ASSOCIATION OF ZOOS & AQUARIUMS

Amphibian Husbandry Resource Guide

Edited by:
Vicky A. Poole, National Aquarium in Baltimore
Shelly Grow, Association of Zoos & Aquariums
Edition 1.0, 5 February 2008

For more information about AZA and its amphibian programs, visit
http://www.aza.org/ConScience/Amphibians_Intro/

ATAG
The Amphibian Taxon Advisory Group

Table of Contents

Foreword

The global zoological community is responding to an unprecedented conservation crisis; amphibian populations are declining and one-third to half of all 6,000 known amphibian species are threatened with the possibility of becoming extinct within the next decade. The threats facing many amphibian species cannot be abated in the wild in time to save them; in these cases, *ex situ* conservation and captive breeding may be their only hope of survival.

To address this crisis as effectively and efficiently as possible, Association of Zoos and Aquariums (AZA) institutions must pool their herpetology expertise and experiences. Most of the species in greatest need of *ex situ* conservation have never been bred in captivity, and facilities are being requested to develop conservation programs from the ground up. The AZA Amphibian Taxon Advisory Group (ATAG) encourages you to lead the charge on behalf of these species and has already developed a number of resources within the past year to help you get started (available at: www.aza.org/ConScience/Amphibians_Intro/).

The ATAG has identified species in Canada, the United States, Mexico, and the Caribbean that are in greatest need of *ex situ* conservation. The list of these species is available in the ***Action Plan for* Ex Situ *Amphibian Conservation in the AZA Community***. The Action Plan also includes a detailed description of current amphibian collections and spaces within the AZA community.

The ATAG also published a ***Conservation Resource Manual*** to help you develop successful amphibian conservation and/or research programs (either *in situ* or *ex situ;* internationally or domestically) that are well-evaluated, fit into your institution's collection plans, are appropriate given your resources, and incorporate complementary education programs. The manual also identifies potential funding sources and provides species-specific action plans and currently available husbandry manuals.

Finally, this ***Amphibian Husbandry Resource Guide*** will help you provide the best care possible to the amphibians under your watch. The exact protocols for maintaining populations of many species in need of *ex situ* conservation are unknown, but this guide will help ensure that you are using some of the best husbandry techniques known, particularly in the face of emerging diseases whose arrival in collections must be minimized and whose emergence and transport must be managed. Chapters on assisted reproduction of amphibians and managing small amphibian populations for genetic purposes will be added to this guide in Spring 2008. To further refine your amphibian husbandry techniques and to benefit from other AZA herpetologists, the ATAG recommends that you attend the AZA Board of Regent's *Amphibian Biology and Management* course (http://www.aza.org/prodev/) and participate in networking opportunities at the annual ATAG meetings.

I thank all of the authors that have dedicated so much time to making these resources available to the AZA and global community, and look forward to hearing about new projects being developed. Together, we can meet this conservation challenge.

The ATAG is here to help you. Feel free to contact me, Diane Barber, ATAG Chair, at dbarber@fortworthzoo.org, or (817) 759-7180.

Good luck!

Diane Barber

Chapter 1

General Amphibian Husbandry

Jennifer B. Pramuk[1] and Ron Gagliardo[2]

[1] *Department of Herpetology, Bronx Zoo/Wildlife Conservation Society*
2300 Southern Blvd. Bronx, NY 10460 USA
jpramuk@wcs.org

[2] *Atlanta Botanical Garden,*
1345 Piedmont Avenue
Atlanta, GA 30309
rgagliardo@atlantabotanicalgarden.org

Lemur leaf frog (*Hylomantis lemur*).
(Photo: J. Larsen Maher/WCS)

INTRODUCTION

There are many reasons to keep amphibians in captivity including for purposes of exhibition, education, conservation, preservation, and for hobby and personal interests. Historically, zoos and aquariums have included amphibians within their herpetology programs and displays; however, as they become more conservation-oriented (versus the menageries of the past), zoos and aquariums will have to alter their collections to reflect their resources and capacities to carry out this work (Rabb, 2004). The financial and spatial requirements necessary to meet conservation goals and propagate critically endangered amphibians are significantly less than those required for larger species (e.g., elephants). The Amphibian Ark (www.AmphibanArk.org) has estimated that approximately 500 species of amphibians are in need of carefully managed *ex situ* help; however, today likely fewer than 10 species are in managed programs (K. Zippel, pers. comm.)

Amphibians compose a group of vertebrates that display an enormous diversity of natural histories. Within the three orders, anurans (frogs and toads), salamanders, and caecilians,

there are 6,218 species (www.amphibiaweb.org) with potentially many hundreds more awaiting discovery and description. Within this group, lifestyles run the gamut from terrestrial to fully aquatic as adults, with some species even adapting and thriving in arid regions of the world. Reproductive modes range from the "typical" amphibian that is terrestrial as an adult but lays aquatic eggs that hatch into aquatic larvae, to species that brood their eggs within their vocal slits or special pouches on their backs, to females that are viviparous (give live birth). Within vertebrates, only fishes rival this wide range of reproductive modes. Because the ecological characteristics and husbandry requirements of amphibians are so diverse, it is impossible to cover specific guidelines for all groups in this document.

This short guide provides very basic information on how to maintain captive amphibians. Good husbandry practices can circumvent many of the health problems encountered in amphibian collections. Where possible, materials and suggested suppliers are listed and in some cases, alternatives are offered for items that may not be available in all areas. At the end of the chapter, an extensive list of Additional Recommended Literature is provided for those who want to fortify their knowledge of amphibian natural history and husbandry techniques. It is recommended that you communicate with others who have worked on your species (or closely related species or genera) in captivity and employ their proven techniques and avoid repeating less fruitful methods. If husbandry experience is unavailable for the target species, methods may have to be tested through trial and error and shared with peers.

Twenty years ago, relatively little was known about amphibian captive care. More recently, a sort of "renaissance" has occurred in the science of amphibian husbandry and breeding. Yet this area of study is still lagging behind the disciplines of mammalian and avian husbandry, especially in the areas of nutrition and veterinary care. It is up to you, the next generation of amphibian scientists to fill in our knowledge gaps and improve a field that is still relatively new. If you would like to learn more, refer to the list of citations at the end of this document. Moreover, attending the *Association of Zoos and Aquarium's (AZA) Professional Training Program: Amphibian Biology and Management* is an invaluable experience (www.aza.org/prodev/). The monograph published by this course is a very useful learning tool and many of the topics covered herein are covered there in greater detail. Finally, AZA husbandry manuals for mountain yellow-legged frogs (*Rana muscosa*), Panamanian golden frogs (*Atelopus zeteki*), and Puerto Rican crested toads (*Peltophryne lemur*) recently have been updated and others will soon follow (Grow and Poole, 2007). These manuals are great starting points for any of these species and also provide information applicable to other amphibian propagation programs.

Planning

It is important to consider the overall purpose and long-term goals of keeping a particular species in captivity. Goals can range from educational exhibits to candidates for reintroduction. In addition, it is imperative to gather as much information about the natural history and environmental parameters of the species of choice before proceeding to acquire animals. Extrapolating from related taxa can be useful in some instances where there exists absolutely no precedent for keeping a species in captivity. Although it is not without its controversy, the latest and most thorough reference on evolutionary history of amphibians is that by Frost et al. (2006). Often there is something available in the literature on the captive husbandry of at least one species within the considered family. Select the most closely related species that is closest geographically to your amphibian of interest.

At the end of this chapter we provide a list of in-print references, reliable web resources, and links to products that have been used with success by the authors. Note that the listing of products and suppliers does not imply an AZA or Amphibian Taxon Advisory Group (ATAG) endorsement.

Acquiring Amphibians

Obtain amphibians from reliable sources, preferably from captive bred stock or animals that have been harvested in a responsible and sustainable manner. Ask the supplier questions about the animals you are obtaining (e.g., how long has the animal been in captivity, what sort of medical treatments, i.e., for parasites, have been administered, etc.). Only collect or receive animals from the wild with approval and documentation from the proper authorities. Many states and most countries now require permits for amphibian collection. Moreover, while in the field, you should take measures to prevent the spread of *Batrachochytrium dendrobatidis (Bd,* the amphibian chytrid fungus) and other potential pathogens from one area to the next or from one individual to another. In the field, latex or vinyl gloves should be worn at all times and changed between animals. Limit handling of animals as much as possible. Keep in mind that harmful pathogens or toxic skin secretions could be transferred easily from one animal to the next. Boots, walking sticks, and other field equipment should be cleaned free of all soil and other debris and if possible, sanitized with household bleach (3-6% sodium hypochlorite) to a 10% dilution for 15 minutes between field sites. This concentration should kill *Bd* along with ranaviruses. Additional fieldwork hygiene protocols can be found in Chapter 2 of this guide, Speare et al. (2004), and Zippel et al. (2006).

Transporting Animals

Collected amphibians should be housed in plastic, disposable deli cups or similar containers with tight fitting lids. Small holes should be cut into lids to allow for gas exchange. Make sure that the holes are sanded down on the inside of the container or punctured from the inside outwards and do not have sharp edges, as amphibian skin abrades easily. The bottoms of the containers should be lined with moist paper towels and/or well-rinsed, moist sphagnum moss. Animals need to be kept cool (generally 65–75 F/18–24 C, depending on the species) during transport, by utilizing insulated packaging such as Styrofoam. First, place animals inside plastic containers inside a small cardboard box and then place this box within a larger insulated box. In extremely warm ambient conditions, sealable plastic lunch bags filled with ice or cool gel packs can protect against overheating. Wrap any temperature packs in newspaper and place within the outer insulated box, but never directly inside the cardboard box containing animals. Animals should be shipped the fastest way possible regardless of expense.[1] Slower, more "cost effective" modes of shipment could mean death to your animals, especially during warmer seasons. It is best to ship animals during the spring or fall, instead of making shipments during extreme hot or cold seasons. Although there is a lack of sufficient data to say with certainty how many amphibians die during international transport (Smart and Bride, 1993), some shipments of amphibians have resulted in high mortality due to improper packing (crushing) of animals, overcrowding, overheating, and lack of access to water (Brookland et al., 1985).

Basic Needs

There are several basic but critically important aspects to keeping amphibians in captivity: 1) enclosures; 2) water (sources and quality); 3) environmental conditions (light, temperature, and humidity); 4) food; 5) natural history and behavior; and 6) veterinary care. Although there are many other nuances of amphibian husbandry to consider, if you master these key parameters for amphibian health, you will be well on your way to maintaining and propagating vigorous animals. This is a general husbandry guide and is merely intended as a starting point for those interested in beginning an amphibian program. Sections addressing breeding methods, larval husbandry, and veterinary care can be found at the end. The majority of the world's amphibian species have never been kept, much less bred, in captivity, so we still have much to learn (Pough, 2007). Keeping new species successfully can take months or even years of careful fine tuning to work out proper husbandry protocols. Remember that you are a pioneer of sorts. Do not get discouraged by setbacks and when you

1 Explore air freight or express shipping (same-day or over-night) options

discover something that works, please share your information with amphibian-minded colleagues. Consider publishing your findings, or else start a blog, join a listserv, whatever it takes to get the word out!

ENCLOSURES

While some amphibian species may survive in a minimal enclosure such as a plastic box with paper towel and a hide box, it is important to consider other aspects of the health and well-being that can be addressed through proper housing, substrate, and refugia. These aspects, combined with light, temperature, and humidity, can directly contribute to the animals' ability to behave normally and thrive in captivity.

Types of Amphibian Enclosures

Commonly used amphibian enclosures are constructed of glass, acrylic, fiberglass, or other synthetic materials. Using non-porous, easily cleaned materials is important. All enclosures should have a tight-fitting (e.g., screen) lid but keep ventilation in mind. The most commonly used enclosures are over-the-counter glass aquariums fitted with screen lids. Recently, some companies have begun manufacturing amphibian-specific terraria; despite their increased cost (ranging from 40–150 USD), initial reports are that they are worth the extra cost for ease of cleaning and access.[2] Initially, acrylic enclosures are more expensive, however they are lighter than glass and more resistant to cracking and breaking; in the long run, they are probably less expensive. The downside of plastic enclosures is that they scratch more readily and require frequent buffing to keep clear. For many terrestrial salamanders and smaller terrestrial frogs, 20 gallon-long aquariums with tight fitting lids work well. For arboreal species, consider purchasing tall aquaria or setting an aquarium on its side to provide more vertical space. Add tree branches and plants to offer sufficient climbing and perching areas. Again, the natural history of the species in question will need to be considered before purchasing an appropriate enclosure.

An alternative to aquaria is using polycarbonate food grade storage containers for amphibian enclosures.[3] The lids can be modified to provide sufficient air exchange. The opening in the lid can be affixed with screen that is glued in with a hot glue gun or silicone aquarium sealant. Although not as aesthetically pleasing as glass enclosures, these serve well as off-exhibit housing. The benefit to using food storage containers is that they are cost effective, stackable, and sturdier than glass. One salamander breeder in the US uses these simple enclosures for an entire colony with great success in maintenance and breeding.

Freestanding Vivaria (i.e., closed systems)

Proper drainage is a key to keep your vivarium healthy and productive and to reduce levels of harmful bacteria. Over-watering can kill your plants so it is important to give excess water a place to drain to keep the soil from becoming saturated and anaerobic. Water level in the gravel should be maintained at least 0.5 inch below the upper level of the substrate (e.g., sphagnum or sheet moss). This water will slowly wick its way into the soil through capillary action, offering moist conditions for the plants and providing sufficient humidity through evaporation. Coco fiber can also be used as a substrate on top of the gravel. Coco fiber is long lasting, holds moisture, and is free of unwanted organisms such as worms or flagellates. Because coco fiber is friable, a substrate divider should be used to keep this layer from mixing with the gravel or lightweight expanded clay aggregate (LECA) layer below. Fiberglass window screen or shade-cloth cut to fit the footprint of your tank work well as dividers for the two layers. On top of the coco fiber, you can use live java or cushion mosses, or hydrated and rinsed sphagnum moss. Sphagnum moss has an advantage over sheet moss

2 ExoTerra® and ZooMed® both sell quality terraria specifically for amphibians
3 Such as Rubbermaid® or Sterlite®

in that the former will usually develop live growth when maintained under high humidity and moderate light.

An appropriate basic vivarium can be created easily by starting with a glass aquarium and adding approximately two inches of pea sized, washed, naturally colored aquarium gravel. There are many inexpensive substrates as well as more costly amphibian-specific ones available on the market; gravel works well as an inexpensive starting substrate. Gravel set upon a false bottom made of egg crate (i.e., sheets of plastic light diffusion grid commonly used as a false ceiling below fluorescent lights, available at any hardware store) covered in fiberglass screen, resting on top of one to two inch (2–5 cm) pieces of PVC works well. This set-up reduces the weight of your tank by allowing you to use less gravel. Alternatively, a similar thickness of LECA[4], a lightweight, uniform substrate that is very popular with dart frog culturists, provides a well-drained lower substrate. LECA is slightly more expensive than aquarium gravel, but it is much lighter and can be used in situations where weight is an issue. Make a three or four inch (7.5–10 cm) square depression in the gravel or other substrate in one corner of the aquarium's floor or grade the substrate to form a small pool at the shallow end for your animals to access. The gravel layer should be completely covered with at least a one-inch (2 cm) thick layer of sphagnum moss or sheet moss. The moss should be soaked overnight and rinsed thoroughly prior to using in an enclosure. Ideally, this sort of environment should be set up at least two weeks prior to introducing animals so that the plants can become established and beneficial bacteria (i.e., natural, biological filtration) will be ready to breakdown wastes. You can add cork bark to the background and plants, but it is recommended that you remove all or most soil from the roots as soil can harbor potentially harmful parasites such as nematodes. Many bare root plants (e.g., pothos) will do well when planted in a semi-aquatic environment. When breaking down and cleaning enclosures they should be rinsed with water and can be disinfected with household bleach (3-6% sodium hypochlorite) to a 10% dilution. If bleach is used, rinse thoroughly and allow to air dry completely or use sodium thiosulfate to neutralize the bleach.

Plumbed Vivaria (i.e., open/semi-closed systems)
There is often a decision to be made between housing an animal within a naturalistic enclosure or, at the other extreme, a sterile box with a paper towel. There may be a happy medium in using a system that incorporates an enclosure fitted with drainage, a false bottom and potted plants (with all potting soil removed). This type of enclosure has proved useful in preventing the parasite buildup that can occur in closed systems or open systems that contain substantial substrates. The simplicity of this set up makes cleaning easy without too much time and effort, also allowing for efficient fecal test monitoring (Figure 1).

Figure 1. A large terrarium set up for highly hygienic conditions. Note the keeper using a spray nozzle to flush feces and other debris from the enclosure. Waste products are flushed through a layer of egg crate, covered in fiberglass window screen, down a drain drilled into the floor of the enclosure. (Photo: R. Gagliardo)

4 Such as Terra Lite®

Another enclosure type utilizes commercially available screen enclosures, known as reptariums. These lightweight screen enclosures are easy to assemble, move around, and clean (Figure 2). They have proved very useful in outdoor applications for phyllomedusine frogs that enjoy basking and for large hylids that would damage themselves by jumping around inside a glass enclosure.

Figure 2. Lightweight, screen enclosures outfitted with potted plants and cork bark work well for large, arboreal frogs and are easy to move between various climate-zoned areas. (Photo: R. Gagliardo)

Arboreal and particularly jumpy species seem very prone to escape and injury from many enclosures. A method employing a ten-gallon aquarium set on end and outfitted with a front opening door and ventilation area serves well for housing glass frogs (centrolenids) and colonies of juvenile phyllomedusines (Figure 3).

Figure 3. Vertical tanks used for housing arboreal frogs. Note the simplistic set-up including a floor with wet paper towels instead of gravel or soil, which can harbor parasites. (Photo: R. Gagliardo)

To accommodate a drain in a glass tank, a hole should be cut into the bottom with a diamond-tipped hole drill bit [approximately 1–1.5 inch (2–4 cm)]. Use a powerful drill (e.g., 14–18 volt) (Figure 4). Make sure to wear proper eye protection when working with power tools. While drilling, run a stream of tepid water over the area where drilling to reduce heat buildup which can crack the glass. If working in an area that cannot be flooded with a hose, use a dam made of duct tape, clay, or putty around the hole to be drilled to create a small

reservoir of water. While drilling, go slowly and firmly to avoid cracking the glass. Fit the hole with an appropriate sized plastic bulkhead, siliconed into place, to be used for a drain.[5]

Figure 4. Demonstration of how to drill a hole in a glass aquarium using a diamond-tipped drill bit. Photographed at the AZA *Amphibian Biology and Management* School where everyone gets to try their hand at drilling a tank. (Photo: J. Pramuk)

Affix a plastic stopcock at the base of the PVC pipe leading from the tank drain to a central drain. The stopcock will allow you to control the rate at which water drains from the tank. This set-up will allow the tank to be flushed with purified or dechlorinated room temperature water. Open systems do not require filtration, as the misters are flushing and dumping the enclosure on a daily basis. Most bulkheads are threaded, allowing the installation of a standpipe of any desired height. This height can also be increased seasonally to provide a deeper breeding pool. Pool depths vary, depending on the needs of each species.

If planning a large collection or for animals that require frequent misting, it is recommended to plumb tanks to a mister system, allowing the excess water to flow into a drain placed at the bottom of the enclosure. Frequent flushing will ensure that wastes do not accumulate in the enclosure. Misters (and lights) can be set up on timers, allowing you to mist as often as required for the species in your collection. This type of vivarium works well for larger species that produce more wastes or in situations where the collection is large and frequent hand misting would be too labor intensive. Plumbed vivaria allow the keeper to maintain a high level of cleanliness. Frequent flushing of the system also may reduce parasite (e.g., nematode) loads in the enclosure. False floors, consisting of a piece of egg crate covered with fiberglass window screen tacked into place with silicone or a soldering iron, work well with plumbed tanks.

Rain Chambers

Many amphibians breed during the rainy season, which can easily be duplicated under controlled conditions *ex situ*. In some cases, relocating specimens from their living enclosures into a dedicated breeding enclosure (i.e., rain chamber) may disrupt the breeding cycle. It is prudent to move either the animals well-prior to initiating pre-breeding conditioning (weeks to months in advance), or else to utilize the specimen's current living enclosure and installing the rain chamber there.

There are a variety of ways to set up a rain chamber. The most common is to use a tank outfitted with a false bottom, drain, and sump container with a submersible pump that drives water up to a spray bar made from a length of PVC pipe. The PVC pipe can be modified with

5 Bulkheads are available from US Plastics, Aquatic Ecosystems, or McMaster-Carr.

different numbers, positions, and sizes of holes to achieve various degrees of coverage and force. The pump is plugged into a timer so that rain cycles can be regulated. Rain chambers can be fashioned from tall aquariums with false bottoms, large plastic trashcans, and even five gallon buckets. If submersible pumps are not available, canister filters can work to run mist bars.

Components of Amphibian Enclosures

All materials within tanks can be used to offer diverse environments based on a species' natural history and wild habitat.

Display Backgrounds

Concrete: Concrete is sturdy and if dyed to match the color of soil or mud, can be naturalistic and long lasting (Figure 5), but there are several drawbacks including its excessive weight and porous nature. Additionally, highly active frogs tend to abrade their noses against concrete while pursuing prey, which can lead to systemic infections and death (J. Pramuk, pers. obs.); during installation, have the fabricator smooth the concrete surface as much as possible before it dries. Concrete leaches lime for a long time after it has cured. Unfortunately, lime can create a dangerously high (basic) pH, which can kill amphibians. Acid washes can resolve this problem, but make sure these environments are well cured and that the pH is tested periodically for several months before introducing amphibians. Sealing the concrete will help with porosity. Consult an experienced contractor if you are inexperienced with acid wash or concrete sealing procedures.

Figure 5. An amphibian exhibit at the Bronx Zoo with a concrete background. This exhibit displays North American species including *Hyla versicolor, Ambystoma maculatum,* and *Lithobates* (*Rana*) *sylvatica.* It is a closed enclosure with a combination mechanical and biological filter that produces a waterfall trickling down the back of the exhibit. Waterfalls are aesthetically pleasing while aerating the water. (Photo: J. Pramuk)

Fiberglass and resin: The benefits of fiberglass and resin-based backgrounds are that these materials are lightweight and can be shaped easily to resemble rockwork, mud banks, etc. Unfortunately, fiberglass and resin-based compounds will release chemical vapors (off gas), which can be toxic to animals or plants. One advantage is that the toxic phase for these substances is shorter than that for concrete. Both of these materials are more difficult to work with and may require specialized training, proper ventilation, and personal protection devices (PPDs), but the results are aesthetic and long lasting.

Substrates

When choosing a substrate, first consider the needs of your animals and determine whether you want an aesthetically pleasing enclosure or one that is easy to clean. The benefit of most naturalistic substrates is that they provide burrowing species places to hide. The pH of the

substrate also should be considered. For example, some mosses (e.g., sphagnum moss) are rather acidic which may irritate some species' skin.

Quarantine or hygienic situations:

- Astroturf: This plastic grass-like material is inert and easy to disinfect (as long as it is rinsed thoroughly afterwards). It can be cut to fit any size or shaped enclosure. Astroturf is mold-resistant and can be maintained in semi-aquatic enclosures. Larger, heartier species such as *Rhinella (Bufo) marinus* that defecate frequently may do best on this substrate.
- Foam rubber: Foam rubber can be purchased in various thicknesses from fabric stores. This material can be cut to any shape and works well for quarantine situations where cleanliness is a priority, as it can be disinfected. It can also be used for isolated specimens that need or require a softer surface for medical purposes (i.e., broken limbs or rostral rubs). There is some concern about the potential release of dioxins with foam and its potential to harbor harmful bacteria. A better option is high quality sphagnum moss; see below for more information.
- Paper towels: Paper towels work well in quarantine situations, but because they dry out quickly, they should be monitored several times a day to ensure that they are sufficiently moist. They also become saturated with water easily and become soiled, providing a substrate perfect for bacterial growth. Daily changes can keep problems to a minimum. Try to use unbleached paper towels, as white or bleached paper towels may contain traces of chemicals such as dioxin. Change at least every 48 hours if not sooner, depending on the situation.
- Pulp fiber pet bedding[6]: This option is reported as a suitable alternative to soil for caecilians and other burrowing amphibians (D. Fenolio, pers. comm.). This substrate should be rehydrated with excess water squeezed out prior to use.

