



Amphibian Conservation Resource Manual

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14 September 2007

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

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Welcome!

Roughly one-third to half of all 6,000 known amphibian species will more than likely become extinct within the next decade, an extinction crisis unprecedented since that of the dinosaurs. The combined effects of habitat loss, climate change, water and air contamination, and the chytrid fungus are the apparent culprits behind this conservation crisis. What was once considered a last resort conservation strategy – the capture and captive breeding of individual species outside of their natural habitat– has now become the only way to spare numerous species from their otherwise-certain extinction.

Association of Zoos and Aquariums (AZA)-accredited facilities have been called on by The World Conservation Union (IUCN) to each become responsible for saving one amphibian species through emergency collection and on-grounds captive rearing and by supporting international captive rearing efforts that maintain species in-range. While this may seem like an idealistic goal, AZA is asking its members to go one step further and become the leading education and awareness-raising voice on this matter, making each of our 140+ million visitors aware of the crisis and presenting them with ways to get involved.

This crisis demands an unprecedented response, far beyond the zoological community's previous experience with any taxa. The only way we are going to be successful is if individuals and institutions take the lead and develop new conservation programs for and partnerships on behalf of species in need. We cannot afford to be passive. We must be proactive and use our resources to the best of our abilities. The AZA Amphibian Taxon Advisory Group (ATAG) will provide assistance to AZA-accredited zoos and aquariums ready to respond to this conservation crisis, but cannot create programs for the hundreds of amphibians that are disappearing without your active participation.

This *Conservation Resource Manual* will help you make informed decisions about how you can most successfully contribute to amphibian conservation, based on your facility's resources, expertise, and expectations. This *Manual* shows you both why and how you can get involved. It shares the experience and expertise of your AZA colleagues in developing domestic and international captive rearing programs, in evaluating these programs in terms of conservation goals, and in developing effective and complementary education and research programs. This *Manual* also steers you to additional resources, including existing husbandry protocols and action plans, funding opportunities, and key contacts. The target audience for this *Manual* is two-fold; it is directed both at decision-makers with the power to determine whether amphibian conservation action will be one of your facility's priorities, and even more to those individuals who will serve as project implementer(s), if resources are committed to this effort.

In 2008, the ATAG will publish a *Best Practices in Amphibian Conservation Resource Manual* covering quarantine, hygiene and disease management, assisted reproduction, general amphibian husbandry, and construction of isolation facilities.

I thank all of the authors for making these manuals useful resources and look forward to hearing from throughout the AZA community as new projects are developed. Together, we can help protect an entire class of organisms. The ATAG is here to help you. Feel free to contact me, Diane Barber, ATAG Chair, at: dbarber@fortworthzoo.org; (817) 759-7180.

Enjoy and Good Luck!

Diane Barber

Curator of Ectotherms and ATAG Chair
Fort Worth Zoo

Chapter 1: Seven Reasons for Your Facility to Participate in Amphibian Conservation

Shelly Grow, Association of Zoos & Aquariums

There are plenty of reasons to get involved in amphibian conservation; the following list provides just a few:

1. The Crucial Role Zoos and Aquariums Must Play Is Clear

Experts in the amphibian research community and The World Conservation Union (IUCN) agree that the only way to save numerous amphibians from extinction is to gather sustainable, healthy populations from the wild, rear them in captivity, and safeguard them until it is safe to release them into the wild. This proactive effort will protect many amphibians from the chytrid fungus, whose devastating spread is currently impossible to prevent or treat in the wild. **AZA-accredited zoos and aquariums have the amphibian husbandry experience, technical expertise, and facilities that are desperately needed at this time.** While both husbandry techniques and holding/rearing space may need to be adapted to accommodate new and little-understood species and to meet high bio-security standards, the basic infrastructure and expertise are already in place at many zoological facilities.

The only way to ensure that the public adopts amphibian-friendly behavior and that government agencies enact amphibian-friendly measures is to educate the public about the imminent nature of this conservation threat. Within a decade, one-third to one-half of an entire class could disappear. People need to act immediately so that the necessary regulatory changes and personal behaviors that reduce the impact of pollution, habitat, and climate change will be adopted; funds will be raised for captive rearing, habitat restoration, and eventual reintroduction actions; and resources will be committed to monitoring amphibian population changes so that we know what species are where, which are stable, which are decreasing, and what has been lost. With strong education departments, diverse teaching methodologies and techniques, and access to vast numbers of visitors, **zoos and aquariums can lead this important public awareness campaign.**

2. Species of Concern Have Already Been Identified

The IUCN has asked regional zoological associations such as AZA to prioritize the amphibians in their regions as targets of conservation action, so that none of their species goes extinct. This North American focus will allow AZA facilities to work within domestic infrastructure, permitting, and partnership confines, and to share the financial resources required for effective conservation. In addition, a regional focus allows AZA members to demonstrate commitment to local communities, and minimizes any cultural and public relations conflicts related to the export of biodiversity from other regions.

In July 2007, the AZA Amphibian Taxon Advisory Group (ATAG) developed lists of **Canadian, US, Caribbean, and Mexican species whose conservation status would benefit from captive rearing programs at AZA zoos and aquariums.** This list (Appendix 1) was generated through a process developed by the global Amphibian Ark that incorporates conservation threat, mitigation potential, phylogenetic uniqueness, and other considerations. The first steps to get involved are to review these lists and contact the designated species coordinator (Appendix 2) about the status of captive rearing programs for that species. If no species coordinator is listed, consider volunteering to initiate a program on a species' behalf. To do this, contact the ATAG and your state wildlife agency.

If interested in international amphibian conservation, review the Mexican and Caribbean species lists and contact local experts to ensure that project ideas complement local priorities. Commit to expanding capacity in range countries so that *ex situ* populations can be

maintained in-country. The ATAG will assist local efforts to host similar workshops with regional experts to help prioritize Central and South American amphibian species suited for *ex situ* conservation. This will allow AZA facilities to continue growing their existing partnerships and build new ones throughout the hemisphere. Ongoing efforts led and/or supported by AZA institutions are well underway in Mexico and Panama (see Appendices 2 and 4).

3. Your Institution Can Save a Species from Extinction

Due to their relatively small sizes, short generation length, and limited home ranges, **it is possible to safeguard an entire healthy and sustainable amphibian population *ex situ*.**

Given the current crisis facing amphibians, it is wishful thinking to say that no additional amphibian species will become extinct in the wild over the next decade, but that does not mean that the species must be lost forever. Careful population management tied with appropriate research, habitat restoration, and long-term commitment can lead to the reintroduction of an “extinct in the wild” species in the future. This approach may be the only hope for many vulnerable amphibians today. The IUCN is calling for all zoos and aquariums to “save” one amphibian species. Which will you save?

The payback for your efforts will be visible in the short- and medium-terms. As a dramatic example, well-timed field collection efforts in Panama secured some amphibian species just before the wild sources went extinct. *Ex situ* husbandry protocol development and growth in captive assurance populations can progress at a time scale not imaginable for most other vertebrates. Public awareness can also increase rapidly, leading to immediate positive impacts on land management plans, fundraising, and human attitudes and behaviors.

4. Public Relations and Fundraising Opportunities Abound

Amphibian declines are seen in virtually all communities, and provide an opportunity to teach visitors about animals and science, land use and habitat alteration, use of pesticides and lawn chemicals, and the importance of stopping the spread of non-native, invasive species. Amphibian conservation messaging is your opportunity to highlight the positive impact your zoo or aquarium can have on local, national, and international biodiversity, and to engage your visitors in monitoring, changing behaviors, and supporting the expansion of amphibian conservation facilities.

Amphibian conservation is one more way to prove that you are a trusted, pioneering, and powerful conservation organization and can be leveraged to build additional relationships with land stewards, government agencies, and universities. This is an opportunity to become the epicenter of local and regional amphibian conservation dialogues by offering space for the meetings of groups such as herpetological societies, the Partners in Amphibian and Reptile Conservation (PARC) network, and the trainings in citizen-science amphibian monitoring programs, such as those offered through the US Geological Survey, National Wildlife Federation, and others. Bring in speakers, hold amphibian-themed celebrations, and keep your amphibian conservation efforts in the local news and zoo/aquarium member publications.

Leverage your institution’s commitment to community involvement, significant conservation impact, partnerships, and visitor engagement into funds by approaching local and national government agencies and foundations for support for your amphibian conservation efforts. Identify sponsors for specific projects and ask for additional emergency response funds. Engagement in amphibian conservation demonstrates your institution’s dedication to proactive conservation and rapid response to environmental crises.

5. Conservation Action Fulfills Core AZA Accreditation Requirement

Amphibian conservation ties into the conservation elements of your facility’s mission statement and meets the general conservation considerations of the AZA 2007 Accreditation Standards and Related Policies: “Interpretive programs and publications should include

information on the conservation of wildlife and their habitats to foster concern for disappearing biodiversity and to elevate the environmental knowledge of individuals in the field, in the zoo, and the visiting public.”

Amphibian conservation provides an ideal venue for participation and leadership in AZA and the ATAG, as well as other wildlife conservation programs, such as the Conservation Breeding Specialist Group (CBSG), Amphibian Specialist Group (ASG), the Amphibian Ark, and others. For facilities holding Wyoming toads or Puerto Rican Crested toads at their facilities, participation in the associated Species Survival Plans (SSP) is required for AZA accreditation. Chapter 5 has more information about tying amphibian conservation to institutional objectives.

6. Prime Opportunities for Staff Development and Scientific Contributions

The AZA Board of Regents’ offers an *Amphibian Biology and Management* course to help your facility ramp up its amphibian conservation work. In 2007, the Board of Regents added an additional *ABM* course – an unprecedented response to the urgency of this crisis and the enthusiasm of AZA members. By the end of this calendar year, 106 people will have received cutting-edge training in amphibian conservation and husbandry through this course since 2004. *ABM* will be offered again in April 2008.

Many of the world’s amphibians’ life histories and population status are poorly understood. Both *in situ* and *ex situ* research are required for better species management in captivity and in the wild. Engaged AZA-accredited zoos and aquariums will both participate in field research and **use their captive assurance populations to further scientific knowledge about a host of amphibian species. Scientific inquiry and reporting provides information to the larger scientific community, offers professional development opportunities for staff, and increases the ability of all AZA facilities to respond to amphibian population declines** worldwide. Your skilled staff can also become mentors for other novice zoo and aquarium professionals, extending the reach of your institution’s commitment to amphibian conservation action. For more information on research, see Chapter 4.

7. Amphibian Conservation Action Provides Diverse Opportunities for Engagement

To raise public awareness and foster effective captive rearing programs, AZA-accredited facilities need to engage in education, field research, development of both *in situ* and *ex situ* captive assurance facilities, laboratory research, monitoring, captive rearing, capacity building in local and distant communities, and fundraising to support field and *ex situ* efforts. Through each of these actions, **facilities can tailor their amphibian conservation contribution to reflect their unique resources, interests, and expertise.**

Amphibian conservation does not require zoos and aquariums to alter their master plans or delay other parts of their facility’s development. Amphibian conservation can be low cost and can require minimal space. Amphibian conservation costs range from as little as a few hours of staff time per week to small financial contributions to existing AZA amphibian conservation programs, to outfitting climate-controlled shipping containers for housing captive assurance populations for ~\$20,000, to maintaining a captive assurance population for around \$35,000 per year, to doing even more. For the space-constrained, amphibian conservation can be done off-site by supporting the ongoing efforts of AZA or international colleagues or by engaging in monitoring and population surveys. Biosecure captive rearing can be done in spaces as small as 8x8 feet or in moveable shipping containers of varying sizes. For those facilities with more available space, captive rearing done in larger facilities allows the possibility of housing multiple species, offering public viewing areas, and utilizing underused areas of your campus.

The most important first step in every aspect of amphibian conservation is to *get creative and get involved*. This manual is designed to help you do just that.

Chapter 2: Integrating Amphibian Conservation Objectives with Institutional Collection Plans (ICPs)

Andy Snider, Fresno Chaffee Zoo

There are as many ways to do an Institutional Collection Plan (ICP) as there are people to do them. Many methodologies utilize a matrix to determine a “purpose” for each species in the collection, while others choose species based on availability and institutional space. However an ICP is done, specific conservation objectives should be integrated into the document.

What does that mean specifically for amphibians? Basically, it means that we need to stop thinking of amphibians as “fillers” in and around our traditional reptile facilities. Unfortunately, too many of our facilities still view frogs, toads, and salamanders in this way, never even considering the need for conservation of these interesting and increasingly endangered animals. The amphibians may be the most endangered class of vertebrates on the face of the planet, and it cannot be overstated that all institutions have a role to play in their conservation.

While many institutions cannot easily dedicate enough space for a conservation program for one of the charismatic megavertebrate species, all institutions have enough space to dedicate to one or more amphibian species. Does that mean that a 5-gallon aquarium with a frog in it qualifies as a conservation initiative? No, it does not. However, most institutions have some unprogrammed space available in one or more facilities that could be relatively easily retrofitted for amphibians. See Chapter 6 for a description of isolated amphibian facilities in the US and Panama that are ideal for a variety of conservation programs.

So how does someone go about deciding which amphibian species may be right for their institution? First, the AZA Amphibian Taxon Advisory Group (ATAG) is a valuable resource, and currently maintains a list of species that may be considered for conservation initiatives. The ATAG has been given the responsibility to maintain a list of Caribbean, North, Central, and South American species that qualify for *ex situ* conservation work, and this list is currently being compiled for many of these areas. In addition, institutions are encouraged to contact their regional U.S. Fish and Wildlife offices and/or their local Department of Natural Resources office to establish relationships. Oftentimes, these organizations can offer assistance and advice on species in the local area that might benefit from conservation efforts. Remember, all conservation efforts do not have to be in the captive-breeding realm. Simple surveys of local populations are often of great benefit; there is relatively little known about the natural history and population structure of many species.

It should be kept in mind that captive breeding should not be reserved exclusively for endangered or otherwise threatened animals. It is often very beneficial to have experience with a closely related yet more common species whenever a program is being contemplated for a more endangered form. This can be thought of as a “surrogate program.” Working with a common local plethodontid salamander in captivity, for example, could yield extremely important information needed for a successful program to occur for a closely related more-endangered form. As with all programs, however, if captive-bred progeny are going to be released into the wild, the species should be kept isolated from all other animals in the collection to minimize the potential for the release of harmful pathogens into the wild.

All directors and curatorial staff should include amphibian conservation objectives in their respective collection plans. It is our duty as AZA-accredited facilities and good stewards of the world’s biodiversity to make sure no more amphibian species go extinct in our lifetime. Only through our individual efforts and cooperative programs will we make sure frogs do not go the way of the dinosaurs to extinction.

Chapter 3: Assessing What Your Facility has to Offer

What to consider prior to involvement in an amphibian program

Andy Snider and Dustin Smith

With the challenge presented to zoo and aquarium facilities to secure the survival of as many North American, Caribbean, and Latin American amphibian species as possible, each AZA institution will need to review their existing resources realistically before committing to help. The available space, experience, time, and financial support that would be needed should be considered by each facility

Although there are many existing amphibian projects that AZA facilities have been supporting for years, institutions should be prepared to take on new frogs, toads, salamanders, and caecilian species for which nothing may be known about their natural history, or will anybody (AZA institutions or other) have experience with in captivity. Having space designated, financial resources ready, trained staff involved and accessible, and a network of professional contacts in place improves the odds for a new program succeeding.

Providing space within an institution for *ex situ* programs

Each institution has two major types of physical holding space: on-exhibit and off-exhibit.

Exhibits, and their associated interpretive elements and graphics, have the potential to show visitors the wonderful diversity of the amphibian world. The animals within these exhibits become ambassadors for all others. Most facilities have at least a few amphibian exhibits, but there is often much more space available; this can be referred to as “**hidden space**.” How many times have you seen a herp exhibit that contains one or two sedentary, terrestrial snakes, yet has a lot of unused space within the exhibit? In many cases, amphibians can be mixed with other species, including reptiles. Why have one species in an exhibit when it can easily hold two? Of course, not all species will work well together, but many will. Experimentation is the key, and can be fun too! There is sometimes the potential for disease transmission between different species, but these issues can usually be overcome. Be sure to discuss plans with your veterinarian to determine any potential cross-contamination issues.

Sufficient **off-exhibit space** is essential for successful amphibian husbandry and propagation (see Appendix 5 to review available husbandry manuals). Although many amphibians are relatively small, it does not mean they need only a small amount of space. Numerous species are kept and reproduced in small-to-medium-sized colonies, which requires ample space allocation. Most require additional space for the proper husbandry of aquatic tadpoles as well, sometimes necessitating multiple aquatic set-ups. In terms of a facility’s entire collection, this is still a fairly minimal investment of space; one species of great ape could easily hold many dozens of endangered amphibian species.

The concept of **quarantine** is certainly not new and happens with virtually all zoo and aquarium animal arrivals. But in this case, the concept should really be broken into two separate entities: quarantine as animals are entering a collection, and permanent quarantine. As new animals enter a collection, they should be isolated off-exhibit from the general collection for a certain length of time in order to look for signs of illness or stress, watch for normal behaviors such as eating and defecating, and take fecal samples to determine parasite load. This period of time usually ranges from 30 to 90 days, depending on the veterinary requirements from facility to facility. As needed, diagnostic tests such as chytrid fungus swabs can also be taken to determine the presence of this potentially devastating disease.

Permanent quarantine is exactly what it sounds like; animals enter a quarantine facility and are never integrated into the permanent animal collection, preventing transfer of infectious diseases or parasites to/from the rest of the collection. This is often done for specimens whose progeny may be released back into the wild. Ideally, these species (or species assemblage from the same area and habitat) should be housed in a completely isolated environment (be it on- or off-exhibit); utilize separate air-handling systems, offer water treatment in and out of the isolation room, and have either a showering capability or separate clothing for staff working each room. The treated entry water is especially important where there is the potential for disease elements, such as chytrid, to enter via the water system. Treated drainage is equally important if the species within the room has the potential to contaminate the neighboring water systems with non-local pathogens and/or parasites. Heat or ultraviolet sterilization systems may be used to sterilize all outgoing water, or the water may be treated with sodium hypochlorite (bleach) in a holding vat before entering the sewage system.

As opposed to the existing space discussed above, consider **new spaces** designed and built specifically for amphibians or space that once held other types of animals. Virtually all zoos have some currently unused space that could be retrofitted for amphibians. If every institution were to dedicate one unoccupied room to a local amphibian project, especially if permanent quarantine were a possibility, the benefits to amphibian conservation would be incredible. Several institutions have designed specific facilities for amphibians from the ground up. This extraordinary dedication to amphibian conservation should be commended and emulated wherever possible. While we are well aware that the “charismatic mega-vertebrates” help to bring people through our gates, the consequences of zoos’ and aquariums’ inactivity with amphibians is unimaginable – the potential loss of hundreds of critically endangered species.

Kids love amphibians, it is a fact, and it is hard to imagine a more kid-friendly group of animals. **Graphics and interpretive elements** should try to appeal to the “kid” in all of us, regardless of age. Brightly-colored, bold designs and photographs capture the eye, and hopefully more people will pay attention to our messages than with dull, fact-ridden graphics. An easy way to get our messages across is for each facility to design and install one large, attractive graphic panel written in easily understood language that shows what the general visitor can do to help amphibian conservation. For more information on educational initiatives, see Chapter 7.

Financial support for *in situ* amphibian research, conservation, and education

There are many ways an institution can support *in situ* amphibian research, conservation, and education financially, including through staff participation, direct funding, and support for training and meetings related to global amphibian conservation efforts.

Participation with *in situ* efforts is one of the most interesting and rewarding aspects of an amphibian keeper’s job, whether with local or international programs. Due to the large numbers of species in need of conservation efforts and the general lack of baseline, life-history information, there is a demand for involvement. Conservation efforts include creating artificial breeding-site wetlands, educating local persons, or protecting a habitat. In order to understand many of these species, research is needed on every life-stage and aspect of each species’ life, and data is needed regarding abundance, habitat-use, behaviors, reproduction, and more. Researching the habitats, ecosystems, watershed and environmental issues also play a large role in these projects, as amphibians are so dependent on their surroundings. Obviously, the related costs of these research opportunities depend on many factors, especially location. Current AZA projects that can serve as models for new programs include:

- The Puerto Rican Crested Toad SSP has offered staff opportunities for travel to Puerto Rico to paint fences, build breeding ponds, assist with tadpole reintroductions, construct educational displays, and other activities that created lasting impressions on those involved and prepared staff for leadership in other conservation programs.

- Project Golden Frog utilized staff from several AZA-accredited zoos and aquariums and universities in Panama and the US for field studies that supported the *ex situ* efforts. Data was gathered for two years on habitat, water quality, seasonal temperatures and rainfall, and other environmental factors influencing the success of housing them in captivity, including identifying the local diatoms that the tadpoles eat.

When becoming directly involved in the *in situ* portion of any project, there are specific needs, especially when working in the field. Contact the project coordinator to acquire a specific list of supplies needed when participating in field projects. For more information on *in situ* and *ex situ* research and field gear, see Chapter 4.

Institutions that may not have the ability to assist by sending staff to help with *in situ* field efforts can **support new or ongoing *in situ* programs financially**. Selecting species, habitats, or localities that align with your institutional goals, mission, and collection plans will be easier to justify funding. For example, if your facility only displays North American species or has a Smokey Mountain exhibit, then consider funding research on southeast salamanders; likewise if an aquarium mission relates to aquatic environments, support the conservation of a wetland that is a breeding site for amphibian species.

Researchers and conservationists in need of support can range from other AZA institution staff to local/foreign professors and/or students. These persons will be in search of funding for all aspects of their projects including travel, data collection supplies, and project materials. Offering direct funding through a competitive grants program allows projects to be evaluated for appropriateness and scientific/conservation merit. Working through a cooperative program like Zoo Conservation Outreach Group (ZCOG), a coalition of North American zoos and aquariums, corporate sponsors, and individuals working together to support conservation projects of Latin American zoological institutions, increases the impact of one's funding dollar. Personal relationships with researchers can lead to ongoing support of programs, such as the association that has been developed between the St. Louis Zoo and Dr. Luis Coloma's De Los Sapos amphibian research and conservation program in Ecuador.

There are also **international amphibian meetings, conservation workshops, and training courses** that need support, both financially and logistically. In response to the global amphibian decline, there have been many workshops and conferences dedicated to preparing the international amphibian community about how biologists, veterinary pathologists, conservationists, government agencies, and zoos and aquariums should respond. These meetings discuss species selection, husbandry, conservation, and many other important topics. Because of the diverse international participants in attendance, many unique ideas are developed and new contacts formed. A great example of this type of workshop was a meeting hosted by the Conservation Breeding Specialist Group (CBSG) and the World Association of Zoos and Aquaria (WAZA) that took place in February 2006 in El Valle, Panama. This amphibian *ex situ* conservation planning workshop had over 50 individuals in attendance from 14 different countries. These individuals developed working groups to discuss organization, best practices, species selection, and rapid response techniques for the international response to the global amphibian decline.

There have been numerous workshops and classes offered internationally on amphibian biology, husbandry, and conservation to prepare the global community for the challenges ahead. Additional training programs should be sponsored around the globe. In addition, each year the AZA offers its *Amphibian Biology and Management (ABM)* professional training course, currently hosted by the Toledo Zoo. This course teaches amphibian keepers about the biology, captive care, and conservation of amphibians. International participants are

encouraged to attend, and often are supported by an AZA institution which partners with them on an amphibian research or conservation program in their country of origin.

And last but not least, an AZA facility can fulfill its educational mission by **developing and distributing educational materials**, which are utilized in many ways when working with *in situ* projects. After collaborating with amphibian project managers, institutions can assist with creating and funding the fabrication of classroom supplies, brochures, bulletins, posters, graphics, displays, and websites, to name a few. Developing brochures or other educational pamphlets can be provided for awareness, identification, or other purposes. Some projects have used flyers to find new species populations, while others have developed warning notices to help minimize the spread of chytrid. There are also many materials that can be developed to educate schoolchildren in the classroom. Simple educational activity and coloring books are a great way of informing children of the plight facing amphibians and make them happy at the same time. Many of these ideas have been utilized by current amphibian projects.

Staff Expertise and Time

In the past, most zoos believed that “a keeper is only a keeper,” regardless of the animals for which they cared. Today we realize that is not necessarily the case; even some talented and long-term reptile-focused keepers are not particularly interested or adept at keeping amphibians happy and healthy. There are unique skills required to be a successful amphibian keeper; attention to detail and water quality is absolutely essential. Therefore, it is essential that more dedicated amphibian keepers and/or aquarists be recruited, and that they receive proper training, practical experience, time, and resources necessary when participating in amphibian conservation. Additionally, the goodwill impact of spending resources on professional development and committing to the future of our industry cannot be dismissed.

As there are relatively few experienced amphibian keepers out there, advanced **training in amphibian-specific biology, husbandry, and health issues** for keepers, medical personnel, and researchers prior to program participation is essential. Sending staff to established facilities with large amphibian collections for hands-on learning with their skilled staff often helps a great deal and is a cost-effective way to increase staff development opportunities; often traveling staff can lodge with one of the host facility employees to save additional funds. This “shadowing” method also has the added advantage of creating professional contacts between institutions, and often aids staff at both facilities in becoming more excited about these critical animals and their personal contribution. The AZA’s *ABM* course was designed to help teach the basics of amphibian husbandry and management, while presenting basic biology, conservation, and medical information about these animals. The Amphibian Taxon Advisory Group (ATAG) highly recommends this accredited course for all staff members interested in becoming more proficient with amphibian species. The *ABM* course is usually offered once each year in the spring however, due to the urgent demand for trained staff, AZA has offered this course twice in 2007 and may offer two classes annually, as needed (visit <http://www.aza.org/prodev/> for Professional Training course listings).

