Chapter 3

Amphibian Quarantine Guidelines

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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

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Heat sterilized to 160 F (71 C) for 15-20 minutes under pressure is the *preferred* method.

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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*

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

Sanitation

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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.

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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.

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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.

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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.

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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

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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.

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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.
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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.

o Perform at least one full blood panel in Q4 and Q1 animals.

o 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.

o 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.

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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).

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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

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Bd prophylaxis and treatment

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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.

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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.

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- 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.