Naturalistic substrates:

- Coco fiber: This substrate has grown in popularity because it is resistant to breakdown, lasts for a year or more, and makes an environmentally friendly alternative to peat moss. It comes dried and compressed into bricks for easy shipping. Soak the coco fiber brick over night in water and squeeze out excess moisture prior to laying at the bottom of your enclosure. If you use it on top of a layer of gravel, use a piece of shade cloth or fiberglass screen cut to fit between the gravel and the fiber. This will prevent the fiber from mixing with the gravel.
- Moss (sheet and sphagnum): Sphagnum moss can be used as an alternative to foam rubber, and is just as soft, provides more burrowing/hiding opportunities, and is anti-fungal/bacterial. New Zealand or Chilean sphagnum moss is superior to other types of sphagnum, such as Wisconsin Sphagnum. Moss should be soaked for 24 hours and rinsed thoroughly before use. This produces a soft, moist substrate that is easily changed. In quarantine situations or others where the moss is not used for long, it is possible to heat sterilize the moss and recycle it for horticultural applications. Live mosses can be collected locally, but it is possible for these moist mosses to retain *Bd* that could infect your collection (*Bd* spores are most easily transferred from one moist surface to another). Some culturists treat the live moss with a diluted itraconazole (0.01%) solution prior to use in amphibian enclosures but this may not effectively reach every zoospore of the fungus. Considering the risks and consequences to a collection, collecting live moss for the terrarium is not recommended.
- Modified Orchid Substrate: For long-term (3 years +) use in terrariums, a modified orchid media developed at the Atlanta Botanical Garden has shown some promise. This mixture was developed for growing tropical epiphytes where moist, acidic, and well-drained conditions are needed. With proper drainage below, epiphytes such as

6 Such as Carefresh Pet Bedding®

bromeliads, aroids, and even some orchids can be grown terrestrially within the amphibian enclosure. The recipe for the mixture follows:

Modified Orchid Substrate (Atlanta Botanical Garden)
1 part peat moss
1 part fine horticultural charcoal
2 parts fine orchid (fir) bark
2 parts milled sphagnum
1 part medium tree fern fiber

Mix, Moisten well for 24 hours before use (if possible) as components tend to be very dry!

Ecologically friendly substitutes:
- *Ground Coconut* (coco-peat) in place of peat moss (see www.peatmoss.com for information on environmental restoration practices from peat moss producers).
- *Bruc Fiber* (an ericaceous weed harvested in the Pacific Northwest) in place of tree fern fiber.

- Potting soil: In general, soil is not a good choice as this industry is poorly regulated in terms of components. Potting soil tends to become compacted and become permanently oversaturated with water under terrarium conditions. If there is no other alternative, use only steam-sterilized potting mix without vermiculite, perlite, or other artificial additives such as fertilizers. Potting soil can harbor and encourage the establishment of nematodes and other parasites, so its use should be limited. However, some fossorial amphibians (e.g., spadefoot toads and many salamanders) may do best on a soil substrate.
- Rocks and Gravel: Gravel is a useful, inexpensive, and relatively easy-to-clean substrate. It is widely available from most pet supply dealers and comes in a variety of sizes and colors. However, it is heavy and can lend an unnatural look to your vivarium if the appearance is overly uniform. Be careful that your animals will not ingest the gravel by accident as this can cause impaction. In particular, aggressive feeders such as horned frogs (*Ceratophrys* spp.) are known to ingest gravel when feeding. A generous layer of moss on top of the gravel layer can reduce the risk of accidental gravel ingestion.
- Sand: Play sand is relatively inexpensive and usually well rinsed prior to packaging. Pick a grain that is not powdery but that has some substance to it. Note that sand, if consumed in substantial quantities, can pose an impaction hazard. A calcium-fortified sand[7] has been employed to aestivate species such as Budgett's frogs (*Lepidobatrachus laevis*).

Enclosure Furnishings
Furniture, refugia, and landscaping are important to the wellbeing of your animals. It can be simple (a wet paper towel as substrate and an inverted dish with a door cut into it as a hut) or as naturalistic as your imagination will allow. Regardless, all amphibians should be offered plenty of hiding spots in their home to provide sanctuary. In the wild, with the exception of relatively few usually toxic diurnal species, most amphibians are nocturnal and considered prey by just about every other carnivore in their habitat. Providing a few accoutrements to give them some security will pay off with more stress-free, healthier animals. Cork bark; dried leaves; coconut huts (coconut halves turned upside down with a little door cut into it); inverted, opaque plastic tubs with an opening cut into one side; scrap PVC pieces; and film

7 Such as Calisand®, Vitasand®, or Repilite®

canisters can all make great hiding places (Figure 6). Cork tubes work well as refugia for species that dwell and breed in tree holes. For burrowing species (e.g., gopher frogs) you can use PVC tubes buried partially in the substrate to simulate a burrow.

Figure 6. An army of *Mantella pulchra* using one of their corkbark hiding spots. The dish on the left is an insect feeding station, which prevents insects from dispersing too rapidly and lets the frogs take their time feeding. A short piece of PVC on the lower right offers an alternative refuge. (Photo: J. Pramuk)

Refugia need to be opaque. Be sure not to use an object that is heavy or unstable as it can fall and crush your animals. Some herpetoculturists also obscure one outside wall of the enclosure to increase seclusion and to provide visual barriers between tanks. Avoid black covers which makes glass more reflective on the inside, encouraging frogs to jump against the wall, potentially causing trauma to the rostrum. Attractive false rock walls can be made from a wall of cork bark affixed with silicone onto the back of the enclosure. Be cautious of potential gaps where frogs can become wedged between cork and glass. A quick and easy method to obscure the inside walls of an enclosure while also providing a substrate for plants utilizes only silicone, peat moss (or coco peat), and crushed tree fern (or substitute bruc fiber). Place the enclosure on its side and spread a thin layer of silicone over the entire surface with a wide putty knife. Wearing gloves, sprinkle tree fern or bruc fiber over the surface to desired effect and carefully press into silicone to insure good adhesion. Next, sprinkle dry coco peat or peat moss liberally over entire surface, filling in spaces among the tree fern or bruc. Pat down and allow the silicone to cure for 12 hours before turning enclosure over to allow excess peat to fall away. This makes an excellent surface for mosses and other creeping plants such as *Ficus pumila* or *Philodendron* spp. to grow on to. Taking this a step further, customized "rock" walls can be created by laying down a layer of expanding spray foam[8] carving it to resemble rock once dry, and coating it with a layer of black aquarium silicone infused with coco fiber or waterproof epoxy resin. Pockets can be carved into the foam to hold small plants. Silicones for aquatic applications[9] are slightly more expensive than aquarium or household silicone, but are more durable. All silicones used need to be 100% silicone with no potentially toxic additives.

Be sure that everything that goes into your enclosure (both organic and inorganic) is not contaminated with soap, bleach, pesticides, or other chemicals. If you add items such as gravel, rocks, decorations, or plants, make sure they too are not contaminated with anything. Occasionally, rocks and gravel contain toxic residuals from processing. Some plastic plants are not intended for use in water and may contaminate your enclosure. Be careful when collecting natural items from an area where amphibians abound. Moist surfaces (e.g., mosses, soils, wet leaves) can harbor parasites and *Bd* spores that can infect your captive animals. Only collect material from areas where *Bd* fungus or ranavirus have not been reported in wild populations. Remember that it can take only one *Bd* spore to infect an amphibian. If you do collect wild materials, make sure they dry out completely, or are heat treated and dried, prior

8 Such as Great Stuff Gaps and Cracks®
9 Such as Dow Corning® 795

to use. It is suggested that soil or other organic objects are microwaved or steam sterilized prior to placing them into the enclosure.

Wood pieces are beautiful and naturalistic, providing climbing, hiding, or basking areas for your amphibians. Epiphytic (i.e., air) plants or vines can be mounted to the wood, adding a naturalistic flair. Many types of readily available wood will work fine in most situations; however, in environments with greater humidity, some woods will rapidly begin to decay. Some of the best choices for wet enclosures include cypress, cork, and ghost wood. Terraces can be created using cork or other wood, which can then be planted with vines, java moss, or other plants. Terraces also partition the space which be controlled by territorial males of certain frog species such as mantellas and dendrobatids. Most terrestrial salamanders will require plenty of cover such as bark and wooden pieces and a thick layer of dried leaves, which they will use to burrow underneath and hide.

Plants
Artificial (e.g., plastic or "silk") plants can be used for amphibian tanks and work well in situations where tank objects need to be cleaned thoroughly and frequently. An added benefit to artificial plants is that they will not transmit *Bd* fungus from a supplier to collection animals as live plants have been suspected of doing (C. Peeling, pers. comm.). However, there is some anecdotal evidence that amphibians prefer live plants. In addition, living greenery acts as a biological filter by converting nitrogenous wastes effectively in your enclosure. Consider the following factors when selecting plants: 1) adaptability to high-humidity conditions; 2) non-toxic to animals; 3) compatibility with the species' natural history; and 4) native to the same region as your animal(s) (if you are a stickler for biological accuracy).

High-humidity species: Pothos species (e.g., *Scindapsus aureum*); ferns; tropical and temperate ivys; *Selaginella* and other club mosses; java moss; *Tillandsia* and other bromeliads (plants of the pineapple family whose water-filled axils are essential for leaf axil breeders such as *Oophaga (Dendrobates) pumilio*); creeping or other species of *Ficus*; and aroids of the family Araceae are commonly used plants in terraria. Peperomias, begonias, and calatheas work well, too. Orchids are increasing in popularity in general and their use in the terrarium is becoming more common. Terrestrial jewel orchids are susceptible to rotting in moist environments and may not be the best choice for a beginner. More success has been seen with smaller, epiphytic species mounted on branches and placed in areas where good air-circulation and drainage allows proper drying of root systems. Submerged, aquatic plants should be provided for salamanders, as many species adhere their eggs either singly or in clusters to submerged plant leaves. Species of aquatic plants that can be cultured easily include java fern, java moss, and the ubiquitous *Elodea*.

Some species of potted plants purchased at the local greenhouse contain toxins that may be unwittingly consumed by your animals when crickets or other prey items ingest the plant material. For example, oxalate-producing plants [e.g., silver queen (*Aglaonema roebelinii*)] have been linked to subcutaneous edema and lethargy in waxy frogs (*Phyllomedusa sauvagii*). Feed crickets were suspected of eating the plants in the terrarium before the frogs consumed the crickets (Wright and Whitaker, 2001). Avoid using other oxalate-producing plants such as aroids [e.g., dumb cane (*Dieffenbachia*)]. There is also some evidence that some plants in the Commeleniaceae (wandering Jew) family are toxic (R. Gagliardo, pers. obs.). A little research on appropriate plants will pay off in the long run for the well being of your animals.

WATER
Both quantity and quality of water are important considerations and are among the most important factors aiding an amphibian's survival. Unlike reptiles, amphibians do not have a

shelled (amniotic) egg. Their relatively unprotected eggs are essentially part of the aquatic environment in which they are bathed and the developing embryo is subjected to whatever water quality problems are present. Amphibians are perhaps even more sensitive to water quality than many fishes; consequently, aquarists, because they are aware of water quality issues, often make the best amphibian caretakers. Amphibians do not drink from their mouths but instead absorb all or most of their moisture through their highly permeable skin (in anurans, water intake is primarily through a "drink patch", located on the posterior portion of their belly). They also absorb a significant portion of their oxygen through their skin. If their skin desiccates, amphibians will lose the ability to exchange gases through their skin and will effectively suffocate. Unfortunately, an amphibian's amazing adaptations and strong ties to an aquatic environ also mean that they are particularly sensitive to changes in water quality and quantity.

Water temperature, pH, ammonia, and nitrite should be tested daily in new enclosures to ensure that conditions are appropriate for your new amphibian inhabitants. Relatively inexpensive colorimetric water quality chemical test kits can be purchased for less than 20 USD at most pet stores (Figure 7). Specific reagents in each test kit react with a sample of water, resulting in a color change that is compared to a chart indicating the amount of each chemical in your water. Additionally, more expensive but more accurate spectrophotometers can be used to measure these parameters. Testing results are more accurate, but not necessarily more useful, as colorimetric methods are significantly more cost effective and will usually be sufficient to tell you if your water is within normal ranges or not. Newly constructed terraria often will undergo an ammonia spike several days following set-up and you should ensure that equilibrium has been maintained prior to introducing amphibians to the enclosure. It is recommended that you set up an enclosure several weeks to a month prior to introducing animals. This will allow natural bacteria to become established in the substrate and plant growth to become lush. Both the bacteria and plants will act as biological filters. After animals are introduced, water quality should be tested periodically to troubleshoot the cause of death whenever mortalities occur. Keeping a daily water quality log will provide baseline data and can target problems before they become harmful to your animals.

The importance of water quality cannot be overstated. For more information refer to Whitaker (2001), Browne et al. (2007), and Kevin Zippel's website (http://home.att.net/~kczippel/waterqual.html), or register for the AZA Board of Regents' *Amphibian Biology and Management* course, from which much of the below information is summarized (Odum and Zippel, 2004).

Figure 7. Water testing chemistry: Colorimetric water testing kits can provide an inexpensive and easy way to troubleshoot and monitor the health of your enclosures. (Photo: J. Pramuk)

Chlorine

Chlorine is the most toxic substance that you will have to contend with in your source water supply. Unfortunately, you likely will encounter this chemical because most municipal water treatment plants use it to kill bacteria in our drinking water. Even minute amounts can cause distress or death in fishes and amphibians; sensitivity varies greatly across species and life stages. For example, tadpoles are more sensitive to chlorine and other water quality issues

than adult amphibians because they breathe through their gills. It is recommended that all chlorine be removed from water prior to its use on your collection. Chlorine test kits are commercially available, and source water should be tested on a routine schedule at various times of day, as chlorine concentrations in municipal water supplies fluctuate.

Aging the water (having it sit for a day or two) prior to use, allows free chlorine (Cl_2) to dissipate in the form of gas. The process of dechlorinating can be accelerated through aerating (using an air stone), heating the water, or using an activated carbon pre-filter on source water. However, this will not get rid of chloramines, if they are used in place of chlorine as the antibacterial agent in your municipal water source. In this case, using a water conditioner (e.g., sodium thiosulfate) is recommended if you are not using a reverse osmosis or other filtration device. Sodium thiosulfate can be purchased in bulk and made into a supersaturated solution by mixing crystals with room temperature water until no more will go into solution. The solution can be stored in chemists' 250 ml transparent squeeze bottles for convenience. Be aware that when thiosulfate reacts with chloramines, toxic ammonia is produced in small amounts, which will need to be handled with appropriate filtration.

Many older cities add phosphates to their municipal water to chelate (bind) lead used in older pipes from the water supply. Unfortunately, excess phosphates are bad for amphibians as they bind calcium. A high phosphorus:calcium ratio can lead to serious neurological and osteological problems (e.g., paralysis) and even death. Phosphates usually are too small to be removed via reverse osmosis (RO), but they can be removed via phosphate sponges[10], arsenic filters (Figure 8), or other chemical filtration methods.

Figure 8. Water filtration system for one of the Kihansi spray toad rooms at the Bronx Zoo. A) Air handling unit; B) Air filter placed over intake window of the room; C) Pre filter and carbon filter assembly; D) Arsenic/phosphate filter manufactured by BASF and distributed by Aquasana in Houston, Texas; E) Filtered water reservoir; F) Reconstitution barrel where minerals and salts are added back in to the RO water; G) ProMist® mister motor that delivers spray to each of the enclosures in the room. The entrance of this room is equipped with a footbath filled with Virkon-S® disinfectant. (Photo: J. Pramuk)

Dissolved Oxygen

Amphibian larvae absorb oxygen through their gills and skin and/or by gulping air. The amount of oxygen required by an aquatic amphibian depends to a great part on its natural history. For example, lentic (pond-dwelling) species require less oxygen than lotic (stream-dwelling) species. For stream-dwelling species you will have to employ a filter and/or air tube with air stone to increase the amount of dissolved oxygen (DO) in the enclosure.

DO is the amount of oxygen present in freshwater. Freshwater is said to be saturated with oxygen when it holds the theoretical maximum for a given temperature and atmospheric

10 Phos-Zorb®, Aquatic Eco-systems, Inc. phosphate sponges, and Poly Filters® work well for phosphate removal. Another option would be to use a Tide Pool® sump filter and to place a phosphate pad in it. Cycling the water with the same pad (i.e., having a closed system) will remove more phosphate from your water source over time. The effluent of the Tide Pool® filter should run through a strong ultraviolet sterilizer to kill bacteria. A powerful aquarium pump (e.g., 600 gallons/minute) is required to return filtered water to the tanks above.

pressure. The warmer the water and the lower the atmospheric pressure, the less oxygen water can hold. DO concentrations must be sufficient to support the enclosure's aerobic community, including the amphibians, their food source, and the biofilter bacteria. DO concentrations are dependent upon the enclosure's water volume and surface area; stocking density and organic load; and the efficiency of the biofilter. Insufficient levels of DO (<80%), accelerate the decomposition of organic matter, releasing poisonous gases such as hydrogen sulfide (detectable by its signature sulfurous smell of rotten eggs and thereby serving as a warning of low DO levels), carbon monoxide, and methane (Odum and Zippel, 2004). DO levels can be increased by aerating and circulating the water by use of a standard air pump and air stone and should be monitored by using test kits or electronic meters (i.e., a spectrophotometer). A simple and attractive way to increase DO is to use a water pump to create a water feature (i.e., waterfall).

Too much DO in the water can lead to supersaturation. Often municipal tap water, due to high pressure and temperature changes, has high levels of dissolved gases, which can come out of solution upon contact with submerged animals. If bubbles are apparent on the skin of your animals or on the surfaces of submerged objects in your tank, the water may be supersaturated. This phenomenon can lead to "gas bubble disease" which can lead to erythema, hemorrhage, and death (Whitaker, 2001). Supersaturation of water can be prevented by aging the water, bringing it to room temperature, or using an air stone and aquarium pump to aerate the water for a day or two prior to use. Aeration is important to break the water surface tension allowing the gases to dissipate.

General Hardness

General hardness (also called Total Water Hardness) is the amount of salts dissolved in freshwater and is measured through chemical titration to degrees of hardness (dGH) (Table 1) or via electrical conductivity in micro Siemens (μS) (Andrews et al., 1988). Primarily, minerals that contribute to hardness are calcium and magnesium, but also include copper, zinc, iron, boron, and silicone. Soft water contains up to 75 mg/L calcium carbonate ($CaCO_3$) while hard water, in comparison, contains 150–300 mg/L $CaCO_3$. In nature, rainwater usually is quite soft and species that live in microclimates fed by rainwater (e.g., ephemeral pools and leaf axils of plants) will do best in softer water. Generally, the water hardness for amphibians should not exceed 150 mg/L $CaCO_3$ (~8.5 dGH). Calcium and magnesium salts can be added to harden water or deionized, distilled, or reverse osmosis (RO) water can be added to soften water (Odum and Zippel, 2004).

Water Softness (dGH)	Mineral Saturation (ppm)	Softness
0–4	0 –70	very soft
4–8	70–140	soft
8–2	140–210	medium hard
12–18	210–320	fairly hard
18–30	320–530	hard
>30		liquid rock

Table 1. Degrees of general hardness and their corresponding water softness.

Alkalinity

The alkalinity of water is a measure of the ability of a solution to neutralize acids or its buffering capacity.

Nitrogen

Unlike most terrestrial organisms such as reptiles, which secrete concentrated uric acid or other relatively nontoxic forms of ammonia, nearly all aquatic amphibians excrete nitrogenous waste as ammonia. This mode of waste excretion is energy efficient however the waste product is highly toxic and is dependent on a healthy external environment to keep ammonia levels in check. Ammonia has two forms in water: the highly toxic un-ionized ammonia molecule (NH_3), and the less-dangerous ionized ammonium (NH_4^+) (Odum and Zippel, 2004). Dependent on temperature and pH, aqueous ammonia and ammonium will exist in equilibrium:

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

The concentration of toxic ammonia (NH_3) will rise with increases in temperature and pH and will fall and be converted to the less-toxic ammonium ion (NH_4^+) as temperatures and pH decline. Most water quality tests measure the total ammonia nitrogen (TAN) as the total amount of ammonia plus ammonium, however pH and temperature will also need to be evaluated to determine the actual value of toxic ammonia in your water. Table 2 can be used to determine the percentage of un-ionized ammonia (i.e., the more toxic ammonia) in your water at a given pH and temperature:

Temperature (C)	**pH**						
	Acidic		*Neutral*	*Basic*			
	6.0	**6.5**	**7.0**	**7.5**	**8.0**	**8.5**	**9.0**
5	0.0125	0.0395	0.125	0.395	1.23	3.80	11.1
10	0.0186	0.0589	0.186	0.586	1.83	5.56	15.7
15	0.0274	0.0865	0.273	0.859	2.67	7.97	21.5
20	0.0397	0.125	0.369	1.24	3.82	11.2	28.4
25	0.0569	0.18	0.566	1.77	5.38	15.3	36.3
30	0.0805	0.254	0.799	2.48	7.46	20.3	44.6

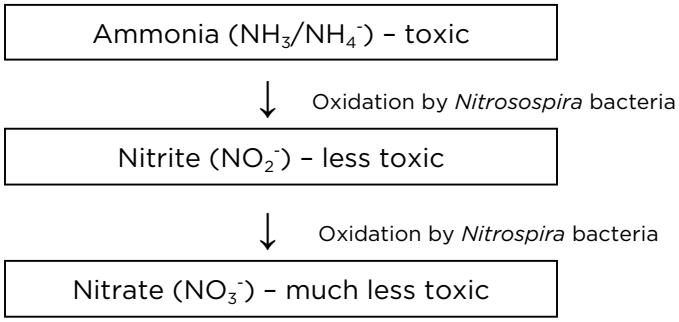
Table 2. Ammonia relative to pH and temperature (adapted from Emerson et al, 1975).

Amphibian waste and uneaten food produce toxic waste products (ammonia and nitrite). Clinical signs of ammonia intoxication include color change, increased mucous production, erythema, and lethargy (Whitaker, 2001). Long-term exposure to elevated ammonia levels can lead to a compromised immune function and secondary infection. Weekly water tests should be performed, followed by a thorough workup when illness is suspected. A water quality log should be maintained by keepers to track chemistry changes over time and should measure temperature, pH ammonia, nitrite/nitrate, hardness, and alkalinity of the water. A mechanical filter, weekly partial water changes, and good aeration and circulation will help keep water quality in balance. In a pinch, ammonia levels can be regulated by using an additive such as AmRid® or AmLock®.

Filters designed for fishes and available from any pet supplier work well for amphibians. A good filter will provide both mechanical removal of large organic matter and denitrification through biological processes. Chemical compounds such as carbon filters, phosphate sponges, ammonia-absorbing clays, or resins[11] may be used to target removal of specific chemicals. Several types of filters are available on the market and include types that employ polyester fiber, canisters or cartridges, or sand as methods of mechanical filtration. Air, rotary magnetic motors, or water pumps drive filtration mechanisms. After a few weeks or months of use, efficiency of all filters will decrease as debris inhibits water flow through the media. Regular cleaning schedules should be followed to ensure efficiency of the filter.

11 Such as Zeolite® or Ammo-Chips®

Beneficial bacteria in the tank convert ammonia toxins from waste products to less-toxic forms (nitrate and nitrite) through a process called biological filtration or *biofiltration*. Biological filtration takes advantage of naturally occurring communities of *Nitrosospira* and *Nitrospira* bacteria working in concert to oxidize reactions and convert toxic forms of nitrogen to safer forms. The process can be explained by following chemical reactions that are a portion of the nitrogen cycle:



A tank that is well established with denitrifying bacterial organisms will be a healthy microcosm that maintains waste levels naturally. Be sure to preserve the bacterial colonies by not using chlorinated water to clean the filter and by not being too physically vigorous in cleaning. Denitrifying bacteria live on all aquatic surfaces, and it is important to maintain this bacterial layer as much as possible to recolonize surfaces after cleaning.

The biofilter requires two critical things for proper health and maintenance: suitable substrate and ample oxygen. Appropriate substrates consist of ceramics or aquarium gravel and provide increased surface area upon which the nitrifying bacteria can thrive. The beneficial species of bacteria are aerobic, requiring sufficient oxygen to survive. Oxygenation is accomplished by employing a pump that circulates oxygenated water through the biofilter. Water should flow through the filter media slowly enough for the bacteria to absorb the nitrogenous wastes, but fast enough to maintain an aerobic environment. An anaerobic filter environment, created by slow or stagnant water, also produces ammonia and other toxic byproducts, such as hydrogen sulfide. This situation can be fatal to amphibians.