Most everyone working in a zoo or aquarium environment wants conservation programs to succeed, and staff from non-biologically related areas of an institution may offer extra hands and perspectives that the animal department’s staff may not consider. Herpetology departments should welcome the aid of educators, facilities maintenance staff, accountants, government affair lobbyists, graphics and information technology crew, and others in the offices for their help with projects, paperwork, and facilitation of program goals. Their word-of-mouth and personal investment will further staff understanding. Plus, most employees are happy to be asked to participate in institutionally-supported projects.

Research on captive species requires time, passion, and energy, and is a burgeoning area with amphibians. There is so little known about so many species that *ex situ* research can take

many different avenues, most of which are neither difficult nor expensive to accomplish. Research into basic husbandry, reproduction, nutrition, and behavior are all possible to carry out, and there are often established programs that can be used as templates for initiating a project. Many species of amphibians have yet to be successfully maintained in a captive situation, so virtually anything we learn is beneficial. Others that have been maintained successfully still have not reproduced in captivity, creating yet another avenue for research possibilities. Our knowledge of amphibian nutritional needs is still in its infancy, and behavioral considerations are rarely addressed in an organized way. In addition, while zoos are often rabid in their need and/or desire to concentrate on enrichment activities with mammals, this is rarely considered with amphibians. Contrary to popular belief, the lives of amphibians can be enriched by fairly simple means, and there is no doubt that many other ideas and concepts can and will be developed when institutions become more heavily involved with these species. Staff time will be necessary to provide logistical project coordination and implementation, as well as support institutional infrastructure during any project.

Oftentimes, institutions are hesitant to initiate research projects on their own, in the belief that ideas or concepts may not be fully realized. In these cases, collaboration with one or more other institutions should be considered. Good research often leads to more questions than answers, and each collaborating institution can take on the task of answering an individual question as it arises. Duplication of efforts, rather than being a hindrance or a waste of resources, is very important, especially when so little is currently known. When the same or similar results are noted at multiple facilities, the overall result is more credible.

Staff should be supported and encouraged to publish or present their findings from research endeavors. With such a small pool of knowledge currently available on amphibians in captivity, sharing what is learned may lead to advances with other species elsewhere. Research can be shared via herpetological publications and/or reporting presentations/posters at professional meetings, conferences, or symposia. It is recommended that the mode of dissemination be peer-reviewed so that the research will hold up under scrutiny and be more valuable to science. See Chapter 4 for more information on research program needs and considerations.

With the large number of amphibian species in need of research and captive efforts, **program coordination and management** for all species involved will require a lot of time and committed individuals to facilitate and assist with logistics. Institutions, whenever possible, should allow staff both the time and resources to accomplish those coordination tasks. AZA-wide initiatives only succeed with the help of program volunteers. Some projects may need help from an organized group of individuals and institutions, but smaller, more restricted species with fewer specimens may require only a single person to focus on managing a program, both within zoos and to act as a liaison with local government agencies.

The first step for program participation is to make sure that an institution has assigned an Institutional Representative (IR) to the ATAG. This group and its steering committee offer the most essential peer-based communication program for amphibians in North American zoos and aquariums. By participating in annual meetings and IR listserv communications, IRs at each AZA facility have input on steering committee elections and ATAG decisions on species issues, population management, conservation programs, and strategies to achieve the combined goals of all parties involved. The IR is also responsible for communicating relevant information back to directors and staff at their home institution. Contact the ATAG secretary with any questions regarding the designation of your facility's IR. Additionally, the general ATAG listserv is open to all interested personnel as a timely venue for amphibian-related communications on husbandry, research, and conservation.

As more species and populations are managed for conservation and genetic/demographic reasons, more species program coordinators and studbook keepers/population managers will

be needed. While most institutions would love to have studbook keepers, PMP managers, SSP or Taxon Management Plan (TMP) coordinators on staff, they are often unwilling and/or unable to provide the time necessary for database management and to facilitate action plans. Institutions need to dedicate the time and money necessary for these talented individuals to get the necessary training, including AZA Population Management I and II classes, to fulfill their obligations in a reasonable amount of time. In fact, the Director of their institution must sign an agreement stating that with his/her granted approval the institution fully commits to support the staff member. It is highly recommended that institutions provide work-time for these endeavors, rather than requiring individuals to complete these projects on their personal time. Relying on staff to volunteer their time to these important programs leads to low morale and individuals that are unable to complete all their endeavors. Everyone needs a life outside of work to be personally happy and healthy.

Professional contacts

Whether an institution focuses on native species or exotic species, **utilizing existing government or professional contacts** with which your institution already has a relationship is ideal for amphibian program start-up. Look for prior staff or institutional experience that may lead to networking opportunities. Also, other zoos and aquariums may need your contacts in order to facilitate their project goals. New projects can begin this way, or you can join an existing project that may need components that a new institution can offer. For example, Omaha's Henry Doorly Zoo had a relationship with the Johannesburg Zoo through some previous mammalian reproductive research. Their utilization of this relationship has evolved into an impressive amphibian conservation project with a facility at the Johannesburg Zoo dedicated to conserving amphibians of South Africa.

If an AZA facility needs assistance developing new relationships for desired programs, contact the ATAG steering committee members for ideas. A request to other institutions through the ATAG listserv or references to researchers, government officials, or species specialists may establish those contacts. Do not be afraid to contact local, state, or federal wildlife agencies, as well as colleges or universities in order to get involved with local species; zoos and aquariums have much to offer to conservation of wildlife that is generally welcomed. As institutions continue to expand on their amphibian collection, especially with species that have significant conservation value, more of these contacts will follow.

Summary

In the struggle to secure as many declining amphibian species for the future as possible, awareness of each institution's existing resources is only Step One. Once the space, time, financial resources, trained and dedicated staff, and communication network are in place, participation is ultimately up to each institution. Consider initiating *ex situ* programs with new species and supporting efforts to broaden our limited knowledge of these amazing animals.

If your facility still is not ready for the challenge of a new program, continue to improve upon your amphibian resources and training, and gain experience through a smaller, model, or existing program. Regardless, begin with basic peer-communication by becoming active with the ATAG, confirm that your institution has an ATAG IR, and see if others can offer solutions to the gaps slowing down initiation of your own novel amphibian programs.

As stated elsewhere, research requires time, energy, and passion. If an institution dedicates the time portion, the energy and passion comes more readily. When individuals are required to dedicate much of their own time to these endeavors, as well as their passion and energy, they often become disenchanted and burned out quickly. We not only want and need to recruit more people to our amphibian mission, but we want them to remain committed to the effort for many years to come!

Chapter 4: Developing Amphibian Research Projects Both *In situ* and *Ex situ*

Vicky A. Poole, National Aquarium in Baltimore

Introduction

As zoos and aquariums are places of captive environments, the general concept of providing a haven for declining species is well established. However, housing a group of animals outside its natural range is not conservation in and of itself, but rather part of the whole picture of habitat and species preservation in the wild. Without understanding and preserving the entire ecosystem in which a species exists and its natural history, we are merely maintaining species in our institutions that may never be able to supplement or replace wild populations, should that become necessary.

In situ research describes any project undertaken within the natural environment where a studied species is found. These projects can also include the surrounding habitat and environmental conditions, as well as other species within the range of a studied area. With the expertise of zoos and aquariums, a project may also include an *ex situ* (outside the natural range) component, including captivity and laboratory settings.

In addition to results that a research project can generate, directly impacting species and habitat management *in situ*, participation in these efforts support the conservation and research end of all of our institutional missions while offering opportunities for staff development, positive public relations, and support for our captive collections. When considering beginning or participating in an existing research effort, evaluate your resources (funding, staff time and interest, facilities, etc.) and make sure the effort fits with your institution's goals, mission, collection plans, and experience for maximum returns on investment. See Chapter 2 on amphibian conservation and ICPs.

There are many different types of research projects, most of which have established standards for monitoring and evaluating that an experienced researcher will be able to perform. For the novice, it is important to partner with qualified personnel during the planning and evaluating stages as well as in the field or laboratory. Methods for running a research project as well as some examples of *in situ* and *ex situ* amphibian research projects from zoos and aquariums will be presented.

What does amphibian research involve?

Opportunities for increasing our knowledge about amphibians present themselves almost daily. Determining the specific types of research for zoos and aquariums to undertake is ideally driven by a conservation application for species within our collections and information desired, such as life history studies or population assessments prior to reintroductions. Often, many types of projects are utilized in order to obtain the most complete picture possible.

Taxa specific studies for single species or assemblages *in situ* include collection of distribution range, genetics, life history data (i.e., reproduction, behaviors, and habitat use), intra- and interspecific interactions, skin pharmacology, as well as population and health assessments. Common techniques for sampling include trapping, visual or audio monitoring programs, various transect methods, tracking via telemetry or pigment trails, breeding site surveys, and larval sampling. Using more than one method and applying the appropriate ones during different times of year, such as during breeding or dry seasons, increase the likelihood of collecting relevant information. Whether monitoring population declines (Mendelson, et al., 2005) or testing new field or conservation techniques (Lindquist, et al., 2007), AZA professionals are leaders in *in situ* research.

As amphibians are so closely linked to their natural surroundings due to their dependency on water, it is virtually impossible to study any amphibian species in detail without closely examining their place within the related ecological complex. **Ecological based studies** include habitat assessments such as floral and faunal diversity, food-chain complexes, climate (i.e., temperature, precipitation, barometric pressure) and the environment (especially water quality parameters), as well as the specific issues that impact local natural community changes. Before Panamanian golden frogs were ever collected to begin the *ex situ* population in AZA facilities, data was gathered for two years on the habitat, water quality parameters, seasonal temperatures and rainfall from data loggers, and other environmental factors that would influence the success of housing them in captivity, including identifying the local diatoms which the tadpoles eat (Zippel, 2002; Poole, 2006).

Baseline background information is quite useful when assessing the successes of restoration and habitat alteration projects. The process of the Adaptive Management Feedback Loop utilizes the information gained from **restoration or habitat alteration research** to constantly influence the land management decisions, such as hydrologic improvements, prescribed burning, or restricted grazing of a field site. As a public model of their Adopt-a-Pond Program, the Toronto Zoo restored an on-grounds wetland. They established experimental plots to test the vernal pool design and function as a breeding site for treefrogs to see if additional modifications were necessary.

Reintroductions offer many opportunities for *in situ* projects involving home range comparisons to wild specimens, habitat utilization, behaviors, and program success. In addition to surveying froglets post-release, the Puerto Rican Crested Toad SSP has also researched the diets and growth of wild tadpoles and froglets and compared them to cohorts in captivity. The St Louis Zoo is currently comparing the home ranges of Ozark hellbenders and their survival by radio-tracking released specimens.

Since much of the known veterinary research on amphibians has come from *ex situ* zoo/aquarium collections, **study of health issues** impacting amphibian species *in situ*, such as chytridiomycosis and ranavirus, should be performed for monitoring purposes or prior to any attempts at either species collection or reintroduction (Gascon, et al., 2007). Additional studies into the natural history of the pathogens themselves is critical, including persistence, transmission, climate influence, resistance, and ultimately, suppression. Amphibian researchers will have to continue to monitor *in situ* infectious disease at their sites so that the impact from pathogens is constantly updated and valid.

Research on *ex situ* specimens in our own institutions or capacity facilities can provide important information about basic health and husbandry parameters to supplement information gathered from fieldwork. Studies on diet, predator-prey responses, medical, genetics, habitat and environmental preferences, and assisted or natural reproduction can be relevant to a field researcher when seeking new populations or to a conservationist when determining potential release sites for species reintroduction. By simply utilizing medical specialists within our own organizations such as nutritionists (Browne, 2004), reproductive physiologists (Obringer, et al., 2000; Browne et al., 2006), geneticists (Chiari, et al., 2006), and pathologists (Davidson, 2003) zoos/aquariums have led the way in amphibian research for years, including the original description of *Batrachochytrium dendrobatidis* (B.d.), more commonly referred to as the chytrid fungus (Longcore et al., 1999).

Studying the **impacts of wildlife/habitats on local and regional human communities** is not commonly considered by biologists planning a field project. However, research by a social scientist can assess all stakeholder needs that may have long-term impacts on the success or failure of any resulting conservation efforts. This area may be one of the most important areas

of research, since anthropogenic causes of species decline must be identified. Studies should examine the impacts species and projects have on local persons and livelihoods, so that negative attitudes might be improved through education, public awareness, and alternative economic strategies.

Any of these types of research are even more relevant when studied over an extended time period and can include or compare both data collected before, during, and after population decline events, known causes of decline have occurred, or habitat alteration is completed. The goal is to have a foundation on which to build conservation efforts.

How to determine which project to do

Participation in any conservation or research endeavor is generally driven by the passion of the persons involved. Look to prior staff or institutional experience that may lead to networking opportunities. Consider starting your own novel project, expanding upon other projects to include an additional research focus, or offering your experiences to other projects.

Local vs. International?

Where to work is as big of a question as what to study. Focus on projects that are a good fit for your institution. If your facility only exhibits North American species, then working in Asia is not as appropriate as considering a local salamander project. The World Conservation Union (IUCN) recommends focusing on regional priority species before tackling international issues.

There is still so much to learn about amphibians in our own country that justifies taking on a local project. Every state has its own list of threatened and endangered species some of these which may actually occur naturally on your institution's grounds! Most institutions that have international projects tend to gain their experience with field projects locally, where the physical and cultural environment is much more familiar.

International projects have their value and intrigue; however the logistics and costs go up when working abroad, while the available time in the field is usually limited. Consider expanding existing institutional relationships abroad or work in a sister city to where you are located.

Impacts and costs

Participating in a project involves funding and staff time, both of which are limited in almost every institution. Matching your institutional commitment to your resources is key. Be honest with what you have to offer, funding shortfalls and staff shortages during field projects are hard to prioritize when a crisis happens. Your institutional financial and staffing needs must always be met first and foremost.

Funding sources vary, depending on the scope or duration of the research. For smaller/shorter projects, an institution's operational budget may be able to absorb these limited expenses. Projects of a longer duration or larger scope will often require outside funding through grants or a benefactor. On-going projects can usually be funded by several funding sources in order to cover expenses.

You do not have to be a development wiz to apply for grants. There are a plethora of grants available for research projects, however the range of what each will cover financially will help select which grants are appropriate for your specific needs (Appendix 3). Be sure to always acknowledge the funding sources during project reporting.

Unless an institution has dedicated researchers, the amount of time spent working on projects generally takes staff away from their routine duties. The workload must often be shared by coworkers and subordinates due to time constraints. This added responsibility can have

negative impacts if clear communication of the purpose of the project is not related from the beginning. Consider funding additional supplementary field positions or temporary staff at your facility during that time, which may add an additional funding constraint as well.

The staff development opportunities offered by participation in projects is wonderful, but not priceless. It is an invested cost that an institution must incur for undertaking research and is considered part of an institution's financial commitment to a project. Most grants will not cover staff salaries of investigators, however you can usually include the outside costs of field assistants/technicians or contracted procedures performed by specialists such as pathologists, geneticists, translators, or surveyors, as long as it is relevant to the project. And hours put in by paid staff (plus an 18-23% overhead for benefits, etc) do count towards *non-grant funding* or *match support* on applications.

Depending on the project, involvement can be shared by many in an institution from employees to volunteers. Students or youth groups can also be encouraged to participate if possible which will foster a desire to preserve wildlife and wild lands, familiarize them with current technologies and techniques, and provide them career-path opportunities.

If you have limited staffing for your facility, than sending your staff to participate in projects with other organizations offers a valuable staff development opportunity that only involves a limited time and financial resource.

Partnerships/Teambuilding

There are different group dynamics when considering research projects, each with their own sets of costs and benefits as outlined below, although the most significant goal should be the project results.

- **Solo institutional effort** where the financial and time investment is higher, resources may be somewhat limited, but in-house decision making process is familiar and less cumbersome.
- **Partnered institutional efforts** show cooperation for a common goal and are very popular, especially when seeking outside funding and media attention. Mutually beneficial collaborations allow partner institutions to pool their resources and expertise, spread costs, address several aspects of a project simultaneously, multiply impact for public awareness and promotional opportunities, and benefit from many perspectives. Logistics and communication time increases with additional personnel.

Obvious potential partners include other zoos, aquariums, botanical gardens, and nature centers. Local, state or federal government agencies or research organizations, such as natural resources departments, US Fish and Wildlife Service, or National Institutes of Health benefit from zoo/aquarium involvement in research programs. For example, the Santa Barbara Zoo is working in partnership with the US Forest Service to survey streams for California red-legged frog (*Rana draytonii*) and Arroyo toad (*Bufo californicus*) eggs and larvae (Foster, et al., 2007), and the Puerto Rican Crested Toad SSP works closely with the USFWS on both *in situ* and *ex situ* research and conservation efforts.

Contact can also be made with non-governmental organizations (NGOs) like natural history museums, herpetology or natural history societies, and other conservation organizations which are full of individuals who focus on amphibian biology, habitats, or other related research. These groups can also be a potential source of volunteers, staff, and project funding, and in turn, mutually gain from the experience and direction zoo professionals can provide.

Universities and veterinary schools can provide partners with access to many other disciplines within their institutions that can be quite necessary to any project. Academic professionals such as biologists, entomologists, geneticists, pathologists, botanists, to name a few, are

experienced with research design and can usually help with planning project methodology. Contacting a statistician before implementing research will help guarantee that the data to be gathered is appropriate and accounts for randomness, as well as assisting with data analysis. A social scientist such as a professor of women's studies, religion, or anthropology will be able to assess local and regional anthropogenic factors/attitudes impacting taxa or habitat involved when preparing a project in an unfamiliar culture. When research technicians are needed, bright students are accessible and are willing to gain experience while obtaining academic credit via internships.

Networking is key to forming your team. When selecting collaborators for your project, the following guidelines should prove valuable:

- Seek out individuals who share your interests and are as passionate about the project as you are.
- Work with individuals that are able to keep an open mind and can be attentive to the validity and reliability of the research.
- Define roles and establish recognition agreements *in advance* to minimize miscommunication and to keep the project running smoothly.
- Approach organizations that would benefit from having your institution serve as a venue for promotion of their projects and educational messages.
- Involve other departments within your own institutions, with supervisory approval.
- Be aware of the physical needs that your particular research may involve and select individuals that are capable of working in varied climates and terrain.
- Include someone on your team with medical or first aid/CPR skills qualified to respond to emergencies, including snakebites, if appropriate and depending on the remoteness of your research site.
- Communicate and meet periodically with all team members throughout the project to keep it on track, and at completion so that all goals have been accomplished.

There has been valid amphibian research performed by AZA facilities both *in situ* and *ex situ* for over 20 years. Beyond the research information given in the individual Species Action Plans in Appendix 4, here is an example of zoo/aquarium research to illustrate the partnerships, evolution of projects, and the resulting work:

The National Aquarium in Baltimore and The Baltimore Zoo (now The Maryland Zoo in Baltimore) worked in partnership on several studies with the National Institutes of Health (NIH) based on the alkaloids found in the skin secretions of poison dart-frogs (Dendrobatidae). These concentrated on the roles of genetic and environmental determinants in the inter- and intra-specific variability in alkaloid profiles (Daly, et al., 1994), and studies of the uptake system for dietary alkaloids present in most genera of this family (Daly, et al., 1992), by utilizing dart frogs in captivity and sampling wild specimens in Central America.

During a field expedition to collect specimens for these studies, a population of the granular poison-dart frog (*Dendrobates granuliferus*) was found on the Atlantic coast of Costa Rica, well outside of its known range, living sympatrically with the strawberry poison-dart frog (*D. pumilio*). This discovery was of great interest to researchers at the American Museum of Natural History, which led to a second expedition to the locality and a publication (Myers, et al., 1995).

Several other collaborative research projects have resulted over the years from this continued relationship (Daly, et al., 1997a; Daly et al., 1997b).

How to get started with a research endeavor

Once you have decided on the subject of your research project, determined the level of involvement you can provide, established your team and defined roles, you are ready to begin!

If you are **starting a project from scratch**, the type of research design (experimental hypothesis testing with or without controls, or non-experimental designs, for example) will be dependent on the data you are considering gathering or the question to be answered.

The basic parts of the research process include the following distinctive phases, which are not necessarily linear, but can occur concurrently and often lead to new aspects of research.

- **Define Problem to be Studied:** The question to be answered will determine if you will need to pursue exploratory (of unknown scope or unclear definition) or conclusive (results that lead to decision-making, generally quantifiable) research.
- **Literature Review:** Thoroughly research the issue/species including current literature, status and legal issues, habitat, and the potential impact to other stakeholders in the region that you may impact, or visa versa.
- **Design and Plan Research Project:** During this process you will need to prepare a Research Proposal to have on hand for funding requests, permit applications, and other review purposes.
 - Review Measuring and Monitoring Biological Diversity, Standard Methods for Amphibians (Heyer, et al., 1994) for field techniques specific for amphibian research.
 - Establish contacts with agencies (local, state, federal, and/or international) regarding any necessary permits.
 - Acquire any Institutional Animal Care and Use Committee (IACUC) approval(s) required from participating institutions for issues of animal welfare, if relevant. The American Society of Ichthyologists and Herpetologists (ASIH) have developed standard animal welfare recommendations for using amphibians in research and is available on-line (Beaupre, et al., 2004).
 - Seek and acquire funding once the project is designed and methodology is planned. Be prepared to write grants in order to fund all or select portions of your research project.
 - Select subjects: Determine if the entire population is to be researched (census) or a sample of the population is to be used. Analysis of data collected from the latter group will depend upon whether the sample is representative (probability testing) or non-representative (non-probability testing) of the entire group.
 - Determine methods and data collection techniques. Plan project with definable (and quantifiable) results, if possible.
 - Solicit qualified independent authorities to critique your project design for merits of scientific research and soundness of methods before implementation.
 - Establish a timeline for the project and divide into discrete phases if necessary. A pilot study may be necessary before the full research project begins to test feasibility and appropriateness, and evaluate logistics.
 - Gather equipment needed for research (if a field project, see Checklists A and B for suggestions) and organize logistics for field research with team members.
- **Data Gathering:** Project implementation stage.
- **Data Processing and Analysis:** This is where data gathered is evaluated to show trends or to produce results to be discussed.
- **Project Conclusions and Recommendations:** Bringing the research together and interpreting the results from the analysis leads to critical conclusions, and possible strategic recommendations for further research or management.
- **Project Evaluation and Dissemination:** It is always a good idea to have independent authorities and team members evaluate projects after they are concluded to see if they were effective and/or modifications should be performed. In addition, the research should be shared with the public via publications and/or reporting presentations/posters at professional meetings, conferences, or symposia. It is

recommended that the mode of dissemination be peer-reviewed so that the research will hold up under scrutiny and be more valuable to science. Some other factors to consider include the following:

- Always acknowledge ALL partner institutions and help from supporting organizations and funding agencies.
- Allow time for review by team members prior to release of results and reporting publications.
- Translate resulting publications and developed materials for distribution to participants, agencies, and stakeholders in foreign-speaking range countries.

If unable to perform a start-up project, **consider contributing to an existing project.** Have your Amphibian Institutional Representative (IR) contact the Amphibian Taxon Advisory Group (ATAG) Chair for current projects with AZA institutions (See the AZA Amphibian Action Plans in Appendix 4 for some suggestions), or contact local, state, or federal agencies for work needed to be performed. Other existing amphibian projects programs are always looking for additional participants, including:

- Local university research
- Frogwatch USA monitoring program of the National Wildlife Federation
- Regional initiatives of conservation groups like natural history societies or scouts.

Expand current projects into other regions or onto your institution's grounds. Some projects can be further enhanced by additional research that your facility may be able to provide, for example, nutritional or genetic research.

Through networking at conferences, maintaining memberships in professional organizations, and joining Internet listservs, you can usually find a project that meshes with your mission and interests.

If your facility is unable to provide sufficient staff time for a project, **providing financial support** to external research is always a great way to participate. In addition to direct institutional funding, smaller fundraising efforts (T-shirt sales, bake sales, etc.) for specific projects often get staff and volunteers from many areas interacting towards a common goal. Consider developing an endowed granting program to help provide continuous support for amphibian research projects.

In-kind support, such as providing equipment or supplies, meeting facilities, or professional services support (i.e., administrative, advertising/PR, legislative, or translation), as well as allowing a research site on your institution's property, are all potentially helpful to any under-funded project. Consider what your institution can bring to the table to help ongoing efforts succeed.

Field research concerns

There are added logistics and specific concerns for doing research in a species' wild habitat. Inquiries about the location for lodging, transportation to and within, facilities for research activities not performed in the field, animal housing and veterinary needs, safety and security, access to clean water take extra time and increases with the remoteness of field sites.

How field personnel and the research impacts the environment must be considered. Researchers, ecotourists, and others are moving into areas where humans have never been in the past and are perhaps further spreading diseases to naïve amphibian populations. It is recommended to wash and disinfect all equipment with alcohol or a mild bleach solution, including footwear and clothing, between sites to reduce the likelihood of carrying fungal spores, viruses, or bacteria from one area to another. When working in areas with known chytrid, ranavirus, or other pathogens, reduce chances for spreading diseases by beginning work in unexposed areas before moving into "hot zones." Similarly, it is not wise to use

equipment from an institution's animal collection in the field without disinfecting before and after use. If hand washing is not possible between handling individuals, specimens should be handled with disposable gloves only. Speare (2001) has listed recommendations on minimizing the risk of disease to wild amphibians.

