistance, and inhibits reproduction (Belden et al. 2003). Although there are few studies of the effect of stress on amphibians, studies in fish have indicated immediate and long-term detrimental effects of stress (Bly et al. 1997). The biochemical responses to stress in amphibians are similar to those in fish, and amphibians probably suffer similar detrimental effects to stress. For this reason and to reduce stress, it is essential to handle these animals minimally and gently and to avoid disturbing them if possible. Some terrestrial amphibians are prone to violent jumping and damage to their snouts. It is possible to reduce jumping injuries by covering the exterior walls of glass tanks with shade cloth or paint. Injuries to larger species can be prevented by using bubble wrap or sponge matting on the sides and roofs of enclosures. With aquatic species in glass tanks held on metallic mesh racks, it is advisable to paint the underneath area of the tank bottom to prevent rostral abrasions from the amphibians' attempts to swim down.

During quarantine, it is important to minimize environmental stress. Lids on enclosures should provide adequate ventilation while preserving a relative humidity similar to that of the species' natural habitat. The room should include the appropriate humidifier if possible, especially when cold dry air is heated. Otherwise, it is advisable to maintain the humidity of individual containers by using hand sprays or aerators in water bowls. If commercial suppliers have shipped captive-reared amphibians such as *Xenopus* spp. and *A. mexicanum* in large quantities, we recommend quarantining the animals in groups and housing them in single enclosures. The risk with group quarantine is that one sick amphibian can infect the group and precipitate the need for common treatment. It is also necessary to dispose of waste that includes natural substrates and disposable plastic containers in a manner that will prevent transmission of pathogens to the natural environment such as burning, chlorination, or heating.

Hibernaculum

Amphibians that hibernate include many species of frogs and salamanders from the northern continental climates of North America and Eurasia and also from extreme southern hemisphere climates. These amphibians slow their metabolism during winter to avoid unsuitable conditions, including freezing. A hibernaculum, which is an artificial environment designed to simulate the natural hibernation environment, should provide the following:

- Optimum temperatures;
- Adjustment of temperature during hibernation;
- Habitats for species that hibernate under water;
- Maintenance of oxygen and humidity levels;
- Low-intensity, short-photoperiod lighting;
- Minimum disturbance of amphibians; and
- Sanitary removal of wastes and dead amphibians without causing stress to the living amphibians.

In most cases, it is customary for the hibernation environment to have above-zero temperatures and to provide a substrate within which hibernating amphibians will rest at optimum humidities and air exchange. With all species, it is important to stop feeding 1 week before lowering temperatures. Species from cool continental climates hibernate at temperatures from 0 to 4°C. Some species will not survive hibernation at temperatures that exceed 4°C. A standard refrigerator operates at about 4°C and can be used as a "cold room" if the air is exchanged frequently. Some anurans benefit by an acclimatization period at approximately 20°C before hibernation. This temperature is that of air conditioning.

For amphibians that hibernate under ice, it is necessary to submerge the animals in water that is 10 to 15 cm deep and to maintain the temperature between 2 and 3°C and not above 4°C. Water must be well aerated, with low-intensity light levels maintained for 8 to 10 hours and minimal disturbance of the amphibians. For the maintenance of small numbers of amphibians, it is perhaps most economical to use disposable plastic containers held in a refrigerator. Water must be chlorine free, and personnel should change the water approximately once a week or three times in 2 weeks using prechilled water. It is important to design the water-changing schedule to minimize agitation of the amphibians and yet maintain bacterial counts below pathogenic levels. Do not place these amphibians in refrigerators containing volatile materials. For the hibernacula of hundreds of amphibians that hibernate under ice it is possible to use large fiberglass tubs. It is essential to provide aeration and filtering devices equipped with a cooling-circulating device that removes wastes yet allows gentle water flow. We recommend keeping amphibians such as *L. sylvatica*, which hibernate under forest litter at 7 to 10°C, in enclosures that include sterilized leaves and forest litter. Personnel should frequently sprinkle the enclosure with water.

Cool and Warm Rooms

Some species of amphibians such as aquatic species from cool temperate climates or mountain streams have physiologies that are adapted to cool to cold temperatures. For these species, a room kept at a temperature between 7 and

12°C with a similar water temperature is suitable. For other species from more moderate climates, which hibernate at 7 to 12°C, it is possible to have a cool room as a hibernaculum. Warm temperate and tropical species of amphibians require a warm room maintained between 25 and 34°C (Nace et al. 1974). If the facility has only a few tropical amphibians, heat lamps and immersion heaters in their pools can provide the higher temperatures that they require. Similar warm temperatures are optimal for invertebrate maintenance and culture. However, with invertebrate rooms, it is necessary to implement strong ventilation coupled with a stable temperature to minimize dust and potential allergies.