You can set up a tank with external biofilters attached to it so that you have biologically active filters ready to go at a moment's notice. Nitrogen consuming bacteria can be encouraged to grow by seeding the tank with household (non-scented) ammonia.

Ultraviolet light sterilizers work well for reducing levels of harmful bacterial in your water. The ultraviolet bulbs lose effectiveness over time and should be changed every six months.

Water changes are critical and should be performed as often as your particular system requires. There is no universal formula for frequency of water changes, as it depends on the number and size of animals in your enclosure, the volume of water, frequency of feeding, efficiency of the filters, and plants associated with each tank.

pH

The pH of water is basically the proportion of hydrogen (H^+) and hydroxide (OH^-) ions in solution. The pH scale is logarithmic with each pH unit representing a 10-fold change in the number of hydrogen ions. For example, a pH of 6 is 10 times lower than a pH of 7 and is 100 times lower than a pH of 8. When the number of hydrogen ions is greater than the number of hydroxide ions present, the water is acidic and its pH value falls below 7, conversely when hydroxide exceeds hydrogen, the water becomes basic (alkaline) with a pH value above 7. With a neutral pH of 7, hydrogen ions are equal to hydroxide ions. Natural pristine water sources generally have a pH between 6.5 and 8.5 (Ultsch et al., 1999) that can be dramatically

influenced by air, water, or soil contamination. Many amphibians prefer a pH that is slightly basic. However, as pH requirements vary by species, a pH of 7 is recommended as a good starting point if the optimal pH is unknown. Some amphibians prefer slightly acidic water, such as aquatic caecilians and peat bog-breeding species such as Pine Barrens treefrogs (*Hyla andersonii)*. Salamanders that dwell in limestone aquifers, such as the Barton Springs salamander (*Eurycea sosorum),* require a slightly alkaline (basic) environment.

As indicated in Table 2 (above), the level of toxic ammonia (NH_3) is relative to pH and temperature. The ammonia produced by the accumulation of decomposing detritus and the products of animal and bacterial respiration progressively causes the pH to fall (become acidic) within an enclosure. The pH can be kept near neutral through regular cleaning of your mechanical filter, routine water changes, and good aeration. If a high pH requires immediate correction, you can increase its acidity relatively safely in two ways: 1) add a commercially prepared blackwater treatment or 2) add tannins in the form of peat or sphagnum moss, placed within a piece of cloth or knee-high hosiery and set inside the tank (much like a tea bag). Adding reverse osmosis, deionized, or distilled water will also lower the pH, however the resulting pH may not be stable (Odum and Zippel, 2004). Make the system more basic (raise the pH) by slowly adding small amounts of sodium bicarbonate (baking soda) to the system (approximately 1/8 teaspoon per 20 gallons of water), and wait for 24 hours prior to testing and, if needed, readjusting. When changing pH, go slowly to minimize impact on the physiology of your animals; aim for no more than a 0.5 pH change per 24 hours.

Source Water Treatment

Reverse osmosis (RO) or carbon filters are the types of water purification most widely used by herpetoculturists to improve source water for use with amphibians. RO systems employ a semi-permeable membrane that selectively allows some atoms or molecules to pass through but not others. The membrane leaves behind impurities such as salts, but is not effective in removing some smaller compounds such as phosphate from the water. RO water is free of solutes and is purer than what can be tolerated by most amphibians. Its high purity (low level of solutes) means that in an attempt to reach osmotic equilibrium, water will move from the relatively pure surrounding water into the body tissues of an amphibian, which contain higher concentrations of solutes. Over time, this may result in edemic (bloated) animals and kidney problems. To compensate for the water's purity, salts and minerals should be added back to the RO water to create a solution that is isotonic with amphibians. Below is a recipe for making reconstituted RO water:

Recipe for Reconstitution of RO Water (K. Zippel)
(http://home.att.net/~kczippel/waterqual.html)
100 gallons RO water
15.0 g $CaCl_2$ (Calcium Chloride)
17.6 g $MgSO_4$ -$7H_2O$ (Magnesium sulfate)
13.6 g $KHCO_3$ (Potassium bicarbonate)
11.3 g $NaHCO_3$ (Sodium bicarbonate)
0.5 g commercial trace element mix[12]

Dissolve crystals in a jar of water and add to storage vat. Blend thoroughly before use.

Final composition:
General Hardness: 3 degrees
Carbonate Hardness: 2 degrees

12 Available through hydroponic gardening suppliers (i.e., #6 Chelate Trace Element from Homegrown Hydroponics)

Ca:Mg (3:1)
$Na:Ca^{+}Mg^{+}K$ (1:4)
pH ~ 7.4 depending on aeration

Pure RO (un-reconstituted) water is ideal for misting systems for display tanks where unsightly mineral deposits are not desired. However, inside the enclosure other sources (pools) of balanced water need to be accessible to animals to prevent osmotic imbalance within their bodies.

If you live in an area not prone to acid rain or excessive pollution, rainwater can provide a good alternative to municipal water. Rainwater collection barrels can be attached to downspouts on buildings, however copper, galvanized, or asphalt roof surfaces can contaminate rainwater with metals and other chemicals. Collection barrels are also open to contamination from amphibians that may dwell in your gutters. Rainwater usually will be slightly soft and is particularly useful for keeping leaf axil breeding amphibians such as strawberry poison dart frog [*Oophaga* (*Dendrobates*) *pumilio*].

Seven Commandments of Healthy Water

These seven key points for ensuring good water quality are adapted from Odum and Zippel (2004):

1. Start with high-quality water and test water quality parameters on a routine schedule.
2. Keep water fresh through frequent partial or complete water changes, proper flow-through, and/or filtration.
3. Clean mechanical filter media at least weekly.
4. Replace chemical media regularly.
5. Treat biological media as living creatures. They need oxygen and food (nitrogen).
6. Do not overcrowd or overfeed animals.
7. Incorporate living plants as much as possible.

ENVIRONMENTAL CONDITIONS

The large diversity of amphibian species is a result of the variety of habitats they have successfully colonized and the broad range of environmental parameters in which they live. Accommodating amphibians will require attention to temperature (air and water), light, and humidity, which can vary significantly between specialized natural habitats and microhabitats. Additionally, diligence for replicating naturally occurring annual temperature, humidity, and rain cycles may be key for the captive-survival or reproduction of some species.

Air Temperature

Maintaining appropriate temperatures for your amphibians is one of the most important considerations for their overall health. As ectotherms, amphibians are unable to produce substantial amounts of body heat and instead rely on environmental temperatures and behavioral modifications (e.g., basking or hibernation) to meet their thermal requirements. Temperature requirements for amphibians are as important as they are for reptiles or fishes. A popular misconception is that all amphibians need to be kept cool but again, this is where natural history research is important. If you are keeping tropical montane species, their median temperature requirements will be lower than tropical lowland species. Invest in an infrared temperature gun and monitor the temperature gradient throughout your enclosures often, making sure temperatures are within the range acceptable for your species.

The microhabitat of each species' native habitat should be considered. Some amphibian species live in microhabitats that are different from that expected of the geographic area in which they live. For example, temperate species usually do well at temperatures in the range of 65–75 F (18–24 C); however, cooler temperatures or a brumation period may be required for the autumn and winter seasons. Tropical lowland frogs can be maintained at 75–85 F (24–

30 C). Tropical montane frogs generally do well when kept from 65–75 F (18–24 C) (Whitaker, 2001). Eggs and larvae of most Neotropical hylids and dendrobatids can be maintained from 77–80 F (25–27 C) (Cover et al., 1994; Whitaker, 2001). Aquatic caecilians (of which all species are tropical) can be kept at relatively high temperatures from 80–85 F (27–30 C).

It is usually easier to add heat to an enclosure than to remove it. It is therefore recommended to maintain amphibian rooms slightly cooler than the average temperature required by collection animals. Heating individual enclosures with basking lights or submersible heaters to create thermal-gradients allows specimens to move about the enclosure in order to regulate their internal body temperatures. Ideally, it is preferable to alter the temperature of the room rather than using heat pads or heat lamps, as these methods can dry out enclosures quickly, unpredictably affect humidity levels, or overheat animals which can quickly lead to death. However, this luxury usually is not viable with cosmopolitan collections housed together in a single room.

If amphibians are confined to a small room or greenhouse-like structure, a portable room AC/heating unit can be installed to regulate the ambient temperature.[13] These units are rather expensive (~700 USD) but have been used at Omaha's Henry Doorly Zoo to maintain temperature in small rooms with great success (See Appendix 1).

Water Temperature

Water temperature will generally mirror the ambient temperature of your enclosure. Make certain that your incoming water has reached room temperature prior to use on your animals. A good way of ensuring water temperature equilibrium is by having a reservoir to acclimate the water prior to use. Depending on conditions, you may need to heat or cool the water in the reservoir or even in an aquatic enclosure. As a general rule, choose a submersible heater that can provide 5–10 watts of output per gallon of water being heated. Water heaters are fairly inexpensive (~15 USD), while chiller units for aquariums are relatively expensive (~700 USD). Water chillers, however, are particularly important for culturing coldwater salamanders such as hellbenders (*Cryptobranchus* spp.).

Lighting

While much more research is needed to document the specific requirements and benefits, quality and quantity of light are both important to amphibians. Some species require ultraviolet light for calcium metabolism, normal behavior, and reproduction. Amphibians (and reptiles) manufacture vitamin D_3 from exposure to ultraviolet-B (UVB) radiation through a process in which Vitamin D_2 is converted to D_3. Vitamin D_3 is critical for proper absorption of the calcium necessary for building and strengthening bone. Many species of frogs such as harlequin frogs (*Atelopus* spp.) and some leaf frog species (*Phyllomedusa* spp.) bask regularly and thus would seem to require a stronger source of ultraviolet light than many other species. Even non-basking species receive some reflected ultraviolet light; it is important to their physiology and development, as well.

The sun emits two types of ultraviolet radiation: ultraviolet-A (UVA) and UVB. UVA includes long-wave solar rays of 320–400 nanometers (billionths of a meter). UVB radiation is comprised of short-wave solar rays of 290–320 nanometers. Both types of ultraviolet radiation can cause point mutations in DNA and in excess doses can cause cancers or other problems. In humans, UVB rays are more potent than are those of UVA in producing sunburn. Natural light is by far the best option for captive animals, but is not always available. In lieu of natural daylight, timers can be used to maintain the ambient photoperiod in a natural cycle. Because glass does not transmit middle-wavelength ultraviolet light, it should not be used for

13 Such as the Sunpentown® 12,000 BTU unit

cage tops; instead, choose wire mesh. Acrylic and fluoroplastics transmit some short wavelength ultraviolet light (Gehrmann, 1987), but wire mesh is best.

Artificial lighting has come a long way towards replicating the real thing, but it is still far from replacing natural light. Lights should be placed a sufficient distance from your animals so as not to cause burns or other problems, but close enough for the ultraviolet to be effective. The UVB output of fluorescent bulbs decreases substantially after a few hundred to a few thousand hours of use. Unfortunately, the gradual change in ultraviolet-effectiveness is not apparent to the human eye. Obtain bulb-life information from the manufacturers and periodically measure ultraviolet-output with a light meter[14] to monitor bulb-life, changing them accordingly.

Many ultraviolet-emitting lights are now available on the market and have been designed specifically for amphibians and reptiles.[15] Note that there are very few data indicating the precise UVB requirements for amphibians, nor how species may differ in their ultraviolet uptake (Pough, 2007). However, there is some anecdotal evidence that ultraviolet lighting has improved amphibian husbandry, especially the health of diurnal species. For example, modified Eiko® halogen bulbs (Eiko® Supreme Ext/Su/10K, 12V 50W; with the lens carefully removed using a Dremel® tool) are widely used by several zoos and provide full-spectrum ultraviolet light. Evidence suggests that these lights allow a 5- to 20-fold increase in the conversion of vitamin D_3 from UVB among some amphibians, compared to other light sources (Browne et al., 2007). The bulbs last more than two years, cost less than 3 USD apiece and therefore are more cost-effective than many brands of *reptile bulbs* currently available. Track-light kits compatible with these bulbs can be purchased from local building-supply stores (Figure 9).

Figure 9. Left, a terrarium set up for Kihansi spray toads (*Nectophrynoides asperginis*) at the Bronx Zoo. This tank was manufactured by All Glass Aquariums to our specifications. This tank is on an open system and is affixed with mist nozzles and full spectrum lighting. Right, Eiko® bulbs and tracks used for lighting spray toads and basking amphibians in the collection. (Photos: J. Pramuk)

Humidity

Relative humidity (RH) is the percentage of water vapor in a given area; the proportion of water in the atmosphere relative to the amount of moisture the atmosphere can hold. It is a relative measure because the absolute value changes with the air temperature. In other words, warmer air will contain more water vapor than will cooler air with the same RH, thus a direct correlation with temperature.

14 Such as SolarTech's solarmeter® UV(A+B) model 5.7 or UVB model 6.2
15 Some of these brands include ZooMed® ReptiSun®, Coralife® Incandescent Reptile Bulb, etc.

Humidity is extremely important because it will determine how quickly an animal will lose water from its body to the surrounding air. Lower humidity, such as in a desert environment will ensure that water is lost at a faster rate. For this reason, many amphibians will only become active at night, during rainstorms, or during rainier times of the year.

Humidity levels in rooms will fluctuate depending on the time of year. In warmer months, if air conditioners or forced heating are used, the RH will decrease and moisture will be more rapidly lost from enclosures and animals. Humidifiers can be used to increase RH within a room. Many types of room humidifiers are available commercially and are relatively inexpensive (30–150 USD). Some portable humidifiers have been reported to release microorganisms and minerals from their reservoir into the air, which can lead to health problems. Use of steam vaporizers create high heat and are also not recommended for amphibians. Evaporative humidifiers may be the best type of humidifier to use, but they need to be kept clean or else scale (accumulated mineral deposits) can build up in the water reservoir and create problems. Although expensive at approximately 1 USD/gallon, distilled water can be used, which greatly reduces the release of microorganisms and minerals into the air.

While maintaining ambient humidity in a room is helpful, what is more crucial will be maintaining humidity within an enclosure containing amphibians. There are many ways to add or regulate humidity within enclosures ranging from automatic misting systems, to hand spraying, to regulating the amount of ventilation. Automatic misting systems are excellent for maintaining constant levels of humidity within large numbers of enclosures and come in a variety of sizes, brands, and configurations. Although labor intensive, hand misting with small hand sprayers or larger "pump" sprayers allows more opportunities for observation by the keeper and can aid in overall monitoring of animals.

Breeding behaviors in some species may be stimulated by increases in humidity, rather than heavy rain. Simulate this by venting a humidifier into the tank with PVC pipes or flexible tubing (Figure 10). Sealing the tank with plastic wrap will help hold in the humidity, although small air vents should be included to allow for dissipation.

Figure 10. Cool-mist humidifiers offer another way to achieve a sudden increase in humidity that may stimulate breeding. (Photo: R. Gagliardo)

Tools

Prior to placing animals in an enclosure, monitor the environmental parameters every few hours for a few days. It is very easy to overheat your animals so ensure that the highest temperatures fall within the acceptable range for your species. There are a number of tools that facilitate the monitoring of conditions.

Figure 11. Digital thermometer. (Photo: J. Pramuk)

Digital and analog thermometers are essential for measuring the temperature both inside and outside your enclosures. A simple maximum/minimum (max/min) or digital thermometer (Figure 11) serves well for obtaining basic information on daily temperature fluctuation. Infrared temperature guns are ideal for measuring the temperature inside an exhibit or enclosure without disturbing your animals or opening the enclosure. For detailed records, portable data loggers work well for measuring environmental conditions within your enclosures or amphibian rooms (Figure 12).[16] These digital devices can measure RH, temperature, and even light levels. Depending on the model, you can store up to 65,000 measurements. These devices come with an easy-to-use software program that allows intervals to be logged, start times selected, and recorded data to be downloaded to a spreadsheet program such as Microsoft® Excel to produce detailed charts and graphs.

Figure 12. A HOBO® temperature and humidity data logger. (Photo: J. Pramuk)

FOOD

Feed amphibians a diet that resembles what they would eat in the wild as closely as possible and offer them the greatest variety available. Malnutrition of amphibians can lead to developmental (e.g., the dreaded spindly-leg) and reproductive problems, metabolic bone disease, tetany and paralysis, failure to thrive, and death.

Important things to consider when selecting food for your animals are: 1) the calcium:phosphorous ratio; 2) lipid (fat) content; and 3) size of the prey item. Frequency of feeding also is critical and will depend on the natural history of the species being maintained. For example, dart frogs are highly energetic and require frequent feedings (at least three times per week). A good rule to use for energetic species is that there should be insects remaining in the enclosure between feedings so that the animals can feed *ad lib*. More sedentary species such as some members of the frog genera *Ceratophrys, Dyscophus, Litoria,* and *Pyxicephalus* and salamanders such as *Ambystoma* are prone to obesity and feedings

16 Such as HOBO® (manufactured by Onset)

should be monitored accordingly. Avoid feeding too many fatty prey items (e.g., pinky mice) to these and other ambush predators as their metabolisms are slow. A good vitamin supplement with a calcium:phosphorous ratio close to 1:1 should be offered several times a week with feedings. It is critical to consider the natural history of the species in deciding how and when to feed. Feedings should be based on the animal's natural feeding times, rather than on the convenience of the keeper's schedule. Feeding nocturnal species early in the day can give the food items time to hide and elude predation before the lights go off. Using feeding bowls or hand-feeding techniques for critical species can aid monitoring health and proper nutrition.

Nutrition

There are several herp vitamins on the market that are quite good including Reptocal® mixed 1:1 with calcium carbonate ($CaCO_3$), Reptimin®, Nekton® products, etc. (Figure 13). The Food and Drug Administration (FDA) does not test and regulate animal vitamins, therefore the percentages of vitamins and minerals in these products are not monitored and therefore, there is no guarantee of what is in the final product. One way of circumventing this problem is by using human vitamins.[17] Make sure you use vitamins that have Vitamin A rather than Vitamin A as beta-carotene. Amphibian nutrition is covered in detail elsewhere (Wright and Toddes, 2004).

Figure 13. Two examples of commercially prepared amphibian vitamins. (Photo: J. Pramuk)

Culturing Food Animals

The advantage of culturing your own live foods is that it gives you control over the cleanliness of your cultures. Often this will be the most cost-effective method of obtaining insects. It is time-consuming and labor intensive, and in the case of some colonies, quite odiferous. If possible, catching appropriately sized insects from the wild is a great way to supplement animals' diets; however, this may introduce parasites and chemical contaminants such as pesticides.

Insect Collecting

Many insects can be collected locally by sweeping butterfly nets in tall vegetation. Other effective methods of collection include employing insect traps at night that are attached to black or mercury vapor lights.[18] However, ensure that the collection area is chemical-free (i.e., without pesticides or herbicides).

One easy way to collect nutritious insects is by trapping termites, a commonly available and nutritious food source in many areas of the world. Roll moist, pesticide-free corrugated cardboard and place into a two foot (0.6 m) long piece of PVC pipe with a cap on one end. Drill some holes into the lower half of the pipe. Bury the open half of the pipe into the ground. After a week or two, periodically check the trap for termites. If occupied, shake the open pipe

17 Such as One a Day Men's Plus®
18 These types of traps and butterfly nets are available from BioQuip®

end into a bucket to collect termites. Animals that particularly relish termites include natural ant consumers such as microhylid, dendrobatid, and mantella frogs.

Crickets
Crickets, as well as mealworms (beetle larvae), spikes, mousies (crane fly larvae), and reportedly high-calcium soldier fly larvae (e.g., Phoenix Worms®) can be purchased from U.S. suppliers. Some of the calcium reports should be considered carefully, as the raw calcium content of prey does not relate directly to availability for your amphibians. In other words, calcium may remain unavailable to an animal.

The most common insect used to feed amphibians in captivity is the domestic cricket, *Acheta domestica.* Crickets can be cultured at your facility or purchased from a bait or animal food supplier. Usually, suppliers provide various sizes suitable for feeding animals. Hatchling crickets (pinheads) are appropriate for feeding dart frogs, mantellas, and smaller species of salamanders. Adult crickets are suitable for larger species of amphibians such as marine toads, most ranids, and *Dicamptodon* salamanders. Crickets that are too large for an animal will either not be consumed, or can rupture the digestive tract of the amphibian that is able to swallow something larger than it can accommodate. Crickets can be housed in a plastic utility sink or garbage can, available from hardware stores. Use a layer of petroleum jelly in a ring or a band of smooth packing tape around the top of the enclosure to minimize cricket escapes from the enclosure. Provide stacked cardboard egg crates to increase surface area for your crickets and moisture via a chick feeder[19] or sliced oranges. Crickets do not have an optimal calcium:phosphorus ratio and should always be gut-loaded with a high-calcium cricket diet[20] for a minimum of 48 hours prior to feeding to your animals. Gut-loading is the practice of feeding, or "loading" food animals with a nutritious food prior to feeing them out to a predator. Gut-loaded crickets are closer to the ideal calcium:phosphorus ratio of 1.5:1 (Wright and Whitaker, 2001). They also should be dusted with a premium vitamin powder immediately prior to feeding out (see Nutrition section above).

Crickets can be cultured easily by offering an adult colony a shallow plate or tub filled with a mixture of moist sand mixed with sterile soil or peat moss in which the females may oviposit (lay their eggs). After three or four days, the egg-filled container can be removed and placed into an aquarium. The eggs are small, white, and ovoid (appearing like tiny grains of rice). Ten to fourteen days later, the pinheads should hatch. Some institutions place breeding tubs in with newly purchased adult crickets during the gut-loading time period to increase culturing potential.

To separate the pinhead crickets from the substrate, place the tray on a piece of egg crate that is suspended on top of an aquarium. When the pinheads hatch, they will fall through the grid into the clean enclosure below. Use a moist sponge on a plate or an inverted test tube with a sponge on one end to provide water to the pinheads. Make sure crickets are maintained at moderately high temperatures 80–85 F (27–29 C) at relatively high humidity. A maintenance diet of high-calcium feed or chicken scratch can be used to feed growing crickets.

As with all insects, place a rock or other small emergent object in all amphibian water dishes so that crickets will not drown. Moreover, be aware that crickets have been known to chew on the skin of amphibians. Placing a bottle cap with cricket feed in your terrarium will help prevent hungry crickets from injuring amphibians.

19 Available from feed stores or the internet
20 Such as Mazuri® high-calcium feed or Zeigler's® Hi-Cal cricket diet

Fruit Flies

Fruit flies are an excellent food source for smaller and recently metamorphosed amphibians (e.g., they have relatively more calcium than pinhead crickets). Culturing fruit flies takes practice and a bit of finesse, but the concept is straightforward. Yeast grown on a sugar/starch source produces a sugar-alcohol that in turn supports the complete life cycle of the flies. A dry potato-based medium is hydrated in the bottom of a container by adding an approximately equal part of water (by volume) to the medium (Figure 14). There are two different species of fruit flies commercially available to culture: *Drosophila hydei* (the larger of the two species) and *Drosophila melanogaster*. Both species are sold as wingless or apterous (mutant) forms, making them easier to culture and for amphibians to catch. If both species are raised in your facility, raise the cultures separately and do not mix them together.

Figure 14. Left, a mature fruit fly culture made with the Carolina Biological Formula 4-24 (blue). Note the paper towel piece inside the jar used to increase surface area for the flies, maggots, and pupae. A muslin top allows the flies to breathe. Right: A Metro rack containing fruit fly cultures for the collection. Cultures last for approximately one month after which time they should be retired. Each crate is labeled with the date the culture was made. Crates should be rotated regularly. Jars should be well cleaned with hot water and bleach, rinsed thoroughly and allowed to dry before making new cultures. (Photos: J. Pramuk)

Fruit fly medium can be made out of readily available materials or purchased commercially.

Staten Island Zoo's Homemade Fruit Fly Medium (C. Eser)

3 cups instant potatoes flakes (without butter or other flavor additives)[21]
2 teaspoons brewer's yeast
Mix above dry ingredients together

4 cups of boiled water
2 teaspoons molasses
Mix above wet ingredients together

Add dry and liquid contents together and stir. Divide the mixture equally into containers (e.g., one liter plastic deli containers or Ball jars). Sprinkle top of each mixture with methyl paraben[22] (a commercially available preservative) and brewer's yeast. Use about one teaspoon per container. For the brewer's yeast, add approximately 1/2 teaspoon per container. Yield: enough for 6 one liter containers.