Some other tips for field research, especially when researching amphibians are as follows:

- When working in the field, consider swabbing all amphibians encountered for chytrid testing. Boyle, et al. (2004) has outlined a chytrid swabbing method.
- It may also be necessary to prophylactically treat amphibians for *B.d.* as they are removed from the wild (Nichols and Lamirande, 2001).
- Plan an itinerary and share with team members in advance including setting meeting times and locations, sites for contacting family and work, and plan breaks if time/funds allow (recuperation and logistical flexibility).
- Establish a safety plan. Prepare a safety kit, know where the closest hospitals are to your field site, and be prepared for the possibility of a snakebite. Also consider having emergency cards for everyone on the field team including emergency contacts, insurance information, allergies, and other relevant medical information.
- If the location is remote, plan an extra overnight on the way back from the field before the travel day. This layover stop allows field teams a chance to clean up and rest before traveling, or if necessary, additional time for dealing with unexpected logistics.
- Consider fitness for grueling fieldwork. Start exercising and working out to prepare for the rigors of fieldwork.
- Be sure to get permission for access to field sites from local agencies if public lands or from private landholders, well in advance if possible. Confirm each time the site is accessed so managers are apprised of activities on their property.
- Have a reliable, appropriate field vehicle to use. The limiting factor for field team size is usually the vehicle (typically 4 persons can sit in a SUV with room for field gear). If the team is larger, project expenses increase to provide additional vehicles, so fill the vehicles to capacity to get the most out of your investment.
- If housing live animals while in a field setting, their care and comfort must be considered. While the rest of the team is gathering data, having dedicated husbandry personnel may be necessary to move animals into appropriate environments (i.e., out of direct sunlight or soaking cooling bags in streams), collecting field sweepings or other food items, cleaning enclosures, providing water from the site in which the animals were captured, or performing medical treatments.

International Research Concerns

In addition to the field issues listed above, there are other issues that may further complicate working abroad. Some suggestions to improve planning are listed below:

- If personnel need passports or other documentation – don't delay! It's now taking several months to secure a passport in the USA. And check with the consulate for the research country to see if you need any visas (including research), which also may take some time, have unexpected restrictions, and require you to travel within a specific timeframe.
- If required, acquire USFWS C.I.T.E.S. Import Permit in advance of trip (it may take 6-9 months to receive permits or renewals depending on request and backlog).
- Be safe! Check out the State Department's Travel Warning for the country in which you'll be working.
- Consult your physician and/or a travel medicine specialist for necessary shots, anti-malarials, and other recommended prophylaxis.
- Include bi-lingual personnel or hire translators experienced in fieldwork, if necessary.
- It helps speed things up at the airport to get permission from the airlines in advance if flying back with specimens/samples that need to fly in the cabin along with you.

- Acquire equipment well in advance since some stuff will not be available in the research locality.
- Plan and distribute itinerary to all participants in advance so that team members can arrange travel and fully understand where and when to meet.
- Secure lodging in advance for all team members together. Have everyone meet at a common hotel the evening before work is to begin, so that the entire team can get an early start the next morning.
- Consider hiring local persons as added field guides and technicians. Not only can they serve as translators and are familiar with the habitat, but it empowers them to become stewards of their environment, supports the local economy, offers experience, and may help alleviate negative attitudes in the local community towards the project if one of them is involved. Seek out grants that cover expenses related to hiring local guides.
- If working in a foreign speaking area, be sure to get materials translated in advance for local field personnel.
- If traveling back with samples or specimens, allow a few extra days for export permits, customs, and other necessary paperwork acquisition.

Summary

With the current state of global amphibian population decline, the end goal of any research effort should be species and habitat conservation applications. Whenever possible or appropriate all taxa specific projects should also expand into habitat. After a population has been surveyed and the habitat data been analyzed, perform a Population-Habitat Viability Assessment (PHVA) and use the information as a tool to help guide conservation decisions by determining what impacts causing decline can be eliminated or reduced.

Be mindful of the social impacts amphibian species and habitats have on the local communities, especially when declines may be a factor. Documented research into this area can be additionally beneficial when conservation strategies are considered.

And finally, project evaluation and dissemination of results is extremely important and allows the research findings to be fully utilized by others. Use peer-reviewed journals when possible since the findings are critical to establishing a reliable body of research and knowledge on amphibians and their environment.

Hopefully, this chapter has inspired folks to consider accepting the challenge of an amphibian-related research project. Filling in the science and natural history gaps before populations or even entire species disappear, will help not only captive management, but conservation decisions as well.

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Checklist A: Equipment for working in the field

Pack as light as possible, especially if hiking to remote sites and select gear that is appropriate to the terrain in which you are working. A secure staging spot or field vehicle for leaving some equipment that is needed rarely or periodically (i.e., extra food or travel clothes) is helpful.

Place everything in plastic bags in case of rain or being tied to the top of a vehicle for space. A suggested list of field equipment is as follows:

- o Collection materials, or other needs for your research
- o Flagging tape to mark path or other research locations.
- o Global Positioning System (GPS) receiver (one per field team)
- o Good cell phone (one per field team)
- o Camera with extra batteries and film/memory cards
- o Laptop, or else internet cafés for maintaining contact
- o First aid kit, including Sawyer Venom Extractor® (and antivenin in extremely remote) and an Epi-pen®. Other supplemental supplies to the kit can include vet wrap, mole skin, skin adhesive, latex gloves, gel hand sterilizer, iodine, triple antibiotic, hydrocortisone ointments, Tylenol®, Imodium®, antibiotics, and sterile saline (eye/wound flush).
- o Whistle for locating lost individuals.
- o Backpacks and/or daypacks
- o Sleeping gear, including tent and sleeping bags or jungle hammock
- o Cooking gear and mess kit
- o Food for the duration of field time, including energy bars if unable to cook due to weather, etc.
- o Water, or water purification tablets or portable water filter. Have a personal water bottle, as well.
- o Gatorade or other electrolyte-replacing powder.
- o Water storage container(s) for transporting water to campsite
- o Clothes, including comfortable footwear, hat sunglasses, and inclement-weather gear.
- o Towels
- o Toiletries
- o Toilet paper
- o Sun block and bug spray
- o Flashlight and/or camp light. Headlamps are very handy for fieldwork and at the camp site.
- o EXTRA BATTERIES. Bring 3 times the amount you think you will need in the field.
- o Multi-tool and/or field knife
- o Duct tape
- o Waterproof notebooks and permanent markers.
- o Money

Checklist B: Additional equipment for international field expeditions

Field research in foreign countries may require some of the following supplies in addition to the list in Checklist A:

- o Phone cards or reliable international cell phones
- o Pocket translating dictionary
- o Duplicates of passport and plane tickets (left in an accessible secure location if needed).
- o Foreign currency

Chapter 5: Developing *In situ* Conservation Projects

Bob Johnson, Toronto Zoo

Introduction

Most zoos and aquariums include conservation as one of their mission statement pillars. Previously, this simply meant informing zoo visitors of the threats to the world's wildlife and the loss and fragmentation of habitats. The scientific management of animal populations required collaborative programs to ensure that at least the genetic component of biodiversity was preserved within the relatively small captive populations. As animal managers realized that their role in conservation could not be divorced from the threats to populations in the wild, cooperatively managed species programs provided zoo based expertise and support to assist in the recovery of wild populations. Having an animal management group within SSPs and TAGs facilitated the development of working groups to make recommendations for conservation programs.

More recently, zoos and aquarium have reaffirmed their roles as holistic conservation organizations. Their captive animal ambassadors are now linked to their wild counterparts, and often the protection or restoration of the habitats in which they live. Sustainable programs require an inclusive process with the people that live with wildlife or depend on competing resources. Now *ex situ* conservation has come to mean working to increase the capacity of *in situ* partners to protect their natural heritage.

Developing *in situ* conservation projects, either regionally or internationally, mirrors the requirements in developing *in situ* research projects. Consult Chapter 5 on *in situ* and *ex situ* research programs, particularly the sections on networking, international research planning, and how to get started with a research endeavor. The impetus for such programs may be opportunistic or strategic, but in either case there are similar steps in developing a course of action.

What are *in situ* conservation projects?

As suggested, *in situ* refers to projects that take place in the location where the conservation action is to occur. This may be at a single site, but the program may extend beyond the site itself if conservation programming is warranted in the region or across the country. For example, although the Puerto Rican crested toad conservation program focused on protection of a single wetland breeding habitat, recovery of the species required an island-wide awareness program involving partnerships from other regions. Projects may involve more than one species, particularly if program components address the threats common to a number of species.

It is important to clarify your institution's goals. Whether initiating a project or partnering with existing projects, your *in situ* conservation program should satisfy institutional conservation objectives through its strategic or collection plans, both of which should have Board-level approval. In addition, these programs must compete for limited resources through a facility's standard evaluation process. Funders are more supportive of projects that meet strategic goals. Conservation projects may initiate in animal departments but grow to represent core institutional priorities.

In situ projects require partnerships and a mandate from the species' range country. Projects in the past have often involved a single institution perhaps working unilaterally for the recovery of a species at risk. However, rarely is this the case today. Now institutions initiate projects based on recommendations from evaluating authorities, such as IUCN or AZA conservation committees, or opportunistically explore projects that can then be partnered

with other institutions. Programs that have had greatest success and have been sustained have multiple partners and engage in-country partners in program development. Where skills or equipment are required, conservation partners share the expenses to build in-country capacity.

Conservation projects are multi-layered, dealing with both species management and habitat restoration or protection, and often incorporate a research component along with increasing public awareness. Projects of this nature, involving multiple disciplines and extending over several years, become programs with a more holistic approach to conservation than individual projects. As with research projects, *in situ* conservation projects may simply require an infusion of funding, training or equipment to assist highly skilled and motivated national partners. Often partners are able to promote a project to the level of national concern because the conservation issue has received broader attention. Beyond funding and research skills, zoological management expertise includes genetic and demographic management of small populations, education, nutrition, pathology, veterinary medicine, habitat restoration, construction and infrastructure development, fundraising, promotion and marketing, and even more simply, labor. In any case, *in situ* conservation often requires the assistance of other departments and serves as a team-building and marketing opportunity for your whole facility.

Getting started

In the past, North American zoos and aquariums have been involved in projects around the globe. However, the recent Global Amphibian Assessment (GAA) recommends that zoos and aquariums partition their programs on a regional basis simply to deal with the overwhelming scope of the decline of a whole taxon. Thus, AZA institutions are encouraged to focus upon *in situ* conservation projects from the Caribbean and Americas.

To get started, take stock of your resource commitment. Develop an action plan (see Appendix 4 for examples) that will provide a short or long-term focus while guiding you through important steps. Deviating from the action plan will happen as opportunities present themselves, but it is important to know how opportunities mesh with the goal. There are large differences in resources and obligations if starting a program or contributing to an established program with clear expectations of need and results.

There are many resources to help you get started. To maximize results with limited conservation resources, tapping into existing conservation projects provides you with a set of already established priorities and goals. Usually these programs have an existing action plan (Appendix 4), partners and stakeholders identified, some sort of mandate, and offer differing levels of involvement. Within the AZA, the ATAG has identified coordinators to spearhead species-specific actions (Appendix 2), and Amphibian Ark has a directory of projects requiring additional help (www.amphibianark.org). Each program coordinator can help identify projects of greatest need to meet your institutional budget or strategic directive. All AZA programs are in need of funds to sustain *in situ* conservation projects and this is the simplest start for many project partners. The funding criteria of the Conservation Endowment Fund (CEF) grants are a good starting point in determining the components that are important to consider. An institution can rotate staff and resources through projects as priorities shift. Your own local government wildlife agencies also have conservation plans for at-risk species. Sending staff to the AZA *Amphibian Biology and Management* course provides additional skills for developing *in situ* conservation projects and networking contacts.

Lastly, not all amphibian conservation programs require a species focus. Conservation of ecosystem complexes through support of protected areas captures biodiversity hotspots or whole amphibian species assemblages. Indeed, conservation of priority taxa may be the

impetus for establishing or maintaining protected areas. Stewardship programs with local communities or landowners will be required for non-protected areas.

There are several steps before conservation plans are implemented:

1. Stakeholder input-

Stakeholder meetings provide an opportunity to review existing resources or gaps and identify personnel and their institutions already working on conservation programs. Often these contacts provide entry and additional contacts with established communication networks. Stakeholder meetings ensure that all potential partners have an opportunity to become involved and to identify resource needs. Involvement at the formative stages ensures buy-in and program growth at a speed compatible with in-country decision-making processes. This will avoid potential partners from feeling left out of a process and that a diversity of approaches are incorporated in the action plan.

2. Resource evaluation-

Be sure to assess your space, financial, and personnel limitations, as well as your existing partnerships to ensure that your commitment does not exceed your resources (See Chapter 3).

3. Action plan development-

Action plans provide a step-by-step approach to the implementation of a conservation project. The stakeholder meetings will have identified a host of issues, possible solutions, and potential partners. Group similar projects or steps into five or six large categories (such as research needs, fundraising, habitat restoration, *ex situ* role, education/outreach, and administration to name a few). Each category would capture the diversity of ideas from all stakeholders and participants through this all-inclusive process, which then become goals. Identify the necessary actions to achieve the goals ("action steps") and then review to ensure that all possible solutions are documented, goals are adequate to address conservation needs, and gaps are addressed.

Assign priorities for each action. Timelines can then be assigned based on priority or logical progression, with individuals or organizations identified as leaders. Leaders are responsible for coordinating group responses and reporting at subsequent meetings.

I prefer to develop three-year plans with a detailed budget for each year. Unspent funds can be directed towards the next year's budget or any funding shortfalls will have less impact because they can be recovered within a year. Over the three years, funds are being spent in Year One, while resources are being allocated for Year Two and raised for Year Three. Annual reports will provide a summary of each year's results and outline the budget and plans for the upcoming year. This allows me to plan beyond a single year and to adjust as necessary with the knowledge that there is time to revisit issues. Having the project planned out over three years demonstrates commitment and is an honest appraisal of the resources and timeframe required for program implementation.

At this stage you should have identified all potential partners, stakeholder needs, resources, conservation concerns and remedies, needs and gaps, steps to address goals, leaders and a time-frame for action and reporting. The end result is an Action Plan that has been generated in-country. Buy-in from all participants in an open forum develops credibility for the process and the newly formed partnerships. The goal is now to develop capacity so that skills are sufficient to make programs sustainable beyond the involvement of outside input. Making programs self-sufficient is the goal of *ex situ* conservation.

Starting with a pilot project provides an opportunity to explore and evaluate partnerships and working relationships without committing long-term resources. As pilot projects evolve into

partnerships with larger expectations, they require guidance from action plans that have stakeholder consensus. Funders also like pilot projects that have tested opportunities and techniques with the expectation that lessons learned will increase the likelihood of success of their investment. Starting small with some early success builds confidence in working relationships and demonstrates to funders that the operational framework of the project is viable and worthy of continued support. It is important to realize at the outset that you are building working relationships and trust that will be necessary as challenges mount and crises are experienced. Moving too rapidly may jeopardize the time required to build dispute resolution mechanisms that have the support of all partners. It may be useful to support passionate individuals with the greatest likelihood of success, but it is equally important to be equitable in resource allocation as long as action plan and reporting goals are met. As the project progresses all partners will understand that they will be involved as their expertise is required and the action has a priority for that aspect of the project. With time this approach results in all partners sharing the success of others and the measuring stick becomes less of what they are allocated and more the shared accomplishment of meeting project goals.

Despite the temptation to develop programs unilaterally, they can only be sustainable by growing them within the capacity of in-country participants. In the past, the temptation was to provide program components that were not available in-country. Involvement of in-country participants can fulfill those roles if a commitment is made to train them (i.e., capacity building). While some zoos focus their skills on implementing action plans, others can step in to fund or provide the training/skills development required for these and future conservation initiatives.

As programs evolve, it is advisable to seek additional supporters to sustain programs. These may include university faculties and students seeking projects, industry, community groups, land owners, other NGOs, educators and educational institutions, marketing agencies, and media. These potential partners can provide in-kind support, expertise, or fund unique components of the action plan. Be careful of partnership dependency by avoiding exclusive reliance on your input. It is essential that stakeholders take ownership of the project, understanding that you will be leaving at some point and its long-term success is in their hands.

Action plans developed without stakeholder buy-in fail as in-country partners do not share your priorities or understand methods imported from afar. Often passionate and skilled individuals *in situ* know what is to be done but need resources to be empowered to act. Local partnerships will ensure that *ex situ* programs are culturally relevant. They also train and empower partners to sustain programs in your absence. Without a cadre of committed individuals, *ex situ* programs falter when you are not there to provide leadership.

Do not overlook your own institution's campus or surrounding communities for *in situ* or backyard conservation programs; it is important to raise awareness of how conservation action makes a difference. Participation in local conservation also demonstrates how zoos and aquariums are responding to emerging local and international conservation challenges. Creating opportunities for personal experiences to keep the familiar *familiar* will translate into community support for *ex situ* conservation in far way places with unfamiliar species. The skills and ethics that underpin local conservation programs are similar to those required internationally.

It is essential that we inform visitors and staff of our conservation successes and challenges. Each exhibit can be linked to a field conservation experience with actions that your institution has taken and how the public can become involved. Publishing the methods used to meet conservation goals and evaluating results provides a resource for all conservation practitioners. For Year of the Frog, AZA is developing consistent conservation messages that

can applied to our *ex situ* involvement (www.aza.org/yearofthefrog). As well, the AZA Annual Report on Conservation and Science (ARCS) is a compendium of the diversity of projects and approaches to *ex situ* conservation (www.aza.org/ConScience/ARCS).

Summary checklist:

- *Ex situ* conservation fulfills goals of your institution's strategic plan
- Explore programs already prioritized by AZA conservation committees
- Stakeholder meetings
- Funders
- Action plan
- Pilot project
- Evaluation
- Taking time to be part of the community
- Inform peers and zoo visitors

Chapter 6: Building *Ex situ* Facilities within Range Countries

Scott Pfaff, Riverbanks Zoo and Gardens
Paul Crump, Houston Zoo, Inc.

A number of reasons support the development of *ex situ* facilities within the country of the species occurrence. The number of amphibian species that require *ex situ* conservation tactics far exceeds our carrying capacity within the major, regional zoological associations Association of Zoos and Aquariums (AZA), European Association of Zoos and Aquaria (EAZA), and Australasian Regional Association Zoological Parks and Aquaria (ARAZPA). Capacities in terms of facilities, trained personal, and infrastructure need to be developed across the globe to deal with the extinction crisis. The risk of the introduction of novel diseases into a naïve amphibian community from an “alien” species exists when working with a species well outside of its natural range. This threat can be eliminated or greatly reduced by working in-country. The bureaucratic and logistic hurdles associated with the exportation of adequate numbers of endangered species will be avoided.

The authors of this chapter have both developed *ex situ* amphibian facilities within the range of the species of concern. The Riverbanks Zoo and Garden developed a facility within the grounds of the zoo in the southeast United States and the Houston Zoo developed a facility in partnership with the El Nispero Zoo in El Valle de Anton, Panama. The development of these facilities had obvious differences and challenges that were unique to each country. The details of the two projects will be discussed in detail.

Establishing *ex situ* populations of native amphibians at Riverbanks Zoo and Garden

The IUCN's Technical Guidelines on the Management of *Ex situ* Populations for Conservation stipulates that all critically endangered and extinct in the wild taxa should be subject to *ex situ* management. When the *ex situ* management of an amphibian species is considered necessary, the priority should be to establish the initiative within the range state of ecological origin. Because there are now so many species of amphibians that will require *ex situ* management, a severe strain will be placed on labor, space, facilities and other resources needed to meet the challenge. The need to move away from traditional multi-species *ex situ* facilities to *in situ* single species facilities further strains an already limited resource base.

Riverbanks Zoo and Garden established an *ex situ* initiative for rare amphibians native to South Carolina that is simple and minimizes the use of labor and other resources while maximizing the use of space. The support of range state government entities is considered absolutely crucial to the eventual success of an *ex situ* program. In this case, the South Carolina Department of Natural Resources actually suggested that Riverbanks consider establishing captive populations of rare native amphibians to establish refuge populations and to attempt to ascertain basic captive husbandry regimes, reproductive techniques and gather basic, but hitherto unknown, life history data.

Four species of native amphibians were selected – Pine Barrens treefrogs (*Hyla andersonii*), Carolina gopher frog (*Rana c. capito*), broad-striped dwarf sirens (*Pseudobranchius striatus*) and flatwoods salamanders (*Ambystoma cingulatum*). These species were selected because they were considered to be a threatened species or species of special concern in South Carolina whose populations were thought to be in decline.

All of the specimens obtained were to be collected from the wild while still in the larval stage. Small captive colonies of *Pseudobranchius* and *Hyla andersonii* were established in the first year of the project. Establishing a managed population of *Rana capito* proved to be more problematic. Although a few tadpoles and young adults were collected a captive colony could

not be established and we failed to locate any additional specimens. *Ambystoma cingulatum* also proved to be very difficult to locate and not a single specimen was located in South Carolina in twelve years.

Because Riverbanks is located within or very near the range of the target species it was decided to maintain the captive populations outdoors and not in the reptile and amphibian facility that already contained populations of non-native species. The advantages to maintaining the colonies outdoors were isolation from the rest of the living collection, utilizing secure, unused space suitable for amphibian enclosures, exposure to natural seasonal thermal and diel cycling, precipitation and other environmental conditions and having the capability to maintain the amphibians in semi-natural conditions that greatly reduce labor. We were not certain that the target species could be maintained outdoors year round since the difference between winter low and summer high temperatures can exceed 100° F /55°C, however, wild populations of amphibians are also subjected to such fluctuations and we were confident that captive colonies could as well if they were provided with appropriately complex enclosures.



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The area where the colonies were to be established was enclosed by a 3.5 m high chain link fence to provide security and predator exclusion. Three different sources of water were available; municipal city water, dechlorinated water that had been run through a carbon filter and water that is drawn directly from the Saluda River, a “class A scenic river” with clean, relatively uncontaminated water. The river water system is used almost exclusively for the amphibian colonies. The river water has a pH of ~6.5 and the filtered water ~pH 7.0. Because all of the specimens were collected locally and no novel pathogens or parasites would be introduced to the environment, wastewater is not disinfected.

Totally aquatic amphibians, like *Pseudobranchius*, are housed in 227 L plastic aquaria fitted with a mesh top. The aquaria are densely planted with bladderwort (*Utricularia* sp.) and duckweed (*Lemna* sp.). A thin layer of dead leaves, pine needles and other detritus is provided on the bottom of the tank. The tanks are occasionally topped off to replace water lost through evaporation, but other wise, water changes are not made and there is no mechanical filtration. The water in the outdoor tanks have a pH of ~ 5.7. As many as 70 individual *Pseudobranchius* have been maintained in a single tank, however, 15-20 individuals would be a more typical number of specimens maintained per tank.

Terrestrial amphibians were housed in screen cages that were .6 x .6 x 1 meters. These screen enclosure were elevated off of the ground on wooded shelves or tables to limit their exposure to fire ants. The screen enclosures are provided with deep layers of sphagnum moss, plastic tubs of standing water ~200mm deep and heavily planted with live plants. The water tubs also supported growths of duckweed.

All of the enclosures could be placed in full sun, full shade or semi-shade to manipulate thermal gradients to some degree.

The colonies were provided with feeder invertebrates appropriate to the species 2x per week except during the winter months. The enclosures, especially the aquatic enclosures, were

colonized by aquatic and other invertebrates that also served as a food source for the amphibians.

All of the incoming specimens were subjected to a 30-day quarantine period before being established in the managed populations. Severe habitat loss and climate change rather than disease issues are thought to be the causative factor in the decline of the species selected. Since the colonies were established within the range of the selected species, the release of pathogens into the environment was not a primary concern. Nor was the entrance of pathogens from amphibians extant on the Riverbanks site into the managed populations a major concern. The colonies never came into contact with other captive amphibians at Riverbanks Zoo.

Reproduction in the two taxa that were established was not difficult and large numbers of offspring were easily produced to second generation. Hundreds of *Hyla andersonii* could be produced in a single year. Tadpoles were left in the enclosure water tubs and were supplementally fed commercial dry flake fish food and grape leaves. Newly metamorphosed treefrogs could also be left in the enclosures with the adults although some were established in their own enclosure to demographically manage the population and track growth rates and age of first reproduction. The F¹ offspring attained sexual maturity and produced F² offspring in as little as eleven months. *Pseudobranchius* were slightly less prolific with a group of four adults producing more than 70 surviving offspring during one reproductive season. The founder adults at the time of first reproduction were five years old and the F¹ offspring, which were isolated into their own separate enclosures, did not reproduce until their fifth year indicating that the species may require a generation time of at least five years. Neither of the two established species, *Pseudobranchius striatus* and *Hyla andersonii*, had reproduced in captivity before and basic natural history data was lacking. Data for growth rates, size and age at first reproduction, and many aspects of the juvenile and adult stages was able to be collected.