Reproduction

It is difficult to generalize about conditions for the husbandry of amphibian larvae due to their wide variety of ecological niches and physiological requirements (Alford 1999; Feder and Burggren 1992; Hilken et al. 1995; Ultsch et al. 1999). However, in a well-appointed facility, it is important to provide a room that is dedicated to reproduction. A bench area for ovulation and in vitro fertilization is desirable as appropriate protocols become available for the many species that reproduce naturally only with difficulty (Browne et al. 2006b; Michael et al. 2004; Toro and Michael 2004; Wright and Whitaker 2001). Separate racks or spaces for each quarantine group of larvae trays or tanks will help prevent disease transmission. A large double sink will enable the cleaning and sanitation of trays and tanks. We refer interested readers to the section Egg Development and Larval Rearing in Browne and Zippel (2007), which appears elsewhere in this issue.

Laboratories: Space and Equipment

Laboratories should have well-separated bench areas for necropsy and for reproduction. The minimal requirements are an optical and dissecting microscope, adequate bench space (Figure 3), a sink, the provision of distilled water, and safe storage of hazardous chemicals, including inflammable liquids (http://safety.science.tamu.edu/chemstorage.html).

Administration and General Storage

The administration room must have the following accessories to fulfill its multiple purposes:

- Shelf and drawer space for the storage of record sheets, files, articles, and books;
- Computer facilities for the keeping and analysis of records;
- General storage space for feed and cages and for services that involve washing equipment, tools, and so forth; and
- A refrigerator and a deep freezer for the storage of feed, therapeutics, media, and other perishable items.

Figure 3 One of the important functional considerations in laboratories is the provision of adequate bench space. Especially as reproduction technology becomes more generally used, it is essential to provide space for egg-hatching trays and for the induction of adults.

Adult Enclosures

A huge variety of pre-existing enclosures and materials can be used to construct enclosures for amphibians. When planning a facility, the best strategy is to refer to an established facility that has corresponding amphibian care requirements. However, some essential features of enclosures are common to the keeping of all amphibians. In general, enclosures should provide a suitable environment to satisfy the physiological and behavioral requirements of the species housed, to prevent the escape of amphibians and their live food, to enable easy maintenance and monitoring, and to be amenable to stacking in racks and other structures.

Physiological and Behavioral Factors

A number of specific physiological and behavioral factors affect enclosure design. For example, amphibian species have optimum temperature, humidity, and lighting requirements. Enclosure design will sometimes vary dramatically to satisfy variations in physiological needs at different life stages or when natural seasons are simulated. The most dramatic examples of variations in temperature and humidity requirements are the cold temperatures required for the hibernation of some species and the drying regimes required for promoting aestivation of particular species. In some cases, adults may require only moderate humidity. In contrast, recently metamorphosed individuals may need very high humidity.

The behavior of a species is central to its enclosure design, particularly for species that have sophisticated reproductive behaviors (Hayes et al. 1998) such as the dendrobatids and the mantellas. Some species including dendrobatids engage in complex courtship behavior and, in general, complex maternal care (Schmidt and Henkel 2004). These species need enclosures with structures (normally including suitable vegetation) that provide substrates for their behaviors. For example, the males of some species engage in territorial and agonistic behaviors, therefore these animals must be provided with adequate space. The maintenance of these enclosures will also be different from enclosures with simplified structures and the much lower density of animals. Other examples of the influence of behavior on the design and maintenance of enclosures are cases of amphibians for which the presence of scent marking reduces stress and for which excessive sanitation may be detrimental. Nevertheless, there are species that may thrive at high densities and in enclosures with frequent cleaning, including many temperate climate toads (Browne et al. 2006a,b; Browne et al. 1998) and some ranids (Longo 1987; Rodriguez-Serna et al. 1996).

Types and Materials

Adult enclosures should constitute the most simple layout possible after consideration of any special needs of the species. For aquatic species, enclosures may vary from aquariums for members of the genera *Xenopus*, *Ambystoma*, or *Pipa* to large flow-through systems for species such as the hellbender (*Cryptobranchus alleganiensis*) or giant salamander (*Andrias japonicus*). For terrariums that house groups of amphibians, the design must accommodate territoriality and provide the size needed for natural reproduction, for example for some small continuous breeders such as the dendrobatid and mantella species. For larger species, it is often possible to keep the animals at high densities if personnel can eliminate feeding hierarchies and can provide adequate sanitation. The numbers of each species will also influence the choice of enclosure types.