21 Such as Potato Buds
22 Available from Ed's Fly Meat or Carolina Biological Supply

Atlanta Botanical Garden Fruit Fly Culture (R. Gagliardo)

Dry mix: 28 oz potato flakes, 3 cups powdered sugar, 8 oz Brewer's yeast

Liquid mix: 1/2 and 1/2 water and white vinegar

By volume, mix 1 part dry mix and 1 part liquid. Sprinkle about 10 grains of baker's yeast on surface. Rinse sides of container with water and while doing so, wet the baker's yeast. Wait 2 minutes for initial ethanol release from hydrated yeast to dissipate, then inoculate surface of the mixture with a solid layer of fruit flies.

There are consistent, commercially available fruit fly media that come in white or blue.[23] They are identical except for blue dye is added to aid in seeing larvae as they burrow in the substrate. Antifungal agents are incorporated into the mix already, but if cultures are kept in hot, humid conditions (or if cultures suddenly become moldy during the warmer summer months), it may be necessary to add a little additional methyl paraben or other food preservative to each culture.

Recipe for Fruit Fly Culture (I. Hiler)

1. Harvest flies from active cultures into a mason jar. Make sure jars you will use for new cultures are clean and dry. Cover with a secure lid. Select healthy cultures that are fungus and mite free. Harvest enough to add approximately 50 flies per new jar. Set aside.
2. Clean off workspace with disinfectant and dry thoroughly. Set up jars. Put 1/3 cup fruit fly medium at the bottom of each jar.
3. Add 1/3 cup (or slightly more) water to each jar. There should be no dry flakes left (dry flakes indicate you have not added enough water).
4. Sprinkle a small amount (approximately 1/8 teaspoon each) of fresh yeast and vitamin powder on top of the moist medium. To keep them fresh, both of these additives should be stored in a fridge. Note: The yeast provided with the medium is usually stale and should not be used. Too much yeast will create an excess of CO_2 and kill the colony.
5. Add a piece of slightly crumpled, moist paper towel (approximately 1/3 of a sheet) or a moist coffee filter on top of the medium (unbleached paper is preferable). This will allow more surface area for the flies and their offspring.
6. Sprinkle approximately 50 flies into the jar and immediately place a clean piece of muslin or coffee filter on top and close with threaded brass ring lid to prevent flies from escaping.
7. Place jars on a shelf, set in a warm area clear of draft, and labeled with a date.

If your cultures crash and you live in the US or Canada, you can always order your entire fruit fly supply from Ed's Fly Meat. This is an expensive option, but they do have a reduced rate if you have a standing order (ask for *Monthly Meat*).

23 Such as Formula 4-24 Drosophila Medium®, available from Carolina Biological Supply.

House Flies
Houseflies (*Musca domestica*) can be easily reared out from maggots purchased from any bait company. The maggots pupate within a matter of days at room temperature. Once the flies emerge from the pupae, they can be refrigerated for a few hours to calm them down prior to placing them in an amphibian enclosure. Tree frogs in particular relish houseflies. You also can culture your own, but the person-hours needed and stench that emits from housefly cultures makes it easier to purchase the larvae from commercial producers.

Mealworms
Mealworms are commonly available from most insect suppliers. There are two commonly available species, *Tenebrio molitor* (a smaller larva) and *Zoophobas morio* ("giant" mealworms or "Super" worms). Both are the larval stage of tenebrionid beetles, which are hearty, easy-to-keep insects. Larvae can be maintained in cricket meal or bran in a large plastic open container (e.g., a plastic dishwashing pan). To prevent escapes, use packing tape or petroleum jelly in a band around the top of the container. Slices of apple and/or potato make good sources of moisture. Adult beetles will lay eggs in the substrate and your next generation will start anew. The larvae can be easily harvested from the media by using a kitty litter scoop or similar device. The media will need to be replaced once it has been consumed by the larvae and replaced with powdery frass (insect poop). Note that many people are or become allergic to the powdery frass, so wearing a dust mask is recommended when cleaning out the mealworm tub. Larvae can be harvested manually or with forceps. Note that these beetle larvae have a thick exoskeleton, which is difficult for many amphibians to digest. The giant mealworms should only be fed to the largest species of amphibians [e.g., African bullfrogs (*Pyxicephalus adspersus*)]. Additional information on rearing mealworms is available elsewhere (Nehring, 1996).

Wax Moth (wax worm) Larvae
Wax moth (*Galleria mellonella*) larvae are parasites of beehives and eat the wax and honey of the hive. Wax moth larvae are a very rich source of lipids (fats). They are good to feed to animals that are underweight or those being bulked-up for breeding. Do not over-feed wax moth larvae, as too much fat can lead to lipidosis (fatty deposits on the lens of the eye or liver) or death.

Wax Moth Culture Medium (I. Hiler)
16 oz. Gerber Dry Cereal®
16 oz. dry oatmeal
32 oz. honey
4 oz. glycerin

Put dry cereal and oats in a blender or food processor and grind into a fine powder. Put the powder in a large mixing bowl with glycerin. Add honey and hand mix to incorporate all ingredients until a sticky-dough forms. To start a colony, add a dozen or so late-age larvae or moths. Their life cycle is about ten weeks. Usable worms for small frogs will be available from the culture for about three and half weeks. Yield: approximately 30 portions.

Roaches
Roaches can be easily cultured in a well-secured aquarium by placing a layer of newspaper on the bottom and providing cork bark pieces for increased surface area. The roaches are fed a diet of vegetables and fruits, and although they are relatively slow to reproduce they provide a nice alternative to crickets. While conventional feeder insects such as domestic crickets and mealworms are readily available to amphibian keepers, roaches can be easily cultivated and

can offer an important supplement to a quality and varied amphibian diet. Three species are currently propagated in large numbers as food items:

Lobster Roach (*Nauphoeta cinerea*)
Probably the most commonly-bred of the feeder roach species. Adults are comparable in size to large crickets (23–26 mm), although they have a greater *meat* to exoskeleton ratio than adult crickets. Adults of both sexes are winged, but flightless. Lobster roaches can easily climb glass, so measures to keep them contained should be employed. A 1-2 inch wide band of petroleum jelly or a product called Bug Stop[24] will work perfectly well. This species is a very prolific breeder with a short time between generations. A newly hatched nymph can reach breeding age within three months. Adult females produce clutches of 20–30 nymphs at 30–60 day intervals. The female produces an ootheca (egg case); however, she pulls it back into her body for incubation. Individual roaches can live for 12–24 months.

Discoid Roach (*Blaberus discoidalis*)
The discoid roach is also easily propagated. The adults (35-45 mm) are ideal for larger species of amphibians, but the nymphs are useful for smaller and medium-sized species. Both sexes are winged, but flightless, and they cannot scale smooth surfaces like glass. This species is not as prolific as the smaller lobster roach and colonies can take some time to become established; however, once they are established they can be quite prolific. Breeding age is reached within 4–5 months and the life span is 12–18 months. Young are born live, remain hidden under the mother for several hours or days, and then disperse.

Orange-Headed Roach (*Eublaberus prosticus*)
This is a larger species (38–48 mm) that is a prolific breeder. Sexual maturity occurs between 3-5 months. These roaches can live up to 24 months. Due to aggression, house these insects in as large an enclosure as possible. If not provided with adequate space, moisture, and high-protein food, orange-headed roaches become cannibalistic, biting the wings of other adults and eating freshly-shed adults or nymphs. Ensure that plenty of water is available at all times in the form of chopped fruits or vegetables. These roaches are winged, but flightless and incapable of climbing glass or smooth surfaces.

Depending on the production needs, colonies may be established in containers ranging from 10-gallon aquariums to plastic containers (30 gallons or larger). Cardboard egg-crates may be stacked in multiple layers for furnishings. No substrate is necessary, and in fact may make collection more difficult. The roaches will make refugia out of the multiple layers of egg-crate.

Temperatures should be kept at 80–90 F (27-32 C). All three roach species discussed can handle temperatures lower than this, however reproduction declines dramatically at temperatures lower than 80 F (27 C), or may cease completely.

Finely ground premium dry dog food or crushed high-quality tropical fish flakes should be offered at all times in a shallow dish. This part of the diet should be kept dry at all times to prevent potentially harmful mold growth. As a source of moisture and vitamins, a variety of chopped vegetables should be offered at least three times per week. Remove unconsumed vegetables after 24 hours to prevent mold in the colony.

Smaller roach colonies should be cleaned weekly, and more frequently for larger ones. Due to their usually dry fecal pellets, sweeping out the enclosure is often sufficient, although

24 Available from Pro Exotics

disinfecting the container should be undertaken every 1–3 months depending on the number of roaches in a colony. Egg-crates should also be replaced as they become coated in feces.

Springtails
Springtails are essential to culture if you are rearing small species or recently metamorphosed amphibians. Springtails (*Collembola* spp.) are white or grey-colored hexapods, small insects that live in the soil and eat fungi, bacteria, and plant materials. Some species are carnivorous, eating nematodes and other springtails. These tiny insects are often seen in the soil of potted plants. Starter cultures can be purchased commercially.[25] Springtails can be cultured in a plastic sweater box with a tight-fitting lid. Add the starter culture to a 1.5-inch (4 cm) layer of commercially prepared, sterilized potting soil (free of added fertilizers). Mix soil with sphagnum moss and keep it wet. Sprinkle brewer's yeast and a little fish flake food on the top of the soil. Place clumps of tree fern or cork bark on top of the soil (Figure 15). The springtails will colonize the pieces of bark, which can then be rotated through the animal enclosures and back into the springtail colonies. Springtails can be kept at a range of temperatures and usually will do fine at regular room temperatures. For more information about springtails refer to Emmer (1993).

Figure 15. A springtail culture in a plastic shoebox. Cork bark pieces or fern fiber can be used to inoculate amphibian enclosures with springtails, by simply transferring pieces of bark into a terrarium. (Photo: J. Pramuk)

Confused Flour Beetles (*Tribolium* spp.)
These beetles can be fed as larvae or adults to amphibians and are very easy to rear. Starter cultures can be obtained commercially.[26] To set up a culture of beetles, fill a plastic shoe or sweater box halfway with unbleached, enriched flour. Introduce a starter culture of beetles and larvae. Within a month or two, larvae and beetles can be harvested by using a screen or flour sifter. Offer the beetles and larvae on a shallow dish on the floor of the amphibian tank. Not all animals will eat beetles; however, they are a good alternative to pinhead crickets and fruit flies for smaller amphibians.

Earthworms and White (Grindal) Worms
Earthworms and white worms are a rich source of lipids and protein and are a great food item for underweight amphibians. Earthworms have one of the best calcium:phosphorous ratios of any feeder invertebrate. Earthworms are readily available from any bait supplier in a variety of sizes. A relative of earthworms, white worm starter cultures can be obtained commercially.[27] To set up white worms, use the substrate and enclosure described above for springtail cultures. White worms should be kept cooler than most cultures (approximately 60-68 F/15–20 C). To culture white worms, place a slice of wheat bread on top of the soil after adding worms. Set a small piece of plate glass or acrylic on top of the bread, allowing worms

25 Available from Ed's Fly Meat or LFS Cultures
26 Available from Ed's Fly Meat or LFS Cultures
27 Available from Aquabid.com and LFS Cultures

to congregate between the bread and glass. Worms can be rinsed or scooped off onto an amphibian-feeding dish. If the room where the culture is housed is warm, consider acquiring an electronic wine cooler, which can be maintained at 60-68 F (15–20 C).

Blackworms (*Lumbriculus variegatus*)
Blackworms are a nice, nutritional food source to feed salamanders and smaller frogs. They can be ordered from several suppliers or acquired at local pet stores. The worms can be maintained in a plastic sweater or shoebox with a secure lid, covered with just enough water to submerge the worms, and stored within a refrigerator. The worms need to be rinsed with fresh water everyday. Dead worms will float out with the effluent water and can be rinsed down the drain. The worms will clump together and are easy to harvest. Worms can be placed on a shallow feeding dish for terrestrial animals.

Fish and Rodents
Fish such as minnows, goldfish, or rosy reds can be fed to larger species of amphibians.[28] Feeding frozen, thawed fish to amphibians exclusively can lead to thiamine (Vitamin B_1) deficiency. It is better to vary the diet or avoid feeding frozen fish altogether.

Rodents include the following sized mice or rats: neonates (pinkies), fuzzies, hoppers, and adults. They can be fed to larger amphibians, although they should be offered sparingly due to their very high fat content. Neonates, in part because of the mother's milk in their stomachs, are particularly fatty and cholesterol-laden. Overfeeding these items to amphibians can lead to obesity and related health problems such as fatty liver, kidney failure, gout, and lipidosis (often manifested in amphibian eyes as unsightly opaque deposits on the lenses). However, if an animal is underweight, offering neonate mice or wax moth larvae are effective for rapidly boosting weight. Rodents should be offered dead rather than alive to reduce chances of amphibians being injured.

NATURAL HISTORY AND BEHAVIOR

Behavioral considerations cannot be overstated, promoting normal behavior in captive animals ultimately leads to greater longevity and breeding. Replicating natural habitats and parameters, providing natural food items, and utilizing behavioral enrichment techniques encourages natural activities (i.e., basking, perching, feeding, and reproduction). Behavioral enrichment is a dynamic process that alters an animal's environment. By providing stimuli to offer choices and to encourage natural behaviors, an animal's welfare is enhanced. Although amphibian brains lack the highly developed cerebrum that is common to mammals, amphibians have natural behaviors that can be encouraged in captivity and that are thought to increase their quality of life and overall health.

Behavioral enrichment for amphibians focuses on methods that replicate the natural environment as much as possible, inducing natural behaviors, and does not focus exclusively on modifying their enclosure or environment. One of the most important natural behaviors for all animals is feeding and the feeding response. One form of enrichment is to offer a wide variety of prey items, providing a broader nutritional base and creating a more complex captive existence. However, not only the variety of food but also the way food is offered can promote natural behavior. Food items can be hidden or scattered throughout enclosures to encourage foraging and hunting behaviors; conversely offering food at times when the animals are not active (sleeping) will allow food items to evade predation. Most amphibians are nocturnal, making them difficult to exhibit, feed, and monitor during normal daylight hours. Reverse lighting can be employed using light timers, although it can be difficult to

28 Such as cryptobranchid salamanders, Surinam toads (*Pipa* spp.), horned frogs (*Ceratophrys* spp.), bullfrog species, and large toads (*Rhinella* spp. or *Rhaebo* spp.), etc. Use forceps for feeding horned frogs and African bullfrogs to avoid being bitten by their odontoids (fang-like projections on the lower jaw).

force the biological clock of an amphibian to mirror that of diurnal hominids. It is important to keep in mind "what," "how," and "when" we feed different species and keep in mind the difference between "keeper comforts" and "critter comforts." Cricket and fruit fly feeders can be created to release prey items into an enclosure slowly. These feeders are most useful for ant feeders such as dendrobatids and microhylids, which often hang out by ant mounds in the wild in an ambush strategy to catch their food. Another form of behavioral enrichment is training amphibians to *station* (to come to a designated location), enabling veterinarian and keeper staff to weigh or examine the animals without being handled, thus lessening potential for trauma and stress. Although this may sound like an impossible task for an animal as small as an amphibian, through hard work, it can be accomplished: keepers at Disney's Animal Kingdom trained dendrobatid frogs to go to a weigh station at the sound of a clicker; while keepers at Louisville Zoo conditioned mandarin newts (*Tylototriton shanjing*) to respond to the sound of tapping metal forceps to be fed.

Reproductive aspects are also of concern. Many amphibians are territorial in the field and in captive situations can form dominance hierarchies. Male dart frogs and *Mantella* species will set up territories and combat for females. Even though dart frogs are usually solitary creatures, both female and male dendrobatids will become territorial during breeding season. Careful observation of collection animals aids in determining sex ratios and monitoring combat levels. Plethodontid salamanders are highly olfactory and use pheromones to mark territories and attract females. Enclosures with residual pheromones from an earlier inhabitant may cause stress to a newly introduced animal. The size and lay out of enclosures can affect behavior. Large or active species, such as some tree frogs (hylids), African goliath frogs (*Conraua* spp.) or Neotropical rain frogs (*Eleutherodactylus* spp.) require a lot of space. Enclosures that are too small can lead to abraded rostrums, other trauma, and even death. Live plants, cork tubes, and other "furnishings" serve as good perching and hiding areas that not only provide a sense of security for the animals and potential egg deposition sites but also a way to prevent long jumps into the sides of the enclosure. PVC tubes partially buried make very secure hiding spots for ranids and some salamanders. In addition, consider substrates as vehicles for stimulating natural behavior. Fossorial species such as caecilians and the Mexican burrowing toad (*Rhinophrynus dorsalis*) are far more at home in several inches of damp peat moss than on paper towels.

Disposition should be considered as well. For example, ambush predators such as African bullfrogs (*Pyxicephalus* spp.) and horned frogs (*Ceratophrys* spp.) cannot be part of a multi-specimen enclosure, because they will consume anything that fits into their mouths, including conspecifics (cannibalism). For the sake of natural history accuracy it is recommended that multiple species be exhibited together only if they coexist in nature. Nothing completes these types of enclosures better than living plants that the species may encounter in nature. When geographically correct plant species are not available, substitute others that fulfill the need, especially in off-exhibit areas. This ultimately leads to more content and healthy amphibians.

Amphibian Breeding

Amphibian reproductive cycles are closely linked to their physical and biological environments. Many effective breeding programs manipulate environmental cues such as temperature, humidity, and photoperiod. Altering these factors on an annual cycle that mimics the species' natural latitude is a good starting point for most amphibians.

Cycling

Cycling, an artificial physical conditioning, is a response to seasonal environmental changes (rainy/dry or cold/warm) that occurs naturally in the wild and varies by species. These physiological changes need to occur to induce viable egg laying or sperm production. A period of one to four months of lowered temperatures followed by a gradual warming period can induce breeding in many species of amphibians. For this to occur, animals should

undergo a period of fasting prior to being cooled down. At the end of the cooling period, the photoperiod and the temperature should be increased gradually. Environmental temperatures can be lowered to minimum of 50 F (10 C) for species from the temperate zone and 64-68 F (18–20 C) for tropical species. Most animals are not torpid under these conditions and remain at least slightly active, requiring clean water every day. In tropical regions of the world, breeding condition is often onset by a rainy season. Misting systems[29] attached to a timer can replicate rainy conditions and synchronization to local rain events cues an animal's instinctual reproductive response to barometric changes.

Hibernation or Brumation
Brumation is a state similar to hibernation in which an amphibian will dramatically reduce its metabolism and food intake although it may still drink. Both hibernation and brumation are a response to cool temperatures but in brumation the animal's response does not exhibit the extreme torpor of a hibernating animal. To simulate winter conditions, many amphibian culturists use small refrigerators or wine coolers to brumate their animals over the winter season. Make sure that sufficient water or moisture is provided during this period, as animals continue to require it.

Assisted Reproduction
If breeding cannot be achieved through cycling methods, another widely used method to induce reproduction is to administer exogenous hormones (e.g., human chorionic gonadotropin or synthetic analogues of luteinizing hormone-releasing hormone injections). Assisted reproduction is covered in detail elsewhere (Whitaker, 2001).

Eggs
Eggs should be treated with utmost care. All amphibian eggs possess layers of semi-permeable membranes that surround the ovum. Because they lack a hardened shell, they are essentially part of their surrounding aquatic environment. Even direct-developing eggs with relatively tough outer capsules uptake moisture from the damp substrate and should be kept moist at all times. If at all possible, avoid the temptation to move egg masses until the larvae hatch. Instances where this may not be possible include environments where the eggs can be consumed by other frogs or animals in an enclosure. Small, wet dip nets or spoons work well for transferring egg masses. Small tadpoles can be transferred with a turkey baster.

Eggs may be laid in water in large clumps, strings, or in small parcels at different sites. Many salamanders lay their eggs attached to sticks or vegetation in the water. There is much interspecific variation in the form and number of eggs laid, and the physiochemical properties of the eggs vary according to where they develop in the environment. See Duellman and Trueb (1994) for a thorough review.

Amphibian eggs have an animal and a vegetal pole (Figure 16A). In most cases, eggs of species whose embryos are exposed to sunlight are pigmented with melanin over the animal hemisphere. In contrast, most eggs that undergo development in concealed sites lack pigment and are light-sensitive (e.g., mantellas), as are the eggs laid on the undersides of leaves (e.g., those of *Phyllomedusa, Afrixalus,* and *Hyperolius*).[30] Yolks may be creamy-yellow, pale-grayish yellow, or in cases where the eggs are laid on leaves, pale green. Within several hours to a few days, early signs of development should be apparent, such as a clearly defined yolk plug (Figure 16B) and elongation of the embryo (Figure 16C). If the entire clutch or an individual egg becomes fuzzy, these eggs are bad and should be discarded. This likely is the result of eggs that were not fertilized by a male. This could be caused by several factors

29 Such as ProMist® products
30 These eggs should be shielded from light and if relocated, set in darkened areas or containers. Avoid flash photography if these eggs are valuable.

including stressed animals (overcrowding), insufficient number of males to incite territorial behavior, poor nutrition, improper environmental conditions, or young inexperienced males.

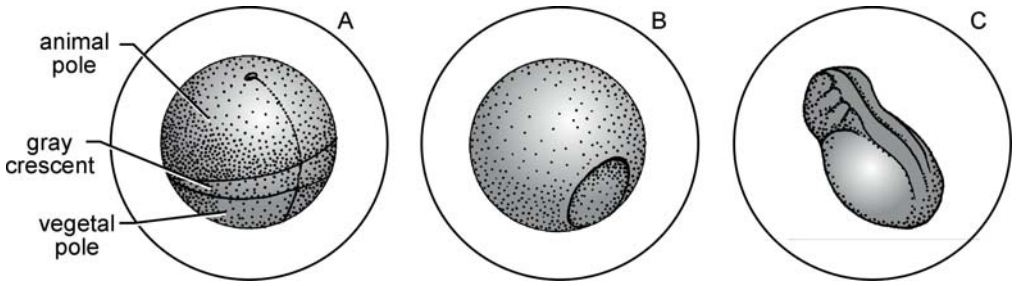


Figure 16. Amphibian eggs and an embryo in early stages of development. Within the first few days or even hours, signs of development should be visible. Earlier signs include clearly defined animal and vegetal poles (A), gastrulation (B) and (C) elongation of the embryo and neural fold development (the neural folds will become the spinal cord of the larva). Moldy (fuzzy) eggs will not develop and should be discarded. (Drawings: J. Pramuk)

The capsules of eggs laid in water will immediately swell by the uptake of water. Oxygenation of eggs is critical to their development and there is a continual increase in oxygen consumption throughout development. It is important to provide sufficient oxygenation, especially if there is a large clutch of eggs in a single enclosure. Amphibian embryos and larvae generally excrete nitrogenous wastes as the most toxic form ammonia. Additionally, amphibian embryos will develop normally only within specific limits of salinity and pH. Generally, development will occur faster in warmer environments but will plateau at a certain point (the ideal temperature) and eventually decrease as temperatures become too warm. Ensure the health of developing eggs and embryos by performing frequent water changes and water quality tests. Again, read up on collection species to ensure that set-up is optimized for the health of eggs and larvae.

Larvae Rearing

Read up on collection species before attempting to breed and rear them. Survival of most free-living amphibian larvae (tadpoles) is density-dependent. Although most tadpoles primarily consume plant material, many are omnivorous and some species are cannibalistic.[31] Larval mouthparts and digestive systems are adapted for specific diets and therefore a plant eater for example, probably will not survive on a diet of animal matter. Plant eaters have a longer digestive tract, which enables them to break down cellulose effectively.

Water quality is extremely important for amphibian larvae. Water should be purified or, at the minimum, de-chlorinated if the municipal water source is relatively clean (see Water section above). Some species of frogs are adapted to living in tannic environments, such as in puddles of water on the floor of a tropical rainforest. These species can be reared in dilute *tadpole tea* that is brewed to mimic these tannic conditions and have natural antibacterial properties to prevent the water from becoming fouled.

Tadpole Tea (Pramuk and Hiler, 1998)
1 oz. Alder cones (*Alnus* spp.) (Figure 17)
1 oz. German peat moss[32]
2 quarts rain water

31 Larval cannibalism may require some species [i.e., green and black poison dart frog (*Dendrobates auratus*)] to be held individually.
32 Such as Eheim Ltd.

Add all three ingredients to a medium saucepan and simmer for about twenty minutes. Let cool. Add 0.5 cup of *tea* to 5 gallons of water. Yield: 40 gallons of tadpole water.