Hyla andersonii

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Maintaining the colonies outdoors in semi-wild yet managed conditions greatly reduced the amount of labor necessary to maintain the colonies. Feeding, enclosure maintenance and other labor-intensive tasks were kept to a minimum. The specimens seemed to thrive in the enclosures with little external manipulation. The outdoor colonies of *Pseudobranchius* have been established for 15 years and individual longevities have exceeded 17 years. Indeed the only known mortalities of captive adult *Pseudobranchius* have been due to predation from *Natrachine* snakes that managed to penetrate the mesh covers over the aquaria.

Our experience has shown that managed populations of native amphibians can be established with a minimal commitment of labor, space and other resources and that these colonies can be very successful in terms of captive reproduction, recruitment and long term survival.

The El Valle Amphibian Conservation Center (EVACC)

In 1999, Project Golden Frog (PGF) was initiated to address declining populations of the Panamanian Golden Frog (*Atelopus zeteki*) due to collection for an illegal pet trade and the anticipated encroachment of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis* or *Bd*). As a result of PGF, United States zoos and aquariums have established a robust captive population (Zippel, 2002). The Golden Frog (*Atelopus zeteki*) holds a rich place in Panamanian culture and serves as a modest economic stimulus for local tourism. El Valle de

Anton is the type locality for the species, but populations inside the valley have been extirpated. The reason for the Golden Frog's disappearance from this region is unsustainable collecting that supplies collectors via an illegal pet trade. Several local hotels, as well as the El Nispero Zoo, have maintained small exhibits for golden frogs over the years, showcasing this species to the public but also contributing at some level to its regional decline. The frogs are much cheaper to buy than they are to breed or care for properly, so they have been treated largely as a disposable commodity with local collectors heading deeper into remote areas each year for access to diminishing populations. Campana National Park, located in the same region of west-central Panama, also has lost its resident population of Golden Frogs. At the same time, other native amphibian species appear to be present in normal abundance both at Campana and in El Valle de Anton. As part of an integrated approach, an objective of PGF was to establish a breeding program for golden frogs in Panama and to distribute captive bred offspring to hotels and other facilities in order to alleviate the threat of collection on wild populations.

The Parque Nacional General de División Omar Torrijos Herrera, near the town of El Copé is situated to the east of El Valle de Anton. *Bd* arrived in El Copé at the beginning of 2005 and began to cause population declines for resident amphibians (Lips et al., 2006). Concerned amphibian biologists called emergency meetings in the zoo and academic communities, resulting in formation of the IUCN-sponsored Amphibian Rescue and Recovery Coalition (ARCC). The ARCC began post- and pre-infected site collection to salvage some of the unique amphibian fauna of the Talamancan highlands of western central Panama. Thus, the creation of the EVACC was a direct response to the need identified by PGF to breed Golden Frogs in Panama and to the ARCC who exported rescued amphibians because of the lack of suitable facilities in Panama.

In January 2005, PGF field researchers met with owners of the El Nispero Zoo, during an expedition in the El Valle de Anton region, to discuss husbandry practices for golden frogs on exhibit. At that time, the Houston Zoo was also considering the establishment of an amphibian conservation center at the Summit Zoo just outside Panama City. Due to a lack of suitable infrastructure at that time at the Summit location it was decided that El Nispero was a better choice for location of the center. The latter site was also located within the species' historic range. This fact added a new operational problem of dealing with *Bd* in the region.

In April 2005, Houston Zoo staff conducted a thorough site inspection at the El Nispero Zoo determining the location to be well suited to this project. Mauricio Caballero, Director of the El Nispero Zoo, was then invited to Houston in July 2005 for an informal training program that involved visits to several other Texas zoos and aquariums that maintain significant amphibian collections. In addition, construction plans for the new facility were finalized. In August 2005, ground was broken for the new El Valle Amphibian Conservation Center with a construction team including staff from the El Nispero, Summit, and Houston Zoos.

The EVACC facility is approximately 2400 square feet (800m²) and is sectioned into a public display area, a conservation breeding section, a small laboratory, a storage room, an insect culture area, and a quarantine room. The public area contains twenty displays. All are mixed species exhibits with one grand centerpiece display to feature Panamanian Golden Frogs. The Director of the El Nispero Zoo, Dr. Mauricio Caballero and the EVACC Director Edgardo Griffith put together an exhibit theme based on an altitudinal transect. Plans



Off-exhibit space
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were also developed for a small nocturnal section of the exhibit area. It was decided early on that if EVACC were to only exhibit the target species then the exhibits would not really be true to the amphibian biodiversity of El Valle, thus the off-exhibit areas will remain focused on priority species and the exhibit area will display many more species.

One of the most challenging aspects in the design of this facility involves its water supply. Because the facility is situated within the range of amphibians threatened by *Bd*, the water entering into the facility could potentially transfer zoospores into the facility and infect the specimens housed within it. Three different water sources are available: well, stream, and municipal. During the August 2005 trip, Houston Zoo staff conducted water chemistry tests to determine the best source for this facility. Chlorine concentrations in the municipal water were too low to control the spread of *Bd*, while both stream and well water are exposed to potential vectors. Collection of rainwater via the roof and gutter system was considered, but ultimately



Source water filtration
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rejected. Employment of chlorination and dechlorination systems or fine sediment and carbon block filtration is believed to be the most effective way to treat and dispose of water and ensuring the exclusion of *Bd* and the containment of any other pathogens. A 1% sodium hypochlorite solution applied for one minute will kill the *Bd* (Speare, et al., 2004). Alternatively, *Bd* zoospores are approximately 2Qm in diameter. Fine sediment and carbon block filtration down to 0.5 Qm will also effectively eliminate the fungus from the facility (Gerry Marantelli, pers. comm.). A 120-gallon diaphragm tank provides water pressure for the facility. Exiting water that has been exposed to animal waste (rather than human waste,

drugs, or chemicals) is collected in a 500-gallon tank. No chemical disinfection is employed on exiting water because no novel organisms (either parasites or pathogens) are in the facility; organisms present in the facility are already present outside the facility, although the option exists to treat the wastewater.

In April of 2006, the first chytrid positive polymerase chain reaction (PCR) tests were reported from amphibians in El Valle. As predicted, but somewhat earlier, *Bd* had arrived. EVACC was not yet operational but collection of priority species had to begin. An emergency field center was created and modeled after the ARCC program. Two rooms at Hotel Campestre served as a treatment/quarantine room and as a clean room. Upon entering the collection each specimen was identified to the species, sexed, weighed (in grams), measured SVL (using dial calipers in mm), and swabbed for a PCR chytrid test. Specimens were then immediately entered into a prophylactic treatment course for *Bd* infection by itraconazole treatments [10 minute soak everyday for ten days in 1% itraconazole solution based on Nichols and Larimande, (2000)]. Simultaneously, each specimen was assigned a unique EVACC identification number that we can use to track the individuals chytrid treatment, feeding, and defecation (for retrospective analysis of gut parasites and diet) history, any medical condition or extra treatments the specimen received. Once animals completed their treatment course they were re-weighed, examined, re-swabbed, and usually transferred to the clean room unless under special medical circumstances. Specimens were being fed every day to every other day depending on species and enclosures and substrates being cleaned and disinfected with 10% sodium hypochlorite (bleach) on the days between to allow workers to monitor for fresh fecal material and uneaten food items as well as to maintain maximum hygiene standards. Water

used for the amphibians was potable tap water ran through a 0.5 μ m carbon block filter to remove any chlorine, pesticides, or other harmful substances (after the water was filtered it is stored in a 25 gallon bucket in the bathroom of each room). All animals being housed at the Hotel Campestre were maintained using strict quarantine protocols. Dedicated, disinfected footwear was used for each person entering each room and equipment was not transferred between rooms. A disposable pair of powder-free vinyl gloves was used for every individual enclosure for a single use. All discarded enclosure refuse, used gloves, and collection material was disinfected before it was thrown out as trash to prevent the spread of any pathogens from the collection. The floors of both rooms, and the immediate area outside the rooms, were disinfected after each day's scheduled collection maintenance.

The same high standards of record keeping and biosecurity were transferred to the EVACC once amphibians were moved into the facility in March 2007. As mentioned, correct source water management was necessary to ensure that chytrid-free animals are not reinfected by source water and that parasite and other microorganisms are not transferred between enclosures. The source water at EVACC was filtered in several stages, the last of which excluding any organism larger than 0.5 μ m, specifically chytrid zoospores. Once water has left an enclosure, it is taken directly to a drain. The ends of the waste lines are fitted with strainers and check valves to prevent both water and potential disease vectors (flushed feeder insects, etc.) from reentering the system.

Specimens have been monitored for parasites during the course of their time in captivity. An observant approach has been taken due to our lack of understanding of the beneficial role these microorganisms may play in the biological processes of the animal.

Collection planning and species selection for EVACC was accomplished by hybridizing several existing documents and by taking into account the situational uniqueness of the disease history and the geology of western central Panama. The European Reptile, Amphibian and Invertebrate Taxonomic Advisory Groups role selection criteria was used to determine if species needed *ex situ* conservation measures. Then the draft guidelines developed as part of the global *ex situ* amphibian conservation action plan (Zippel et al., 2006) were used to determine the feasibility of establishing colonies of the species that need *ex situ* conservation measures. In an alternative method, all species that are found around El Valle that are listed as threatened and data deficient in the IUCN's 2006 Global Amphibian Assessment (GAA) were considered. Any species that were also endemic to El Valle were given the highest priority. The assumption being that loss of the El Valle populations would equate to loss of the species. The remaining threatened species were prioritized into groups, those that are Talamanca highland species were given higher priority as opposed to lowland or Chocoan highland species. These were listed as priority species because *Bd* has gone through the highlands and caused many population declines, so again the assumption was that the loss of the El Valle populations would equate to loss of the species because, according to current information, they are extinct elsewhere. The final assessment was done by inviting comments by researchers and discussing some inconsistencies with the GAA. The following list of priority species was then developed:

- Panamanian Golden Frog (*Atelopus zeteki*),
- Lemur Leaf Frog (*Hylomantis lemur*),
- Panamanian Robber Frog (*Eleutherodactylus museosus*),
- Bob's Robber Frog (*Craugastor punctariolus*),
- Rusty Robber Frog (*Craugastor bufoniformis*),
- Heredia Treefrog (*Ecnomihyla "fimbrimembra"*),
- Crowned Treefrog (*Anotheca spinosa*),
- La Loma Treefrog (*Hyloscirtus colymba*),
- Palmer's Treefrog (*Hyloscirtus palmeri*),
- Casqued Treefrog (*Hemiphractus fasciatus*),

- Horned Marsupial Frog (*Gastrotheca cornuta*),
- Panama Poison Frog (*Dendrobates vicentei*),

Working at a hotel presented some unique challenges with respect to biosecurity, e.g., staff would find marine toads (*Bufo marinus*) inside the “clean” (post-chytrid treatment) room. Marine toads are known carriers of *Bd*, (Berger et al. 1998) and the results of swabs taken from toads that were able to gain access to the rooms are eagerly anticipated. Toad feces were also found on top of enclosures. This obviously has implications for the spread of parasites.

There are financial incentives to keep programs in range countries. However, the situation in Panama is likely to be different to other countries. The availability of almost any material needed for construction and Panama’s use of the US dollar as its currency certainly had a positive effect on logistics. EVACC started with a very modest budget and very modest goals. During the course of the project both were allowed to grow. In contrast to other projects, a gradually changing budget likely worked better under these circumstances, as a definitive budget would have been restrictive at the start, as the goals would grow, if it had been established and adhered to, the project may well have failed.

Some specific hurdles of the EVACC program were that staff did not have the advantages of easy access to resources such as ordering of food items or immediate access to veterinary facilities. Coordination of these activities in-country is sometimes made more difficult by host country regulations. For example, importing domestic crickets (a common food item for captive amphibians) has proven very difficult. Collection of wild native food was a large drain on manpower, although the nature of this type of response to a biodiversity crisis excludes the possibility of establishing invertebrate food cultures well ahead of time. This is something that should be done as rapidly as possible once the need is identified. At EVACC staff levels were gradually increased as the number of amphibians in captivity increased. A period of time did exist where staffing level was inadequate. Establishing an appropriate quantity of staff in advance of the need, so that training can be provided under less stressful conditions, is suggested.

In addition to the specifics of capacity issues, there is a more philosophical issue to consider. This is the concept of commitment. The specimens taken in by all three projects need careful stewarding that takes not only individual commitments from keepers and organizers but also institutional commitments by various partners. Individual zoo staff members might come and go, but programs that involve the preservation of species need to continue as seamlessly as possible and this takes real institutional commitment.

It is still too early to declare success or failure. Measurable achievements of the project are simple and primarily concerned with our ability to keep the specimens alive (short term), breed them (medium term), and overcome any issues relating to captive management such as dietary and habitat needs and ability to produce F2’s (long term). Five species have reproduced so far and dozens of captive bred amphibians exist as a result. Some priority taxa are yet to breed, and some are yet to be collected. It may turn out to be too late for those species. Amphibians that have died have been submitted for comprehensive necropsy to Dr. Allan Pessier at the San Diego Zoological Society.

Considerations for a new facility

Each individual project will have its own set of idiosyncratic objectives, complications, and problems and individual methods of measuring success or failure and assessing progress will need to be developed on a case by case basis. However, the overarching outline for how these types of facilities should be approached during the planning stages will be roughly universal. This approach will be discussed in detail in the following sections.

1) Determining Objectives

The first, probably most important, component of any project is to clearly state well-defined project objectives. Once the objectives of the project are defined in a finite manner it becomes easier to find solutions to complete these objectives, it provides an endpoint to the project, and it provides a benchmark with which progress can be measured and success or failure can be determined. Objectives should be consistent with regional and international goals, such as the AZA, EAZA, and ARAZPA's regional amphibian actions plans, the Amphibian Ark's critically endangered mandate, or a national *ex situ* action plan. It is worth noting that at this stage there probably will not be many complete failures, lessons can be learned from the worst attempts and will be applicable to others provided the information is disseminated.

2) Ensuring the Facility Design Can Achieve Objectives

This may seem rudimentary but too often *ex situ* amphibian conservationists are relegated to making goals work within a given space or resources. Institutions who are designing new in-range facilities are encouraged to ensure that objectives are translated into species spatial requirements and facilities are designed around these parameters rather than predetermined "random" estimates of floor space. This will require at least a rough estimate of the spatial, husbandry and dietary requirements of the species, often unknown in rescue operations, but likely possible to be inferred from known taxa. It is important to remember to think in holistic terms as the space a specimen requires for *ex situ* management stretches beyond its enclosure. For example, a single mature male (100mm) Horned Marsupial Frog (*Gastrotheca cornuta*) requires, at the minimum, a 20G tall (or equivalent) enclosure. Upon arrival the specimen will need to be quarantined in an area separate from the main collection. Once cleared from quarantine and moved to a permanent enclosure, the enclosure needs to be outfitted with plants, damp terrestrial and arboreal refugia, in the form of sphagnum moss and cork bark. A specimen of this species of this size will consume around 2-4 large grasshoppers every week. These grasshoppers can be collected inconsistently from the wild or cultured. Culturing requires a series of large vertical enclosures and separate space for egg incubation and rearing of juveniles. In total the physical, permanent spatial needs for the specimen are only about 25-50% of the total spatial needs for the specimens *ex situ* management.

Some facilities, especially those being built in the US, Europe, or Australia, do not need to create self-sustaining structures (biosecurity parameters aside). A diverse assortment of supplies can be acquired relatively rapidly and invertebrate food items can be ordered regularly and reliably en masse (if considered appropriate). Facilities being created in range countries where appropriate supplies are not so easily acquired and where invertebrate food items will have to be cultured are likely have to put more space and resources aside for storage and insect culture.

Biosecurity considerations need to be factored into the facility design and these will vary depending upon known and unknown disease risks. As this is such a significant component to facility design it will be covered in its own section.

3) Acquisition of Resources and Contacts

Ultimately the scope and success of the project depends upon the availability of the appropriate resources. Resources are defined here primarily as monetary, personnel, and property, but also include supplies (medical, husbandry, etc.) and access to pertinent information.

Financially, most projects will benefit from a rigorous budget assembled by a diverse group of people with specific skill sets with knowledge of the logistics of the country in which the facility will be created. It is important to include range-country personnel with knowledge of local laws, procedures, and codes. Procedures such as environmental impact surveys, building inspections, electrical code regulations are easier to factor into the plan if they are known prior

to project initiation. Their regulations will likely vary depending on multiple factors including but not limited to country of operation, public access to exhibits, situated on private or public property, and site access to existing utilities. Raising the funds to create a facility and maintain it long-term will probably be the most challenging aspect of *ex situ* facility management. It should be anticipated that with increased awareness of the global amphibian crisis granting organizations and institutions would be more supportive of *ex situ* management projects. However, from a long-term sustainability point of view, the operating costs of an *ex situ* facility should not come from “soft” money from outside the country of operation. Every effort should be made to make a business plan, designed by host country nationals from a regional perspective to ensure the continued operation of the project from inside the country of operation.

Employing the appropriate personnel to operate the *ex situ* facility will also be a challenge. There is obviously no training to replace natural ability and dedication, but people with a general interest in biology and conservation can be motivated into action. The most likely and useful avenue for finding interested and qualified individuals to manage and operate *ex situ* facilities outside of the regions with large zoological associations are by utilizing pre-existing contacts within universities.

The choice of location of the facility will ultimately have consequences. Institutions looking at starting projects outside their country are encouraged to build or place facilities upon the grounds of pre-existing institutions or organizations that share the same interests and have common objectives for preserving global biodiversity. This has multiple advantages over an organization (either from within or out of the country) purchasing land. This partnership approach can help increase interest in *ex situ* amphibian conservation in the country of operation. Frequently, the costs of the land, utilities, and sometimes even staff will be provided by the in-country partner, whereas the capital costs of the facility will be covered by the out-of-country partner. It is important to have a mutually agreed upon Memorandum of Understanding (MOU) prior to the initiation of such a partnership. The MOU should clearly state the responsibilities of both parties and seek to minimize any “grey” areas to avoid potential conflicts later.

Construction supplies of varying quality can be located in all countries. Shipping containers provide a valuable and often more practical alternative to conventional buildings. Even if the appropriate materials exist and are available, this option will be worth exploring for reasons that can be examined at <http://frogs.org.au/arc/container.php>.

Husbandry supplies, starter invertebrate cultures, and the other irreplaceable items needed for the operation of the facility should be first sought in-country. If suitable alternatives are unavailable cost-efficient methods of shipping should be explored, corporate links and in-kind donations of assistance from airlines and shipping companies have occurred and new links are encouraged to reduce costs.

Contacts within the appropriate government regulatory bodies are essential to an *ex situ* project either in or out of country. The development of these relationships is beneficial to all. Permits are needed to collect and maintain endangered species of amphibians and fulfilling the requirements and limitations of the permit exactly are very important. Often some individuals with government conservation and wildlife agencies place *ex situ* conservation in the same category as captive breeding or collection for the pet trade.

4) Implementation

Once the objectives have been solidified, personnel have been identified, the appropriate budget has been calculated and funded, and all logistics are in place to start management of

the process is key. The partnership approach, or augmentation of an existing program at a zoological institution or university will make this process more efficient. The spectrum of locations and scenarios for the implementation of the creation of the facility are endless and general principles are minimal.

5) Biosecurity

The general principles relating to making biosecure facilities to prevent the spread of non-native amphibian pathogens into the environment and from the environment into the *ex situ* colony come in two forms. The first is the design of the facility. All aspects of the environment need to be controlled in a manner that both contains non-native pathogens within the facility to prevent their escape and infection of native naïve amphibian species, and to prevent the introduction of native amphibian pathogens into the facility thus affecting the *ex situ* colony within. The second form of biosecurity comes in husbandry set-ups and keeper routine. Keeping all enclosures isolated from each other to prevent the spread of microorganisms via vectors such as escaped feeder invertebrates or vermin will help keep disease outbreaks from affecting the whole colony. Keeper routines, such as services the enclosures in a certain pattern will also help trace the source of diseases.

Projects in different geographic locations and disease issues will require their own set of biosecurity protocols. For example, in Panama, EVACC houses only native El Valle amphibians so the risk of the amphibians within the facility affecting the extant amphibian fauna outside the facility is minimal. However, as the chytrid fungus is prevalent in the region and assumed to be in the source water for the facility, extreme caution is taken when an amphibian, a keeper, water, or any other material enters the facility. Water is cleared of potential chytrid zoospores by serial mechanical filtration down to 0.5 Qm. Upon entering the facility, biologists wear dedicated footwear, coveralls that never leave the facility and are laundered regularly. The decision to shower in is made upon returning from the field or from areas likely to make the biologist a potential disease vector.

Biosecurity options will be explored in greater detail in the Best Practices Resource Manual to be released by the AZA in 2008. Additional details can be found at <http://frogs.org.au/arc/conference.html> and http://www.amphibianark.org/CBSG_WAZA_AmphibianExSituConservationPlanningWorkshopFinalReport.pdf.

6) Long-term Sustainability

The end goal of developing a facility either at another institution in a different country or assisting with the augmentation of an existing facility is self-sustainability. A resource umbilical cord of a pre determined time is essential but steps should be taken to establish a long-term plan that will perpetuate, in the absence of a financial sponsor, the goals and stability of the facility.

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Chapter 7: Amphibian Resource Manual Education Initiatives

Cindi Collins, Fort Worth Zoo

Education is of critical importance to the recovery of many vulnerable and critically endangered amphibian species. A concentrated effort of public awareness and programming is needed to increase awareness and encourage community involvement in conservation efforts. To address the amphibian crisis, existing AZA education departments are encouraged to design outreach programs and educational project components for both *in situ* and *ex situ* projects, facility visitors and school programs.

The sheer number of amphibian species that need programming can be overwhelming. Institutions should select methods that are helpful and logical for each individual institution and community. Below are some ideas to help with creating and implementing educational programs as well as disseminating information regarding the amphibian crisis:

Initiate a large-scale marketing campaign to increase public awareness of amphibians.

Coordinate with your Public Relations and Marketing departments to determine the most effective method to reach the intended audience. Through a variety of media tools, such as education materials and programs, graphics and media releases, explore partnerships and sponsors to help promote programs and distribute information. Many institutions already have established tools for sharing information in the form of brochures, flyers, and posters. In addition, use mailing lists, online systems and e-mail listservs to send out information. In response to the challenge, explain that this is a worldwide effort among organizations to develop and implement a global action plan for amphibian conservation such as the Amphibian Ark, The World Conservation Union (IUCN), World Association of Zoos and Aquariums, Conservation Breeding Specialist Group, and Amphibian Specialist Group. Amphibian conservation is a tremendous task and it will take a great effort to be effective.

Contact School District Administrators, Science Coordinators, Principals and Educators.

Share your programming information as well as marketing and educational materials with the people who touch millions everyday – teachers! Educators have lots of material to teach their students, and limited time to do it. Provide them with what they need so they can share easily with students and parents including adopt-an-animal information so they can be involved in school and in their own communities. Offer to conduct teacher workshops for the district or offer workshops at your facility to share information regarding amphibians and conservation efforts. Check with your local school districts for other opportunities. Many districts have teacher in-service days or conferences and invite local organizations to share their materials. Boost school group visits by offering them materials during their visit that highlights the amphibians at your institution and connect to local learning standards. Many teachers have to justify the educational element of their field trip, so make it easy and do it for them.

Strive to create a conservation ethic to help persuade citizens to promote stewardship of the environment and determine an amphibian's role as a bio-indicator in the environment.

Provide ideas and suggestions of how participants can create a beneficial environment for amphibians. Explore how amphibians are bio-indicators for a healthy environment, particularly water sources. The existence of, or lack of, an amphibian population is a great resource to determine the health of a habitat. Amphibians are sometimes overlooked for megavertebrates and many people are unaware of the vital role they play in the environment. Provide a list of ideas and suggestions of how people can do small things to help make the environment healthy and amphibian friendly. The overall task of saving wildlife can be overwhelming. By offering a list of small tasks or explaining that even small conservation efforts could influence the health of an entire species, more people will feel like they can make a difference.

Explore the role of amphibians in the ecosystem, their link in the food chain, as well as their bio-medical importance.

Visitors, patrons and program participants should understand why amphibians are important to the environment and that the loss of a species could create a chain reaction. Create scenarios and share information of how the loss of amphibians could affect other animals as well as entire ecosystems. Educate the public about threats to amphibians such as habitat loss, pollution, climate change and chytrid. Provide information regarding the role of many amphibians in the medical field such as studying frog toxins and compounds for HIV research, development of medications, and the treatment of burn victims. However, with the decline of many of these species, the source of the research and the research itself becomes more challenged. Many institutions already have exhibits and graphics that address these issues. Utilize these existing materials to highlight amphibians and create more awareness among visitors and zoo patrons. Once visitors understand the importance of amphibians, they will be more motivated to contribute to the solution.

Programs should establish and share methods of protecting amphibians through various resources.

It is hard for one person or a few people to do it all and reach everyone. Explore state and local agencies to help disseminate amphibian information. Share programming information with local organizations such as Boy Scouts of America, Girl Scouts of America, the YMCA and home school organizations, and offer an assortment of programs that includes a wide range of ages. Many groups are looking for group projects and a way to contribute to animals and the environment for badges and curriculum objectives.

Support and expand existing conservation efforts, such as rescue programs, field surveys, regional programs and local amphibian conservation projects.