However, in addition to any special accommodations, all designs for adult enclosures must provide the following components:

- Fresh water—preferably through automatic systems;
- Lighting and temperature control;
- Ease of cleaning;
- Accessibility to individual amphibians;
- Security of captive amphibians and their live feed;
- Safety for both the amphibians and the animal care personnel;
- Efficient utilization of space;
- Preferably a modular design; and
- Low cost.

Standard aquariums and accessories held in commercial racks can provide the requirements listed above. Personnel can modify plastic containers and glass aquariums for use as enclosures. Plastic pet tanks are especially valuable because they stack in a small volume, are relatively robust, and are insect proof (see Figure 2). Other materials such as fiberglass and concrete afford the possibility for fabricating large tanks, troughs, and flyways. To avoid toxicity from metals, it is important to construct water supply systems from high-density polyethylene, polypropylene, or nylon. However, because fiberglass, concrete, some plastics, and other materials can leach harmful compounds, it is essential to ascertain their toxicity, to wash the material appropriately, and, if necessary, to allow the compounds to "weather."

The minimum requirements for lids are that they (1) prevent the escape of amphibians and their live feed, (2) provide adequate ventilation for both respiration and the escape of heat, and (3) allow for the passage of ultraviolet (UV^2) light. The escape of amphibians can result in death, and the escape of both amphibians and their live feed can result in the spread of pathogens. In humidified rooms, ventilation rates in tanks are balanced between the maintenance of humidity and the removal of excess heat. Humidification of the whole room enables the use of open mesh lids to maximize ventilation (see Figure 1).

Size and Numbers

The most valuable resources for decisions about the size of enclosures are the experiences of other individuals and institutions. As a specific example, in the case of the medium-sized adult Wyoming toad (*B. baxteri*), Browne et al. (2006b) kept four individuals in 45-L glass aquariums with a 25-cm-thick sponge mat and a cork bark slab for shelter or basking. Standard fluorescent lights in the aquarium and lights set on a timer simulated natural daylight hours. A water tray (10 cm diameter × 2 cm depth) was placed at one end. The tanks and sponge mats were cleaned daily. Each aquarium housed four female or six male toads (Figure 4).

Arrays and Racks

Most commercial reptile racks are suitable for toads and ground frogs that are poor climbers. However, many frogs, especially tree frogs, and burrowing amphibians including many salamanders and caecilians need tight-fitting covers to prevent their escape. Although a problem with all racks is that disease easily spreads between enclosures, it is possible to minimize this problem by using partly independent drainage systems for each tank and by wearing gloves and using other barrier techniques during servicing (Gutleb et al. 2001).

Artificial Lighting, Heating, and Humidity

It is customary to program lighting to simulate natural light cycles. As the latitudes move from the equator, the seasonal differences in the daily cycle become more pronounced. Tropical amphibians generally thrive with 12 hours of light-

Figure 4 Arrays of standard commercial glass aquariums with fluorescent and other lighting often offer the best housing system. It is necessary to place the aquariums in well-quarantined areas and, if in zoos, in well-quarantined rooms. From Browne RK, Seratt J, Vance C, Kouba A. 2006b. Hormonal induction with priming and in vitro fertilisation increases egg numbers and quality in the Wyoming toad (*Bufo baxteri*). Reprod Biol Endocrinol 4:43. Available online (Biomed Central: The Open Access Publisher).

ing. Substrate temperature gradients produced by heating elements beneath the cage floor may be more effective than photothermal gradients, especially for nocturnal or secretive amphibians. Many adult and larval amphibians bask in the sun or use temperature gradients to regulate body temperatures above ambient levels to increase growth rates of digestion and development and to fight infection (Browne and Edwards 2003; Feder and Burggren 1992; Maniero and Carey 1997; Ultsch et al. 1999). Basking amphibians have mechanisms to prevent water loss through the skin and tend to be more tolerant of low humidity (Withers et al. 1984).

