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DEVELOPING NEW AMPHIBIAN QUARANTINE STANDARDS AND PRE-RELEASE SCREENING PROTOCOLS

In February 2006, the Conservation Breeding Specialist Group (CBSG) and World Association of Zoos and Aquariums (WAZA) held an Amphibian *Ex-Situ* Conservation Planning Workshop in Panama. During this workshop, recommendations where made to upgrade housing and quarantine standards currently in place at zoological institutions. These recommendations were initially criticized by many as being too impractical or extreme for AZA institutions to follow due to lack of resources and funding. However, given the global spread of chytrid fungus and the potential for new pathogens to do the same, the Amphibian Taxon Advisory Group is strongly encouraging institutions (especially those holding species designated for reintroduction) to modify their current husbandry and quarantine standards to comply with the new recommendations.

AMPHIBIAN QUARANTINE AND PREVENTIVE MEDICINE

Dr. Ryan De Voe, *Senior Veterinarian*, North Carolina Zoological Park

Two essential parts of a successful captive amphibian program are appropriate quarantine and preventative medicine protocols. Vigilance in these areas helps avoid introduction of infectious disease into an existing captive population (or to new specimens from the existing population). Currently, the standard protocols practiced by most zoos for amphibian quarantine include a 30 to 90 day observation period in a location remote from the main population, fecal screening and treatment for parasites, and a physical examination. In addition, blood collection and analysis is conducted when possible. Historically, little specific infectious disease testing has been performed on quarantined amphibians. With the emergence of chytridiomycosis and ranaviral infections as recognized amphibian pathogens, more institutions are taking measures to specifically screen for these diseases.

To screen for chytrid fungus, skin scrapings or skin biopsies can be collected and examined microscopically or processed and screened with a molecular test via polymerase chain reaction. Molecular diagnostics for chytrid fungus are available commercially through a number of laboratories. Ranavirus diagnostics are a little trickier and specific molecular tests are not routinely available, except through research laboratories. Other options for ranaviral diagnosis include viral isolation and microscopic examination of tissue samples with immunostaining. Quarantine and preventative medicine programs become even more important when dealing with captive amphibian populations involved in release programs.

A question that has plagued many program managers is how to ensure that captive produced animals do not introduce pathogens into wild populations. A novel pathogen can be devastating in a naïve population of animals, so this situation needs to be avoided at all cost. The dilemma is that we have an incomplete understanding of what microorganisms are carried by and/or are capable of causing disease in amphibians, thus there is absolutely no way anyone can guarantee an individual animal is free of infectious disease. This reality dictates that if we are going conduct reintroduction programs, we need to look at the scenario from a risk assessment standpoint. It is essential to screen for known pathogens in animals prior to release, as well as avoid contact between animals used for reintroduction and other collection animals. Furthermore, we should be vigilant about identifying new potential pathogens.

An important point to understand is that most potential pathogens, such as parasites and viruses, cause few problems in their natural host. Severe disease can occur when these organisms are transmitted to non-adapted species. A good example that many in the zoo field are familiar with is cercopithecine herpesvirus 1 (CHV1) or "Herpes B virus." In macaques CHV1 causes minimal disease, limited usually to mild ulcerative lesions of mucosal surfaces in adult animals. In non-adapted species, including humans and other non-human primates, CHV1 can cause severe or fatal encephalitis. This principle can be applied to all taxa, so great care should be taken mixing species, especially those from different regions. We know that chytridiomycosis and ranaviral infections cause few problems in some amphibian species, but have been implicated in causing local population declines or even extinctions in others. For these reasons it is appropriate to maintain captive populations of amphibians that produce offspring for release to the wild in permanent quarantine situations.

The Puerto Rican Crested Toad SSP has historically released tadpoles into ponds on Puerto Rico and allowed them to metamorph and disseminate on their own. This presents a challenge for prerelease infectious disease testing. The individual tadpoles are extremely small and any screening tests need to be rapidly completed so the larvae can be shipped to the field and release prior to metamorphosis. The advent of molecular diagnostics makes rapid turn around of tests for specific pathogens possible, though the small size of the larvae necessitates that individuals be sacrificed for testing. Currently, the Puerto Rican Crested Toad SSP is trying to develop standardized PCR testing for chytrid fungus and ranavirus that can be employed prior to sending groups of tadpoles for release. Adult animals in breeding populations should be monitored for evidence of infectious diseases including parasitism.

In summary, it is paramount that those working with captive amphibian populations be familiar with appropriate quarantine and preventative medicine protocols. Population managers directing wild release programs should continually reassess practices to ensure that all possible measures are taken to avoid introduction of pathogens into wild populations of amphibians.

A PRACTICAL LOOK AT COMPLYING WITH HIGH QUARANTINE AND HUSBANDRY STANDARDS FOR RELEASE AMPHIBIANS

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The Omaha's Henry Doorly Zoo has recently developed an amphibian quarantine facility that follows preferred and minimum standards developed at the CBSG/WAZA husbandry workshop. The following information is meant to guide institutions to develop their own quarantine facilities

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using existing space, as well as give examples of how to utilize that space.

CBSG/WAZA Recommended Minimum Quarantine Standards: Location of Quarantine Facility

"Preferred standard for location of the Amphibian Quarantine Facility: Quarantine facility is a completely separate building from the cosmopolitan animal collection. Only a single species or species assemblage (an amphibian faunal group that 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) may have advantages over a dedicated building.

Minimum standard for location of Amphibian Quarantine Facility: Dedicated space in a cosmopolitan animal facility must consist of isolated rooms, containing only a single species or species assemblages as described for the preferred standard (above). Animals need to be taken care of first in the day before servicing of animals in the cosmopolitan collection. It is important for managers to understand that this constitutes the Amphibian Quarantine Facility and "shower-out" or minimum equivalent must occur prior to handling non-quarantine collection animals."