Provide information regarding existing conservation efforts to involve patrons and zoo visitors. Although the fate for some species may be rather gloomy, there are many heroic efforts being made to ensure species survival. For some amphibians, captive breeding programs are vital for the continual existence of a species. Many facilities are already involved in conservation projects in their facility or throughout the world. Take this opportunity to highlight the creative and dedicated efforts of keepers, supervisors, and conservationists. Many of these programs occur behind-the-scenes and the public is not even aware they exist. Also, encourage visitors to become involved through volunteering, joining a monitoring program, or making a monetary donation. Appeal to the electronic-savvy crowd and explain how Global Positioning Systems (GPS) and other electronics are used to monitor animal movement and determine habitats to increase interest and understanding.

Train staff and support training courses or internships at institutions with existing programs.

Educating the staff is a critical role in this effort. Understanding and performing proven husbandry practices will increase success rates. Create more interest and share ideas with other staff members by highlighting existing conservation efforts to promote individual efforts as well as team efforts. Share the workload by providing opportunities to involve additional staff or volunteers with internships and volunteer support roles. Involve zoo visitors or sponsors by creating a scholarship program to send zoo staff to relevant courses such as the *Amphibian Biology and Management* course, to support conservation efforts and to share established husbandry practices.

Encourage your facility to participate in the 2008 Year of the Frog global public awareness campaign through educational materials and media releases.

To promote amphibian conservation, a Year of the Frog Task Force comprised of members from multiple AZA committees is developing and compiling materials and information for each participating facility's use. Materials for the campaign will be available in an assortment of

formats to be used in conjunction with the Conservation Made Simple toolkit to be utilized at organized events such as Leap Day on February 29, Earth Day on (or around) April 22 or during other programming opportunities. Among these materials is a list of Eight Amphibian Conservation Actions for 2008. For more information, visit <http://www.aza.org/YearoftheFrog>.

Use existing materials to enhance your education programming and disperse information.

Do not feel you have to reinvent the wheel. Amphibian conservation has been a concern for several years so many institutions have already created educational materials and resources to provide program ideas. Visit the following sites for resources to use in your own educational programming. Share these sites with educators and other organizations that can promote the amphibian awareness campaign.

- Puerto Rican Crested Toad Teacher Curriculum and Activity Book
<http://www.puertoricancrestedtoadssp.org>
- Project Golden Frog
<http://www.ranadorada.org>
- Amphibian Alert curriculum/Sedgewick County Zoo
http://www.scz.org/amphibian_alert.html
- For ideas on how to design programming and materials, use the Butterfly Festival Manual and Activity Guide as a resource.
<http://www.butterflyrecovery.org/education>

Appendix 1. List of prioritized species from Canada and the US

Genus	Species	Common name	Distribution	Extinction Risk (IUCN, 2006)	Prioritization Score
<i>Cryptobranchus</i>	<i>bishopi</i>	Ozark hellbender	United States of America	Near Threatened (NT)	68
<i>Rana</i>	<i>muscosa</i>	Mountain Yellow-Legged Frog	United States of America	Critically Endangered (CR)	66
<i>Rana</i>	<i>sierrae</i>	Sierra Nevada Yellow-legged Frog	United States of America	Critically Endangered (CR)	66
<i>Rana</i>	<i>sp.</i>	North of Mogollon Rim	United States of America	Critically Endangered (CR)	66
<i>Necturus</i>	<i>alabamensis</i>	Alabama Waterdog	United States of America	Endangered (EN)	59
<i>Bufo</i>	<i>californicus</i>	Arroyo Toad	Mexico, United States of America	Endangered (EN)	58
<i>Rana</i>	<i>sevosa</i>	Dusky Gopher Frog	United States of America	Critically Endangered (CR)	56
<i>Rana</i>	<i>subaquavocalis</i>	Ramsey Canyon Leopard Frog	United States of America	Critically Endangered (CR)	51
<i>Bufo</i>	<i>baxteri</i>	Wyoming Toad	United States of America (Extinct)	Extinct in the Wild (EW)	50
<i>Cryptobranchus</i>	<i>alleganiensis</i>	Hellbender	United States of America	Near Threatened (NT)	50
<i>Eurycea</i>	<i>tonkawae</i>	Jollyville Plateau Salamander	United States of America	Endangered (EN)	50
<i>Eurycea</i>	<i>chisholmensis</i>	Chisholm Trail Salamander	United States of America	Vulnerable (VU)	48
<i>Eurycea</i>	<i>sosorum</i>	Barton Springs Salamander	United States of America	Vulnerable (VU)	46
<i>Eurycea</i>	<i>waterlooensis</i>	Austin Blind Salamander	United States of America	Vulnerable (VU)	46
<i>Rhyacotriton</i>	<i>olympicus</i>	Olympic Torrent Salamander	United States of America	Vulnerable (VU)	45
<i>Amphiuma</i>	<i>pholeter</i>	One-Toed Amphiuma	United States of America	Near Threatened (NT)	44
<i>Eurycea</i>	<i>naufregia</i>	San Gabriel Springs Salamander	United States of America	Endangered (EN)	42
<i>Rana</i>	<i>chiricahuensis</i>	Chiricahua Leopard Frog	Mexico, United States of America	Vulnerable (VU)	38
<i>Rana</i>	<i>pretiosa</i>	Oregon Spotted Frog	Canada, United States of America	Vulnerable (VU)	38
<i>Eurycea</i>	<i>nana</i>	San Marcos Salamander	United States of America	Vulnerable (VU)	38
<i>Eurycea</i>	<i>rathbuni</i>	Texas Blind Salamander	United States of America	Vulnerable (VU)	38
<i>Dicamptodon</i>	<i>ensatus</i>	California Giant Salamander	United States of America	Near Threatened (NT)	37
<i>Rhyacotriton</i>	<i>cascadae</i>	Cascade Torrent Salamander	United States of America	Near Threatened (NT)	37
<i>Rhyacotriton</i>	<i>kezeri</i>	Columbia Torrent Salamander	United States of America	Near Threatened (NT)	37
<i>Ascaphus</i>	<i>truei</i>	Coastal Tailed Frog	Canada, United States of America	Least Concern (LC)	34
<i>Bufo</i>	<i>canorus</i>	Yosemite Toad	United States of America	Endangered (EN)	34
<i>Rana</i>	<i>onca</i>	Relict Leopard Frog	United States of America	Endangered (EN)	34

<i>Ambystoma</i>	<i>californiense</i>	California Tiger Salamander	United States of America	Vulnerable (VU)	33
<i>Dicamptodon</i>	<i>aterrimus</i>	Idaho Giant Salamander	United States of America	Least Concern (LC)	33
<i>Dicamptodon</i>	<i>copei</i>	Cope's Giant Salamander	United States of America	Least Concern (LC)	33
<i>Batrachoseps</i>	<i>aridus</i>	Desert slender salamander	United States of America	Critically Endangered (CR)	33
<i>Bufo</i>	<i>houstonensis</i>	Houston Toad	United States of America	Endangered (EN)	32
<i>Pseudobranchius</i>	<i>axanthus</i>	Narrow-Striped Dwarf Siren	United States of America	Least Concern (LC)	31
<i>Pseudobranchius</i>	<i>striatus</i>	Dwarf Siren	United States of America	Least Concern (LC)	31
<i>Ambystoma</i>	<i>cingulatum</i>	Flatwoods Salamander	United States of America	Vulnerable (VU)	28
<i>Ascaphus</i>	<i>montanus</i>		Canada, United States of America	Least Concern (LC)	26
<i>Rana</i>	<i>luteiventris</i>	Columbia Spotted Frog	Canada, United States of America	Least Concern (LC)	26
<i>Phaeognathus</i>	<i>hubrichti</i>	Red Hills Salamander	United States of America	Endangered (EN)	24
<i>Necturus</i>	<i>lewisi</i>	Neuse River Waterdog	United States of America	Near Threatened (NT)	24
<i>Necturus</i>	<i>punctatus</i>	Dwarf Waterdog	United States of America	Least Concern (LC)	23
<i>Rhyacotriton</i>	<i>variegatus</i>	Southern Torrent Salamander	United States of America	Least Concern (LC)	23
<i>Ambystoma</i>	<i>bishopi</i>	Frosted flatwoods salamander	United States of America	Vulnerable (VU)	20
<i>Amphiuma</i>	<i>means</i>	Two-Toed Amphiuma	United States of America	Least Concern (LC)	20
<i>Amphiuma</i>	<i>tridactylum</i>	Three-Toed Amphiuma	United States of America	Least Concern (LC)	20
<i>Rana</i>	<i>draytonii</i>	California Red-Legged Frog	United States	Near Threatened (NT)	19
<i>Desmognathus</i>	<i>folkerti</i>	Dwarf Black-Bellied Salamander	United States of America	Data Deficient (DD)	19
<i>Gyrinophilus</i>	<i>gulolineatus</i>	Berry Cave Salamander	United States of America	Endangered (EN)	19
<i>Gyrinophilus</i>	<i>subterraneus</i>	West Virginia Spring Salamander	United States of America	Endangered (EN)	19
<i>Plethodon</i>	<i>stormi</i>	Siskiyou Mountains Salamander	United States of America	Endangered (EN)	19
<i>Ambystoma</i>	<i>tigrinum stebbinsi</i>	Sonora tiger salamander	United States of America (Native and Introduced)	Least Concern (LC)	18
<i>Batrachoseps</i>	<i>diabolicus</i>	Hell Hollow Slender Salamander	United States of America	Data Deficient (DD)	18
<i>Batrachoseps</i>	<i>gabrieli</i>	San Gabriel Slender Salamander	United States of America	Data Deficient (DD)	18
<i>Batrachoseps</i>	<i>incognitus</i>	San Simeon Slender Salamander	United States of America	Data Deficient (DD)	18
<i>Batrachoseps</i>	<i>kawia</i>	Sequoia Slender Salamander	United States of America	Data Deficient (DD)	18
<i>Batrachoseps</i>	<i>minor</i>	Lesser Slender Salamander	United States of America	Data Deficient (DD)	18
<i>Batrachoseps</i>	<i>relictus</i>	Relictual Slender Salamander	United States of America	Data Deficient (DD)	18

<i>Eurycea</i>	<i>chamberlaini</i>	Chamberlain's Dwarf Salamander	United States of America	Data Deficient (DD)	18
<i>Eurycea</i>	<i>pterochila</i>	Blanco River Springs Salamander	United States of America	Data Deficient (DD)	18
<i>Eurycea</i>	<i>robusta</i>	Blanco Blind Salamander	United States of America	Data Deficient (DD)	18
<i>Eurycea</i>	<i>troglydites</i>	Eurycea Troglydites Complex	United States of America	Data Deficient (DD)	18
<i>Plethodon</i>	<i>aureolus</i>	Tellico Salamander	United States of America	Data Deficient (DD)	18
<i>Plethodon</i>	<i>sequoyah</i>	Sequoyah Slimy Salamander	United States of America	Data Deficient (DD)	18
<i>Plethodon</i>	<i>shenandoah</i>	Shenandoah Salamander	United States of America	Vulnerable (VU)	17
<i>Bufo</i>	<i>nelsoni</i>		United States of America	Endangered (EN)	16
<i>Batrachoseps</i>	<i>campi</i>	Inyo Mountains Salamander	United States of America	Endangered (EN)	16
<i>Plethodon</i>	<i>welleri</i>	Weller's Salamander	United States of America	Endangered (EN)	16
<i>Notophthalmus</i>	<i>meridionalis</i>	Black-Spotted Newt	Mexico, United States of America	Endangered (EN)	16
<i>Necturus</i>	<i>beyeri</i>	Gulf Coast Waterdog	United States of America	Least Concern (LC)	15
<i>Ambystoma</i>	<i>macrodictylum croceum</i>	Santa Cruz Long-Toed Salamander	United States of America	Least Concern (LC)	13
<i>Plethodon</i>	<i>nettingi</i>	Cheat Mountain Salamander	United States of America	Near Threatened (NT)	9
<i>Ambystoma</i>	<i>tigrinum ssp.</i>	Dickinson Co, Iowa Tiger Salamander	United States of America		8
<i>Plethodon</i>	<i>kiamichi</i>	Kiamichi Slimy Salamander	United States of America	Data Deficient (DD)	8
<i>Rana</i>	<i>catesbeiana</i>	American bullfrog	Canada and United States of America (Native and Introduced), Puerto Rico (Introduced)	Least Concern (LC)	5
<i>Hyla</i>	<i>andersonii</i>	Pine Barrens Treefrog	United States of America	Near Threatened (NT)	4

List of prioritized species from the Caribbean

Genus	Species	Distribution	Extinction Risk (IUCN, 2006)	Prioritization Score
<i>Eleutherodactylus</i>	<i>schmidti</i>	Dominican Republic, Haiti	Critically Endangered (CR)	76
<i>Eleutherodactylus</i>	<i>semipalmatus</i>	Haiti	Critically Endangered (CR)	76
<i>Eleutherodactylus</i>	<i>albipes</i>	Cuba	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>amadeus</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>apostates</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>bakeri</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>brevirostris</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>caribe</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>chlorophenax</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>corona</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>cubanus</i>	Cuba	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>darlingtoni</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>dolomedes</i>	Haiti	Critically Endangered (CR)	72

<i>Eleutherodactylus</i>	<i>eunaster</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>fowleri</i>	Dominican Republic, Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>furcyensis</i>	Dominican Republic, Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>glandulifer</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>glanduliferoides</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>jugans</i>	Dominican Republic, Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>lamprotes</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>leoncei</i>	Dominican Republic, Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>mariposa</i>	Cuba	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>nortoni</i>	Dominican Republic, Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>oxyrhynchus</i>	Dominican Republic, Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>parabates</i>	Dominican Republic, Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>parapelates</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>paulsoni</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>poolei</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>rufifemoralis</i>	Dominican Republic	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>sciagraphus</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>tetajulia</i>	Cuba	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>thorectes</i>	Haiti	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>turquinensis</i>	Cuba	Critically Endangered (CR)	72
<i>Eleutherodactylus</i>	<i>ventrilineatus</i>	Haiti	Critically Endangered (CR)	72
<i>Leptodactylus</i>	<i>fallax</i>	Dominica, Martinique, Montserrat, Saint Kitts and Nevis	Critically Endangered (CR)	71
<i>Eleutherodactylus</i>	<i>juanriveroi</i>	Puerto Rico	Critically Endangered (CR)	61
<i>Eleutherodactylus</i>	<i>junori</i>	Jamaica	Critically Endangered (CR)	61
<i>Eleutherodactylus</i>	<i>locustus</i>	Puerto Rico	Critically Endangered (CR)	61
<i>Eleutherodactylus</i>	<i>richmondi</i>	Puerto Rico	Critically Endangered (CR)	61
<i>Eleutherodactylus</i>	<i>unicolor</i>	Puerto Rico	Critically Endangered (CR)	61
<i>Eleutherodactylus</i>	<i>orcutti</i>	Jamaica	Critically Endangered (CR)	60
<i>Eleutherodactylus</i>	<i>wightmanae</i>	Puerto Rico	Critically Endangered (CR)	57
<i>Bufo</i>	<i>fluviaticus</i>	Dominican Republic	Critically Endangered (CR)	56
<i>Mannophryne</i>	<i>olmonae</i>		Critically Endangered (CR)	56
<i>Phyllodytes</i>	<i>auratus</i>	Trinidad	Critically Endangered (CR)	56
<i>Plectrohyla</i>	<i>acanthodes</i>	Guatemala, Mexico	Critically Endangered (CR)	56
<i>Plectrohyla</i>	<i>avia</i>	Guatemala, Mexico	Critically Endangered (CR)	56
<i>Plectrohyla</i>	<i>ixil</i>	Guatemala, Mexico	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>alticola</i>	Jamaica	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>bartonsmithi</i>	Cuba	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>blairhedgesi</i>	Cuba	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>bresslerae</i>	Cuba	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>cavernicola</i>	Jamaica	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>fuscus</i>	Jamaica	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>griphus</i>	Jamaica	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>iberia</i>	Cuba	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>jaumei</i>	Cuba	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>lucioi</i>	Haiti	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>orientalis</i>	Cuba	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>pezopetrus</i>	Cuba	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>rhodesi</i>	Haiti	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>rivularis</i>	Cuba	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>sisyphodemus</i>	Jamaica	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>symingtoni</i>	Cuba	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>tonyi</i>	Cuba	Critically Endangered (CR)	56

<i>Eleutherodactylus</i>	<i>warreni</i>	Haiti	Critically Endangered (CR)	56
<i>Eleutherodactylus</i>	<i>jasperi</i>	Puerto Rico	Critically Endangered (CR)	55
<i>Eleutherodactylus</i>	<i>eneidae</i>	Puerto Rico	Critically Endangered (CR)	50
<i>Eleutherodactylus</i>	<i>karlschmidti</i>	Puerto Rico	Critically Endangered (CR)	50
<i>Plectrohyla</i>	<i>hartwegi</i>	Guatemala, Mexico	Critically Endangered (CR)	49
<i>Bufo</i>	<i>lemur</i>	Virgin Islands (British), Puerto Rico	Critically Endangered (CR)	46
<i>Bufo</i>	<i>tacanensis</i>	Guatemala, Mexico	Endangered (EN)	41
<i>Bufo</i>	<i>tutelarius</i>	Guatemala, Mexico	Endangered (EN)	37
<i>Eleutherodactylus</i>	<i>cooki</i>	Puerto Rico	Endangered (EN)	21

List of prioritized species from Mexico*

Genus	Species	Common name	Extinction Risk (IUCN, 2006)	Prioritization Score
<i>Bufo</i>	<i>cristatus</i>	Large-Crested Toad	Critically Endangered (CR)	76
<i>Rana</i>	<i>pueblae</i>		Critically Endangered (CR)	61
<i>Charadrahyla</i>	<i>trux</i>		Critically Endangered (CR)	60
<i>Ambystoma</i>	<i>mexicanum</i>	Axolotl	Critically Endangered (CR)	59
<i>Plectrohyla</i>	<i>calthula</i>		Critically Endangered (CR)	56
<i>Plectrohyla</i>	<i>ephemera</i>		Critically Endangered (CR)	56
<i>Plectrohyla</i>	<i>psarosema</i>		Critically Endangered (CR)	56
<i>Plectrohyla</i>	<i>thorectes</i>		Critically Endangered (CR)	56
<i>Ambystoma</i>	<i>taylori</i>	Taylor's Salamander	Critically Endangered (CR)	54
<i>Plectrohyla</i>	<i>siopela</i>		Critically Endangered (CR)	53
<i>Bromeliahyla</i>	<i>dendroscarta</i>		Critically Endangered (CR)	45
<i>Charadrahyla</i>	<i>altipotens</i>		Critically Endangered (CR)	45
<i>Ecnomihyla</i>	<i>echinata</i>		Critically Endangered (CR)	45
<i>Ecnomihyla</i>	<i>valancifer</i>		Critically Endangered (CR)	45
<i>Megastomahyla</i>	<i>mixe</i>		Critically Endangered (CR)	45
<i>Megastomahyla</i>	<i>pellita</i>		Critically Endangered (CR)	45
<i>Plectrohyla</i>	<i>calvicollina</i>		Critically Endangered (CR)	45
<i>Plectrohyla</i>	<i>celata</i>		Critically Endangered (CR)	45
<i>Plectrohyla</i>	<i>cembra</i>		Critically Endangered (CR)	45
<i>Plectrohyla</i>	<i>crassa</i>		Critically Endangered (CR)	45
<i>Plectrohyla</i>	<i>cyanomma</i>		Critically Endangered (CR)	45
<i>Plectrohyla</i>	<i>hazela</i>		Critically Endangered (CR)	45
<i>Plectrohyla</i>	<i>pachyderma</i>		Critically Endangered (CR)	45
<i>Plectrohyla</i>	<i>pycnochila</i>		Critically Endangered (CR)	45
<i>Rana</i>	<i>omiltemana</i>	Guerreran Leopard Frog	Critically Endangered (CR)	45
<i>Rana</i>	<i>tlaloci</i>	Tlaloc's Leopard Frog	Critically Endangered (CR)	45
<i>Ambystoma</i>	<i>amblycephalum</i>		Critically Endangered (CR)	44
<i>Ambystoma</i>	<i>andersoni</i>		Critically Endangered (CR)	44
<i>Ambystoma</i>	<i>dumerilii</i>		Critically Endangered (CR)	44
<i>Ambystoma</i>	<i>leorae</i>	Leora's Stream Salamander	Critically Endangered (CR)	44
<i>Ambystoma</i>	<i>lermaense</i>	Lake Lerma Salamander	Critically Endangered (CR)	44
<i>Bufo</i>	<i>gemmifer</i>	Jeweled Toad	Endangered (EN)	41
<i>Plectrohyla</i>	<i>chryses</i>		Critically Endangered (CR)	41
<i>Plectrohyla</i>	<i>cyclada</i>		Endangered (EN)	41
<i>Ambystoma</i>	<i>bombypellum</i>	Axolotl	Critically Endangered (CR)	39
<i>Ambystoma</i>	<i>granulosum</i>	Granular Salamander	Critically Endangered (CR)	39
<i>Bufo</i>	<i>cavifrons</i>	Mountain Toad	Endangered (EN)	37

<i>Bufo</i>	<i>spiculatus</i>		Endangered (EN)	37
<i>Charadrahyla</i>	<i>chaneque</i>		Endangered (EN)	37
<i>Plectrohyla</i>	<i>mykter</i>		Endangered (EN)	37
<i>Ptychohyla</i>	<i>erythromma</i>		Endangered (EN)	37
<i>Ambystoma</i>	<i>rivulare</i>		Data Deficient (DD)	37
<i>Rana</i>	<i>chichicuahutla</i>		Critically Endangered (CR)	36
<i>Plectrohyla</i>	<i>labedactyla</i>		Data Deficient (DD)	33
<i>Bufo</i>	<i>perplexus</i>		Endangered (EN)	32
<i>Plectrohyla</i>	<i>ameibothalame</i>		Data Deficient (DD)	29
<i>Ptychohyla</i>	<i>acrochorda</i>		Data Deficient (DD)	29
<i>Ptychohyla</i>	<i>zophodes</i>		Data Deficient (DD)	29
<i>Rana</i>	<i>dunni</i>		Endangered (EN)	25
<i>Ptychohyla</i>	<i>leonhardschultzei</i>		Endangered (EN)	21
<i>Plectrohyla</i>	<i>sabrina</i>		Critically Endangered (CR)	20
<i>Exerodonta</i>	<i>chimalapa</i>		Endangered (EN)	16
<i>Bufo</i>	<i>pisinnus</i>		Data Deficient (DD)	8
<i>Rana</i>	<i>catesbeiana</i>	American bullfrog	Least Concern (LC)	5

* Excludes prioritization of the *Plectrohyla* and *Thorius* genera

Appendix 2: Species Coordinators

If interested in working with any of the following species, all identified as high priorities for *ex situ* conservation programs, contact the listed coordinator. If no coordinator is listed, consider taking on the challenge of developing this program – and its related partnerships – at your facility.

Species: *Ambystoma californiense*
Common name: California tiger salamander
Coordinator: Andy Snider
Fresno Chaffee Zoo
Director of Animal Care and Conservation
559-498-5914
asnider@fresnochaffeezoo.com

Species: *Ambystoma mexicanum*
Common name: California tiger salamander
Coordinator: TBD

Species: *Ambystoma taylori*, *A. amblycephalum*, *A. andersoni*, *A. dumerilii*, *A. leorae*, *A. lermaense*
Coordinator: Luis Carrillo
Africam Safari
Curator of Reptiles and Amphibians
52-222-281-7000 x231
lcarrillo@africamsafari.com.mx

Species: *Ascaphus truei*
Common name: Coastal tailed frog
Coordinator: TBD

Species: *Atelopus zeteki*
Common name: Panamanian golden frog
Coordinator: Vicky Poole
Exhibit Manager - *FROGS! A Chorus of Color*
National Aquarium in Baltimore
Phone: (410) 576-1193
vpooles@aqua.org

Species: *Batrachoseps aridus*
Common name: Desert slender salamander
Coordinator: Andy Snider
Fresno Chaffee Zoo
Director of Animal Care and Conservation
559-498-5914
asnider@fresnochaffeezoo.com

Species: *Bufo baxteri*
Common name: Wyoming toad
Coordinator: Bruce Foster, SSP
Coordinator
Central Park Zoo, Wildlife Conservation Society
Collection Manager - Animal Department
212-439-6505
bfoster@wcs.org

Species: *Bufo canorus*
Common name: Yosemite toad
Coordinator: TBD

Species: *Bufo houstonensis*
Common name: Houston toad
Coordinator: Paul Crump
Houston Zoo, Inc.
Reptile and Amphibian Keeper
713-533-6655
pcrump@houstonzoo.org

Species: *Bufo lemur*
Common name: Puerto Rican crested toad
Coordinator: Diane Barber, SSP
Coordinator
Fort Worth Zoo
Curator of Ectotherms
817-759-7180
dbarber@fortworthzoo.org

Species: *Cryptobranchus bishopi*
Common name: Ozark hellbender
Coordinator: Mark Wanner
Saint Louis Zoo
Zoological Manager
(314) 781-0900 x272
wanner@stlzoo.org

Species: *Eleutherodactylus locustis*, *E. richmondi*, *E. wightmanae*
Coordinator: Dr. Rafael Joglar
University of Puerto Rico - Rio Piedras
Professor
787-764-0000 x3567
rjoglar@uprrp.edu

Species: *Eurycea chisholmensis*
Common name: Salado Springs salamander
Coordinator: Jessica Crowley
Dallas Zoo
Reptile and Amphibian Keeper
(214) 670-7573
yessie12@hotmail.com

Species: *Eurycea naufragia*
Common name: Georgetown salamanders
Coordinator: Jessica Crowley
Dallas Zoo
Reptile and Amphibian Keeper
(214) 670-7573
yessie12@hotmail.com

Species: *Eurycea sosorum, waterlooensis, nana, rathbuni, tonkawae*
Common name: Barton Springs, Austin Blind, San Marcos, Jollyville Texas Blind salamanders
Coordinator: Paul Crump
Houston Zoo, Inc.
Reptile and Amphibian Keeper
713-533-6655
pcrump@houstonzoo.org

Species: *Necturus alabamensis*
Common name: Black warrior water dog
Coordinator: Erik Keyster
Cincinnati Zoo
Lead Amphibian Keeper
513-487-3306
erik.keyster@cincinnati zoo.org

Species: *Rana chiricahuensis*
Common name: Chiricahua Leopard frog
Coordinator: Paula Swanson
Phoenix Zoo
Manager of Reptiles
602-273-1341 x7610
Pswanson@thephxzoo.com

Species: *Rana muscosa*
Common name: Mountain yellow-legged frog
Coordinator: Andy Snider
Fresno Chaffee Zoo
Director of Animal Care and Conservation
559-498-5914
asnider@fresnochaffeezoo.com

Species: *Rana onca*
Common name: Relict leopard frog
Coordinator: Paula Swanson
Phoenix Zoo
Manager of Reptiles
602-273-1341 x7610
Pswanson@thephxzoo.com

Species: *Rana pretiosa*
Coordinator: TBD
Species: *Rana sevosa*
Common name: Mississippi gopher Frog
Coordinator: Steve Reichling
Memphis Zoo
Curator of Reptiles, Amphibians, Nocturnal
901-333-6711
sreichling@memphis zoo.org

Species: *Rana sp.*
Common name: Mogollon Rim form leopard frog
Coordinator: Paula Swanson
Phoenix Zoo
Manager of Reptiles
602-273-1341 x7610
Pswanson@thephxzoo.com

Species: *Rana subaquavocalis*
Common name: Ramsey Canyon leopard Frog
Coordinator: Paula Swanson
Phoenix Zoo
Manager of Reptiles
602-273-1341 x7610

Appendix 3: Amphibian Conservation Funding Sources

American Association of Zoo Keepers Grants

Primary Purpose of Grant:

To encourage research conducted by animal keepers at zoos and aquariums.