There is surprisingly little information on the effect of different combinations of visual and UV light on amphibian health. Middle-wavelength ultraviolet light (UVB[2]) from 285 to 320 nm penetrates the skin and enables the synthesis of vitamin D_3 in many vertebrates (Holick 1989), with peak conversion at 297 nm (MacLaughlin et al. 1982). Some species of amphibians may require UVB light for calcium metabolism, normal behavior, and reproduction. The requirement of UVB for cryptic nocturnal rainforest species is not known. However, Carmen et al. (2000) have shown that nocturnal geckos are more sensitive to UVB and convert D_3 with limited exposure at dusk/dawn. In addition, many cryptic nocturnal frogs hide in plain sight where they would receive substantial UVB exposure during daylight hours during periods of inactivity. Consequently, it is important to provide UVB to all captive amphibians by exposing the animals to one of the following: to natural sunlight, through specialized skylights manufactured from clear polymers that are transparent to UVB (e.g., Polycast Solacryl®); or to artificial lighting sources that produce UVB.

A wide variety of artificial lighting systems are available for the provision of UVB for captive husbandry of amphibians and reptiles. It is customary to classify these systems as (1) fluorescent, (2) mercury vapor, and (3) halogen, on the basis of the lighting technology used to generate UVB. The following brief summaries of these systems include the pros and cons of each.

1. **Fluorescent lamps** currently comprise the most widely used technology for the provision of UVB for captive amphibian husbandry. These lamps contain mercury vapor at very low pressure, which produces short-wave ultraviolet light (UVC[2]) (at 185 and 254 nm), when an electrical arc passes through the bulb. This UVC is converted to longer UVB, UVA (ultraviolet light from 320 to 400 nm in wavelength), and visible wavelengths (> 400 nm) after striking a mixture of phosphors that coats the inside of the lamp. Commercial suppliers market fluorescent lamps in a variety of forms such as linear T8 and T12 bulbs and U-shaped or spring-shaped compact fluorescent bulbs. Advantages include high-energy efficiency, low heat output, and moderate cost. Disadvantages include low heat output (if one seeks a basking thermal gradient), frequently low levels of UVB emission, and relatively short bulb life (6 months to 1 year before replacement due to decreasing UVB production).
2. **Mercury vapor lamps** utilize the same principle as fluorescent lamps but at a higher pressure of mercury vapor, resulting in longer wavelength emission without the use of phosphors. Suppliers frequently market these lamps in a "self-ballasted" design, which operates on normal line voltage in standard incandescent fixtures. Advantages include higher emissions of UVB, the potential for illuminating larger areas, and substantial heat output for creating thermal gradients for larger animals in large enclosures. Disadvantages include high cost, high wattage of available lamps, excessive heat output for smaller enclosures, and short lamp life of self-ballasted models.
3. **Halogen lamps** function similarly to that of a standard incandescent bulb, whereby a resistive filament is heated until it glows and emits light. In halogen versus standard incandescent lamps, a mixture of halogen gases within the bulb extends bulb life. We believe that halogen lamps are applicable for use with captive animals based on our coauthor's recent evaluation (T.H., manuscript in preparation) of two lamp models manufactured by Eiko, Ltd. (Shawnee, KS) for provitamin D_3 conversion and UVB irradiance relative to other lamp technologies available on the market. The results of this evaluation (T.H., manuscript in preparation) indicate substantial advantages over other available lighting for UVB production, including a 5- to 20-fold increase in conversion of provitamin D_3 relative to other lamps, very low cost of bulbs, versatile fixtures that allow for directed and focused illumination, a potential 2-year-plus lamp life, and moderate heat output that is suitable

for creating thermal gradients in small enclosures. Disadvantages include the need for specialized fixtures to run the bulbs (12V low voltage lamp) and limited utility in very large enclosures.

Readers should also note that UVB will not penetrate through normal glass or through most clear plastics. Thus, it is necessary to situate lighting from any UVB source so that it directly illuminates the enclosure and does not pass through any transparent materials. One should use screening—typically either aluminum or vinyl-coated fiberglass—in the lid of enclosures to provide direct transmission of light to amphibians. Finally, to maximize the passage of light, one should choose the largest mesh size that prevents the escape of prey items.

Water, Substrates, and Shelter

Amphibians do not drink through their mouths. Instead, aquatic species absorb water through the skin, and some terrestrial anurans have a specialized area of skin in the pelvic region to absorb water. If flow-through systems are not available, shallow water dishes, moist substrates, and spraying are appropriate for terrestrial amphibians.