Figure 17. Left, Indian almond leaves and right, alder cones. Both of these plant products can be used to increase tannins in tadpole water or in the substrate of an enclosure to limit bacterial growth and mimic tannic conditions seen in tropical forests. Dendrobatid tadpoles in particular do well reared in tannic water. (Photo: J. Pramuk)

Another trick adopted more recently by dart frog breeders is the use of Indian almond leaves in tadpole culture (Figure 17).[33] Fish hobbyists breeding *Betta* have used these leaves to reduce bacterial load in the water. Indian almond leaves also are attractive and can be used on the floor of a tropical frog enclosure either crushed or whole to provide antimicrobial tannins to the enclosure. These have been particularly effective with mossy frogs (*Theloderma corticale*).

Water cleanliness can be maintained via mechanical and/or biological filtration, frequent partial water changes, or both. This is directly correlated with container size and volume.

Figure 18. Plastic shoebox-type containers are perfect for raising small groups of dendrobatid, centrolenid, or hylid larvae. These containers are not hooked up to filters and the water therefore will need to be changed at least partially on a regular basis. (Photo: R. Gagliardo)

Water-changes should utilize water of the same temperature as found in the tadpoles' enclosure. Tadpole enclosures can range from an aquarium with a filter for an entire clutch of offspring, to individual plastic shoebox containers (Figure 18), or deli cups used to house individual larvae. Tadpoles can also be held in a partitioned plastic tray (buttoner organizer) with the bottom replaced with screen (glued into place with silicone or a hot glue gun). Sheets of perforated PVC available from hardware stores can also be used to construct partitioned tadpole trays (Figure 19). These trays serve as a sieve that can separate the

33 Leaves can be obtained through dart frog breeders or in bulk from commercial vendors like Aquabid.com

tadpoles from one another, but can allow them to be held in a common filtered body of water. This set-up offers the advantage of reducing cleaning duty, but risks sharing pathogens across the whole clutch (i.e., if one gets sick, the whole group is exposed).

Figure 19. A tadpole tray constructed of perforated sheets of PVC. The tray is sitting in an aquarium that is connected to an aquarium filter. This set up was photographed at the Toledo Zoo. While easy to maintain larger numbers of tadpoles separately, the re-circulating water could allow one diseased tadpole to infect the entire system. (Photo: J. Pramuk)

Regardless of whether the tadpole aquarium has a filter, regular water quality tests need to be performed and water will need to be changed periodically to reduce waste buildup. It is recommended that partial water changes are performed whenever the water is slightly cloudy, fouled, tadpoles are lingering near the surface, and/or waste is settling at the bottom of the tank. Replace approximately half to one-third of the water per cleaning event. If a filter is used, ensure that the current is not strong enough to draw the tadpoles up into the filter. A filter sponge can be formed around the influent end of the filter to slow intake and to prevent tadpoles from being killed. Once the hind limbs are well developed and there is evidence of forelimb development, provide a way for metamorphosing froglets to climb onto land, such as by reducing water volume and tilting the tank or providing a sloped substrate so that froglets can crawl onto a gradually exposed surface. Not providing a land surface for froglets or newly metamorphosed salamanders may result in drowning.

Larval Foods

Improper or insufficient tadpole nutrition can lead to metabolic and developmental problems such as spindly leg syndrome, which leads to permanently deformed and often crippled adults. Too much food at one time can foul the water and kill larvae. Research the feeding method employed and natural diet for the targeted species in advance.

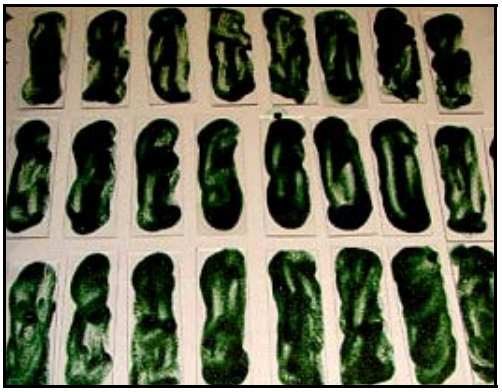


Figure 20. Feeding plates made from Sera Micron® paste smeared onto microscope slides and allowed to air dry prior to feeding. (Photo: R. Gagliardo)

Appropriate larval food includes the use of:

- Tetramin® tropical fish food flakes and tablets for dart frog tadpoles and those of many other species. Break tablets into quarters or smaller chunks depending on the portion tadpoles can eat within approximately ten minutes. Larger food items can be ground with an inexpensive coffee grinder.
- Sera Micron® and/or finely ground spirulina (blue green algae) for filter feeders such as some hylid tadpoles.
- Sera Micron® smeared onto a feeding plate (i.e., microscope slide, Petri dish, rocks, or other inert material) allowing it to dry, and placing it at the bottom of the tank for grazing species (Figure 20).
- Mazuri® Amphibian and Carnivorous Reptile Gel is a nutritionally-complete gel diet that is prepared from a powder. Some amphibian tadpoles will consume the gel, but use sparingly as it fouls the water.
- Rangen, Inc. Salmon Diet: Used with great success by the Indiana University *Axolotl* Colony for feeding axolotls (*Ambystoma mexicanum*). Larvae are fed from 4 cm to adult.
- Although extremely labor intensive, diatoms (green-brown algae) have been used at the Detroit Zoo's National Amphibian Conservation Center as a tadpole food. A protocol for rearing diatoms is presented in Poole (2006).
- Homemade *Zippy Flakes* tadpole food is an excellent larval amphibian food. See recipe below:

> ***ZIPPY FLAKES* TADPOLE FOOD** (K. Zippel)
>
> Mix the following ingredients:
>
> 16 g Sera Micron® powder
> 8 g Klamath Lake blue-green algae (cyanobacteria)[34]
> 2 g Reptile/amphibian powdered vitamins such as Reptocal® mixed 1:1 with calcium carbonate.
>
> Add water slowly until powder just forms a thick paste. Spread onto a sheet of glass or flat plastic dish. Dehydrate in refrigerator for two days. Scrape off flakes with a knife into an airtight storage container.

VETERINARY MEDICINE

Quarantine

New animals coming from outside the collection should be quarantined for a minimum of 30 days (preferred quarantine duration is 60 days) ideally in a building separate from the rest of the collection. A quarantine period should provide sufficient time for any symptoms to appear that are not related to the stresses of transport. At the very minimum, there should be a dedicated room for amphibian quarantine. New acquisitions may appear healthy and exhibit normal behaviors, but may be harboring fugitive pathogens to which other animals in the collection could be naïve and susceptible. Moreover, undergoing transport is stressful to most animals, leading to dehydration, overheating, starvation, stress from cage mates, or physical trauma to skin or internal organs. These stresses may depress the immunity of specimens and make them more susceptible to infection. Ideally, refrain from handling animals for the first few weeks when they arrive to quarantine. Minimizing the amount of contact to that which is absolutely necessary provides animals with a relatively stress-free and much-need period for acclimation. Collect and analyze fecal samples during this period.

34 Available at most health food stores

NOTE: Wild-caught animals from areas of the world known to have *Bd* (e.g., many parts of the United States, Latin America, Africa, Australia, Europe, and Asia) or those that have had exposure to a cosmopolitan collection sometime during their transport or captive history should be tested and treated for this pathogen upon arrival (Nichols and Lamirande, 2000). Recently, a collection of *Hyla cinerea* received from a dealer in Florida appeared perfectly healthy yet tested positive for *Bd*. When the dealer was called to inform them that their animals were positive, they had not heard of *Bd*, nor were they particularly interested in learning how to cure it. One has to wonder how many animals in this dealer's warehouse were exposed to *Bd* and subsequently spread to other institutions and hobbyists throughout the world.

Small plastic aquaria work well for temporary quarantine enclosures.[35] Paper towels can be used as a relatively sterile and disposable substrate; this will allow quick visual examination of the isolated animals as well as ease of fecal collections. The substrate should be changed daily and retain enough water that the enclosure remains sufficiently moist between cleanings. Paper towels can dry out alarmingly quickly in low humidity environments so check several times throughout the day to ensure that sufficient water is present. Enclosures can be partially covered in plastic food wrap to increase humidity. Throughout the quarantine period, handling of animals and human activity in the room should be kept to a minimum. Ideally, these animals should be serviced by personnel without other amphibians in their daily routine, or else these animals should be serviced at the end of the workday to reduce risk of cross-contamination. For more information on quarantine protocols, see Chapter 3.

Parasites

Wild-caught and even captive-born and -raised amphibians likely will harbor parasites. Often, animal dealers will hold captive-born animals in a cosmopolitan collection prior to shipment. This situation can lead to infected animals that are shipped to unsuspecting recipients. Fecal samples should be examined throughout the duration of quarantine to assure that incoming amphibians are free of parasites and are healthy before being placed in a room with any other animals in the collection. Make sure that fecal samples are submitted to a veterinarian for examination. *Direct* and *float* fecal examinations can be performed in-house with a microscope and a few basic pieces of equipment. Three negative fecal exams should be observed prior to releasing an animal from quarantine. Keepers should routinely check fecals as a matter of good husbandry practice. Various parasite treatments may include fenbendazole-treated (Panacur®) food items, Drontal Plus® (praziquantel/pyrantel pamoate/febantel), or another medication administered orally (see Chapter 3 and Wright and Whitaker, 2001).

Biosecurity

Biosecurity involves three equally important aspects: 1) safety of the humans and scientists in an area; 2) decontamination/disinfection of field equipment (especially boots and nets) to prevent spread of a possible infectious agent to other sites and other animal populations; and 3) careful quarantining (isolation) of live, sick animals from all other populations in the field and in laboratory animal colonies (USGS, 2007). Strict antiseptic and quarantine-like measures should be taken to keep collections free of amphibian diseases and to reduce the potential spread of pathogens. This is important for all animals in the collection regardless of life stage, but is especially critical for animals in quarantine. Use a disinfectant such as household bleach (3-6% sodium hypochlorite) to a 10% dilution to clean tools, empty enclosures, etc. and make sure that all surfaces are rinsed thoroughly and are free of chemical residue [use bleach in well-ventilated areas and wear a personal protection device (PPD)]. Powder-free rubber, vinyl, or latex gloves should be worn when handling all amphibians and need to be changed between enclosures. Gloves should be moistened with a spray bottle or

35 Such as Critter Keepers® and Small Pal Pens®

other source of clean water before handling amphibians. Footbaths should be provided at the entry and exit of each room to reduce the risk of pathogens being transferred between rooms. Footbaths should be filled with an antiseptic such as 10% household bleach and should be replaced daily. Make every effort to eliminate pest animals (e.g., cockroaches, flies, rodents, or feral geckos) from amphibian rooms, which can be carriers of disease. Ideally, staff should wear a dedicated outfit or at least a dedicated lab-coat that for each amphibian room, which is washed daily. Plastic shoes or boots also work well as dedicated quarantine space footwear. See Chapters 2 and 3 for more information about this topic.

Amphibian Diseases and Treatment

There is no substitute for careful observation, so monitor animals closely everyday. These baseline data will provide invaluable information for evaluating the health of animals. At the first sign of unusual behavior or symptoms, contact a veterinarian with amphibian expertise. Often, hesitating even by a day to treat a sick amphibian will be too late for treatment.

Fecal tests may indicate the presence of parasites in the animals. Bloody stools also can be an indicator of parasite-infected animals. Most wild amphibians harbor parasites that are symbiotic and do not harm their hosts; however, bringing animals with a parasite load into captivity can disrupt this equilibrium, and the parasites, if unchecked, can kill their host. Effective anthelminthic and antiprotozoal treatments that can be prescribed by a veterinarian include levamisole, ivermectin, fenbendazole (Panacur®), praziquantel (Droncit®), pyrantel (Strongid-T®), and metronidazole (Flagyl®). *Bd* is a recently discovered disease that can be devastating to a captive collection and wipe out an entire collection in a matter of days. In some species however, symptoms may not be expressed and carriers of *Bd* can remain unidentified in a collection for years. The most commonly prescribed treatment is 0.01% Itraconazole (Sporonox®) soaks for ten minutes daily for ten days (Nichols and Lamirande, 2000).

For more information on controlling amphibian diseases in collections, refer to Chapter 2.

CONCLUSIONS

This guide is provided as a starting point for general amphibian husbandry. There are many other good resources available including those additionally cited below. We wish you luck in your amphibian propagation endeavors. Your work may be the last hope for some species to avoid extinction.

ACKNOWLEDGMENTS

The authors would like to thank Shelly Grow (AZA) and Vicky A. Poole (National Aquarium in Baltimore) for their invaluable assistance in formatting and editing this chapter. We also thank William Holmstrom (Bronx Zoo) and Joseph R. Mendelson, III (Zoo Atlanta) for valuable comments on the manuscript. Cathy Eser (Staten Island Zoo) and B. Ian Hiler (Aquarium of the Americas) kindly provided valuable information on insect diets, while Kevin Zippel and R. Andrew Odum (Toledo Zoo) provided expert information on water quality, culturing, and in other areas. Robert Hill (Atlanta Botanical Gardens) generously provided information on roach culturing.

RECOMMENDED AMPHIBIAN NATURAL HISTORY WEBSITES

www.amphibiaweb.org
A useful website maintained by UC Berkeley on taxonomy of amphibians. Usually has the most current list of amphibian species.

http://research.amnh.org/herpetology/amphibia/index.php
The American Museum of Natural History's Amphibian Species of the World.

Unfortunately, the taxonomy of the two above websites is often in conflict; however, the information in both is useful regardless of disagreements on which is the most widely-accepted amphibian taxonomy.

RECOMMENDED AMPHIBIAN CARE WEBSITES

www.amphibiacare.com
A great site for general amphibian care.

www.caudata.org
A detailed and informative site for salamander husbandry.

http://home.att.net/~kczippel/waterqual.html
A nice site on water quality as it relates to amphibian husbandry.

PRODUCT LIST AND SUPPLIER CONTACT INFORMATION

ROOM HEATING/COOLING UNITS

Sunpentown International
(800) 330-0388
www.sunpentown.com/wa12poacwihe.html

TERRARIUM SUPPLIES:

Twin Oaks/Glasscages.com: Custom enclosures
(615) 446-8877
www.glasscages.com

ExoTerra®, ZooMed®, and Kritter Keeper®: Enclosures available at pet supply dealers.

Autograph Foliages: Artificial plants
(216) 426-6151
3631 Perkins Avenue, Cleveland, OH 44114
www.autographfoliages.com/custom_made/floor_plants.html

Aquabid.com: Aquarium supplies, including Indian almond leaves
www.aquabid.com

HORTICULTURAL SUPPLIES

Tropical Plant Products, Inc.: Live tropical plants and supplies
(407) 293-2451
www.tropicalplantproducts.com

Tropiflora: Live tropical plants
(800) 613-7520
3530 Tallevast Road, Sarasota, FL 34243-3890
www.tropiflora.com

Agristarts Inc. (I-IV): Tropical plant tissue culture liners (plugs)
(407) 889-8055
1728 Kelly Park Road, Apopka, FL 32712
www.agristarts.com

Casa Flora, Inc.: Tissue culture liners of many types, natives, tropical, and ferns.
(972) 225-5210
P.O. Box 41140, Dallas, TX 75241
www.casaflora.com

Deroose Plants, Inc.: Bromeliads and other tropical plants
(407) 889-5228
4601 N. Rock Springs Road, Apopka, FL 32712
www.derooseplants.com

OFE International, Inc.: Moss and supplies for orchids and bromeliads
(305) 253-7080
P.O. Box 161081, Miami, FL 33116-1081
www.ofe-intl.com

Discoveries in Gardening: Premium quality sphagnum moss
New Zealand
(866) 241 9653 (international call free)
www.discoveriesingardening.com

Calwest Orchid Supplies: Sphagnum moss, cork bark, fern fiber
(800) 301-9009
11614 Sterling Avenue, Riverside, CA 92503
www.orchid-supplies.com

Black Jungle Terrarium Supply: Terra-Lite LECA, coco fiber, aquarium furnishings, plants, and ExoTerra® enclosures
(800) 268-1813
370 Avenue A, Turners Falls, MA 01376
www.blackjungle.com/

Hummert International: Horticultural supplies
(800) 325-3055
www.hummert.com

LARVAL AMPHIBIAN FOODS
Mazuri: Amphibian and Carnivorous Reptile Gel diet
www.mazuri.com

Ambystoma Genetic Stock Center: Rangen, Inc's. salmon pellets for axolotls
(859) 323-5679
101 TH Morgan Building, Lexington, KY 40506-0225
www.ambystoma.org/AGSC/food.htm

Sera-Micron: Powdered fry fish food (only available in small 0.6 oz jars)
(800) 659-1970
158 Keystone Dr., Montgomeryville, PA 18936
www.sera-usa.com

TetraMin® Tropical Tablets and flake foods can be obtained from many pet food suppliers.

VITAMIN SUPPLEMENTS
Arcata Pets: Reptocal®
(800) 822-9085
www.arcatapet.com

Drs. Foster and Smith: Reptocal®
(800) 381-7179
www.drsfosterandsmith.com

Guenter Enderle: Nekton®-Rep and Nekton®-MSA
(727) 741-3386
2340 State Rd., Clearwater, FL 33763
www.nekton.de

Vitamin-B Complex and One a Day® Men's Vitamins available from any drugstore

INSECT CULTURE
Aubuchon Hardware/Hardwarestore.com: 1 pint wide-mouth glass Ball jars
www.hardwarestore.com

Sefar America: Fine-mesh polypropylene screen for permanently mounting in fruit fly jar lids
(800) 995-0531

Ed's Fly Meat: Wingless fruit fly and springtail cultures, and deli cups
(877) 359-6328
www.edsflymeat.com

Carolina Biological Supply: Fruit fly cultures, prepared medium (Formula 4-24® - order by the case to receive a discount), jars, and miscellaneous supplies
(800) 334-5551
www2.carolina.com

Bioquip: Invertebrate collection equipment (e.g., black lights, butterfly nets)
(310) 667-8800
2321 Gladwick Street, Rancho Dominguez, CA 90220
www.bioquip.com

Armstrong's Crickets: Crickets, mealworms, wax worms, red worms, and nightcrawlers
(800) 345-8778
PO Box 125 West Monroe, LA 71294
www.armstrongcrickets.com

Bassett's Cricket Ranch, Inc.: Crickets and mealworms
(800) 634-2445
365 Mariposa, Visalia, CA 93292
www.bcrcricket.com

Fluker Farms: Crickets, fruit flies, and meal worms
(800) 735-8537
www.flukerfarms.com

Grubco: Crickets, fly larvae, mealworms, and wax worms

(800) 222-3563
P.O. Box 15001 Hamilton, OH 45015
www.grubco.com

L.F.S. Cultures: Springtails, microworms, tubifex worms, grindal worms, white worms, red worms, fruit flies, flour beetles, and mealworms
(662) 236-4687
P.O. Box 607 University, MS 38677
www.lfscultures.com

New York Worms: Crickets, earthworms, wax worms, butter worms, and fruit flies
(516) 759-3538
7 Germaine Street, Glen Cove, NY 11542
www.nyworms.com

Worm Man's Worm Farm: Butterworms, nightcrawlers, crickets, fruit flies, mealworms, Phoenix worms, roaches, soldier grubs, fly larvae, and wax worms
(732) 656-0369
PO Box 6947, Monroe Township, NJ 08831
www.wormman.com

PLUMBING

Pro Products: Specialized habitat control products including misting systems and heat panels
36 Split Rock Road Mahopac, NY 10541
(845) 628-8960
www.pro-products.com

Aquatic Eco-systems: Water filtration, pumps, RO systems, phosphate absorbing media, and glass drill bits
(407) 886-3939
www.aquaticeco.com

McMaster-Carr: Bulkhead fittings, ball valves, tubing, and miscellaneous plumbing
(330) 342-6100
www.mcmaster.com

U.S. Plastics: Plastics, bulkhead fittings, ball valves, tubing, and miscellaneous plumbing
1390 Neubrecht Rd., Lima, OH 45801-3196
(800) 809-4217
www.usplastic.com

North Coast Pets: Diamond-tipped glass drill bits and bulkhead fittings
(877) 231-7416
www.northcoastmarines.com

Spectrapure: RO systems and other filters
(800) 685-2783
2167 E. 5TH Street, Tempe, AZ 85281
www.spectrapure.com

Ecologic Technologies, Inc.: Rainmaker® misting system supplies
(410) 431-7106
P.O. Box 1038, Pasadena, MD 21123-1038
www.cloudtops.com/misting_system_index.htm

MISCELLANEOUS:

Forestry Supply: Equipment including data loggers
(800) 647-5368
205 West Rankin Street, Jackson, MS 39284-8397
www.forestry-suppliers.com

Ben Meadows: Equipment including data loggers
(800) 241-6401
Janesville, WI 53547-5277
www.benmeadows.com

Precision Weighing Balances: Weight scales
(978) 521-7095
www.balances.com

LITERATURE CITED

Andrews, C., A. Exell, and N. Carrington. 1988. The Manual of Fish Health. Tetra Press, Morris Plains, NJ. Pp 44-45.

AZA Amphibian Biology and Management Monograph: Compiled for students of AZA's Board of Regents *Amphibian Biology and Management* course. Learn more about this class at www.aza.org/prodev/.

Barnett, S. L., J. F. Cover, and K. M. Wright. 2001. Amphibian Husbandry and Housing *In* K. M. Wright and B. R. Whitaker (Eds.): Amphibian Medicine and Captive Husbandry. Krieger Publishing Company, Malabar, Florida. Pp 35–61.

Browne, R.K., R.A. Odum, T. Herman, and K. Zippel. 2007. Facility design and associated services for the study of amphibians. ILAR Journal 48(3):188-202.

Speare, R., L. Berger, Skerratt, L. F., R. Alford, D. Mendez, S. Cashins, N. Kenyon, K. Hauselberger, and J. Rowley. 2004. Hygiene protocol for handling amphibians in field studies. Amphibian Diseases Group, James Cook University, Townsville 4811, Australia. Pp. 4,

Berns, M. W. 1965. Mortality caused by kidney stones in spinach-fed frogs (*Rana pipiens*). BioScience 15:297–8.

Brookland, J., C. Hora, and N. Carter. 1985. Injury, damage to health and cruel treatment: present conditions in the shipment of live fauna. A Report by the Environmental Investigation Agency. Animal Welfare Institute and Humane Society of the United States, Washington. Pp. 36.

Cover, J. F. Jr., S. L. Barnett, and R. L. Saunders. 1994. Captive management and breeding of denrobatid and neotropical hylid frogs at the National Aquarium in Baltimore. *In* J. B. Murphy, K. Adler, and J. T. Collins (Eds.): Captive Management and Conservation of Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles, St. Louis, MO. Pp. 267–273.

Duellman W. E. and L. Trueb. 1986. Biology of Amphibians. McGraw-Hill Book Company, New York. Pp. 670

Emerson, K., R.C. Russo, R.E. Lund, and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. J. Fish Res. Board Can. 32(12):2379-2383.

Emmer, R. E. 1993. How to culture springtails. AAZPA 1993. Regional Proceedings. Pp. 520–524.

Frost, D. R., T. Grant, J. Faivovich, R. H. Bain, A. Haas, C. F. B. Haddad, R. O. De Sá, A. Channing, M. Wilkinson, S. C. Donnellan, C. J. Raxworthy, J. A. Campbell, B. L. Blotto, P. Moler, R. C. Drewes, R. A. Nussbaum, J. D. Lynch, D. M. Green, and W. C. Wheeler. 2006. The Amphibian Tree of Life. Bulletin of the American Museum of Natural History. Pp. 370.

Gerhmann, W. B. 1987. Ultraviolet irradiances of various lamps used in animal husbandry. Zoo Biology 6:117–127.

Grow, S. and V.A. Poole. 2007. Amphibian Conservation Resource Manual. Association of Zoos and Aquariums. Silver Spring, Maryland. Pp. 208. www.aza.org.

Lillywhite, H. B. 1975. Physiological correlates of basking in amphibians. Comp. Biochem. Physiol. 52A:323–330.

Nehring, N. 1996. Raising Mealworms. Learn how to create your own colony at home. Reptiles 7:108–115.

Nichols, D.K. and E.W. Lamirande. 2000. Treatment of cutaneous chytridiomycosis in blue-and-yellow poison dart frogs (*Dendrobates tinctorius*) (abstract). In Proceedings: Getting the Jump on Amphibian Disease, Cairns, Australia, 26–30 August 2000. Pp. 51.

Odum, R. A. and K. Zippel. 2004. Water Quality. Monograph for Amphibian Biology and Management. AZA 2004. Pp. 26.

Poole, V. A. 2006. Husbandry Manual: Panamanian Golden Frog, *Atelopus zeteki.* 2nd Edition. www.ranadorada.org.