Qualifications:

Applicant must be a full-time permanent keeper at an AZA-member institution with at least two years animal-keeping experience.

Amount and Term of Grant:

Four grants available at \$1,000 each:

- AAZK-Geraldine Meyer Professional Travel Grant
- AAZK/ AZA Advances in Animal Keeping Course Grant
- Conservation, Preservation, and Restoration Grant
- Research Grant

Funding Restrictions:

Grant cannot be used for the purchase of live specimens, nor to offset or pay wages for a keeper or other participant.

Additional Information:

See <http://www.aazk.org/grants/grants.php> for details and applications.

Shelly Roach
Columbus Zoo and Aquarium
P.O. Box 400
Powell, Ohio 43065
shelly.roach@columbuszoo.org
AAZK, Inc. Administrative Offices
3601 S.W. 29th Street, Suite 133
Topeka, Kansas 66614-2054

Closing Dates:

- 1 February and 1 July for the AAZK-Geraldine Meyer Professional Travel Grant
- 1 February for the AAZK/ AZA Advances in Animal Keeping Course Grant
- 1 June for the Conservation, Preservation, and Restoration Grant
- 1 June for the Research Grant

Amphibian Taxon Advisory Group (ATAG) grant

Primary Purpose of Grants:

Support amphibian conservation within the AZA community, particularly projects that address ATAG Regional Collection Plan Conservation or Education Goals.

Qualifications:

None specified.

Amount and Term of Grant:

Annual awards average \$1000.

Funding Restrictions:

None specified.

Additional Information and/or Application:

Diane Barber, ATAG Chair
Curator of Ectotherms
Fort Worth Zoo
1989 Colonial Parkway
Fort Worth, TX 76110
Phone: (817) 759-7180
dbarber@fortworthzoo.org

Closing Dates:

1 January 2008

Association of Reptilian and Amphibian Veterinarians

Primary Purpose of Grants:

To fund clinically oriented and/or conservation research projects involving either reptiles and/or amphibians. Proposals will be evaluated based on clinical significance, conservation relevance, feasibility of proposal, and research methodology.

Qualifications:

Clinically oriented proposals and proposals submitted by ARAV members will be given priority.

Amount and Term of Grant:

Typical awards are in the range of \$2000-\$3000.

Funding Restrictions:

It is expected that the research should be completed in 12-18 months. Recipients of funding will be asked to either present their results at the ARAV annual conference or publish them in the Journal of Reptilian Medicine and Surgery.

Additional Information and/or Application:

See <http://www.arav.org/Grants.htm> or contact:

Byron JS de la Navarre, DVM

Animal House of Chicago, Complete Veterinary Care, Inc.

2752 W. Lawrence

Chicago, IL 60625

(773)878-8002 (Fax)773-878-0546

E-mail: exotxdr@aol.com

Closing Dates:

1 September 2006 (no information for 2008 as of Aug 2007)

AZA Conservation Endowment Fund Grants

Primary Purpose of Grants:

To support cooperative conservation and related scientific and educational activities of AZA member institutions and their collaborators.

Qualifications:

All principal investigators (i.e., those responsible for completing the project) must be individual members of AZA. All others should be listed as collaborators in the narrative of the proposal.

Amount and Term of Grant:

Most awards fall in the range of \$10,000 to \$20,000. Larger awards are considered on a case-by-case basis. There is no time limit for project completion.

Funding Restrictions:

Grant monies cannot be used for overhead or salaries, except for graduate student stipends or technicians. Travel costs unrelated to the completion of the project (e.g., conference presentation expenses) also are not supported. Ownership of major equipment (e.g., computers) may revert to the AZA Conservation and Science Department at the completion of the project.

Additional Information and/or Application:

See <http://www.aza.org/ConScience/WhatIsCEF/> for program description and application information or contact:

Director of Conservation and Science

Association of Zoos and Aquariums

8403 Colesville Road, Suite 710

Silver Spring, MD 20910-3314

Closing Dates:

1 April 2008

The Bullitt Foundation Grants**Primary Purpose of Grant:**

To support the protection and restoration of the environment of the Pacific Northwest. Priority areas include energy and climate change; forests and land ecosystems; growth management and transportation; public outreach, education, and capacity building; rivers, wetlands, and estuaries.

Qualifications

The Foundation invites proposals from nonprofit organizations that serve Washington, Oregon, Idaho, British Columbia, western Montana (including the Rocky Mountain range), and the rain forest region of southern Alaska. The majority of grantees are citizen groups located in the Northwest that are working to build and strengthen the environmental movement and to educate the broader public about the importance of protecting and restoring the environment.

Amount and Term of Grant:

Not specified.

Funding Restrictions:

See regional restrictions listed above.

Additional Information

See <http://www.bullitt.org/grants/grantseeking> or contact:

1212 Minor Avenue

Seattle, WA 98101-2825

206-343-0807 (tel.)

206-343-0822 (fax)

info@bullitt.org

Closing Date:

1 May and 1 November (may vary from year to year)

Chicago Zoological Society – Chicago Board of Trade Endangered Species Fund**Primary Purpose of Grants:**

Proposals should focus on a threatened, vulnerable, or endangered species, or a habitat that is of high biological value or is substantially threatened (IUCN Red List Status). This includes projects that will quantitatively assess population and environmental status with indications of best conservation strategy and projects that will help achieve sustainable relations between local people, and the species of concern. The development of educational materials and training of local people that promote these aims are very welcome components of the projects.

Qualifications:

Grants are open to SSC Specialist Group Chairs and Officers, AZA/WAZA Chairs and Officers, and all interested researchers.

Amount and Term of Grant:

Projects up to \$5,000 (smaller requests will fare better)

Funding Restrictions:

Travel from U.S. and Europe to field sites in developing countries will not be funded, but travel within target countries will be considered. There is the expectation that some kinds of equipment will be standard and will already be in the possession of qualified investigators.

Additional Information and/or Application:

Carla Owens

Manager, Library Services

Chicago Zoological Society - Brookfield Zoo

caowens@brookfieldzoo.org

Closing Dates:

4 September 2007 (no information for 2008, as of Aug 2007)

Cleveland Zoological Society: Conservation and Research Small Grants Program**Primary Purpose of Grants:**

To provide financial support to conservation and research projects involving animals and their habitat, and/or educational or cultural activities which involve or impact animals and their habitat. Ideal projects will have clear, long-term conservation implications, positively impact local people and create opportunities for capacity building in country.

Qualifications:

None listed.

Amount and Term of Grant:

Annual awards range from \$1000 - \$3000.

Funding Restrictions:

None listed.

Additional Information:

For more information about eligibility criteria and proposal guidelines, see

<http://www.clemetzoo.com/conservation/grants/small/> or contact

Sonia Di Fiore

216-635-2526

sld@clevelandmetroparks.com.

Closing Dates:

Pre-proposal deadline: 2 March 2007 (no information for 2008 as of Aug 2007)

Cleveland Zoological Society Scott Neotropical Fund**Primary Purpose of Grants:**

The Scott Neotropical Fund supports the work of neotropical residents in their countries through direct project support, training opportunities, and/or technical assistance that will continue to benefit the local people, wildlife and habitats of the neotropics into the future. The primary beneficiary of the funds as well as the impact of the project must be within the neotropics (Mexico, Central America, South America and the Caribbean).

Qualifications:

None listed.

Amount and Term of Grant:

Annual awards range from \$3000 - \$5000.

Funding Restrictions:

Recipients must be living and working in Latin America.

Additional Information:

For more information about eligibility criteria and proposal guidelines, see

<http://www.clemetzoo.com/conservation/grants/small/> or contact

Sonia Di Fiore

216-635-2526

sld@clevelandmetroparks.com.

Closing Dates:

Pre-proposal deadline: 25 May 2007 (no information for 2008 as of Aug 2007)

Compton Foundation**Primary Purpose of Grant:**

The prevention of environmental deterioration and the protection of natural resources. The Foundation's priorities in the environmental field include:

- Land, river and watershed protection and management for purposes of long term habitat and ecosystem preservation and restoration.
- Changing the relationship between people and the natural environment in order to promote a sustainable and just balance between meeting present human needs and conserving natural systems for future generations.

Qualifications

Priority is given to projects which have more than local application, are replicable, and are likely to be taken over and managed by a long-term funding source. If appropriate and warranted, a project may receive more than one year of support. In all program areas, the Foundation has a special interest in providing support for minorities.

Amount and Term of Grant:

None listed.

Funding Restrictions:

No grants are awarded to individuals.

Additional Information

See <http://www.comptonfoundation.org/priorities.html> or contact:

Compton Foundation

545 Middlefield Rd, Ste. 178

Menlo Park, CA 94025

Phone: (650) 328-0101 Fax: (650) 328-0171

info@ComptonFoundation.org

Closing Date:

7 March for consideration by Board (June meeting).

28 March for RFPs responding to the Emergency Contraception Initiative.

7 September for consideration by Board (December meeting).

(no information for 2008 as of Aug 2007)

Cryptobranchid Interest Group**Primary Purpose of Grant:**

To support giant salamander projects.

Qualifications

None listed.

Amount and Term of Grant:

Up to \$1,000.

Funding Restrictions:

None listed.

Additional Information:

See <http://www.caudata.org/cig/>

Diane Barber

Curator of Ectotherms

Fort Worth Zoo

1989 Colonial Parkway

Fort Worth, TX 76110

817-759-7180

817-759-7183 FAX

dbarber@fortworthzoo.org

Closing Date:

5 October 2007

Disney Wildlife Conservation Fund**Primary Purpose of Grant:**

Supporting in situ projects that focus on protecting and studying endangered and threatened animals and their habitats. Special focus on tropical, marine, African, and Asian animals and ecosystems.

Qualifications

AZA institutions should apply via the AZA Conservation Endowment Fund, which historically has received support from the Disney Wildlife Conservation Fund. Open to US non-profit

organizations, which should send a brief letter of inquiry to the address below, outlining the project and scope.

Amount and Term of Grant:

Applications to the Disney Wildlife Conservation Fund directly are accepted through an annual invitation-only process. Awards generally do not exceed \$20,000 per project.

Funding Restrictions:

Overhead and capital funds are not eligible.

Additional Information

See <http://www.dwcf-rfp.com/>

Disney Wildlife Conservation Fund

P.O. Box 10000

Lake Buena Vista, FL 32830

Closing Date:

1 March (subject to change)

Earthwatch Center for Field Research Grants

Primary Purpose of Grant:

To support field-based research and to promote sustainable conservation of our natural resources and cultural heritage by creating partnerships between scientists, educators, and the general public.

Qualifications

Grants are available for field-based research only, ranging typically from \$7,000-\$80,000 per year using 20 or more volunteers per year, spread throughout the season over multiple 7-16 day teams (grant levels vary with the number of volunteers used, the length of teams, the relative appeal of the research site and topic, and the quality of accommodations available).

Amount and Term of Grant:

See Qualifications.

Funding Restrictions:

Field research projects only.

Additional Information

See <http://www.earthwatch.org/site/pp.asp?c=dsJSK6PFJnH&b=421835> or contact:

David Lowe, Program Director for Life Sciences

Earthwatch Institute

3 Clocktower Place, Suite 100

P.O. Box 75

Maynard, MA 01754

800-776-0188 or 978-461-0081, ext. 127 (tel.)

978-461-2332 (fax)

dlowe@earthwatch.org

Closing Date:

Submit to Earthwatch Institute at least one year in advance of the anticipated fieldwork.

US Environmental Protection Agency (EPA) - Environmental Education Grants

Primary Purpose of Grant:

To support environmental education projects that enhance the public's awareness, knowledge, and skills to make informed decisions that affect environmental quality.

Qualifications

Applicant organizations must be located in the United States and the majority of the educational activities must take place in the United States, Canada and/or Mexico. A teacher's school district, an educator's nonprofit organization, or a faculty member's college or university may apply, but an individual teacher, educator, or faculty member may not.

Amount and Term of Grant:

Because of limited funds, EPA prefers to award smaller grants to more recipients. Also, Congress requires that at least 25% will go to small grants of \$5,000 or less. The notice states that chances of being funded are significantly increased if you request \$5,000 or less from an EPA regional office or \$100,000 or less from EPA Headquarters.

Funding Restrictions:

Grants require non-federal matching funds for at least 25% of the total cost of the project.

Additional Information

See <http://www.epa.gov/enviroed/grants.html>

Closing Date:

EPA anticipates posting a new Solicitation Notice in September 2007 and will welcome new grant proposals at that time.

Environmental Systems Research Institute - Conservation Program**Primary Purpose of Grant:**

To provide an on-going GIS software donation program for non-profit conservation and environmental organizations.

Qualifications

None listed.

Amount and Term of Grant:

Software donations only.

Funding Restrictions:

None listed.

Additional Information

For more information, send a blank email message to ecpgrant@esri.com. You will receive guidelines and application instructions by return email. Information is also available on ESRI's Web site at <http://www.conservationgis.org/aaesrigrants.html>

Closing Date:

On-going program.

International Herpetological Symposium, Inc. Grants Program**Primary Purpose of Grant:**

To provide financial assistance to individuals or organizations conducting herpetological research, conservation, and education.

Qualifications

Projects should represent one of the following categories:

Herpetological Natural History

Proposals in this category should address new field research in areas such as population distribution, behavioral ecology, and life history strategies of amphibians or reptiles.

Herpetological Conservation Biology

Proposals in this category should address new research on endangered or threatened amphibian or reptile species or the phenomena that affect the maintenance, decline, and restoration of their natural habitat.

Captive Propagation

Proposals in this category should address research in captive behavioral studies or new techniques in captive maintenance and breeding of amphibians or reptiles.

Herpetological Education

Proposals in this category should address starting and/or maintaining an educational program pertaining to amphibians or reptiles at a facility available to the public, such as a zoological park, school, or community center.

Applicants may be anyone from the herpetological community. When a grant is awarded, the recipient agrees to abide all local, state and federal laws. Recipients will be encouraged to

present their findings at the next year's symposium. Recipients will also agree to mention the IHS in any publications resulting from this grant award.

Amount and Term of Grant:

Grants are initially in the amount of up to \$500 and will be awarded to applicants whose projects represent a significant contribution to herpetoculture.

The total number of grants awarded will depend solely upon the balance of the grant fund in any given year. Depending on the applications received, an attempt will be made to award a grant to each category and some categories may receive more than one award. The committee reserves the right to reassign the category under which a given proposal is submitted.

Funding Restrictions:

Grants may not be used to support salary, tuition, or publication expenses.

Additional Information

See <http://www.kingsnake.com/ihs/grant.html> for instructions on how to apply.

Closing Date:

All applications should be postmarked no later than 31 December. Grants will be awarded by 1 March.

Jessie B. Cox Charitable Trust

Primary Purpose of Grant:

To protect and enhance the natural and urban environment, and to conserve New England's natural resources, including:

- protection of critical natural resources;
- energy conservation;
- public awareness of the critical environmental issues facing the region.

Qualifications

The geographic focus of the Trust is New England.

Amount and Term of Grant:

The average annual grant is about \$35,000, with grants generally in the range of \$25,000 to \$75,000. Although the Trustees occasionally award grants outside this range, the Trust ordinarily does not consider grant requests for less than \$20,000. Project support is available for multiple years.

Funding Restrictions:

See regional restrictions listed above.

Additional Information

See <http://www.hembar.com/selectsrv/jbcox/cox.html> or contact:

Susan M. Fish, Grants Administrator

Donor Services Office

Hemenway & Barnes

60 State Street

Boston, MA 02109-1899

617/557-9775

sfish@hembar.com

Closing Date:

Concept papers may be submitted at any time, but must be received by 15 January, 15 April, 15 July or 15 October to be considered at the next regular meeting of the Trustees.

John Ball Zoo Society Wildlife Conservation Fund

Primary Purpose of Grant:

Support in situ and ex situ wildlife conservation, captive animal management, and educational programs.

Qualifications

None listed.

Amount and Term of Grant:

Grants range from \$750 to \$2,500, no time table for project completion is given. Awards are made in June.

Funding Restrictions:

None listed.

Additional Information

See <http://www.johnballzoosociety.org/conservation.php> or call 616-336-4301.

Closing Date:

20 April 2007 (no information for 2008 as of Aug 2007)

Lincoln Park Zoological Garden - Ed Almandarz Science Fellowship in Herpetology**Primary Purpose of Fellowship:**

Scientific research on reptiles and amphibians to be conducted at the Lincoln Park Zoo.

Qualifications

These grants are available to undergraduates or individuals who have received their undergraduate degree within six months of the beginning of the fellowship. Selection is based on applicant's academic and/or research record, letters of recommendation, statement of research interests, and availability of appropriate research project.

Amount and Term of Fellowship:

Stipend of \$2,500 per individual for a 12 week period. Project must be completed by the end of the twelve week period.

Funding Restrictions:

There are no restrictions on the stipend; living arrangements and expenses are the responsibility of the applicant.

Additional Information

See <http://www.lpzoo.com/conservation/>

or contact:

Science Fellowships

Director of Conservation and Science

Lincoln Park Zoological Gardens

2001 North Clark Street

Chicago, IL 60614

conservation@lpzoo.org

Closing Date:

14 March (may vary from year to year)

Memphis Zoo Conservation Action Network Grants**Primary Purpose of Grants:**

To support original conservation research projects, which have a clear and direct application to the Memphis Zoo's mission of preserving wildlife.

Qualifications:

Applicants must be Memphis Zoo staff and/or research affiliates of the Memphis Zoo.

Amount and Term of Grant:

Not specified.

Funding Restrictions:

Indirect costs are not supported. Exceptions to this rule are made for grants over \$30,000 that have as their primary component a graduate student stipend that is being paid for by the Memphis Zoo.

Additional Information:

Contact Dr. Andy Kouba, Curator of Research and Conservation to ensure that your proposed project falls within the scope of the Memphis Zoo's conservation goals. Memphis Zoo, 2000 Prentiss Place, Memphis TN, 38112. Phone (901) 725-3410, Fax (901) 725-9305, akouba@memphiszoo.org

Closing Dates:

Applications will be accepted between 1 May and 14 June (may vary from year to year).

Memphis Zoo Conservation Action Network - Emergency Conservation Grants**Primary Purpose of Grants:**

To provide financial support for crucial emergency conservation action unencumbered by traditional grant cycles.

Qualifications:

Applicants must be Memphis Zoo staff and/or research affiliates of the Memphis Zoo.

Amount and Term of Grant:

Grants will be awarded on an as-needed basis, and are open year round. The awards will be for less than \$3,000.

Funding Restrictions:

None specified.

Additional Information:

Applicants must submit a letter of intent to the Memphis Zoo's Curator of Research and Conservation, Dr. Andy Kouba (Memphis Zoo, 2000 Prentiss Place, Memphis TN, 38112. Phone (901) 725-3410, Fax (901) 725-9305, akouba@memphiszoo.org).

Closing Dates:

None; applications are reviewed on an as-needed basis.

Miami Metrozoo Conservation and Research Fund**Primary Purpose of Grants:**

To support wildlife conservation efforts. Evaluation criteria are based on maximum impact on wildlife conservation.

Qualifications:

None listed

Amount and Term of Grant:

Up to \$5,000.

Funding Restrictions:

Applications will be accepted from individuals, non-profit organizations or municipalities.

Additional Information:

For guidelines and application forms, contact Linda Cunningham by phone at (305) 253-5050 or fax at (305) 378-6381.

Closing Dates:

31 August (may vary from year to year)

National Ocean and Atmospheric Administration (NOAA) - Community-Based Restoration Program**Primary Purpose of Grant:**

To bring together citizen groups, public and nonprofit organizations, industry, corporations and businesses, students, landowners, local government, and state and Federal agencies to restore fishery habitat around the coastal U.S.

Qualifications:

Varies by program

Amount and Term of Grant:

Variable

Funding Restrictions:

Variable

Additional Information

www.nmfs.noaa.gov/habitat/restoration/funding_opportunities/funding.html

Closing Dates:

Variable

NEW Zoological Society Conservation Fund

Primary Purpose of Grant:

Support wildlife conservation and research efforts directly benefiting wildlife in their native ranges worldwide.

Qualifications

Both organizations and individuals are eligible. Award monies will be given to those projects dedicated to results-based solutions. Applications from other AZA-accredited facilities will be accepted, however projects should focus directly on wildlife within their native habitats.

Amount and Term of Grant:

The Fund has no set maximum or minimum awards and funding will be for a one-year term only. In the past, however, the Fund has supported projects ranging from \$250 to \$1000 for a one-year term.

Funding Restrictions:

No capital expenditures. The fund cannot underwrite the captive breeding efforts of other animal management facilities. See [web page](#) for additional information.

Additional Information

Individuals interested in applying for a conservation grant can find all application-related materials on-line at: <http://www.thenewzoo.com/society/conservation-fund.php>

Donations can be made directly to the conservation fund by contacting the N.E.W. Zoological Society, Inc. at (920) 434-6814.

Contacts

Neil Anderson, Zoo Director, (920) 434-8597

N.E.W. Zoological Society, Inc. (920) 434-6814

Kathy Walker, Edu. Coordinator (920) 434-4162

Closing Dates:

Applications must be received in an electronic format (via email or on CD) by 1 May 2007

Oregon Zoo – Future for Wildlife Grants Program

Primary Purpose of Grants:

To support field conservation programs in the Pacific Northwest and abroad. Funded projects must directly contribute to the survival of populations of free-living animal species and the ecosystems in which they are found.

Qualifications:

Funds are only distributed to organizations, not individuals.

Amount and Term of Grant:

Small grants, averaging \$2,000

Funding Restrictions:

The grant is not for/does not include/allow overhead costs.

Additional Information:

<http://www.oregonzoo.org/ConservationNew/grants.htm>

Anne Warner

Conservation Manager

Future for Wildlife Grants Program

Oregon Zoo

4001 SW Canyon Road

Portland, OR 97221

Closing Dates:

Proposals must be received by 30 June and 31 December, respectively.

Applications are reviewed in July and January of each calendar year.

Pittsburgh Zoo and PPG Aquarium Conservation Fund**Primary Purpose of Grants:**

To support the preservation of wildlife and wild habitat. A wide variety of projects will be considered, but field studies and cross-disciplinary approaches to conservation are especially encouraged.

Qualifications:

None listed.

Amount and Term of Grant:

Annual awards of from \$1,000 to \$3,000.

Funding Restrictions:

Visitor surveys, travel and training (except to bring foreign researchers to meetings) and seed money for technique development are not appropriate for this fund.

Additional Information:

<http://zoo.pgh.pa.us/zoo.asp?ContentID=346>

Administrative Assistant

Department of Science & Conservation

Pittsburgh Zoo

One Wild Place

Pittsburgh, PA 15206

412-665-3766

email: conservation@zoo.pgh.pa.us

Closing Dates:

21 November (may vary from year to year)

Project Golden Frog/Atelopus Conservation Trust Grant**Primary Purpose of Grant:**

The PGF/ACT Grant supports conservation programs that seek to increase our basis of knowledge or continue to protect the endangered Panamanian golden frog and other *Atelopus* species throughout their range and in captivity.