Terrestrial amphibians tend to ingest substrates when feeding, and they sometimes can develop impactions (blockages) or punctures of the stomach or intestine that are frequently lethal. The provision of wood chips and artificial potting soil is appropriate and effective as long as it is free of fertilizer, which can be particularly harmful to amphibians. Suitable materials for substrates are green moss, sphagnum moss, peat moss with sand, leaf litter, gravel, loamy soil, ground coconut pith, and plastic grid. Plastic grids work well with amphibians that forage over dry ground, including toads and other ground anurans. Except for toxic species and mimics, most amphibians rely on camouflage for protection. Providing shelter and hiding places is important with the amount and type dependent on the species, life stage, and density. In nature, ambush predators reside completely or partially under shelter particularly during daylight hours. These species probably suffer stress when exposed. The susceptibility of amphibians to stress from exposure is apparently reduced in toxic species such as bufonids and aposematic dendrobatids. These species quickly adapt to open environments and move about freely (Schmidt and Douglas 2003).

Water Sources and Water Quality

Water from either a natural or a treated source (e.g., municipal water supply) is not a pure substance but is instead a suspension and solution of various organic and inorganic components. These additional substances in the water supply might be required to maintain the organism, might have no effect on the organism, or might be detrimental. The overall concentrations of these substances in a supply of water are customarily grouped together under the term "water quality." This grouping includes all aspects of the water (e.g., pH, inorganic salts, organic compounds, metabolic waste products, dissolved gases, and bacterial suspensions).

It is essential to equip the amphibian facility with a reliable supply of suitable water with appropriate hardness for osmotic regulation, trace elements for metabolic functions, and pH. Municipal water supplies vary considerably in these characteristics depending on the original source of the water and its treatment. The composition of the water may also vary from one time to another depending on the treatment and supply changes. Municipal water generally has chlorine or chloramines added for sanitization, and these compounds may be toxic to both adults and larvae. Municipal water also frequently has low oxygen concentration and is best aerated to remove unwanted volatile compounds and for oxygenation. Aeration also lowers harmful high gas concentrations due to supersaturation.

Although investigators have used municipal water for general services or for the water bowls of terrestrial amphibians, we caution that its use may not be optimal. We advise against using untreated municipal water supplies for aquatic gill-breathing forms. We and DeNardo (1995) believe also that it is beneficial to treat water at a minimum to remove chlorine, chloramines, copper, and organic compounds. A good guideline is to meet the standards prescribed for fish (Boyd 1979; Lucas and Southgate 2004; Wheaton 1977). Most municipal water should be within the acceptable limits of most amphibians for salinity, alkalinity and hardness, fluorides, heavy metals, microorganisms, pH, and toxicants; but the water should undergo periodic testing. It is possible to obtain general values of these qualities from the water supplier. If the source of water is from treated effluent, it may be desirable to remove organic microcontaminants through the use of carbon filters. Municipal water departments may add copper sulfate to water supplies to control algal growth, particularly in the fall and spring. They may also introduce copper and zinc to the supply through facility piping (i.e., copper pipe). Copper and zinc sulfate are toxic, inhibit tadpole growth and can significantly increase mortality, and may have a synergistic toxic effect (Herkovits and Alejandra 1998). The addition of Versene® (EDTA) at 50 mg/L of water will remove copper and zinc sulfate.

Oxygen

The saturation concentration of oxygen increases as temperatures decrease. The very cold waters in mountain streams enable species such as cryptobranchids and the Lake Titicaca frog (*Telmatobius* spp.) to survive by the intake of oxygen through their highly folded increased skin area (Figure 5). It is necessary for the oxygen concentration of warm water amphibians to be > 5 mg/L and for cold water amphibians, 8 mg/L (Table 1).

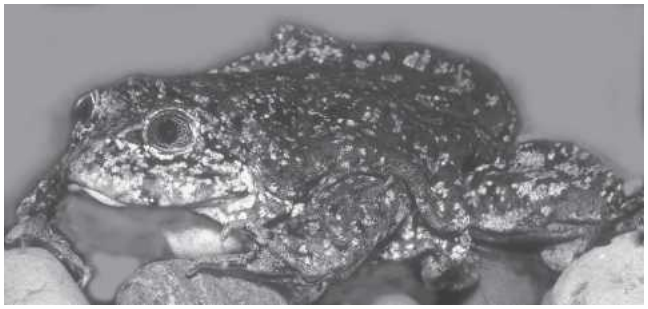

Figure 5 The Lake Titicaca frog (*Telmatobium* spp.) has very wrinkled skin to enable more rapid oxygen uptake during its submerged aquatic life in the frigid waters of Lake Titicaca, Bolivia. Donated to the author by Danté Fenolio, University of Miami, Coral Gables, FL.