Providing dedicated quarantine space for multiple species will be the biggest challenge for institutions. Many zoos and aquariums don't have empty buildings waiting to be filled with endangered amphibians, or it may not be feasible to room off an area for one species. Take some time to look around your institution. Is there an old sea lion building for example, with the potential to house amphibians? Is there a storage room in the basement of your facility that could hold one endangered species? Just one? Modified insulated shipping cargo containers have been used in Australia and Europe for just such a purpose and an unused hallway is being modified to accommodate 20 isolation rooms at Omaha's Zoo.

Our existing space comes in the form of a 4,200 square foot passageway, with and average width of 11 feet and the entire length being 220 feet. This area was originally planed to be utilized as a public viewing area to an orangutan exhibit and a passageway to a planned panda facility. This space was never designed to house animals, so basic necessities such as environmental controls, floor drains, and a water source were not present. It was decided to build individual rooms in the existing space that would house one species or an assemblage of amphibians from the same area. As retrofitting the area with cement block walls would prove to be costly and labor intensive, it was decide to use walls constructed of two inch aluminum tubing and transparent hollow core Lexan. These materials (typically used to construct green houses) are relatively inexpensive and readily available.

Isolation rooms range in size from 8'x8'x8' to 16'x10'x8'. Though some rooms have shared walls, each unit is silicon sealed to prevent water and air seepage. This also minimizes vermin such as cockroaches from moving from room to room and transporting disease. To overcome the lack of area environmental control, each individual room utilizes a freestanding, commercially available, heating and cooling unit. This allows each room to meet the general environmental temperature needs of the designated species. A standard duct work system provides heated\cooled air to the area. Small flexible air ducts and dampers branch off of the main line to provide fresh air and maintain positive pressure for each isolation room. Filters are used on these small lines to prevent pest insects and large particles from getting into the rooms.

Since no water lines were originally run into the amphibian space, an existing cold water line running one floor above the amphibian area was tapped and run to several 300 gallon storage tanks after passing through a Reverse Osmosis unit, eliminating the chance of exposing the amphibians to outside chemical contaminants. While in the storage tanks, water is re-constituted, re-circulated and filtered mechanically, as well as chemically and passed through ultra violent sterilizers. PVC plumbing lines allow the water to be pumped from the storage tanks to individual rooms where it is utilized on demand. Each tank system supplies enough water for two to five isolation rooms.

Amphibian housing in each room consists of movable rack systems. The shelving racks where purchased commercially then modified to roll and utilize a gutter system that allows all waste water to drain to one centralized sump. Recirculation systems, as well as dump and fill systems are used for the racks. Each rack utilizes three shelves with four 16 gallon, or six five gallon food storage polycarbonate boxes. These boxes house individuals or groups of amphibians. Polycarbonate containers where chosen over glass for long-term durability and ease of drilling. Individual lighting needs of each species is also integrated to these rack systems.

CBSG/WAZA Recommended Minimum Quarantine Standards: Guidelines for disposal of water and wastes:

"Facility wastewater must be treated to minimize risk of introduction of foreign pathogens out of facility and into surrounding area. Heat and pressure wastewater treatment is strongly preferred. At minimum, chlorine treatment of wastewater must take place in an amphibian-safe manner (e.g., consider chemical fumes from sterilization agents).

Solid waste disposal, including all substrate, props, gloves, etc., should be decontaminated by way of incineration, disposal by medical waste hauler or heating to a minimum of 160ºF for 20 minutes and discarded.

For carcass disposal, institutions must follow appropriate necropsy procedures. Accepted final tissue disposal options include: incineration, alkaline tissue digestion, formalin or alcohol fixation, or disposal by certified medical waste hauler."

Many people assume that all water heading down a drain will be treated by local waste-water management plants before entering water systems habituated by local amphibian populations. However, it is not uncommon for waste water management facilities to bypass standard treatment processes when faced with a surge of water from events such as spring run off or excessive rainfall, sending potential amphibian pathogens directly into local wetlands, lakes or rivers.

To prevent potential contamination of surrounding wetlands, all waste water from each isolation room is pumped out via a commercial "sump pump," through plumbing lines to centralized fifty-five gallon drums (one drum per room so there is no cross contamination of water). Once a drum is full, the waste water is disinfected, utilizing a 12 hour chlorine treatment before being released into a floor drain that was installed in the common area.

For general security issues, only specified amphibian keeper staff have access to individual rooms and the public is not allowed to visit the area. Dedicated gloves, footwear and coveralls are provided for the keepers to wear while working in the isolation rooms. Keepers also utilize dedicated footwear in the common area to minimize contamination from the other amphibian areas of the zoo. In addition, consistent, directional traffic flow is practiced during maintenance and feeding.

Food items for amphibians are obtained from commercial breeders. Upon arrival, they are taken directly to the individual isolation rooms where they are unpacked and maintained. Food items are not taken from room to room, or obtained from other areas of the Zoo.

Similar technologies and practices have been implemented by the Johannesburg Zoo of South Africa for their Amphibian Conservation Center. These methods have worked very well for them, demonstrating the transferability of the above describe techniques not only to AZA institutions but to foreign countries as well.

With approximately half of the world's amphibians threatened with extinction and with captive assurance colonies as the only immediate hope for survival for many of these species, updating quarantine standards for amphibians is a priority. This material is presented in order to demonstrate how, with a little imagination and planning, institutions can follow stricter quarantine standards without excessive cost and effort.

For further information about products and general costs please contact *jkrebs@omahazoo.com*.