Qualifications:

Applicants must be associated with a recognized organization (e.g. zoo/aquarium, NGO, university, etc.) through which s/he can receive funding. There are no eligibility restrictions on the nationality of the applicant. Students are encouraged to apply.

Amount and Term of Grant:

Maximum annual request not to exceed US\$5,000

Funding Restrictions:

Field studies and other projects that demonstrate a multidisciplinary approach to *Atelopus zeteki* receive highest funding priority, although projects involving *ex situ* conservation of other *Atelopus* species will be considered.

Research and projects that involve range-country collaborators are encouraged.

Environmental education programs, development of techniques that can be used in a natural environment and captive propagation programs that stress an integrative approach to conservation are also appropriate. Projects must directly affect biological conservation.

Additional Information:

www.projectgoldenfrog.org or www.ranadorada.org

Attn: Rick Haeffner, General Curator

Denver Zoological Gardens

2300 E. Steele Street

Denver, CO 80205-4899

USA

FAX: 303-376-4901

Closing Date:

1 July

Riverbanks Conservation Fund**Primary Purpose of Grant:**

To support AZA Conservation Committee (SSP, TAG, CAP, SAG) projects identified as priorities in AZA-endorsed Action Plans; AZA Conservation Committees (other requests); outside agencies (USFWS, state wildlife agencies, etc.); and individual researchers.

Qualifications

None listed.

Amount and Term of Grant:

Awards generally do not exceed \$1,000 per project.

Funding Restrictions:

See Primary Purpose section above.

Additional Information

Ed Diebold, Director of Animal Collections

Riverbanks Zoological Park

P.O. Box 1060

Columbia, SC 29202

803-779-8717 (tel.)

803-256-6463 (fax)

Closing Date:

1 February

Roger Williams Park Zoo/Sophie Danforth Grant**Primary Purpose of Grant:**

Field studies and other projects that demonstrate a multi-disciplinary approach to biodiversity and ecosystem conservation and projects that involve in-country collaborators receive the highest funding priority. Environmental education programs, development of techniques that can be used in a natural environment, and captive propagation programs that stress an integrative approach to conservation are also appropriate. Projects must directly affect biological conservation.

Qualifications

Applicants must be associated with an organization (e.g. NGO, university, etc.) through which s/he can receive funding. Funding checks are provided to organizations, not individuals. There are no eligibility restrictions on the nationality of the applicant.

Amount and Term of Grant:

Maximum annual request is \$1,000

Funding Restrictions:

None listed.

Additional Information

Information on the application process can be found at

<http://www.rwpzoo.org/conservation/danforth.cfm>

Closing Date:

1 June 2007 (no information for 2008 as of Aug 2007)

Rolex Awards for Enterprise**Primary Purpose of Grant:**

To provide visionary men and women worldwide with the financial support and recognition needed to carry out innovative working projects in areas ranging through science, technology, exploration, the environment, and cultural heritage.

Qualifications

Applications may be submitted by individuals only. If a project represents the efforts of several individuals, one person must represent the group and sign the application form.

Amount and Term of Grant:

Not specified.

Funding Restrictions:

The Rolex Awards are not research grants; neither do they constitute a reward for past achievements. Since projects must have a practical application in improving our world, studies or pure research works cannot be considered.

Additional Information

See <http://www.rolexawards.com/awards/how-to-apply/index.html> or contact:

The Secretariat - Rolex Awards for Enterprise

P.O. Box 1311

1211 Geneva 26

Switzerland

+41 22 308 22 00 (tel.)

+41 22 308 25 85 (fax)

secretariat@rolexawards.com

Closing Date:

Awards are presented every two years. The 2008 deadline for the Americas has passed.

Sea World & Busch Gardens Conservation Fund**Primary Purpose of Grant:**

Its primary focus is to support conservation efforts directly benefiting wildlife in their native ranges (in-situ).

Qualifications

None listed.

Amount and Term of Grant:

The Fund has no set minimum or maximum grant amount. In the past, however, the Fund has supported projects ranging from \$5,000 to \$25,000 for a one-year term.

Funding Restrictions:

No capital expenditures. The fund cannot underwrite the captive breeding efforts of other animal management facilities.

Additional Information

See http://www.swbg-conservationfund.org/get_involved.html for more information.

SeaWorld & Busch Gardens Conservation Fund

231 S. Bemiston Ave., Suite 600

Clayton, MO 63105

mail@swbgfund.org.

Closing Date:

Grant applications are accepted year-round. All applications must be received in an electronic format (via email or on CD) by December 1 of the previous year.

Society for the Study of Amphibians and Reptiles Grants**Primary Purpose of Grant:**

Grants are intended to support herpetological research, education or conservation. There are 6 categories of grants; category (4), "Herpetological Education," is the most relevant for member institutions. Projects including improvement of displays, interpretive graphics, husbandry, or behavioral research are supported.

Qualifications

Must be a member of Society for the Study of Amphibians and Reptiles.

Amount and Term of Grant:

Not specified.

Funding Restrictions:

None listed.

Additional Information

<http://www.ssarherps.org/pages/GIH.php>

Society for the Study of Amphibians and Reptiles

Joseph R. Mendelson III, Chair, SSAR Grants-in-Herpetology

Department of Biology

Utah State University

Logan, UT 84322-5305

Closing Date:

31 December 2007

Zoo Boise Conservation Fund**Primary Purpose of Grant:**

- Habitat restoration
- Wildlife conservation and management
- Community-based conservation, including eco-tourism
- Conservation education, including workshops and trainings
- Professional development, including education and scholarships
- Scientific research related to natural history, ecology, habitat, national park inventories or biological assessments.

Amount and Term of Grant:

\$1,000 to \$25,000.

Additional Information

Applications may be obtained by visiting Zoo Boise's website at

<http://www.cityofboise.org/Departments/Parks/ZooBoise/Conservation/index.aspx> or by contacting Monica Hopkins, Director of Development and Communications at (208) 384-4125 ext. 206 or via e-mail at mdhopkins@cityofboise.org.

Closing Date:

1 April – 30 June 2007 (no information for 2008 as of Aug 2007)

Additional Grant-Finding Resources

- Community of Science (<http://www.cos.com/>)
- Foundation Center (<http://foundationcenter.org/>)
- FundsNet (<http://www.fundsnet services.com/>)
- GrantsWeb (<http://www.srainternational.org/sra03/grantsweb/index.cfm>)
- Resources and Advice for Graduate Students
(<http://online.anu.edu.au/BoZo/Scott/Studentresources.html>)
- SchoolGrants (<http://www.schoolgrants.org/>)
- Proposal Writing Short Course - From the Foundation Center
(<http://foundationcenter.org/getstarted/tutorials/shortcourse/index.html>)

Appendix 4. Available Action Plans

The following action plans are included:

- Axolotl salamander (*Ambystoma mexicanum*)
- Hellbender (*Cryptobranchus alleganiensis* Daudin)
- Mountain yellow-legged frog (*Rana muscosa*)
- Panamanian golden frog (*Atelopus zeteki*)
- Puerto Rican crested toad (*Bufo lemur*)
- Wyoming toad (*Bufo baxteri*)

ATAG RECOMMENDATIONS FOR IN-COUNTRY SUPPORT FOR CONSERVATION OF THE ENDEMIC MEXICAN AXOLOTL

The AZA Amphibian TAG has designated the axolotl salamander, *Ambystoma mexicanum*, and its habitat as a focus for an AZA Regional response to amphibian declines.

Prepared by Bob Johnson on behalf of the AZA Amphibian TAG

This report supports an integrated conservation programme using the axolotl as a flagship species for the Lake Xochimilco ecosystem. The goal of this project is provide lake remediation and to save a species from extinction. The axolotl is a Critically Endangered species only found in Lake Xochimilco, in Mexico City. This outline addresses conservation actions developed with Mexican partners in January, 2007. These include long term biological monitoring and research on metapopulation structuring within the lake and captive assurance populations; ecotoxicology of lake sediments; impacts and control of introduced species; zoo based disease screening and pathology studies of captive and wild axolotl populations; zoo and community outreach programs; community based social studies to evaluate stakeholder attitudes and participation; and training for Lake Xochimilco boat operators (remeros) to provide ecotourism income and axolotl conservation awareness.

Background

Xochimilco means place of flowers and since colonial times flowers and agricultural products have been grown on islands or chinampas created in the lake. The chinampas have been created by dredging lake sediments onto raised agricultural beds with the channels representing all that remains of the Lake Xochimilco wetland ecosystem, now recognized as a RAMSAR and World Heritage site. Increasing urbanization in the Mexico City basin has resulted in a dramatic deterioration in water quality as fresh water springs were diverted to support increasing urbanization. As with many lake ecosystems, additional stresses include contamination by heavy metals and agricultural chemicals and the introduction of many invasive species of plants and fish (carp and more recently, tilapia). Introduced fish are problematic as they have replaced the axolotl as top carnivore within the lake ecosystem, destroy native plants, increase water turbidity, prey upon axolotl eggs and larva, and compete for space and food.

In 2001-2003, Toronto Zoo provided funding for CIBAC (Centro de Investigaciones Biologicas y Acuicolas de Cuernavaca) and Chapultepec Zoo. During this period, the initial partnership resulted in life support improvements for the captive axolotl colonies and funding for exhibit graphics and printing of outreach materials developed by educators at Chapultepec Zoo. The following publication resulted from an investigation on status and water quality under the coordination of CIBAC: Jones, C. 2002. *Water quality model for the reintroduction of the axolotl (Ambystoma mexicanum) into the canals of Xochimilco, Mexico City*. Unpublished undergraduate honours theses, Trent University. Peterborough, Canada.

From 2003-06 University of Kent, Durrell Institute for Conservation Biology (DICE), provided support for ecotourism training for remero boat operators and guides; artisans trained as souvenir producers; an axolotl Species and Habitat Action Plan; and the funding for a Mexican MSc student for a degree in Tourism and Conservation from DICE.

The Grupo de Investigacion de Ajolote y Xochimilco (GIAX) partnership, with the support of DICE, initiated a January 2007 meeting to review progress to date. The completion of an “Axolotl Species Habitat Action Plan” provides an opportunity for AZA institutions to contribute to existing in-country led and executed conservation priorities. These in situ projects will support a stakeholder driven Conservation Action Plan and foster newly developed partnerships and collaborations under the umbrella of GIA-X, a multi partner alliance of stakeholders for axolotl and Lake Xochimilco conservation. Partners include Government agencies; two universities; researchers and graduate students; educators, biologists and sociologists; farmers, fishers, remero boat operators; two Mexico City zoos; with participation of the AZA ATAG.

Any of the following initiatives can be supported in response to Priority Action plan objectives to begin Lake Xochimilco restoration and save a species from extinction. .

Action Plan

Ecotourism training

A remero (indigenous Xochimilcan boat operators who pole boats through canals) natural history training program was developed from stakeholder workshops and profiling of Lake Xochimilco visitors. Trips were usually 1-2 hours in small groups. Visitors indicated that they wanted more information on the lake and its wildlife and were willing to pay 15-20 pesos more for such trips. Remeros reported increased tips from the longer natural history tours. Previously considered as only boat operators, seen not heard, with little to no interaction with guests, remeros identified natural history training as a potential source for increased income. Guests that requested natural history tours spent an extra hour on the lake and remero incomes increased. The importance of the Lake Xochimilco ecosystem and the unique relationship of the axolotl to the lake and cultural history of Mexico is the focus of tours for lake visitors. With this background, remeros benefited from the training sessions and visitors knowledge increased and the plight of the axolotl was delivered to a wider audience. Building upon remero training by university students, Remero trainers have requested skills development to provide remero peer to peer training. The remero training program would become sustainable as university based trainers are replaced by remero community leaders.

GIA-X Research

Research projects will examine if axolotl distribution in Lake Xochimilco is related to habitat structure and ecotoxicological contamination. These data along with habitat profiling will determine factors responsible for axolotl population fragmentation and as a basis for lake restoration projects. Support is required for permanent sampling stations, more sampling areas and increase the number of samples analyzed.

The lab of Dr. Luis Zambrano is conducting competition and predation experiments between axolotl, at different life stages, and introduced fish, carp and tilapia. Canal restorations and fish barriers in relation to this research will require funding.

Genetic profiling of metapopulations by Dr. Gabriela Para to determine population structuring and fragmentation of populations in the lake is also part of the GIA-X research collaboration. These data will be used to profile captive colonies to determine origins and genetic sustainability

of captive axolotl colonies as assurance populations. Chapultepec Zoo will serve as a tissue bank for future genetic studies,

Chapultepec Zoo

With over 9 million visitors a year Chapultepec Zoo and the three other Mexico City Zoos have active axolotl education programs developed by zoo educators. Additional support is required for printing of resources to be distributed to zoo visitors and education programs, remeros, and CIBAC the biological station on Lake Xochimilco. Chapultepec Zoo will provide disease screening and pathology for all captive axolotl colonies and as a resource in case of sudden mortalities of axolotl within lake Xochimilco. As well Chapultepec Zoo will develop biosecurity guidelines in light of recently developed standards and new emerging diseases.

Collaborations

Perhaps the most important aspect of support for these projects is the re-enforcement of fledgling conservation partnerships among different and diverse stakeholders. Conservation of the Lake Xochimilco ecosystem in the face of overwhelming ecological challenges will require sustaining such partnerships and integrating results to benefit residents of the Lake Xochimilco community, those earning a living from ecotourism, the health of communities around the lake, removal of invasive species, and remediation of the stressed lake ecosystem itself. To this end, the support of a conservation technician will facilitate stakeholder involvement and cement newly developing scientific and community partnerships in a non threatening way. Indeed, the axolotl and whole Lake Xochimilco ecosystem will benefit from non-traditional sustainable collaborations. Mutual respect among partners has been built on such shared understanding. The GIA-X Working Group will prioritise and oversee reporting mechanisms for all projects. This will facilitate capacity building goals. Updated in 2007, "The conservation of the Axolotl in Xochimilco, Mexico city- A Species/Habitat Action Plan" sets out clear goals and outcomes for annual review. Annual Workshop reporting meetings will provide results to all stakeholders for review and re-evaluation of project results and priorities. Thus, a clear plan developed by stakeholders will guide project prioritization and evaluation.

GLOBAL AMPHIBIAN ASSESMENT <http://www.globalamphibians.org/>

In-Country Action & Funding: 2007-08 Recommendations and Budget

Priority Request: GIAX Co-ordination. To provide Action plan co-ordination to sustain the new GIAX partnerships, UAM-based GIAX co-ordinator will facilitate project implementation and evaluation. Cost for one year at 30 hours per week (\$22,000); two years to provide continuity and long range planning and oversight,(\$44,000)

Wages for GIAX Technician-Immediate need, one year

\$22,000

AP GOAL 1. Biology of the species

a. Increasing knowledge of the species .

\$31,500

- **Long term monitoring stations \$20,000**
- **Tolerance levels of water quality parameters \$5,000**

- Preventive medicine and therapeutic techniques \$1,000
 - Genetic variability of populations \$2,500
 - Captive breeding research in country \$3,000
- b. Ensuring the recovery of the wild population **\$73,000**
- Reduce and control introduced species \$25,000
 - Determine critical habitat \$3,000
 - Determine population size and demographics
 - Create controlled areas for repopulation \$20,000
 - Promote egg laying sites
 - Protect most vulnerable stages
 - Create secure areas of restored habitat without introduced threats \$25,000
- c. Promoting breeding centers for conservation. **\$10,000**
- Provide training and infrastructure, equipment and operating funding \$10,000

AP GOAL 2. Ecological interactions

- a. Understanding the relations of the species with biotic and abiotic factors.
- Research impacts of introduced species and pollution on axolotl **\$25,000**
- b. Controlling and regulating exotic species.
- Determine usage of exotic species by local population
 - Determine impacts of introduced species on Lake ecosystem **\$25,000**

AP GOAL 3. Legislation

- a. Having effective and adequate legislation for the conservation of the species and its habitat. **\$3,500**
- Develop Management Plan for the protected area

AP GOAL 4. Social

- a. Recovering the identity of the community with respect to the axolotl.
- b. Uniting different sectors for conservation.
- c. Increasing awareness of and participation of the community in conservation.
- Develop outreach resources for local community **\$10,000**

AP GOAL 5. Political

- a. Ensuring the participation of the different sectors in the decision making process.
- b. Ensuring that the strategies of conservation are respected by the actions of the government.

AP GOAL 6. Environment

- a. Guaranteeing a suitable habitat for the axolotl. **\$15,000**
- Complete water quality analysis \$15,000**
- b. Identifying and evaluating biotic and abiotic factors that affect the axolotl.

- **Determine abundance of exotic and endemic species and impacts on axolotl** **\$3,000**
- c. Reconciling biological, social and cultural elements.
 - **Environmental education workshops in surrounding community** **\$2,800**

AP GOAL 7. Education

- a. Evaluating the species in cultural and environmental terms (Social issues) **\$4,500**
 - **Determine from stakeholders cultural importance**
 - **Provide resources linking the axolotl with a healthy Lake Xochimilco ecosystem**
- b. Creating widespread awareness and understanding of the problems at all levels (local, tourism, national and international).
 - **Train community educators \$2,600** **\$10,100**
 - **Utilize local fishermen and boat operators in community based educational opportunities \$3,000**
 - **Include axolotl in formal (curriculum) and informal education programmes \$4,500**

AP GOAL 8. Uses

- a. Evaluating the uses of the axolotl.
- b. Evaluating the cultural, social and economic importance of the axolotl. **\$3,500**
 - **Develop the axolotl as a keystone species representative of the whole Lake Xochimilco ecosystem**
- c. Developing means and methods of making sustainable use of the species.

Action Plan for the Conservation of the Hellbender, (*Cryptobranchus alleganiensis* Daudin) in North America

DRAFT

The AZA Amphibian TAG has designated the hellbender, *Cryptobranchus alleganiensis*, and its habitat as a focus for an AZA regional response to amphibian declines.

Prepared by Mark Wanner, Saint Louis Zoo. 1 Government Drive, Saint Louis, MO. 63110, 314-781-0900, wanner@stlzoo.org



Background

The hellbender (*Cryptobranchus alleganiensis*) is currently compromised of two subspecies. The Ozark hellbender (*Cryptobranchus a. bishopi*) and the Eastern hellbender (*Cryptobranchus a. alleganiensis*). *C.a. bishopi* is found only in the Midwestern states of Missouri and Arkansas. In contrast, *C. a. alleganiensis* has a much larger range and is found throughout the Eastern Mountain region of the U.S., from New York to Georgia, with an isolated population in Eastern Missouri. However, populations of both subspecies have experienced substantial population declines due to habitat change and degradation (Nickerson and Mays, 1973a), over-collecting and illegal collecting (Nickerson and Briggler, 2007) and a compounding multitude of other concerning factors. In addition, emerging diseases, such as a chytrid fungus,

(*Batrachochytrium dendrobatidis*), has recently been observed in parts of the *bishopi* range. This fungus may also be contributing to their decline (PHVA 2007). Although there is substantial wild data on Ozark hellbenders for the last several decades, little is known of their captive husbandry and propagation. Captive propagation and habitat conservation of both subspecies are priorities for wild population stability and growth.

Action Plan

As a result of the Population Habitat Viability Assessment held in Saint Louis, August 2006, a list of recommendations was produced to help organize and begin to address the hellbender decline. The majority of participants at this meeting were *C. a. bishopi* stakeholders; however, *C.a. alleganiensis* was discussed at great length. These two subspecies are somewhat different in regards to status and what is known of natural history, but they can be intertwined and some of the same conservation tactics can be used for both. Specific recommendations have been developed for both subspecies. They are:

Ozark hellbender (*C.a. bishopi*)

Recommendations:

Biological/human-induced threats: see PHVA

- 1) Build a baseline understanding of diseases found in wild hellbender populations. (\$20,000.00)
- 2) Develop a post-mortem/protocol/pathology network. (\$5,000.00)
- 3) Standardize and unify monitoring and research efforts/methods. (\$5,000.00)
- 4) Upgrade the protection status of hellbenders and prevent illegal collecting
- 5) Determine possible impacts of predation by native and non-native fishes and native mammals. (\$30,000.00)

Land Use: see PHVA

- 1) Initiate intensive water quality analysis and monitoring programs on all prioritized hellbender streams. (\$50,000.00)
- 2) Conduct a comprehensive threat analysis incorporating stakeholder involvement and comments, GIS analysis, modeling and, where needed, field measurements to protect riparian habitats. (\$15,000.00)
 - a. Implementation – manager salary \$30,000.00)
 - b. Private land purchase, easements, alternative water source, tree, fence material - \$100,000.00
- 3) Standardize survey methodology for conducting meta-population studies and long-term monitoring of life history and population demography, and conduct baseline studies. (\$30,000.00/yr.)
- 4) Measure and correlate sediment deposition rates to hellbender demographics from a wide range of streams; impacted to pristine. (\$10,000.00)

- 5) Using surrogate species, determine acute and chronic toxicity of heavy metals, organophosphates, ammonia, etc. to various life stages (eggs, larvae, and adults) of hellbenders. (\$25,000.00)
- 6) Determine the effects of endocrine disrupters on hellbender eggs, larvae and adults. (\$25,000.00)
- 7) Develop reintroduction, augmentation, and captive husbandry protocols and techniques. (\$1,000.00)
- 8) Develop comprehensive watershed conservation plans and agreements.
- 9) Identify stakeholders within priority watersheds and develop a comprehensive outreach program.
- 10) Develop a comprehensive, consensus-based best management practices manual for hellbenders. (\$1,000.00)
- 11) Federally list the Ozark hellbender and petition for listing the Eastern hellbender as a federally threatened or endangered species.

Public Use: see PHVA

- 1) Educate recreational users regarding effects of habitat disturbance. (\$2,500.00)
- 2) Formulate guidelines for river access construction and bridge placement. (\$2,500.00)
- 3) Inspire local landowners and river users to value and protect the hellbender.
- 4) Lobby for new environmental laws to improve water quality. (\$40,000.00)
- 5) Inform anglers about impacts on hellbenders from releasing bait (disease transmission, habitat, and prey competition). (\$2,500.00)
- 6) Seek legislation regarding issues such as collecting hellbenders and dumping bait. (\$2,500.00)
- 7) Support ongoing and new research on the effects of introduced hormones on the health and immune systems of hellbenders in streams.
- 8) Support research into other potential threats related to public use and recreation such as disease in the bait industry, competition/predation from released bait, and effects of noise from recreation vehicles. (\$2,500.00)

Captive Breeding/Husbandry: see PHVA

- 1) Collect eggs from eastern populations of hellbenders for research and from Midwest hellbender populations (Ozark and Eastern hellbenders) to head-start for release. (\$5,000.00)
- 2) Produce animals for captive assurance colonies to maintain genetic diversity, for experimental release and reintroduction where appropriate, and for research purposes. (\$30,000.00)
- 3) Establish “semi-natural” outdoor breeding facilities for hellbenders within their range. (\$30,000.00)
- 4) Conduct research to determine: (\$50,000.00)
 - a. The effect of rain and lowered light levels on the initiation of breeding activity;
 - b. How water quality affects propagation efforts at hatcheries and other facilities that use potentially contaminated water from rivers;
 - c. The nutrition requirements of healthy hellbenders

- d. The types of nest sites that should be provided to encourage breeding;
- e. The feasibility of applying artificial insemination techniques to hellbenders.

Eastern hellbender (*C.a. alleganiensis*)

Since general census work has not been done to the extent it has with Ozark and Eastern hellbenders in Missouri/Arkansas, the main recommendations for the Eastern hellbender in the Eastern U.S. are:

Research

- 1) Determine status. Search streams with Historic records of hellbenders. States to begin census work are North Carolina, West Virginia, Western Virginia and Western Pennsylvania. Determine stream population sizes in forested areas vs. non-forested areas. (\$100,000.00/per state)
- 2) Develop meta-population genetics study - fragmented populations, abundance and life history. (\$50,000.00)
- 3) Codify survey methods and data collection. (\$5,000.00)
- 4) Determine National Park status. (Dr. Freake – ongoing, \$10,000.00)
- 5) Develop website and emblem for grants and donations. (\$2,000.00)

Education

- 1) Promote awareness. (\$25,000.00)

Policy

- 1) Dissolve loopholes in state laws (specifically West Virginia). (\$1,000.00)
- 2) Once hellbenders are protected in all states within range, pursue Federal regulations.
- 3) Close loopholes involving international trade.