Polychlorinated Biphenyls (PCBs[2]) and Other Toxicants from Plastics

The benefits of using plastic piping, containers, and instruments for many applications in amphibian quarters are their ease of use and favorable cost; however, these products are not without danger, especially when they come into contact with the water supply. Phenolic and acrylic plastics may contribute significant levels of PCBs to the water. It is important to balance the use of polyvinyl chloride with the available alternatives. A flush of the supply the first thing each day will reduce the levels of chlorinated biphenyls or other compounds that may have dissolved in static water in the pipes. In any case, it is necessary to rate all piping for use in potable water supplies. Some municipalities may also add phosphates to minimize the leaching of lead from old pipes, yet phosphates are known to be toxic to amphibians. Some species of amphibians have species-specific sensitivities. In extreme cases, low-level phosphates interfere with calcium metabolism, cause tetanic seizures, and eventually result in death (Stiffler 1993).

Gas Supersaturation

It is possible to supersaturate municipal and well water with dissolved gases (especially nitrogen and carbon dioxide, but sometimes even oxygen). However, in aquatic animals, exposure to these gases can cause gas-bubble disease, a condition similar to the "bends" in divers. Supersaturation is generally problematic only in the winter when the water is especially cold and capable of holding higher levels of dissolved gases. Pressurizing the water for transfer in piping exacerbates this condition. The treatment for supersaturation is degassing, which personnel can accomplish by aerating the water, heating the water, or simply allowing the water to stand until it reaches equilibrium with the surrounding air. Aerating water to remove gas might seem counterintuitive, but remember that the water is supersaturated from being under pressure, and aeration at atmospheric pressure will bring the water back to equilibrium at atmospheric pressure quickly. Heating the water to room temperature will lower its ability to hold dissolved gases.

Copper

Copper is a very common component of potable water plumbing systems. Copper also exists in some well water supplies. It dissolves in many acids and forms chemical complexes with ammonia in water. Copper is also very toxic to many aquatic organisms. When water is allowed to stand in copper piping for any length of time (i.e., overnight), some of the copper will dissolve. When the water is then used in an amphibian enclosure, copper is added to the system, which may have detrimental effects on the inhabitants. Thus, it is essential to flush this static water before using the water. Studies with *R. pipiens* have shown that concentrations as low as 0.15 mg/L will kill 50% of newly hatched tadpoles in 72 hours (Landé and Guttman 1973).

Chlorine as Cl_2 and Chloramines

Municipal water suppliers generally use chlorine as an antibacterial agent, and chlorine may be present in concentrations of > 9 mg/L in some tap water. The concentration of chlorine in municipal water supplies can vary greatly from day to day, or even hour to hour, depending on conditions at water treatment facilities. Arthur and Eaton (1971) have noted that concentrations as low as 0.0034 mg/L have reduced reproduction in fathead minnows (*Pimephales promelas*) within 72 hours, and at 0.15 mg/L, the lethal concentration was 100% (LC_{100}). Esvelt et al. (1971) also reported that the 96-hour median lethal concentration

Table 1 Saturated concentration of dissolved oxygen (mg/L) in fresh water at temperatures from 0°C to 30°C[a]

Temperature (°C)	0	2	4	6	8	10	12	14
O_2 (mg/L)	14.5	13.8	13.0	12.4	11.8	11.3	10.8	10.3
Temperature (°C)	16	18	20	22	24	26	28	30
O_2 (mg/L)	9.9	9.4	9.1	8.7	8.4	8.1	7.8	7.5

[a]Data adapted from Lucas JS, Southgate PC, eds. 2004. Aquaculture: Farming Aquatic Animals and Plants. Oxford: Blackwell.

($LC_{50}{}^1LC_{50}{}^2$) for shiners (*Notemigonus chrysoleucas*) was as low as 0.19 mg/L.

The concentrations of chlorine in municipal water supplies are many times greater than the minimum lethal concentrations for many aquatic life forms. For this reason, it is essential to remove chlorine from the aquatic environment of gill-breathing animals either chemically or by "aging" the water for several days. Aging allows time for the chlorine to dissipate out of the water, and the dissipation proceeds more rapidly when the water is well aerated and warmed. Unfortunately, many municipal water suppliers no longer use simple chlorine as a bactericidal agent. Instead, they are now using chloramines (NH_2Cl, $NHCl_2$, NCl_3), a more stable group of compounds that do not readily dissipate from water, which greatly increases the aging time required. The action and toxicity of chloramines are virtually the same as those of free chlorine (Tompkins and Tsai 1976). It is possible to remove both chlorine and chloramines from water with a variety of chemicals, the most common of which is sodium thiosulfate ($Na_2S_2O_3$). This compound reduces the free chlorine to the nontoxic chloride ion. When thiosulfate reacts with chloramines, there is also a release of toxic ammonia in small quantities, which may present a separate problem.