Pough, F.H. 2007. Amphibian biology and husbandry. ILAR Journal 48(3):203-213.

Pramuk, J. and I. Hiler. 1998. An investigation into the obligate oophagy of *Dendrobates pumilio* tadpoles (Anura: Dendrobatidae). Herpetological Review 30:219–221.

Rabb, G.B. 2004. The Evolution of Zoos From Menageries to Centers of Conservation and Caring. Curator 47:237–246.

Smart, A. C. and I. G. Bride. 1993. The UK Trade in Live Reptiles and Amphibians: A report to the RSPCA on the nature and status of the reptile and amphibian pet trade between 1980 and 1992. The Durrell Institute of Conservation and Ecology, University of Kent at Canterbury, Canterbury, Kent, UK. Pp. 252.

Ultsch, G.R., D.F. Bradford, and J. Freda. 1999. Physiology: Coping with the environment. *In:* McDiarmid R.W. and R. Altig (eds.). Tadpoles: The Biology of Anuran Larvae. University of Chicago Press, Chicago. Pp. 189-214.

USGS. 2007. Collection, preservation and mailing of amphibians for diagnostic examinations. USGS National Wildlife Health Center Publication, Washington, D.C. www.nwhc.usgs.gov/publications/amphibian_research_procedures/specimen_collection.jsp

Wright, K. M. and B. Toddes. 2004. Amphibian Nutrition. Monograph for Amphibian Biology and Management. AZA 2004. Pp. 18.

Wright, K. M. and B. R. Whitaker. 2001 (eds.). Amphibian Medicine and Captive Husbandry. Keieger Publishing Company. Malabar, Florida. Pp. 499.

Whitaker, B.R. 2001. Reproduction. *In:* Wright, K. M. and B. R. Whitaker (eds.). Amphibian Medicine and Captive Husbandry. Keieger Publishing Company. Malabar, Florida. Pp. 499.

Zimmerman, E. 1986. Breeding Terrarium Animals, TFH Publications, Inc., New Jersey, USA. Pp. 384.

Zippel, K., R. Lacy, and O. Byers (eds.). 2006. CBSG/WAZA Amphibian *Ex Situ* Conservation Planning Workshop Final Report. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN 55124, USA. Copies can be ordered through the IUCN/SSC Conservation Breeding Specialist Group, 12101 Johnny Cake Ridge Road, Apple Valley, MN 55124 (www.cbsg.org)

ADDITIONAL RECOMMENDED LITERATURE

Allen, M. E., and O. T. Oftedahl. 1989. Dietary manipulation of the calcium content of feed crickets. Journal Zoo Wildlife Medicine 20:26–33.

Cochran, D. M. 1961. Living Amphibians of the World. Doubleday and Company, Inc., Garden City, NJ. Pp. 199.

Conant, R. and J. T. Collins. 1998. A Field Guide to Reptiles and Amphibians: Eastern and Central North America, 3rd Ed., Houghton-Mifflin, Boston, MA. Pp. 614.

Goncharov, B. F., O. I. Shubravy, I. A. Serbinova, and V. K. Uteshev. 1989. The USSR programme for breeding amphibians, including rare and endangered species. International Zoo Yearbook 28:10–21.

Elinson, R. P., E. M. del Pino, D. S. Townsend, F. C. Cuesta, and P. Eichorn. 1990. A practical guide to the developmental biology of terrestrial-breeding frogs. Biological Bulletin 179:163–177.

Feder, M. E., J. F. Lynch, H. B. Shaffer, and D. B. Wake. 1982. Field body temperatures of tropical and temperate zone salamanders. Smithsonian Herpetological Information Service, No. 52. Smithsonian Institution: Washington, D.C. Pp. 1–23.

Fletcher, C., (ed). 2007. Use of Amphibians in the Research, Laboratory, or Classroom Setting. The National Academies, Washington, DC. ILAR Journal 48(3):179-300.

Halliday, T. R. and K. Adler. 1986. The Encyclopedia of Reptiles and Amphibians. Facts on File, New York. Pp. 143.

Heatwole, H. and G. T. Barthalmus. 1994. Amphibian Biology, Vol. 1: The Integument. Surrey Beatty and Sons Pty. Ltd. Chipping Norton, Australia. Pp. 418.

Heatwole, H. and R. L. Carroll. 2000. Amphibian Biology, Vol. 4: Paleontology: The Evolutionary History. Surrey Beatty and Sons Pty. Ltd. Chipping Norton, Australia. Pp. 536.

Heatwole, H. and B. K. Sullivan. 1994. Amphibian Biology, Vol. 2: Social Behaviour. Surrey Beatty and Sons Pty. Ltd. Chipping Norton, Australia. Pp. 299.

Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster. 1994. Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington, D.C. Pp. 364.

Kaplan, R. H. 1987. Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis.* Oecologia 71:273–279.

Kluger, M. J. 1977. Fever in the frog *Hyla cinerea*. J. Thermal. Biol. 2:79–81.

Lannoo, M. J. (ed.) 1998. Status and Conservation of Midwestern Amphibians. University of Iowa Press, Iowa City. Pp. 526.

Lannoo, M. J. (ed.) 2005. Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley, CA. Pp. 1094.

Lötters, S, K, H, Jungfer, W. Schmidt, and F. W. Henkel. 2007. Poison Frogs Biology, Species and Captive Husbandry. Serpent's Tale/NHBD Edition Chimera Pp. 668.

Lillywhite, H. B., P. Licht, and P. Chelgren. 1973. The role of behavioral thermoregulation in the growth energetics of the toad, *Bufo boreas.* Ecology 54:375–383.

Masters, C. O. 1975. Encyclopedia of Live Foods. T. F. H. Publications, Inc.: Neptune, N.J. Pp. 336.

Mattison, C. 1982. The Care of Reptiles and Amphibians in Captivity. Blanford Press, Poole, England. Pp. 320

Mattison, C. 1987. Frogs and Toads of the World. Blandford Press, New York. Pp. 191.

Mattison, C. 1993. Keeping and Breeding Amphibians. Sterling Publishing Co. Inc., New York, USA. Pp. 224.

McDiarmid, R. W. and R. Altig. 1999. Tadpoles: The Biology of Anuran Larvae. University of Chicago Press, Chicago, IL. Pp. 436.

Moyle, M. 1989. Vitamin D and UV radiation: Guidelines for the herpetoculturist. *In* M. J. Uricheck (Ed.): Proceedings of the 13th International Symposium on Captive Propagation and Husbandry, Western Connecticut State University. Pp. 61–70

Murphy, J. B., K. Adler, and J. T. Collins (eds.). 1994. Captive Management and Conservation of Amphibians and Reptiles. SSAR Publications, Ithaca, NY. Pp. 408.

Myers, C. W., J. W. Daly, and B. Malkin. 1978. A dangerously toxic new frog (*Phyllobates*) used by Ember, Indians of western Colombia, with discussion of blowgun fabrication and dart poisoning. Bull. Amer. Mus. Nat. Hist. 161:307–366.

National Academy of Sciences. 1974. Amphibians: Guidelines for the Breeding, Care and Management of Laboratory Animals. National Academy Press: Washington, DC. Pp. 156. Available at the following link: http://books.nap.edu/openbook.php?record_id=661&page=R1

National Research Council. 1985. Guide for the Care and Use of Laboratory Animals. Washington, D.C.: U.S. Department of Health and Human Services. Pp. 162. Available at the following link: www.nap.edu/catalog.php?record_id=661

Noble, G. K. 1954. Biology of the Amphibia. McGraw-Hill, Dover, NY. Pp. 577.

Norris, D. O., and R. E. Jones (eds.). 1987. Hormones and Reproduction in Fishes, Amphibians, and Reptiles. Plenum Press, New York. Pp. 590.

Obst, F. J., K. Richter, and U. Jacob. 1988. The Completely Illustrated Atlas of Reptiles and Amphibians for the Terrarium. T.F.H. Publications, Neptune, NJ. Pp. 830.

Paine, F. L., J. D. Miller, G. Crawshaw, B. Johnson, R. Lacy, C. F. Smith III, and P. J. Tolson. 1989. Status of the Puerto Rican crested toad, *Peltophryne lemur*. International Zoo Yearbook 28:5–58.

Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C. Pp. 587.

Porter, K. 1972. Herpetology. W.B. Saunders Company, Philadelphia, PA. Pp. 524

Pough, F. H., R. M. Andrews, J. E. Cadle, M. L. Crump, A. H. Savitzky, and K. D. Wells. 2001. Herpetology, 2nd Edition. Prentice Hall, Upper Saddle River, NJ. Pp. 736.

Semlitsch, R. D. (ed.) 2003. Amphibian Conservation. Smithsonian Books, Washington, D.C. Pp. 324.

Staniszewski, M. 1995. Amphibians in Captivity. T.F.H. Publications, Neptune, NJ. Pp. 544.

Stebbins, R. C. 2003. Field Guide to Western Reptiles and Amphibians, 2nd Edition. Houghton-Mifflin, Boston, MA. Pp. 544.

Stebbins, R. C. and N. W. Cohen. 1995. A Natural History of Amphibians. Princeton University Press, Princeton, NJ. Pp. 316.

Taigen, T. L., F. H. Pough, and M. M. Stewart. 1984. Water balance of terrestrial anuran (*Eleutherodactylus coqui*) eggs: Importance of parental care. Ecology 65:248–255.

Tracy, C. R. 1976. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. Ecological Monographs 46:23–326.

Zug, G. R., L. J. Vitt, and J. P. Caldwell. 2001. Herpetology: An Introductory Biology of Amphibians and Reptiles. Academic Press, San Diego, CA. Pp. 630.

Chapter 2

Hygiene and Disease Control: Field and Captivity

John Kast[1] and Nick Hanna[2]

[1]*Assistant Curator of Ectotherms, Fort Worth Zoo*
1989 Colonial Parkway Fort Worth, TX 76110
jkast@fortworthzoo.org
Photos by J. Kast

[2]*Assistant Curator of Reptiles and Amphibians, Audubon Zoo*
P.O. BOX 4327 New Orleans, LA 70178
nhanna@audubooninstitute.org

INTRODUCTION

Amphibian populations are in decline worldwide. Causes of these declines range from direct factors such as habitat destruction, alteration, and fragmentation; introduced species; and over-exploitation to more complex and indirect mechanisms including climate change; ultraviolet-B radiation; chemical contaminants; infectious diseases; and deformities (AmphibiaWeb, 2007). Of all the potential causes for population declines only one, *infectious disease*, has been indicated as a cause for declines in both wild and captive populations (Bradford, 1991; Daszak et al., 2001; Pessier et al., 1999; Young et al., 2007).

There are many infectious diseases in amphibians, including viruses, bacteria, water molds, fungi, and parasitic agents. Several of these diseases have been documented to cause declines in the past. Red-legged disease (*Aeromonas hydrophila*) is a bacterial infection that has been shown to cause massive die-offs in the mountain yellow-legged frog (*Rana muscosa*) in 1979 (Bradford, 1991), while the parasitic fungus *Basidiobolus ranarum* has been implicated as the primary cause of extinctions of Wyoming toads (*Anaxyrus baxteri*) (Taylor

et al., 1999). Recently, emerging amphibian diseases such as the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; *Bd*) and iridoviruses (*Ranavirus* spp.) have become major factors in the deaths of wild and captive populations of amphibians worldwide (Mao et al., 1999; Young et al., 2007; Voigt, 2001; Zupanovic et al., 1998). Whether talking about disease transmission between captive amphibians within a single facility, between different facilities, or between wild and captive populations the conclusion is the same: *Our current husbandry practices for amphibians are no longer acceptable.* In fact, in many cases they may facilitate the spread of disease between different populations of amphibians. This chapter is designed to reframe basic hygiene and disease control in both the field and captive settings with consideration of the evolving threats facing amphibians and knowledge that all amphibian caretakers should be trained and mindful of the pathogens and diseases affecting the animals in their care (Browne et al., 2007; Densmore and Green, 2007). The references and a list of additional sources at the end of this chapter provide more information on amphibian diseases.

FIELD

Site Definition

When working in a field setting, the first precaution against the possible spread of disease among amphibian populations should be the definition of the site or sites. Defining the boundaries of a site or multiple sites can be difficult, and the mechanisms for defining borders may change from one site to the next.

The boundaries of a given site may be obvious and include natural or man-made barriers. Natural boundaries include changes in vegetation; geologic references such as mountaintops or ravines; or discrete bodies of water such as ponds, streams, wetlands, or watersheds. Individual bodies of water should each be treated as a separate site (NSW National Parks and Wildlife Service, 2001). Man-made boundaries consist of roads, development, or delineated protected wildlife areas such as state or federal refuges, parks, and reserves. Site definition is more difficult in instances where natural boundaries or man-made barriers are lacking. In these cases, the site boundary will need to be set by the researcher.

Whenever possible, plans should be made ahead of time to work in only one site per outing or have different groups working at each individual site to avoid cross-contamination. Everybody conducting fieldwork in the given site should be aware of the boundaries and how they are defined.

On-Site Hygiene and Disease Control

When conducting fieldwork, certain precautions should be taken to reduce the risk of spreading diseases. Disease can be transferred from different sites and into captive situations through several vectors including footwear, equipment, vehicles, and specimens.

Hygiene and disease control are controlled to a large extent by proper cleaning, disinfecting, and/or sterilizing. *Cleaning* involves the physical removal of organic and inorganic debris from items. Cleaning will not remove pathogens from the items, but it is a necessary step that allows the disinfecting agent to come into direct contact with pathogens on the actual surfaces of an item. Cleaning is important before disinfecting or sterilizing because most agents are inactivated by organic debris. *Disinfecting* an item by washing it with a proper chemical agent (see Table 1) will reduce the bacteria load or pathogens to a point where they will not serve as a source of infection, but will continue to persist at low levels on the item. *Sterilization* through the use of heat, chemicals, or radiation will remove all life from an item (Wright and Whitaker, 2001).

Once an item is clean it is necessary to determine what level of sterility is acceptable. It can be difficult and time-consuming to sterilize all equipment completely in a field setting. Also,

the necessary equipment to do so may not be available. Therefore, disinfecting equipment with a suitable agent for the appropriate contact time should be adequate for proper disease control in the field (see Table 1). Disinfection of items should always be done at a safe distance from bodies of water so that the solution infiltrates the soil rather than runs directly into the water. In *ex situ* settings, small items can be heat sterilized through the use of an autoclave. As an effective recommendation, heat sterilization under pressure at 160 F (71 C) for 20 minutes will eliminate both ranavirus and *Bd* (Johnson et al, 2003; Langdon, 1989).

All footwear needs to be completely disinfected before, between, and after each site is worked. Rubber boots should be worn when possible, due to the ease of cleaning and disinfecting. Canvas or leather boots/shoes are more difficult to disinfect completely and should only be worn when rubber boots are unavailable. When leaving a field site, footwear should first be cleaned of all debris and then allowed to soak in a disinfecting solution for the appropriate amount of time (see Table 1). After disinfection, footwear should be rinsed well and allowed to dry completely, taking care that the disinfecting solution does not enter any bodies of water. If necessary, having several changes of footwear available and storing used items in plastic bags between sites can be a practical alternative to immediate cleaning. Dedicated footwear should be labeled according to where it is used.

All equipment should be disinfected between sites, and if possible dedicated equipment for individual sites should be used. The use of disposable items further reduces the risk of spreading disease. Non-disposable equipment should be cleaned of all debris, soaked in any one of several disinfecting solutions (Table 1) for the appropriate amount of time, and then rinsed thoroughly, taking care to ensure that none of the disinfecting solution enters any bodies of water. If only one site is being worked, equipment can be brought back to the lab for disinfection.

Vehicles are generally less likely to be a vector for the transmission of disease than footwear and field equipment, but still should be disinfected, especially if used to cross or enter a known contaminated site. The wheels and tires should be cleaned of all debris and disinfected prior to leaving the site, using the same disinfectant that was used on footwear. Always remember to disinfect footwear before getting into a vehicle to prevent pathogens from transferring to the floor or pedals.

Specimen Handling, Collection, and Processing

When handling specimens in the field, even within the same site, precautions should be taken to minimize the risk of spreading pathogens. Non-powdered disposable latex or vinyl gloves are the best choice when handling specimens. Powdered gloves should be rinsed free of powder. A new pair of gloves should be used for each specimen. If gloves are unavailable, wash hands between specimens.

The greatest risk for spreading disease when handling specimens occurs when animals are placed together in the same container or when containers are re-used without being disinfected first. Always use one bag or container per specimen. Do not re-use collecting bags, and utilize a new one for each specimen. Always handle specimens as little as possible. Procedures that are quick, even if potentially painful, may cause less stress than longer procedures (Speare et al., 2004). Amphibians tend not to show signs of stress immediately after handling; however, unnecessary handling should be avoided. Instruments and equipment should always be disinfected between specimens, remembering to rinse thoroughly after the appropriate amount of time. Specimens should only be released at the site of capture, and any sick or dead amphibians found should be preserved and submitted for disease diagnosis. See Chapter 3 for methods to preserve specimens for necropsy and pathology.

CAPTIVITY

Many of the basic hygiene principles used in the field to prevent disease transmission are applicable to the captive setting. However, additional considerations should also be addressed, including bio-security for animals intended for reintroduction; appropriate housing, equipment, and maintenance; water treatment; and staff training, procedures, and protocols.

Biosecure areas

Biosecurity, as defined by the US Geological Survey (USGS) National Wildlife Health Center (2007), involves three equally important aspects: 1) safety of the humans and scientists in the area; 2) decontamination/disinfection of field equipment (especially boots and nets) to prevent spread of possible infectious agent to other sites and other animal populations; and 3) careful quarantining (isolation) of live, sick animals from all other populations in the field and in laboratory colonies.

The recommended biosecurity level for *each species* or *species assemblage* is dependant on the ultimate goal of the captive program. There are four levels of quarantine currently recognized for biosecure areas:

1. **Quarantine 1 (Q1):** for species housed in a facility outside their natural range with the intent to return them to their natural range.
2. **Quarantine 2 (Q2):** for species housed in a facility within their natural range (or similar geography) and with the intent to return them to the wild.
3. **Quarantine 3 (Q3):** for species housed in a facility outside their natural range for display or education, with no possibility for return to the range country.
4. **Quarantine 4 (Q4):** for incoming species to a facility for general display purposes, be it from the wild or another facility.

These quarantine levels and methods for achieving them are discussed in more depth in Chapter 3.

Housing

Ideally, animals obtained at different sites should be housed separately from each other and from other captive animals. This can be done by having individual rooms per species, collection site, or by region (see Appendix I for an example of a Q1 facility; Appendix II for an example of a Q2 facility). Again the level of separation is dependant upon the goals of the captive program. In rooms with multiple species, animals should be housed in individual tanks, species by species. When housing different species from the same collection site or region in the same area, the assumption is made that species from the same site have already been equally exposed to any pathogens currently found at that site.

Rooms housing amphibians should have equipment such as racks, shelves, counters, and floors that are easy to wash/mop, disinfect, and rinse. A cleaning and disinfecting schedule of all exposed surfaces is a facility's baseline defense against cross-contamination (see Table 1).

Tanks housing amphibians need to be made from materials that allow for easy cleaning and disinfecting. Non-porous materials such as glass, fiberglass, or plastic are recommended. Prior to housing any amphibians, these tanks should be cleaned, disinfected, rinsed, and thoroughly dried. The same procedure should be followed when a tank is emptied and stored.

Using automated systems for watering and draining enclosures is ideal. Not only will this decrease keeper workload but it also reduces keeper contact with enclosures and reduces the potential for disease transmission. For more information on housing amphibians and automated systems see Chapter 1.

Equipment

Using proper equipment while servicing amphibians is as important as proper housing types when it comes to hygiene and disease control. Equipment such as tools, gloves, footwear, and clothing should be designated for use on a room-by-room or tank-by-tank basis depending on the desired level of biosecurity.

Non-powdered disposable latex or vinyl gloves are recommended for cleaning enclosures or handling amphibians. If powdered gloves are used they should be rinsed free of powder prior to use. Change gloves between enclosures and store in an easily accessible location within the holding areas. Where higher levels of biosecurity are required, additional specific clothing such as surgical scrubs or Tyvek® jumpsuits (available from most laboratory or protective clothing suppliers) and rubber boots may need to be taken into consideration. Examples of this are discussed further Chapter 3.

Just as with gloves and clothing, any tools used while servicing enclosures should be tank- or room-specific. Tools used should be easy to clean and disinfect. Prior to disinfecting any tools they should be thoroughly cleaned to remove any organic matter. A dual-disinfection [i.e., clean, 1st disinfecting agent (e.g., bleach solution), rinse, 2nd disinfecting agent (e.g., ammonia solution), rinse, dry] routine is best. See Table 1 for disinfecting agents, solutions, and exposure times.

Organic cage furniture such as moss, cork bark, branching, and plants should not be recycled or transferred between enclosures. These items are difficult to disinfect and provide a perfect avenue for pathogen transferal. Non-porous cage furniture such as water bowls, plastic hide huts, and some types of rock can be put through the dual-disinfection routine and reused. See Chapter 1 for more information on enclosure furnishings.

Water Treatment

Water treatment in the captive setting is of utmost importance. In many cases, untreated water is the primary vector for pathogen transmission. For example, *Batrachochytrium dendrobatidis* (*Bd*), the amphibian chytrid fungus, can spread through a single drop of contaminated water (Voigt, 2001). Water treatment should occur in both incoming and outgoing water. Incoming water should be treated for standard chemical contaminants (chlorine, chloramines, etc.) through the use of carbon filtration, water additives, aeration, or reverse osmosis/deionization filtration and reconstitution (see Chapter 1 on *Water*). For captive sites within areas known to have amphibian-related diseases such as *Bd*, more extensive water treatment is necessary. Filtering water through one-micron (1μ) cartridge filters, available relatively inexpensively at hardware stores, is one method that has shown to be successful at removing *Bd* spores.

Wastewater should be transferred to central collecting tanks for treatment prior to being dumped into the local watershed, thereby preventing the introduction of foreign pathogens into the local environment. Before sending water to the central collecting tanks, water should be strained or mechanically filtered through the use of filter baskets, bags, floss, or other means to remove solid wastes. Large amounts of organic material will inhibit the effectiveness of disinfecting agents. Once water is collected it can be treated through the use of heat, ultraviolet sterilization, or a chlorine solution (household bleach) that is neutralized after at least the minimum contact time with sodium thiosulfate. The treatment of wastewater may be incorporated into a keepers' daily schedule such that the wastewater is collected, treated, and kept overnight before discharged. Treatment should be done away from amphibian holding areas to prevent harmful side effects and possible death from chemical fumes.

Staff Procedures

Staff are the primary defense against disease transmission and should receive sufficient training for this role. They should assume that all cages are infected and that pathogens are readily transmissible between enclosures. Amphibians can be disease carriers without showing any signs of infection, thereby infecting naïve populations. Following a few simple routines on a daily basis can go a long way in preventing disease transfer.

Breeding or high-priority animals should be serviced prior to common or non-breeding specimens. Within a room or collection, service the tanks least likely to be infected first such as long-term captives and animals that have tested negative for *Bd* or other diseases and have not exhibited any symptoms. Incoming quarantine animals should be serviced last while permanently quarantined animals should be serviced first. This is not to say the animals should be checked last in the day, but they should be serviced after the main collection to decrease the risk of transmitting a new disease into an existing *clean* (known to be uninfected) collection. See Chapter 3 for more information on staff servicing routines. Observations made early in the day can serve to spot potential problems so they can be dealt with in a timely fashion. Amphibians exhibiting any sings of disease should be dealt with immediately and any that die should have a thorough necropsy performed as soon as possible.

Taking these recommendations into account, facilities can come up with a directional service routine for their amphibian collection. For example, when servicing a room always start at the far end of the room and work towards the door, or always work in a clockwise-rotation around the room. Whatever routine is established, it should be followed in the same order every day. This way should an outbreak occur, it can be tracked and treated more effectively, hopefully with minimal loss of specimens.

CONCLUSIONS

Caretakers should rethink their disease and hygiene control practices as they bring in new species of conservation concern and in the face of emerging diseases (Browne et al., 2007). Following the few simple guidelines discussed in this chapter can lead to effective hygiene and disease control. In doing so, an institution can make great strides in effectively managing their amphibian programs and avoiding catastrophic consequences.

Human error or equipment failure may lead to a breach in protocol and procedures, compromising the desired level of isolation for any given animal. Any biosecurity breakdown should be carefully documented so that new animals are not introduced into uncertain circumstances. These breaches may pose additional challenges for the reintroduction of animals into the wild (those in Q1 or Q2) and should be discussed with peers to determine the best course of action, including members of AZA's Amphibian Taxon Advisory Group (ATAG) and the species' reintroduction team. Striving to incorporate the highest hygiene and disease management and control strategies from the beginning, particularly as new amphibian facilities are being created and new species are brought into *ex situ* programs is strongly encouraged, but breaches may still occur; communication can minimize the negative impacts of these problems.

Table 1. Disinfection strategies suitable for killing *Batrachochytrium dendrobatidis (Bd)* and ranaviruses in field studies (Speare et al., 2004). Concentrations and times given are the minimums shown to be effective. Recommendations for *Bd* are based on Berger (2001) and Johnson et al. (2003). Recommendations for ranaviruses are based on Langdon (1989) and Miocevic et al. (1993).

Purpose	Disinfectant	Concentration	Time	Pathogen killed
Disinfecting surgical equipment and other instruments (e.g., scales)	Ethanol	70%	1 min	*B. dendrobatidis* Ranaviruses
	Vircon	1 mg/ml	1 min	*B. dendrobatidis* Ranaviruses
	Benzalkonium chloride	1 mg/ml	1 min	*B. dendrobatidis*
Disinfecting collection equipment and containers	Sodium hypochlorite (bleach)	1 %	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4 %	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	0.5 min	*B. dendrobatidis*
	Complete drying		3 hrs or greater	*B. dendrobatidis*
	Heat	140 F (60 C)	5 min	*B. dendrobatidis*
			15 min	Ranaviruses
	Heat	98.6 F 37 C	4 hrs	*B. dendrobatidis*
	Sterilizing ultraviolet light		1 min	Ranaviruses only
Disinfecting footwear	Sodium hypochlorite (bleach)	1 %	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4 %	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	1 min	*B. dendrobatidis*
	Complete drying		3 hrs or greater	*B. dendrobatidis*
Disinfecting cloth (e.g., bags, clothes)	Hot wash	140 F (60 C) or greater	5 min	*B. dendrobatidis*
			15 min	Ranaviruses

CHECKLIST: SOURCES FOR INFORMATION ON AMPHIBIAN DISEASES

Amphibian Diseases Homepage, www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm
Focuses on diseases of significance associated with amphibian declines.

Amphibian Specialist Group, www.amphibians.org
The World Conservation Union (IUCN), Species Survival Commission (SSC), Declining Amphibian Populations Task Force (DAPTF), the Global Amphibian Specialist Group (GASG) and the Global Amphibian Assessment (GAA).

Amphibiaweb, www.amphibiaweb.org
Website maintained by UC Berkeley. Includes information on taxonomy and amphibian declines.

Conservation Medicine, www.conservationmedicine.org/amphib.htm

Frog Web, Amphibian Declines and Malformations, www.frogweb.nbii.gov
Center for Biological Informatics of the U.S. Geological Survey.

Global Amphibian Assessment, www.globalamphibians.org
A comprehensive assessment of the conservation status of the world's known species of frogs, toads, salamanders, and caecilians.

REFERENCES

AmphibiaWeb. 2007. Information on amphibian biology and conservation. Berkeley, California. www.amphibiaweb.org

Berger, L. 2001. Diseases in Australian Frogs [PhD thesis]. James Cook University, Townsville, Australia. Pp 330.

Bradford, D.F. 1991. Mass mortality and extinction in a high elevation population of *Rana muscosa*. Journal of Herpetology 25:369-377.

Browne, R.K., R.A. Odum, T. Herman, and K. Zippel. 2007. Facility design and associated services for the study of amphibians. ILAR Journal 48(3):188-202.

Daszak, P., Cunningham, A.A., & Hyatt, A.D. 2001. Draft guidelines for international translocation of amphibians with respect to infectious diseases. Attachment 6. *In* Speare, R and Steering Committee (Eds.): Getting the Jump on Amphibian Disease: Developing management strategies to control amphibian diseases. School of Public Health and Tropical Medicine, James Cook University: Townsville, Australia. Pp. 150-156. www.jcu.edu.au/school/phtm/PHTM/frogs/adms/attach6.pdf

Densmore, C.L. and D.E. Green. 2007. Diseases of Amphibians. ILAR Journal 48(3):235-254.

Johnson, M., L. Berger, L. Philips, and R. Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid, *Batrachochytrium dendrobatidis.* Diseases of Aquatic Organisms 57:255-260.

Langdon, J.S. 1989. Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in red fin perch, *Perca fluviatilis* L., and 11 other teleosts. Journal of Fish Diseases 12:295-310.

Lynch, M. 2001. Amphibian quarantine protocols, Attachment 6. *In* Speare, R and Steering Committee (Eds.): Getting the Jump on Amphibian Disease: Developing management strategies to control amphibian diseases. School of Public Health and Tropical Medicine, James Cook University: Townsville, Australia. Pp. 157-161. www.jcu.edu.au/school/phtm/PHTM/frogs/papers/attach6-lynch-2001.pdf

Mao, J., D.E. Green, G. Fellers, and V.G. Chinchar. 1999. Molecular characterization of iridoviruses isolated from sympatric amphibians and fish. Virus Research 63:45-52.

Miocevic, I., J. Smith, L. Owens, and R. Speare. 1993. Ultraviolet sterilisation of model viruses important to finfish aquaculture in Australia. Australian Veterinary Journal (70):25-27.

NSW National Parks and Wildlife Service. 2001. Hygiene protocol for the control of disease in frogs. Information Circular Number 6, NSW NPWS, Hurstville, NSW. Pp 20. www.nationalparks.nsw.gov.au/pdfs/hyprfrog.pdf

Pessier, A.P., D.K. Nichols, J.E. Longcore, and M.S. Fuller. 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). Journal of Veterinary Diagnostic Investigations 11:194-199.

Speare, R., L. Berger, L.F. Skerratt, R. Alford, D. Mendez, S. Cashins, N. Kenyon, K. Hauselberger, J. Rowley. 2004. Hygiene Protocol for handling amphibians in field studies.

Amphibian Diseases Group, James Cook University, Townsville, Australia. Pp. 4. www.jcu.edu.au/school/phtm/PHTM/frogs/field-hygiene.pdf

Taylor, S.K., E.S. Williams, E.T. Thorne, K.W. Mills, D.I. Withers, and A.C. Pier. 1999. Causes of mortality of the Wyoming toad. Journal of Wildlife Diseases 35:49-57.

USGS. 2007. Collection, preservation and mailing of amphibians for diagnostic examinations. USGS National Wildlife Health Center Publication, Washington, D.C. www.nwhc.usgs.gov/publications/amphibian_research_procedures/specimen_collection.jsp

Voight, L. 2001. Frog hygiene for captive frogs. The Frog and Tadpole Study Group of NSW, Inc., Rockdale, NSW. Pp. 1-4. http://fats.org.au/Publications_files/FF806.pdf

Woodhams, D.C., R.A. Alford, and G. Marantelli. 2003. Emerging disease of amphibians cured by elevated body temperature. Diseases of Aquatic Organisms 55:65-67. www.int-res.com/articles/dao2003/55/d055p065.pdf

Wright, K.M. and B.R. Whitaker. 2001. Amphibian medicine and captive husbandry. Pp. 301-307.

Young, S., L. Berger, and R. Speare. 2007. Amphibian chytridiomycosis: strategies for captive management and conservation. International Zoo Yearbook 41:1-11. www.jcu.edu.au/school/phtm/PHTM/frogs/papers/young-2007.pdf

Zupanovic, Z., C. Musso, G. Lopez, C.L. Louriero, A.D. Hyatt, S. Hengstberger, and A.J. Robinson. 1998. Isolation and characterization of iridoviruses from the giant toad *Bufo marinus* in Venezuela. Diseases of Aquatic Organisms 33:1-9.

Chapter 3

Amphibian Quarantine Guidelines

Shannon T. Ferrell, D.V.M., D.A.B.V.P., D.A.C.Z.M.

Associate Veterinarian, Fort Worth Zoo
1989 Colonial Parkway Fort Worth, TX 76110-6640
sferrell@fortworthzoo.org

Caveat: All the classifications and recommendations below were created to form a baseline of information for amphibian management decisions within AZA facilities. The recommendations represent the optimal quality of care that might not be financially or physically possible given a facility's particular limitations. Therefore, this document should not be construed as being mandated policy, but a set of suggestions that can improve amphibian care and conservation programs within participating institutions. The document can also be used to ensure that the highest recommended standards possible (such as for wastewater treatment and solid waste disposal) are incorporated into plans for new amphibian facilities. Over time, recognition of new diseases and technologies can and should be used to modify the information within this document.

TYPES OF QUARANTINE

- **Quarantine 1 (Q1):** *Out-of-range with intent to return to the wild*
  These animals are not from the locale where the facility is located. The main concerns are both the entrance and exit of pathogens from this quarantine group, as either direction engages a new host/disease interaction with potentially fatal effects.
- **Quarantine 2 (Q2):** *Range country with intent to return to the wild*
  These are wild animals from the general locale where the facility is located. The main concern is entrance of a novel pathogen into this captive group from outside the facility (i.e., a new disease agent has advanced into a geographic range as additional specimens are extracted to a facility, risking exposure to entire captive collection).
- **Quarantine 3 (Q3):** *Out-of-range for display, education, and research; no possibility of return to the wild in range country*
  These are animals in the standard collection of the zoo or aquarium designated for education, display, or research. Although they are not to be released, they can be considered to be in a semi-quarantine state, as they are not exposed to animals outside the collection.
- **Quarantine 4 (Q4):** *Incoming into facility*
  These animals are coming into the collection from the wild or other institutions. They can bring diseases, native or non-native to the range country, into the collection. All specimens entering into the facility should complete a full entry-quarantine regimen (Q4) regardless of ultimate designation (Q1-Q3).

QUARANTINE FACILITIES

Natural history of animal

Prior to the development of a species collection plan and construction of any facility/room, it is important to be familiar with the natural history of the species in question. Knowledge of the temperature, humidity, and light requirements with additional attention given to behavioral temperament can and should heavily influence the construction of the facility. Many species require specific water qualities and temperatures for optimal feeding and breeding that place heavy demands on construction and utilities, and that require advanced planning and budgeting.

Location

- Quarantine 1, 2, and 4 - *Preferred* standard for location of the Amphibian Quarantine Facility
  The quarantine facility is a completely separate building from the cosmopolitan animal collection. Only a single species or species assemblage (an amphibian faunal group that naturally occurs in the range country) is permitted per room. Facilities that house individual species or species assemblages in self-contained units [such as modified shipping containers (Amphibian Research Centre, 2007)] may have advantages over a single dedicated building.
- Quarantine 1, 2, and 4 - *Minimum* standard for location of Amphibian Quarantine Facility
  Dedicated space in a cosmopolitan animal facility should consist of isolated rooms containing only a single species or species assemblages (as described for the preferred standard above). Animals need to be serviced first in the day before caring for animals in the cosmopolitan collection. It is important for managers to understand that these rooms constitute the Amphibian Quarantine Facility; showering upon exit or minimum equivalent should occur **PRIOR** to handling non-quarantine collection animals.

Rooms

- Surfaces
  Walls, floors, and ceilings should be impervious to fluids, creating easier cleaning and enhancing sanitation.
- Electrical
  Water is often splashed around during cleaning of aquatic amphibians such that all electrical outlets should have ground fault circuit interrupters (GFCI).
- Environmental controls (For more information on following topics, see Chapter 1)
  - *Temperature:* Rooms need to be capable of adjusting temperatures to meet the natural historical ranges for the species and be capable of independent variation within a facility such that each room can run at a separate temperature. Temperatures within a room should ideally be warmer during the day with a small nocturnal decrease to simulate environmental fluctuations.
  - *Humidity:* Humidity can be increased by the use of free-standing humidifiers, misting systems, or changing enclosure design to optimize humidity. Non-aquatic amphibians usually need high humidity that can be provided by using a moss substrate to keep the cage environment at an optimal humidity level.
  - *Light:* Rooms and enclosures should be capable of independent light levels based on the required light cycles (most amphibians require at least 8-12 hours of light daily). Full-spectrum lighting is recommend to provide ultraviolet-B (UVB) and UVA.

Enclosures

Glass, fiberglass, or plastic tanks can be used. Acceptable plastics are those used for human food storage as other industrial plastic sources can leach toxicants into the water. Plastic food storage bins (5-15 gallon/19-57 L) with custom-fabricated, ventilated lids are used frequently. Tank dimensions vary with size and number of animals housed. Tanks can be plumbed for constant water flow and drainage, if needed (see *Water* section below for

information on plumbing). Opaque containers and the use of hiding sites (PVC pipe, ceramic tiles, or terra cotta pots, etc.) decrease stress and enhance growth. Cages placed on racks at a tilt promote drainage and hygiene, maximize storage, and improve access through lid on top. As many species can escape by climbing or jumping out of the enclosure, lids should be well-fitted and securable. For more information on enclosures, see Chapter 1.

Water

- Desired types
  - *Disease-free water*: Water acquired from sources determined to be free of amphibian-related diseases
  - *Treated water*: Water treated to safeguard inhabitants against disease transmission
    - Heat sterilized to 160 F (71 C) for 15-20 minutes under pressure is the *preferred* method.
    - Sediment removing mechanical pre-filters with chemical treatments (such as chlorine or chloramines) is the *minimal* method. Improper use of chlorine agents could potentially lead to accidental and catastrophic fatal exposure for resident animals and is also of environmental concern. Aeration of water can be used to remove some chlorine compounds. Other agents (sodium thiosulfate, AmQuel®+, and/or activated charcoal) can be added to chemically-treated water to remove any chlorine compounds. If using sodium thiosulfate to remove chloramines, the water will need further treatment to remove the ammonia (i.e., zeolite or biological filter).
- Sources
  - *City/well*
    Inexpensive and commonly used. Tap water from municipalities contains lethal levels of chlorine or chloramines that should be removed by 24-48 hour aeration, chemical treatments (sodium thiosulfate or AmQuel®+), and/or activated charcoal filtration. Activated charcoal is much more effective at removal of chloramines than aeration. Well-water and tap water might have trace toxic chemicals that could be lethal to amphibians, making the use of activated charcoal preferred for treatment. Both tap water and well-water should have the pH and other water quality levels checked to ensure they are within the parameters for the species maintained. Some water sources will need to have the pH manipulated with chemical additives or buffers to be suitable for use with some amphibian species.
  - *Bottled*: Distilled or reverse-osmosis (RO) treated
    Expensive for a large-scale operation; distilled water and RO water is usually not electrolyte-balanced and can be fatal to amphibians without rebalancing with buffers, electrolytes, and pH adjustments (see Chapter 1 on *Source Water Treatment*).
  - *In-house reverse–osmosis (RO) treated*
    Expensive for a large-scale operation, but provides the highest water purity available. Only moderate volumes are generated at any given time, involving daily production by staff. This method also requires rebalancing and buffering with salts and electrolytes for safe, long-term use with amphibians (See Chapter 1 on *Source Water Treatment*).
- Plumbing/flow system types
  - *Static*: Closed systems with standing water (*dump and fill*)
    Works well for large or small groups. Enclosures should be plumbed for convenient draining and refilling purposes. These systems require daily manual labor to clean and maintain adequate water quality in the confined environment.
  - *Recirculating systems*: Closed systems
    Pumps force the water through mechanical (i.e., sand and/or charcoal) and biological filters to remove debris and nitrogenous wastes, respectively, from enclosures. Filters can become overwhelmed by debris and waste if used for large populations. These

systems require regular maintenance and monitoring to ensure adequate water quality and flow rate.
    - *Continuous flow systems*: Open systems
      A constant stream of water into and out of enclosure usually by a hose, misting system, or other drip source dilutes waste to a non-toxic level in the enclosure water, and removes wastewater and debris continually. A standpipe can be employed to regulate pool depth as well as to drain water from this system. Influent water temperature and quality needs to be regulated and treated to ensure no chlorine compounds or other toxins. Constant monitoring is necessary to prevent temperature fluctuation into extreme ranges or overflow from a blocked drain.
- Water quality testing
  Testing should be performed weekly in Q4 and at least monthly in Q1, Q2, and Q3. Accurate testing equipment is required and staff should be trained for correct use. Electronic colorimetric equipment[1] is highly accurate and should be considered, but is also expensive. Less expensive chemical titration kits and dip-strip tests are available and suitable for non-routine testing, however they are less accurate and precise than electronic colorimeters.
- Modification
  Water chemistries can be manipulated to enhance tadpole growth, breeding, etc. Formulas are available that detail what additives and amounts to add to tank water as needed (Wright and Whitaker, 2001).
- Disposal
  See *Sanitation* section that follows.

HUSBANDRY
Identification

- Morphological identification
  Includes the use of physical characteristics such as size, coloration patterns, sexual dimorphism (i.e., nuptial pads in the males, toe-pad width, etc.), and/or other distinguishing markings to identify individuals within a collection. Photo-documentation is a very valuable tool, but juveniles of some species change dramatically as they age.
- External identification
    - *Toe clips*
      This inexpensive option for marking individuals involves surgical amputation of the end of specific digits based on a coding scheme for marking purposes (Donnelly et al., 1994). The tissue removed can be saved for DNA banking, *Batrachochytrium dendrobatidis* (*Bd*; the amphibian chytrid fungus) polymerase chain reaction (PCR), and/or other disease investigations, if stored correctly.
    - *Attached tags or beads*
      Loose colored wire or elastic bands have been placed around the waist of frogs. Plastic, colored beads have been sewn to the limbs of amphibians using a non-absorbable suture material that passes through a muscle mass and anchors the beads permanently. Placement on animal, added weight, and potential for catching on enclosure furnishings should be taken into consideration for this method.
    - *Ink and branding*
      Traditional tattooing and branding (heat or freeze) have been used to mark amphibians successfully (Kaplan, 1959; Clarke, 1971; Daugherty, 1976). However, application of these methods varies between species and testing should be performed before it is used widely. Select a dye or method that will contrast with skin pigmentation and remain legible over time.
    - *Radiofrequency biocompatible ink*

1 Such as the Hach® DR/890 Colorimeter

This special ink tattoo emits an identification-signal specific to that animal and can be read with radiofrequency.[2] This is new technology and is unknown for use in amphibians.

- Internal identification
  - *Microchip Identification Devices* (PIT tags)
    Subcutaneously implanted microchips function at different frequencies and levels of encryption. Some companies' microchip readers can recognize and/or identify multiple frequencies, but most only read their own frequency. ISO frequency (134.2 kHz, 15-digit numeric identity code) is becoming the world standard, and most US distributers are starting to carry the ISO frequency chips and readers.[3] Surgical glue is recommended to close the implant site.
  - *Injectable elastomers*
    Phosphorescent elastomers are injected underneath the skin or into the muscle superficially (Visible Implant Elastomer or *VIE* Tags).[4] Multiple colors are available, including invisible elastomers that utilize a black light for detection. There are similar pre-cured elastomer tags with individual alphanumeric codes printed on one side (*VI Alpha*). Implanted markers may migrate.
  - *Coded Wire Tags* (CWT)
    An implanted short length of thin magnetized stainless steel wire is marked with rows of coded numbers that can be read under magnification.[4] Implanted markers may migrate.

Nutrition

- Complete, balanced diet
  - *Prey in general*
    Most amphibians will attempt to eat prey items only if they are alive and moving. Prey items need to be the correct size or they will not trigger a feeding response. When possible, offer a varied diet to provide a wider range of nutrients and better simulate a natural diet. See Chapter 1 for more information on amphibian diets.
  - *Insects* – crickets, fruit flies, mealworms, wax moth larvae, springtails, roaches, field-sweepings, etc.
  - *Other invertebrates* – worms or crayfish
  - *Fish* – small minnows, goldfish, shiners, etc.
  - *Small animals* – rodents, lizards, amphibians, birds, or commercially-available sausages[5]
- Supplementation
  Most insects will need to be dusted with a formulated vitamin supplement to ensure proper calcium to phosphorus ratio (Ca:P) in the diet and also provision of certain vitamins, such as vitamin A.
- Feeding schedule
  Varies on needs of animals, but is usually daily for small insectivores and less frequently for larger amphibians (every other or third day). Obesity can be an issue, especially with large terrestrial amphibians, so frequency for offering large meals may range from weekly to monthly; offering smaller live insects between large meals will encourage exercise.
- Presentation/removal
  Ideally, prey items should be fresh and moving. If prey items are not consumed within 24 hours, they should be removed to keep from fouling the environment and possible reverse-predation on the amphibian. Insects such as crickets need a food source (small

2 Somark Innovations, Inc., Saint Louis, MO – available in visible or invisible dyes.
3 AVID® (125 kHz); Banfield® (125 and 134.2 kHz); Biomark®/Destron Fearing™ (125 and 134.2 kHz); and Trovan® (128 and 134.2 kHz).
4 Available from Northwest Marine Technology, Inc.
5 Natural Balance® Reptile Diet sausages.

dish with cricket diet or rodent chow) within the amphibian's tank to keep them from attacking the amphibian.

Sanitation

- Cleaning schedule: *Minimal* standard with frequencies increasing as amphibian biomass and feedings increase
  - Water change frequency is dependent on the natural history of the animal and type of system used. A continuous, low-volume flow with overflow drains is preferred over the static (*dump and fill*) method and reduces stress to the animals. If closed systems are to be used, weekly or more frequent water changes are recommended, depending on if a filtration system is employed. It is advisable to perform a water change two hours post-feeding for aquatic amphibians.
  - General cleaning of all cages should be performed at least weekly.
  - Complete cage break down and cleaning should be performed weekly in Q4 and at least biannually in Q1, Q2, and Q3.
  - Attempt to clean cages at same time of day and in the same directional order to control disease spread.
- Clothing, gloves, and uniform standards
  - Quarantine 1, 2, and 4 - *Preferred* standard for working between species or species assemblages:
    Dedicated clothing and footwear should be available for each species or species assemblage and changed before working with a different group. Disposable protective clothing (e.g. Tyvek® jumpsuits) may be useful in this regard. Ideally, keepers would have appropriate amenities to shower between servicing each species or species assemblage housed in the Amphibian Quarantine Facility. Gloves should be worn while accessing amphibian enclosures, and dedicated glove use may be required per individual container, per species, or per faunal group depending on pathogen risk.
  - Quarantine 1, 2, and 4 - *Minimum* standard for working between species or species assemblages:
    Dedicated clothing and footwear should be available for each species or species assemblage and changed before working with a different group. Disposable protective clothing (e.g. Tyvek® jumpsuits) may be useful in this regard. Gloves should be worn while accessing amphibian enclosures, and dedicated glove use may be required per individual container, per species, or per faunal group depending on pathogen risk.
- Tools
  Ideally, each species or species assemblage will have its own set of tools (nets; forceps; suction tubing; scrub brushes and sponges; etc.) that will not move between cages/rooms. If tools will be used in multiple cages within a room, it is advisable that the tools be soaked in a disinfecting solution for at least 15 minutes. Tools may need to be soaked in specific or multiple disinfectants prior to use depending upon the pathogens to be eliminated (See Chapter 2 for recommendations). After each disinfectant, all tools need to be thoroughly rinsed with fresh water.
- Substrate change frequency
  For substrates that cannot be disinfected (i.e., organic matter and paper towels), complete replacement should be performed daily or weekly in Q4 and at least biannually in Q1, Q2, and Q3.
- Wastewater disposal
  Facility wastewater should be treated to minimize the risk of exporting foreign pathogens out of the facility and introducing them into the surrounding area (Brown et al., 2007). Heat sterilized to 160 F (71 C) for 15-20 minutes under pressure is the *preferred* method and will kill both *Bd* zoosporangia and ranavirus (Johnson et al., 2003; Langdon, 1989). At minimum, chlorine treatment of wastewater with standard household bleach (recommended dilutions and minimum contact time still to be determined) added to the

wastewater should take place in an amphibian-safe manner (e.g., ventilation of chemical fumes and disposal into the sewer system rather than a local watershed). The treatment of wastewater may be incorporated into a keepers' daily schedule such that the wastewater is collected, treated, and kept overnight before discharged.