Nitrogen as Ammonia/Ammonium NH_3/NH_4^+

The majority of metabolic wastes from amphibian larvae and gill-breathing adult amphibians are in the form of ammonia. Ammonia generally enters the aquatic environment as a metabolic waste product of protein respiration, as well as from the bacterial decomposition of organic matter. Ammonia may also be present in municipal water supplies, especially if chloramines are used as an antibacterial agent.

Research has shown that ammonia is an extremely toxic compound with lethal concentrations as low as 0.2 mg/L for rainbow trout fry (*Salmo gairdneri*; Liebmann 1960). Other reports indicate that only slightly higher concentrations kill Atlantic salmon (*Salmo solar*; Herbert and Shurben 1965) and adult rainbow trout (Ball 1967). There is evidence to suggest that many amphibian species may be more tolerant to ammonia than are fish. Leopard frog larvae (*Rana pipiens*) exposed to ammonia chloride concentrations as high as 0.5 mg/L showed little evidence of reduced growth or increased developmental problems (Jofre and Karasov 1999). In comparison, Jofre and Karasov (1999) reported observing toxic effects in green frogs (*Rana clamitans*) only at concentrations > 0.6 mg/L whereas American toads (*Bufo americanus*) tolerated concentrations up to 0.9 mg/L.

In an aqueous solution, ammonia has two forms: the un-ionized ammonia molecule NH_3, which is extremely toxic, and the notably less toxic but still dangerously toxic ionized ammonium in NH_4^+. Tabata (1962) reported that NH_3 was 50 times more toxic to fish than the ionized form of ammonium. These two forms of ammonia are in equilibrium in aqueous solutions that are both temperature and pH dependent, as shown in the following:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

As the temperature increases and the water becomes more basic (pH > 7), the left side of the equation is favored, increasing the concentration of the toxic ammonia molecule. High temperature further exacerbates the situation because of the increased metabolism of animals held in the enclosure. Thurston et al. (1974) experimentally established this relationship (see Table 1). Most assay methods measure the total nitrogen of both ammonia and ammonium ions. To determine the true potential toxicity of the test results, it is also necessary to measure pH and temperature. One can then use Table 2 to establish the amount of the more toxic ammonia in the water environment. Certain bacteria efficiently remove ammonia from the aquatic environment. In an emergency, personnel can implement water changes or use chemical resins like AmLock® or AmRid® or similar products to remove ammonia.

Nitrogen as Nitrite NO_2

Nitrites (NO_2^-) form in the aquatic environment when nitrifying bacteria oxidize ammonia/ammonium (the first step of biological filtration). The toxicity of nitrites in the aquatic environment is similar to that of free molecular ammonia in some species. Russo et al. (1974) experimented extensively with rainbow trout (*S. gairdneri*) and found minimum lethal levels at 0.14 mg/L over a 10-day period. Other investigators have also observed species-specific toxicity of nitrites in amphibians. Blaustein et al. (1999) reported that nitrite concentrations of 0.88 mg/L were seven times more toxic to

Table 2 Percentage of total ammonia in the toxic unionized form for temperatures from 5 to 30°C and pH values from 6 to 9. As the solution becomes alkaline at pH >7, the toxicity of total ammonia rapidly increases as the unionized form increases. Toxicity of total ammonia also increases rapidly with increased temperature[a]

Temperature (°C)	pH						
	6.0	6.5	7.0	7.5	8.0	8.5	9.0
5	0.01	0.04	0.13	0.39	1.23	3.80	11.1
10	0.02	0.06	0.19	0.59	1.83	5.56	15.7
15	0.03	0.09	0.27	0.86	2.67	7.97	21.5
20	0.04	0.13	0.37	1.24	3.82	11.2	28.4
25	0.06	0.18	0.57	1.77	5.38	15.3	36.3
30	0.08	0.25	0.80	2.48	7.46	20.3	44.6

[a]Data adapted from Thurston RV, Russo RC, Emerson K. 1974. Aqueous ammonia equilibrium calculations. Bozeman: Montana State University, Fisheries Bioassay Laboratory Technical Report 74-1. p 18.

the spotted frog (*Rana pretiosa)* than to the red-legged frog *(Rana aurora*) and the Pacific treefrog (*Hyla regilla*) (Blaustein et al. 1999). As with ammonia, bacterial action oxidizes nitrites. In a balanced environment, it is possible to safely remove these toxins without injuring the housed organism. This is the second step in biological filtration. The presence of nitrite warrants corrective action and further monitoring of aquatic systems.