- Solid waste disposal
  Disposal of solid waste from Q1, Q2, and Q4 (and Q3 in the case of a known pathogen outbreak), including all substrate, props, gloves, etc., should be decontaminated by way of incineration or heating to a minimum of 160 F (71 C) for 20 minutes prior to being discarded. Disposal by a medical waste hauler is an alternative.
- Carcass disposal
  For carcass disposal, institutions should follow appropriate necropsy procedures. Accepted final tissue disposal options include: incineration, alkaline tissue digestion, formalin or alcohol fixation, or disposal by a certified medical waste hauler.
- Vermin control
  Vermin in a facility can act as transport hosts for viral, bacterial, and parasitic agents. The use of mechanical trapping methods is preferred over chemical agents as many of the chemical agents (whether sprayed or stored as bait) can adversely affect amphibian health through direct toxic effects or by functioning as endocrine disruptors.
- Disinfectants
  There are no ideal disinfectants that combine wide efficacy against a variety of pathogens; low toxicity; ease of use and disposal; and low cost. A disinfectant should be carefully chosen based on all relevant factors. Reading the product label is highly recommended to use and dispose of the disinfectant compound(s) correctly. Equipment, cages, and surfaces should be cleaned of debris and rinsed prior to the application of any disinfectant. Prior manual removal of debris greatly enhances the efficacy of the applied disinfectant. The following disinfection methods and duration of exposure have been recommended for amphibian settings:
    - 4% sodium hypochlorite (household bleach) for 15 minutes
    - 70% ethanol or 1 mg/ml benzalkonium chloride for 1 minute
    - Desiccation or exposure to 140 F (60 C) heat for 30 minutes

  Rinse all equipment, cages, and surfaces with fresh water after applying a disinfectant (see Chapter 2 for more information on hygiene and disinfection recommendations).

DURATION

- Quarantine 1, 2, and 4 - *Preferred* standard for duration of quarantine
  All animals enter into a facility at the same time and leave at the same time (*all in - all out*). Sixty days are usually needed to detect and treat fully for pathogens, prior to release from a quarantine area. The duration might be extended depending on clinical findings. Animals will not be released from quarantine if mortalities occur from unidentified, unknown causes. If possible and practical, treatment on surviving animals should be initiated. No animals should be released from quarantine until all mortalities have stopped; disease issues are completely eliminated; and the remainder of animals are feeding, defecating, and appear healthy.
- Quarantine 1, 2, and 4 - *Minimum* standard for duration of quarantine
  All animals enter into a facility at the same time and leave at the same time (*all in - all out*). Thirty days is the minimum quarantine period. The duration might be extended depending on clinical findings. Animals will not be released from quarantine if mortalities occur without a cause of death being identified. If possible and practical, treatment should be initiated on surviving animals. No animals should be released from quarantine until all mortalities have stopped; disease issues have been completely addressed or eliminated; and the remainder of animals are feeding, defecating, and appear healthy.

MEDICAL CARE

Records

Daily observations on all animals should be documented. Monitor body weights weekly while animals are in Q4 and monthly for Q1, Q2, and Q3.

Parasites

- Fecals should be tested for parasites weekly while animals are in Q4 and biannually for Q1, Q2, and Q3, if not scheduled for any impending release (i.e., a holding facility). Animals destined for immediate release require two fecal surveys performed in the 30 days prior to release.
- Although many amphibians carry a commensal load of enteric flagellates that do not usually require treatment, the decision to treat will be dependent upon parasite, load level, anti-parasitic agents, species temperament, and ultimate disposition plan. Trying to remove all enteric and systemic parasites via chemotherapeutics can stress the animals, change their enteric biota, and result in the animal's death. A veterinarian and amphibian manager should make a cost/benefit analysis prior to parasite treatments.
- Available medications include fenbendazole, ivermectin, and levamisole. Dosages and route can vary depending on parasite and host species (Wright and Whitaker, 2001).

Medical diagnostics

- Physical examinations by a veterinarian familiar with amphibians
  - *Visual exam and palpation performed at least once in Q4 and Q1.*
  - *Morphometrics:* Record weight and identifying markings.
  - *Clinical:* Document behavior and physical abnormalities
- *Batrachochytrium dendrobatidis* (*Bd*; the amphibian chytrid fungus) screening via DNA probe
  - *Perform prior to any treatments at least once in Q4 and Q1.*
  - *Suggested lab, cost, and collection method:*
    Pisces Molecular LLC, 2200 Central Avenue, Suite F, Boulder, CO 80301-2841, 303-546-9400; 22 USD/sample; Submit skin surface swab or scrape placed into 70% alcohol (contact Pisces for details).
- Ranavirus screening via DNA probe
  - *Perform at least once in Q4 and Q1.*
  - *Suggested lab, cost, and collection method:*
    University of Florida, contact April Childress, 2015 SW 16th Ave, Building 1017 Room V2-238, Gainesville, FL 32608, Phone 352-392-4700 x 5775; 60 USD/sample; Submit swab or tissue (suggested sample for living animal is cloacal swab).
- Hematology/biochemistry
  Dependent upon the specimen's size, it is safe to collect up to 1% of body weight from a healthy animal. Consider not collecting blood from specimens weighing below 50 g due to safety concerns. Correct use of tricaine methanesulfonate (MS-222) can make blood collection easier with reduced stress and adverse problems. Only a veterinarian or trained individual should perform anesthesia, as mortalities can occur.
  - *Perform at least one full blood panel in Q4 and Q1 animals.*
  - *Suggested lab and cost*
    Employ **any veterinary diagnostic laboratory** that runs reptile samples. A hematology and biochemistry panel will cost approximately 30 USD at most national laboratories for an amphibian. Few normal panel values currently exist for most amphibian species in the *International Species Inventory System (ISIS)* database, making interpretation of results somewhat difficult. Based on diagnostic needs, the laboratory may have to design a complete hematology and biochemistry panel, but if limited by cost they apply those existing for reptile species. As more amphibian-specific panels are

designed and submitted to the *ISIS* database, the diagnostic value of any result increases for the population, improving amphibian healthcare overall.

- Necropsy
  All animals receive gross necropsies upon death with a report generated for the medical record. Necropsies should be performed by a veterinarian or trained individual to maximize diagnostic information. Bodies should be immediately refrigerated if there is any delay to the necropsy being performed. Do not freeze the carcass prior to necropsy. If a significant delay will occur prior to necropsy by a veterinarian or trained individual, make an incision into the coelomic cavity and immerse entire carcass in 10% buffered formalin. Animals that are autolyzed and/or desiccated are of little diagnostic value as tissues degrade quickly. Submit recent history and water quality along with the body.
  - *Sample collection for histopathology*
    Samples from a fresh animal are ideal. Samples should be placed into 10% buffered formalin. Small animals (less than 10-20 g) can be placed intact into formalin if a small incision is made into the coelom to allow formalin to permeate the body cavity. Larger animals should have tissues collected by a veterinarian or trained individual. It is suggested that portions of the liver be routinely frozen and saved from all necropsies. If multiple animals die from a disease outbreak at the same time, freeze half of the specimens at –70 F (-57 C) for future ancillary diagnostic tests, and perform necropsies and histopathology on the remaining deceased animals. Tissues will then be forwarded onto a pathologist familiar with amphibian diseases. The pathologist will generate a report for the medical record that is then used to make management decisions.
  - *Sample collection for additional diagnostics*
    - Collect skin sample for *Bd* testing (see *Bd screening via DNA probe* above).
    - Collect cloacal swab or liver sample for ranavirus testing (see *Ranavirus screening via DNA probe* above).
    - If organized by veterinarian, additional samples can be submitted for electron microscopy (in glutaraldehyde fixative) or viral culture (special media required).
  - *Carcass disposal*
    For carcass disposal, institutions should follow appropriate necropsy procedures. Accepted tissue disposal options include formalin or alcohol fixation; incineration; alkaline tissue digestion; or disposal by certified medical waste hauler.

Treatments

- *Bd* prophylaxis and treatment
  - Prophylactic treatment is suggested primarily for amphibians that are coming from a known *Bd* positive collection or field site, or if animals positive for *Bd* are identified through testing. Specimens destined for release from Q1 or Q2 require a minimum 5-day course of *Bd* treatment (listed below) to be completed immediately prior to release. Animals that test positive for *Bd* (and their cage-mates) should be treated and retested one week post-treatment. Multiple treatment cycles may be required to completely eliminate *Bd* infection.
  - Treatment: The author recommends itraconazole diluted to 0.01% concentration (in 0.6% saline) bath for 15 to 60 minutes daily for 5 days as a prophylactic regimen for animals destined for release. For treating those animals that are known positives or exposed to known positives, a 0.01% itraconazole bath for 5 minutes daily for 11 days is recommended (Nichols and Lamirande, 2000). For treatment, animals are placed into a plastic container and allowed to soak with their digits and ventral surface of their abdomen covered with the solution.

- Bacterial therapeutics
  Administer antibiotic with Gram negative (-) bactericidal activity prior to periods of stress. Dosages and routes can vary based on species (Wright and Whitaker, 2001)
- Other pathogens or diseases.
  Consult with staff veterinarian for treatment.

RELEASE SITE ACTIVITY

- Assessment
  Have a veterinarian or skilled person perform a final visual observation of all specimens prior to release and retain any animals with abnormal appearance or behavior.
- Adjustment
  Whether aquatic or terrestrial, animals should have their water and/or cage environments slowly adjusted to the parameters they will be entering upon release. Allow for proper shading and predation protection during the adjustment time post-release.

REFERENCES

Amphibian Research Centre. 2007. Amphibian Research Centre Web tour, ARC Containers: On the Inside. http://frogs.org/au/arc/container.php.

Browne, R.K., R.A. Odum, T. Herman, and K. Zippel. 2007. Facility design and associated services for the study of amphibians. ILAR Journal 48(3):188-202.

Clarke, D.R., Jr. 1971. Branding as a marking technique for amphibians and reptiles. Copeia 1971:148-151.

Daugherty, C.H. 1976. Freeze branding as a technique for marking anurans. Copeia 1976:836-838.

Donnelly, M.A., C. Guyer, J.E. Juterbock and R.A. Alford. 1994. Techniques for marking amphibians. *In* W.R. Heyer, M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster (eds.): Measuring and Monitoring Biological Diversity, Standard Methods for Amphibians. Smithsonian Institutions Press, Washington, D.C. Pp 279-282.

Johnson, M., L. Berger, L. Philips, and R. Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid, *Batrachochytrium dendrobatidis.* Diseases of Aquatic Organisms 57:255-260.

Kaplan, H.M. 1959. Electric tattooing for permanent identification of frogs. Herpetologica 15:126.

Langdon, J.S. 1989. Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in red fin perch, *Perca fluviatilis* L., and 11 other teleosts. Journal of Fish Diseases 12:295-310.

Nichols, D.K. and E.W. Lamirande. 2000. Treatment of cutaneous chytridiomycosis in blue-and-yellow poison dart frogs (*Dendrobates tinctorius*) (abstract). In Proceedings: Getting the Jump on Amphibian Disease, Cairns, Australia, 26-30 August 2000. Pp. 51.

Wright, K.M. and B.R. Whitaker. 2001. Amphibian Medicine and Captive Husbandry. Pp 301-307.

Appendix I

Isolated Amphibian Rooms at Omaha's Henry Doorly Zoo

An Example of Complying with the Quarantine and Husbandry Standards for Amphibians Designated for Reintroduction into the Wild

Jessi Krebs

Supervisor of Reptiles and Amphibians, Omaha's Henry Doorly Zoo
3701 S. 10th St.
Omaha NE 68107
jkrebs@omahazoo.com
Photos by J. Krebs

INTRODUCTION

In February 2006, the IUCN Conservation Breeding Specialist Group (CBSG) and the World Association of Zoos and Aquariums (WAZA) hosted an Amphibian *Ex Situ* Conservation Planning Workshop in El Valle Panama. One of the many purposes of the meeting was to make recommendations for husbandry standards for amphibians that are part of reintroduction programs or captive collections that may be returned to the wild at some point in the future (Zippel et al., 2006). Many of the recommendations made involve upgrading current housing and quarantine standards practiced by many zoological, private, and academic institutions, and have been seen by some as impractical and extreme for zoos and aquariums. Armed with the lessons learned from the global spread of *Batrachochytrium dendrobatidis* (*Bd*; the amphibian chytrid fungus) and the potential for new pathogens to emerge, it would be prudent for institutions that are housing amphibian species designated for repatriation to review their basic husbandry practices and quarantine standards, attempting to comply with the new recommendations.

Omaha's Henry Doorly Zoo responded to this call-to-action and immediately established dedicated amphibian rooms within existing buildings on zoo grounds. The *Isolated Amphibian Rooms* (IARs) have become a working model for the application of the recommended standards in a zoo or aquarium setting. Each of the IARs holds one species or an assemblage of species from the same geographical area. The following list of images, prices, materials, and sources are provided to serve as an early example to others who might consider constructing their own IAR facilities for amphibians.[1]

1 Materials and sources cited are presented based on fabrication at Omaha's Henry Doorly Zoo, not as an endorsement. Contact the author for additional information about any of the products presented.

Figure 1. A typical non-compliant amphibian room.

AMPHIBIAN ROOMS

Not Biosecure Compliant

Most amphibian-holding rooms at zoos and aquariums are not in compliance with new bio-security recommendations. One example of a non-bio-secure amphibian room is one that houses animals from all over the world (Figure 1). Other problems may be that steps were not taken to prevent wastewater from spilling from tanks placed on higher shelves into tanks below, or a wastewater treatment process was not employed to prevent pathogens from exiting the facility and endangering local amphibian populations. Keeper error can never be completely ruled out, and unsecured lids may further increase pathogen spread between animals from different areas of the world.

Figure 2. An example of the Isolated Amphibian Room – size 8 x 8 x 8 ft (2.4 x 2.4 x 2.4 m)

Biosecure Compliant

Each IAR at Omaha's Henry Doorly Zoo holds just one species or one species assemblage from the same area. The IARs are versatile rooms constructed out of commercially available greenhouse materials with all construction completed by zookeepers (Figure 2). IARs at the zoo range from 8 x 4 x 8 ft (2.4 x 1.5 x 2.4 m) in size to 10 x 16 x 8 ft (3 x 4.9 x 2.4 m). The walls are made of 1.5 x 1.5 inch (3.8 x 3.8 cm) hollow-aluminum tubing overlaid with two-ply Lexan® sheeting. Individual walls are joined together with 1inch (2 cm) aluminum angle pieces (Figure 3). Commercially purchased stormdoors are used to access each room. All joints and cracks are sealed with 100% silicone to prevent water from leaking into common areas or into other isolation rooms. Seals are pressure-tested before installation of equipment and animals and visual inspections are ongoing to maintain biosecure levels. The stormdoor is placed at the lowest point and the one-inch threshold allows each room to hold at least 175 gallons (796 L) before overflowing into a common hallway with a drain.

List of items used for the construction of the room in Figure 1 above:

Cap[2]	18 @ 8 ft (2.4 m)
Splice[2]	3 @ 8 ft (2.4 m)
Lexan®[2]	6 @ 6 x 8 ft sheets (1.8 x 2.4 m)
Aluminum Tubing[3]	18 @ 8 ft [1.5 x 1.5 inch (3.8 x 3.8 cm); 1/8 inch (0.3 cm) thick]
Storm door	
Hardware	
Screws	
Washers	

Figure 3. Close-up of the 1inch (2 cm) aluminum angle pieces holding the 1.5 x 1.5 inch (3.8 x 3.8 cm) aluminum tubing and storm door.

2 www.stuppy.com
3 www.statesteel.com/omaha.htm

Figure 4. The portable heating/air condition unit and dedicated footwear placed in each room.

Portable heating/air condition units are used to control the ambient temperature in each room (Figure 4). Units can be purchased with different BTU ratings for different size rooms: 8 x 8 x 8 ft (2.4 x 2.4 x 2.4 m) rooms use 10,000 BTU units; the 10 x 16 x 8 ft (3 x 4.9 x 2.4 m) use 12,000 BTU units. Also visible in Figure 4 is the designated footwear for within this room. Footwear that is easy to disinfect is changed as the keeper crosses the room threshold.

List of items used in the room shown in Figure 4 above:

Heater/AC[4]	10,000 BTU units
Footwear	

Figure 5. Shelving with amphibian enclosures.

4 www.sunpentown.com/wa12poacwihe.html

Tubs used for amphibian enclosures are made from food-grade polycarbonate material to prevent the leaching of toxins sometimes found in plastic materials (Figure 5). Though glass fish-tanks may be a less expensive, the polycarbonate tubs are far more durable and versatile, making them suitable for housing terrestrial or aquatic species. Drilling each tub does not require a specialized drill bit nor do they crack or break as easily as glass. The volume of the tanks used ranges from 5 gallons to 16 gallons.

List of items used for the shelving within the room shown in Figure 5:

Shelving units[5]
Frog tanks[6]
Lids[7]

Figure 6. The over-sized drain system under each shelf being installed in the IAR.

The drain for each enclosure runs into a common drain system located under every shelf. Drain system lines are 2-inch (3 cm) diameter to allow for large volumes of water to pass through them without backing up into adjacent enclosures (Figure 6). The drain systems pipes all run into the wastewater collection tub (Figure 7).

5 www.samsclub.com/shopping/navigate.do?dest=5&item=203424&pCatg=7085 or from materials acquired at local hardware stores
6 www.rcpworksmarter.com/rcp/products/detail.jsp?rcpNum=3328
7 www.habitatsystemsltd.com, custom fabricated

Figure 7. IAR wastewater collection tub with sump pump below.

A sink combination is used to collect all wastewater from each isolation room and is created by stacking two inexpensive utility sinks together (Figure 7). The bottom tub (without legs) is set directly on the floor un-drilled. The second sink (with legs) is set within the tub below, and plumbed to drain into the lower tub without splashing. A sump pump with an automatic on/off switch is set within the lower tub to pump wastewater to the Central Treatment Station (Figure 8). The upper tub can be plumbed for use as a working sink if desired, or else dedicated hose-lines can be run into each room and provide filtered source-water.

List of items used for the wastewater collection tub in Figures 6 and 7 above:

- Two utility sinks
- Sump pump[8]
- PVC pipes, T's, and elbows
- Plumbing

8 www.flotecpump.com/pdf/Page_06_2004.pdf

Figure 8. The building's water storage and central treatment station.

All water is treated coming into and out of the IAR facility at the Central Treatment Station. A large water container is used to hold reconstituted reverse-osmosis (RO) water that can be pumped to each room as needed (right side of Figure 8; See Chapter 1 for additional information on reconstituted RO water). Two barrels are used to collect all wastewater (center of Figure 8), which is then treated with household bleach for 12 hours before being released into the city sewer system. See Chapter 3 for more information on wastewater treatment.

List of items used for influent and effluent water treatment within the room shown in Figure 8 above:

RO water storage vessel	300 gallons (1135 L)
RO filter system	
RO reconstitution feeder	
Wastewater treatment barrel	2 @ 55 gallon (208 L)
Bleach feeder system	
PVC piping	
Plumbing	
Water quality test kits	

Figure 9. Lighting accommodated into the racks.

Lighting on every rack system is provided in two forms: compact florescent lights above each shelf to provide ultraviolet light and small heat lamps on each enclosure to provide basking sites for species requiring higher temperatures (Figure 9).

List of items used for lighting in the room shown in Figure 9 above:

Lighting fixtures[9]
Bulbs[10]

Summary Budget for an 8 x 8 x 8 ft (2.4 x 2.4 x 2.4 m) IAR:

Room materials	1,100 USD
Shelving	270 USD
Heater/AC	700 USD
Frog tanks	145 USD each x 18 = 2,610 USD
Lighting	210 USD each x 9 = 1,890 USD
Plumbing	450 USD
Electrical/duct work	200 USD
TOTAL for one room	7,220 USD

CONCLUSION

The Johannesburg Zoo of South Africa has used similar technologies and practices to develop isolated amphibian rooms in their efforts to meet international biosecurity standards at their Amphibian Conservation Center. At the Johannesburg Zoo, an existing building on zoo grounds was modified to house several endangered species intended for a release program. The methods described above appear to be working very well for them, demonstrating the transferability of Omaha's Henry Doorly Zoo's techniques not only to other AZA-accredited zoos and aquariums, but to international facilities as well.

This chapter demonstrates that with a little imagination, institutions are able to follow the biosecurity recommendations handed down from the CBSG/WAZA Amphibian *Ex Situ* Conservation Planning Workshop with a relatively low investment of space and financial

9 www.drsfostersmith.com/Product/Prod_Display.cfm?pcatid=3773&N=2004+113345
10 www.esuweb.com/cardfile.asp?ItemNumber=55112&IDProductRelationship=281

resources. Hopefully, this will motivate others to consider constructing their own IAR and attempt to save at least one species or one assemblage of amphibians.

REFERENCE

Zippel, K., R. Lacey, O. Byers (eds.) 2006. CBSG/WAZA Amphibian *Ex Situ* Conservation Planning Workshop Final Report. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN 55124, USA. Pp. 65.

Appendix II

Montane Anuran Conservation Center (MACC)

Tara Sprankle

Senior Keeper - Reptiles, The Phoenix Zoo
455 North Galvin Pkwy.
Phoenix, AZ 85008-3431
tsprankle@thephxzoo.com
Photos by T. Sprankle

The Montane Anuran Conservation Center (MACC) facility was recently used at The Phoenix Zoo for the captive rearing of native Arizona leopard frogs (Figures 1, 2, and 3). In use since 1997, the facility consists of two 20 ft (6 m) long refrigerated cargo containers [containers are available in 40 ft (12 m) lengths as well]. The following standards and recommendations are based on the Zoo's experience.

Figure 1. Front View of MACC with shade structure.

Figure 2. Wide view of MACC and its concrete pad stoop.

Figure 3. Back view of the MACC shows where water is plumbed into both buildings via pipe.

To begin, one important adjustment was made to the containers' structure. The original doors (Figure 4) were very heavy and unwieldy and therefore were quickly removed. A simple wooden frame was installed in that area; made and covered with plywood on the inside and wooden paneling on the outside for aesthetics. The seams were sealed with caulking and a regular hollow-core door was installed for entrance to the container (Figure 5).

Figure 4. Original exterior doors before removal.

Figure 5. Inside view of front wall replacing swing doors

A few simple adaptations were made to prepare the interior of the structure for exposure to water and to provide access to source water and electricity. Insulated or refrigerated containers must have either a plastic laminate or aluminum lining in order to avoid rusting and also to help control temperatures. Water and electricity were run to the containers once they were installed on zoo grounds (Figures 6 and 7). The plumbing runs horizontally around both sides of the inside of the building, midway up the wall. There are two hose bibs on each side for a total of five per container. The layout for water and electricity was originally placed with the use of large round plastic aquaculture tubs in mind (Figure 8). We now use a rack system with plastic food storage containers instead, making the existing water and electrical layout impractical.

Figure 6. Water supply and filter into MACC

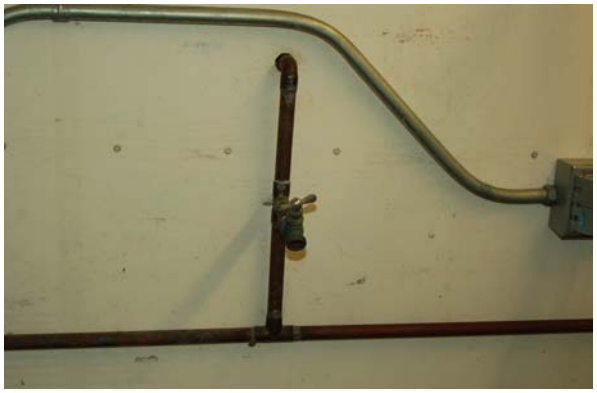


Figure 7. MACC's water pipe and hose bib.

Figure 8. Example of shelving with tadpole tubs.

Additional adjustments were made to maintain constant indoor temperatures and control lighting. The MACC containers came with a refrigeration unit but, because we were not confident it would last, we replaced it with a standard home-use window air conditioning unit (Figure 9). The air conditioning units were installed in the walls (Figure 5); we believe that they have each been replaced once since the facility was built in 1997. Despite our extremely high temperatures in Arizona, we have been able to maintain temperatures between 70-74 F (21-23 C) without too much difficulty. A standard pool timer controls the lights (Figure 10).[1]

Figure 9. A standard household window A/C unit was installed in the wall of each container.

Figure 10. Pool timer used to program lights.

[1] An Intermatic® manual pool timer was used in the MACC.

These buildings were meant to be temporary, but were in use for ten years. We had no designated budget so we could not install floor drains, requiring all of our water to be removed manually and dumped. Since we are raising a native species we have not had to worry about as strict a quarantine situation as would be required if we were working with non-native species. Each container originally cost ~2,000 USD. Because of the age of the facility and staff turnover, the information on original expenses for the facility is unavailable. The overall cost of the project was reduced because animal staff, rather than facilities staff, performed most of the labor.

The leopard frogs have recently moved into a new permanent building that is specifically devoted to conservation and temporary holding for Arizona natives. The container units are currently being utilized for winter housing for other reptiles and amphibians and will be used as emergency holding or storage in the future.