Nitrogen as Nitrate NO_3

Nitrates (NO_3^-), the final product of ammonia nitrogen oxidation in biological filtration, form by the action of nitrifying bacteria on nitrites. Unlike ammonia and the nitrite ion, nitrates are substantially less toxic to aquatic vertebrates. Westin (1974) identified the LC_{50} values of a study group within a certain period of time (in this case, 96 hours) for several fish species, which range from 900 to 2000 mg/L. In the wood frog (*Rana sylvaticus*), Jefferson salamander (*Ambystoma jeffersonianum*), spotted salamander (*Ambystoma maculatum*), and American toad (*Bufo americanus*) eggs, Laposata and Dunson (2004) reported that there was no effect on hatching to 40 mg/L, and the effect was considerably lower than the fraction of the milligram per liter LC_{50} observed for ammonia and nitrites. Based on these data, the presence of low to moderate levels (<1.5 mg/L) of nitrates is not considered a great problem in an aquatic system; as the final product of nitrification, it is possible to use the existing nitrates as a gauge to determine the effectiveness of biofiltration. Still, concentrations > 2.5 mg/L have produced sublethal effects on amphibians (Rouse et al. 1999) and should be considered a problem that needs immediate attention. The buildup of nitrates over time is an indication of insufficient water changes, which personnel should address by increasing water-change frequency.

Carbon Dioxide

The upper limit for carbon dioxide is 6 mg/L. Good aeration should keep the system below this concentration. As carbon dioxide levels increase, the pH tends to decrease.

pH (Acidity/Alkalinity)

pH is a measurement of acidity and alkalinity (range 0-14, with 7 as neutral). In the wild, most unpolluted fresh water that amphibians inhabit has a pH value between 6.5 and 8.5 (Ultsch et al. 1999). The pH must remain relatively constant to preserve aquatic life even though there are continuous amounts of both hydrogen and hydroxide ions entering the aquatic system. The natural aquatic system has pH-stabilizing chemicals called buffers, among which the carbonic acid/carbonate buffer is one of the most important in fresh water systems.

The pH requirements differ from species to species with the majority of pond species, which favor slightly basic water, and those from streams, from peat bogs, in forests, or pools, which have large amounts of decaying vegetation acidic water (Ultsch et al. 1999). Low pH in particular increases the lability and toxicity of metals (Beattie and Tyler-Jones 1992; Glooschenko et al. 1992). However, some species such as those that normally breed in acidic bogs (e.g., Pine Barrens tree frog, *Hyla andersonii*; Conant and Collins 1991) will still reproduce in town water (Riverbanks Zoo breeding program). We advise the amphibian keeper to research pH requirements for each individual species to establish a suitable environment in captivity.

Staff and Management Systems

Trained Staff

Until the amphibian conservation crisis took place, the majority of captive-reared amphibians used in laboratory projects had been species that investigators had selected for their hardiness and ease of reproduction. In those cases, staff trained in general animal husbandry or even as laboratory technicians have typically sufficed. However, in captive breeding programs for threatened amphibians, it has become imperative to employ staff who have specialist training in amphibian husbandry. Both staff and supervisors should be familiar with proper husbandry techniques. Institutions have recently introduced specialist training programs for amphibian keepers. For example, the American Association of Zoos and Aquariums has a model course in Amphibian Biology and Management with the goal to "provide a solid background in amphibian biology as it relates to husbandry, breeding, conservation and cooperative programs" (http://www.aza.org/prodev/Amphibians/). To enable the best staff performance, it is essential to provide well-designed and carefully provisioned facilities that are coupled with unambiguous and efficient management systems.

Management Systems

Management systems should provide the highest possible standards for the care of captive amphibians, especially with regard to quarantine and the ethical implementation of humane treatment. These standards should include the following important components:

1. Strict schedules to guarantee the provision of quarantine, clean water, food, cleaning, and monitoring.
2. Meaningful information systems to record and collate information on the history (including provenance) and condition of each individual. It is also necessary to record information that can be used to further husbandry techniques and reproduction.

3. Individual responsibilities of particular staff for specific aspects of care.
4. Monitoring of health through regular examination and with condition indices and identification of causal factors where problems in health occur (necropsies by veterinary staff).

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