Captive Breeding/Husbandry

- 1) Review PHVA.
- 2) Determine genetics of captive populations; sex captive individuals. (\$1,000.00)
- 3) Identify headstart/egg rearing facilities. (*the Wilds*, Omaha's Henry Doorly Zoo, Dallas MDC, Shepherd of the Hills Hatchery (Mammoth Springs, MO), Saint Louis Zoo)
- 4) Identify which states can collect eggs.
- 5) Write detailed protocol for egg collection, care and transport – in progress. (\$1,000.00)
- 6) Identify 2-3 "breeding" facilities and construct isolated breeding/rearing habitats. (Fort Worth Zoo, Tennessee Aquarium, Nashville Zoo, Milwaukee County Zoo, North Carolina Zoo) (\$300,000.00)

Within each of the Eastern hellbender states there seems to be a range of regulations, laws and policies surrounding the collection/take of hellbenders. *Regs. and state contacts* are as follows:

Alabama – *Priority 1*, permits to handle – Dr. George Cline
Arkansas – *Species of Concern* – few individuals remain in one river – Dr. Stan Trauth
Georgia – *Rare* – no collection without permit – Dr. Jeff Humphries
North Carolina – *Special Concern* – no collection without permit – Dr. Jeff Humphries
 or John Groves
South Carolina – Dr. Jeff Humphries
Illinois – *Endangered (extirpated)* – Chris Phillips
Indiana – *State Endangered* – special permits to collect – Dr. Zack Walker
Kentucky – *Protected* – Louisville Zoo
Missouri – *State Endangered* – Dr. Jeff Briggler
New York – *Special Concern* – illegal to collect without permit – Robin Fosterin
Ohio – Gregg Lipps
Pennsylvania – no take rule – Dr. Peter Petokas
Virginia – no collection allowed – Mike Pinder
Tennessee – permits to collect – Dr. Michael Freake
West Virginia – 100/day possession limit – greatest need of conservation – Joe
Greathouse
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### ***Literature cited***

Briggler, J., J. Utrup, C. Davidson, J. Humphries, J. Groves, T. Johnson, J. Ettling, M. Wanner, K. Traylor-Holzer, D. Reed, V. Lingren, O. Byers (eds.) 2007. *Hellbender Population and Habitat Viability Assessment: Final Report*. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN.

Nickerson, M.A. and J.T. Briggler, 2007. *Applied Herpetology*. **4** (3) pp. 207-216 (10)

Nickerson, M. A. and C. E. Mays. 1973a. The hellbenders: North American “giant salamanders”. *Publ. Biol. Geol., Milwaukee Public Mus.*, **1**:1 – 106.

## **Action Plan for the Conservation of the Endangered Mountain Yellow-Legged Frog (*Rana muscosa*) in Southern California**

The AZA Amphibian TAG has designated the mountain yellow-legged frog, *Rana muscosa*, and its habitat as a focus for an AZA regional response to amphibian declines.

Prepared by: Cynthia J. Hitchcock, Adam R. Backlin, Andrew T. Snider, and Robert N. Fisher

### **Background**

The mountain yellow-legged frog (*Rana muscosa*) was once the most common ranid frog in southern California (Storer, 1925; Schoenherr, 1976; Jennings, 1994). However, populations of this species have been declining throughout the state since the late 1960's (Bradford et al., 1994; Jennings and Hayes, 1994a; Stebbins and Cohen, 1997; Knapp and Matthews, 2000; Vredenburg et al., 2007). Decline causes are largely unknown but several hypotheses appear to be more likely than others for southern California, including habitat loss and degradation, the introduction of invasive exotic species, and possibly chytrid fungus outbreaks. Also, the flood events during the winter of 1968-1969 correlate with a dramatic decrease in vouchered specimens documented after this time (Jennings and Hayes, 1994b; Backlin et al., 2004a).

Recent genetic work on *Rana boylei* species group resulted in a taxonomic revision, naming the southern California clade of *R. muscosa* as a distinct population segment (Macey et al., 2001). This phylogenetic analysis, along with evidence that only a few small extant populations remain in southern California (Jennings, 1993, 1994, 1995, 1998, 1999; Backlin et al., 2001–2002), prompted the listing of the southern California clade of *R. muscosa* as endangered on August 1, 2002 (U.S. Fish and Wildlife Service, 2002). Despite this protective status, this species remains in less than 2% of its former range in southern California and populations are continuing towards extinction (Jennings and Hayes, 1994a; Jennings, 1994, 1995, 1998, 1999; Backlin et al., 2001, 2002, 2004a, 2004b, 2005, Compton et al., 2005a, 2005b; Vredenburg et al., 2007). Further taxonomic revisions now recognize *R. sierrae* and *R. muscosa* as distinct species with the geographic transition zone occurring in the southern Sierra (Vredenburg et al., 2007). Genetic analysis of the remaining frogs in this southern California clade suggests that there are four genetically defined populations remaining within the transverse mountain ranges (Vredenburg, unpublished data).

The four genetically defined populations characterize the remaining eight known localities of *R. muscosa* in southern California. Population sizes at each of these eight localities are estimated to be extremely small (< 41 adults for each population). Our data indicate that several of the remaining localities have fewer than ten pairs of adults. Very small populations, consisting of less than ten pairs, are considered to be highly vulnerable to stochastic events and are likely to become extinct in the short term (Pimm et al., 1988). U.S. Geological Survey (USGS), California Department of Fish and Game (CDFG), U.S. Forest Service, and U.S. Fish and Wildlife Service (USFWS) have been monitoring these eight localities since 2000. Since this time we have documented several severe population crashes. Heavy rains and debris flows following forest fires contributed to at least one of these crashes, demonstrating that the remaining small populations are vulnerable to stochastic events.

During the last several years of actively monitoring these locations it has become clear that more aggressive management is needed to prevent this species from disappearing entirely (Backlin et al., 2005). Local biologists have been suggesting the use of captive breeding and translocation for southern California populations of *R. muscosa* for several years (Stewart et al., 2000; Biological Resource Specialists, 2001). Furthermore, there is recent evidence that captive breeding and translocation can be highly successful for ranid frogs in similar environments, at least in the short term (Fesnock and Scott, 2000; Rorabaugh et al., 2005; Johnson et al., 2005). Therefore it is evident that at the very least, captive breeding and translocation could buy us time by perpetuating the species so that additional measures could be implemented for future recovery throughout its range.

Of the four remaining genetic strains, one is currently being preserved in captivity as an assurance population at the San Diego Zoo Conservation and Research for Endangered Species (CRES), where USGS transported 75 tadpoles last fall. Several of these individuals are now full-grown frogs and many continue to metamorphose. Preservation of each of the four strains is important for the long term success of repatriations as genetic bottlenecks, which are already apparent in our genetic analyses, could decrease survivorship. Therefore, a collaborative effort between agencies, zoos, aquariums, and conservation groups is imperative to preserve each genetic strain and enhance the few populations remaining.

### **Action Plan**

The biology of *R. muscosa* is not well understood. Research is needed to determine population demographics and trends, movement patterns, population genetics, how and where they overwinter, and their tolerances to stressors, which may make them susceptible to disease. In addition we need to investigate environmental factors that likely play a role in the decline of this species. For example, the remaining extant locations are all found in the headwaters of streams. Habitat downstream of the populations may be unsuitable due to factors such as water quality, periodic drying (as many of the streams are spring fed from above), recreational use, or the presence of non-native salmonids. One or more of these factors may be limiting the population sizes and extents at these locations, however additional research is needed to determine this. Baseline biological and environmental information is necessary to ensure that any conservation attempts such as population stabilizations or enhancements have the best chance at success. Captive breeding and translocations would then be the logical next step to stabilize and enhance the remaining populations and prevent extirpation.

During the initial phase of this study, assurance populations need to be assembled to prevent the extinction of unique genetic strains of *R. muscosa*. According to preliminary microsatellite analysis, the four assurance populations representing the remaining genetic composition of *R. muscosa* in southern California include; two groups from the San Gabriel Mountains, one group from the San Bernardino Mountains, and one group from the San Jacinto Mountains. The progeny of these captive populations would be used in repatriation experiments to refine the reintroduction process for this species.

To identify appropriate repatriation sites, additional surveys within the historic footprint of this species need to be completed to evaluate and prioritize the habitat. Many sites will require some form of habitat enhancement. Both potential repatriations sites and sites with extant populations



of *R. muscosa* would benefit from habitat enhancement. The removal of aquatic invasive predators, controlling invasive riparian plants, and constructing fish barriers at strategic locations will improve the likelihood of long term persistence for the frog at these locations.

Due to the protective status of this species and the fact that there are few individuals remaining, collection of wild animals is complex and animals are generally unavailable. One source for the assurance populations could be to obtain animals through emergency salvage efforts. USGS is currently permitted to conduct this activity for this federally endangered frog under the FWS recovery permit TE-045994-6 and through California scientific collecting permits. In 2007 there will likely be opportunities to salvage desiccating tadpoles from wild populations due to a combination of sustaining the lowest recorded rainfall in southern California history and the biological need for this species to overwinter prior to metamorphosis. As the intermittent spring-fed streams this frog inhabits dry, tadpoles become trapped in isolated pools and either become prey for snakes or birds, or perish from desiccation. Because these animals would have perished due to natural processes, they are ideal candidates for removing from the wild without adversely affecting wild populations.

Several limiting factors identified for the persistence of wild populations involve complex ecological interactions and will require in-depth study. Two such factors include interactions between non-native salmonids and *R. muscosa*, and the presence and prevalence of chytrid fungus, an emerging infectious disease. To understand the relationship between *R. muscosa* and introduced salmonids, in-situ experimental fish removal has been implemented to determine if removal of downstream predatory fish will allow *R. muscosa* to expand in to adjacent waters. Continuation of this study is important to establish definitive results. Likewise, skin cells from individuals at each of the extant localities have been analyzed for the presence of chytrid fungus. Additional samples and analyses are required to determine the extent to which this emerging infectious disease is affecting populations and if it has had negative effects in the past.

Lastly, the development of an educational outreach program to supplement our research will give outside parties and the public the knowledge needed to make informed personal choices and have a better understanding of how and why recreational activities might be restricted in some areas. Having the support of the general public will greatly enhance our mission, help preserve the species, and make it easier to obtain our research goals.

### **Collaborations**

Through the process of working to understanding the mountain yellow-legged frog's status, strong partnerships have been developed. The southern California MYLF working group was formed and developed a USDA Forest Service document, Mountain Yellow-legged Frog Conservation Assessment and Strategy, to help guide management decisions. This group includes representatives from the Angeles and San Bernardino National Forests, USGS, USFWS, CDFG, and CRES. In addition to the working group, support for this species has been provided by University of California Berkeley, California State Parks, the Los Angeles Zoo, Agua Caliente Band of Cahuilla Indians, the Western Riverside County Multi Species Habitat Conservation Plan, and the Coachella Valley Association of Governments.

## Goals

### Goal 1. Biology of the species

- Long term monitoring
  - Where is the MYLF current extant
  - Population sizes
  - Sex ratios
- Movement patterns
- Genetic variability
  - Identify management units
- Identify over wintering sites
- Tolerance to environmental stress

### Goal 2. Environment

- Determination of suitable habitat
  - Water quality testing
  - Habitat evaluation
- Identify emergency situations
  - Desiccating tadpoles in dry years
  - Sudden population reductions or extirpations (Bear Gulch)
- Habitat enhancement
  - Fish barriers
  - Trout removal

### Goal 3. Population enhancement

- *In situ*
  - Translocation
  - Head starting
- *Ex situ*
  - Captive breeding
  - Translocation

### Goal 4. Ecological interactions

- Understanding relationship between MYLF and introduced salmonids
- Chytrid fungus assessment

### Goal 5. Social

- Develop outreach and educational programs

## Budget

USGS has the permits and expertise to conduct the field work and data analysis portion of this project and will facilitate and interpret outsourced genetic analyses. We will provide our findings and expertise to our collaborators whom we will depend on to house and care for assurance populations in captivity. We will also rely largely on our partners to facilitate an outreach and education program and will provide expertise and data for our collaborators to do so.

To conduct the field work and data analysis for this project the equivalent of three full-time salaries will be required for each year that the project is funded. This will include two technicians at \$45,000 each and one project manager at \$65,000. Field localities are remote and will require overnight stay in most cases. Equipment and travel for field work will total \$5,000. The polymerase chain reaction (PCR) analyses for genetic interpretation and chytrid fungus testing will cost an additional \$25,000. Agency overhead (50.47%) on these figures equals \$78,228.50 bringing the total dollar amount to \$278,369.50 for the field surveys, data analyses, PCR analyses, and time spent working with our collaborators.

Educational outreach may include the production and dissemination of posters and pamphlets, as well as appropriate signage in appropriate areas. The cost for this outreach effort would probably be approximately \$10,000.

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## Species Action Plan for Panamanian Golden Frogs

### *Atelopus zeteki*/*Atelopus varius*

Prepared by Vicky Poole on behalf of the AZA Amphibian Taxon Advisory Group



### **BACKGROUND**

Golden frogs are a brilliantly-colored, unique Bufonid from the cloud forests of Panama. *Rana dorada*, or the “golden frog,” is culturally significant to the people of Panama. They are as revered as the bald eagle is in the US, with a history dating back to the Mayan civilization. Golden or pottery replications, called huacas (“wa-cas”) were symbols of good fortune and the frogs are still considered lucky to Panamanians. They are used to promote hotels and restaurants, and are such a part of the culture that they show up on their lottery tickets.

True Panamanian golden frogs, *Atelopus zeteki*, were recognized as a distinct species from the similar-looking harlequin frog, *Atelopus varius*, based on a unique skin toxin, zetekitoxin, and bioacoustical differences. In addition to vocalizing, PGFs communicate by semaphoring, a limb-waving phenomenon that is hypothesized to have arisen so that the frogs could locate conspecifics for breeding near the deafening sounds of waterfalls, where their gentle vocalizations are inaudible.

The Panamanian golden frogs, *A. zeteki*, and harlequin frog, *A. varius*, are both listed as Critically Endangered (CR) per the 2004 Global Amphibian Assessment (<http://www.globalamphibians.org/>). The reality is that both species will probably be extinct in the wild (EW) by the end of 2007 due to the pathogenic chytrid fungus.

Chytridiomycosis (*Batrachochytrium dendrobatidis*) was first observed in montane central Costa Rica, where it may have been the cause for extinction of the golden toad, *Bufo periglenes*. It has since become evident that this epizootic is advancing southeast through the cooler mid- to high-elevation mountain forests of Central America, decimating entire populations of amphibians, including *Atelopus senex*, and *A. chiriquensis*. As of 2007, the disease front is documented as far east as El Valle, Panama, the type locality of the Panamanian Golden Frog (PGF), *A. zeteki*.

Other factors impacting golden frogs include collecting for the local Panamanian zoos and hotels, as well as the illegal international pet trade [listings include CITES Appendix I status since 1975; and USFWS Endangered Species Act (ESA) - Endangered species since 1976], deforestation, and habitat alteration by logging and farming that also leads to sedimentation of their pristine waters. Although the local Panamanians were concerned about the future of golden

frogs in the wild, none of the facilities in Panama at that time were equipped or skilled to handle the challenge of a captive breeding effort.

### **CONSERVATION EFFORTS TO DATE**

Unlike many species that are currently imperiled, much effort has already been made to preserve golden frogs through different *ex situ* efforts and several different programs are currently in place. Some of these programs have become models applied to amphibian species globally.



In response to the impending chytrid crisis, a group of concerned biologists convened in 1998 and formed Project Golden Frog/Proyecto Rana Dorada (PGF/PRD), a conservation consortium involving numerous Panamanian and US institutions, that would become a model for other similar efforts worldwide. PGF/PRD's primary goal was to preserve the culturally-significant golden frog which would definitely be lost to the fungus (due to its locality and low-resistance akin to other Atelopine frogs) and use the attractive frog as a flagship species for amphibian decline issues in Panama and worldwide.

Specific initiatives of PGF/PRD included field studies, education, financial support, and captive management. PGF/PRD field studies have yielded natural history information, genetics research, and population monitoring, all of which has been beneficial to management to the captive golden frogs (Lindquist, et al., 2007; Zippel et al., 2007).

Public awareness and education were integrated into the project wherever possible. A bilingual website, [www.projectgoldenfrog.org](http://www.projectgoldenfrog.org), has been an easily accessible method for disseminating information about the species, project, and materials such as the husbandry manual. An AZA group list serve was also established and is moderated by a member of PGF/PRD for quick communications to the entire group and animal keepers. Training opportunities were created for US and Panamanian students and AZA zoo/aquarium staff in field techniques. Classroom curricula were developed and a golden frog conservation workshop was offered to school teachers in Panama in 2003 by members of PGF/PRD to educate school-age children. Graphics were developed and installed at two zoos in Panama, while chytrid warning flyers explaining how to clean field gear and equipment were created and posted in areas where the fungus was found to help minimize the spread. Even preserved frogs from the captive colony were distributed to over a dozen museum collections in the US so researchers will have access to specimens once the species no longer exists in the wild.

Although many AZA institutions and universities in the US and Panama underwrote most of the personnel expenses for PGF/PRD, over 20 individual grants were obtained by members of

PGF/PRD to fund specific field and educational initiatives, including obtaining a designated field vehicle (which was painted with the recognizable color and pattern of the golden frog). Additional funds have been raised over the past few years via t-shirt and plush animal sales for funding vehicle maintenance and acquiring equipment for other golden frog related initiatives. Currently, with the decline of golden frogs in Panama, PGF/PRD is beginning to evolve into a funding program utilizing the surplus from which will offer grants to other atelopine frog initiatives under the umbrella of the Atelopus Conservation Trust (ACT).

With the potential for the wild populations to be eliminated, an *ex situ* population of golden frogs were established in zoos and aquariums in the US. Since 2001, 20 pairs of adult and over 70 juvenile golden frogs have been collected under permits from Autoridad Nacional del Ambiente de Panamá (ANAM) and imported under two USFWS permits issued to the Maryland Zoo in Baltimore (formerly the Baltimore Zoo) and the Cleveland Metroparks Zoo. As a result of breeding at several AZA institutions, there are now more than 2,000 captive bred Panamanian golden frogs at almost 50 AZA institutions in the U.S. and Canada (*A. zeteki* are only available to AZA institutions per the restrictions of their USFWS permit). Breeding recommendations and specimen placement for both species is coordinated by the Population Manager at the Maryland Zoo in Baltimore and a regional Studbook is maintained for 3 distinct populations of golden frogs. The Second Edition of the Panamanian Golden Frog Husbandry Manual including breeding techniques, common medical issues, and environmental parameter data from golden frog habitat was completed in 2006, as well as a brief PowerPoint presentation of golden frog exhibits and holding facilities at AZA institutions (circa 2006) for exhibitry planning purposes.

As predicted in the late 1990s, chytrid fungus has reached the last unaffected areas of the golden frogs' range. With the captive husbandry techniques of golden frogs worked out in the US, it became clear that there should be a facility in Panama that replicated the PGF/PRD efforts for golden frogs, as well as building *in situ* assurance colonies to protect other critical endemic amphibians currently being impacted by chytrid. The Houston Zoo committed to building this much needed facility and serve as a center for rescue, quarantine, treatment, and public education. After a year of design and species prioritization, construction was completed on the 2,400 square-foot El Valle Amphibian Conservation Center (EVACC) in 2007, situated on the grounds of the small, private El Nispero Zoo in the village of El Valle de Anton, the type locality of the Panamanian golden frog. This \$125,000 facility (current value as of June 2007) with an annual operating budget of \$58,000 clearly illustrates the level of support that this amphibian crisis will require globally.





Beginning in the summer of 2006, zoo and aquarium personnel and volunteers came together in El Valle to collect specimens of 13 species that are the highest conservation priorities based on criteria that evaluated known distribution and population sizes, the vulnerability of wild populations to the advancing fungus, and any cultural or political importance. These species include the following:

- **Panamanian golden frog (*A. zeteki*)**
- lemur leaf frog (*Phyllomedusa lemur*)
- Tabasara robber frog (*Craugastor tabasarae*)
- Panamanian robber frog (*C. museosus*)
- Bob's robber frog (*C. punctariolus*)
- rusty robber frog (*C. bufoniformis*)
- crowned treefrog (*Anothea spinosa*)
- Heredia treefrog (*Hyla fimbriembra*)
- La Loma treefrog (*H. colymba*)
- Palmer's treefrog (*H. palmeri*)
- casque-headed treefrog (*Hemiphractus fasciatus*)
- horned marsupial frog (*Gastrotheca cornuta*)
- Panama poison frog (*Dendrobates vicentei*)

All collected specimens for EVACC are tested and treated for chytrid with the anti-fungal drug Itraconazole following the rapid-response collection model developed in Panama by the Atlanta-based Amphibian Recovery and Conservation Coalition (ARCC) in 2005.

These breeding programs will be undertaken to ensure the viability of captive populations and in preparation for reintroduction efforts, should that become feasible. The golden frogs at EVACC will also be included in the regional studbook along with the US specimens and maintained by the Population Manager so that genetic diversity and can be maximized throughout the entire *ex situ* population.

Additional potential roles that this facility may provide once fully established would be as a center for training husbandry personnel within Latin America and as a center for amphibian research. These services are much needed as the response to the amphibian crisis expands and sharing experiences becomes necessary to achieve common goals.

## **ACTION PLAN**

Although much conservation, research, and education work has already been completed on Panamanian golden frogs and the harlequin frog, there is still more work to perform for the long-term survival of these species as well as much that can be learned from them and then applied to other *Atelopus* sp. and amphibians in general.

### **Captive Management**

Continue with the management of the two existing *ex situ* programs, including coordinating information and specimen exchange as needed to maximize survival and long term genetic management. A golden frog Studbook encompassing *A. zeteki* and *A. varius* in both *ex situ* programs should be published by the Population Manager, Meredith Whitney (Maryland Zoo in Baltimore), by October 2007.

If an AZA institution is interested in obtaining a population of golden frogs for exhibit, it is recommended do the following:

1. Download and read the husbandry manual from [www.projectgoldenfrog.org](http://www.projectgoldenfrog.org) website.
2. Contact Meredith Whitney ([mwhitney@marylandzoo.org](mailto:mwhitney@marylandzoo.org)) for specimen assignment and permit paperwork.
3. Contact Vicky Poole ([vpooles@aqua.org](mailto:vpooles@aqua.org)) for a copy of the PGF Exhibits (circa 2006) PowerPoint program for exhibit examples, if desired.

Although the Houston Zoo and several generous supporting institutions such as the Cleveland Zoo and the Buffalo Zoo have made a large commitment to EVACC, they could still use funds to cover the salary for in-country staff for future years, donated laboratory equipment and supplies to outfit and complete the facility, and/or financial support for research projects. Institutions may also be able to offer career development opportunities for their own personnel by sending staff to help work at the center for a week or two or participate in applied research projects. Contact Paul Crump at the Houston Zoo ([pcrump@houstonzoo.org](mailto:pcrump@houstonzoo.org)) for more information.

There is still the need for an additional in-country captive facility within Panama, however efforts to establish a permanent relationships at local zoos and national parks have been difficult due to personnel stability and the influence of political whims within Panama. Beyond EVACC, there are no amphibian experienced facilities that could minimally be enhanced, so an additional effort on the scale of EVACC would have to be pursued. The Summit Zoo just 25 minutes from Panama City has been identified as the site with the best potential and a supporting institution would have to be identified, which Houston Zoo is considering adding a cargo-container facility there, which may be a less-costly option.

### Research Initiatives

Funding opportunities exist for many of these initiatives. If interested in assisting with financial support for any of the projects outlined below, please contact the author for more information.

Unless stated, costs were estimated by the author.

- ***In situ* population monitoring** COST - \$10,000

Periodic assessment of the wild population through the efforts of the staff at Houston Zoo/EVACC and other researchers will continue (recommended every six months At two localities for the next three years). Skin testing for chytrid should continue as well and EVACC plans to be able to run PCR testing for chytrid once they acquire their portable unit in 2007.

A separate full-scale presence/absence survey would be recommended before any repatriation program begins (long-term goal) and is not included as part of this.

Funding needs to be identified.

- **Chytridiomycosis resistance studies**

Introduction of *B. dendrobatidis* into new areas causes mass mortality when the pathogen is novel to the environment (Lips et al., 2006). Although not all species of amphibians are susceptible to the chytrid, golden frogs in both captive collections and the wild have succumbed to chytrid without the recognized Itraconazole treatment.

Since removal of *B. dendrobatidis* from an ecosystem is not realistically possible, attempts at understanding and potentially imposing breeding selection on resistant specimens may be the only way to have fit specimens should a repatriation attempt be

made for golden frogs (Woodhams, et al., 2006). These conservation technologies, if effective, would have the potential to improve amphibian release programs internationally.

Proposed studies include the following:

**Antimicrobial Peptide (AMP) study** COST - \$6,000  
(estimated by P. Dennis, DVM, PhD, Cleveland Metroparks Zoo)

**AMP repeatability study** COST - \$6,000  
(estimated by P. Dennis, DVM, PhD, Cleveland Metroparks Zoo)

Testing the chytrid resistant peptide production over time in individuals.

**AMP controlled breeding heritability study** COST - \$6,000  
(estimated by P. Dennis, DVM, PhD, Cleveland Metroparks Zoo)

To further test the genetic link of the chytrid resistant peptide production and to see if resistant strains of golden frogs can be produced that would be beneficial for repatriation programs.

o **Tadpole diet study** COST - \$1,000  
(estimated by P. Crump, Houston Zoo)

In addition to identifying the species of diatoms that larval golden frogs are consuming, diet selectivity and stream site characteristics in relationship to tadpole density and diatom abundance will also be compared. If golden frog tadpoles are unable to be located in the wild, *A. limosus* from farther east may be used as a model. The application of the information from this study will be used to better understand our knowledge of the Panamanian golden frog in the field, as well as a direct benefit to the captive program.

It is anticipated that a Panamanian undergraduate student at the University of Panama/Smithsonian Tropical Research Institute will undertake the study with the field assistance of Houston Zoo and AZA staff and a specialist at the University of Panama for diatom identification. Once funded and the student is identified, the project should take less than a year to complete.

o **Karyotype study** COST - \$6,200  
(estimated by P. Crump, Houston Zoo)

To compare the karyotype C banding pattern of 4 of the six species described in Panama (*A. certus*, *A. limosus*, *A. varius*, and *A. zeteki*), and an undescribed species found in San Blas (in a locality near Puerto Obaldía). The other two Panamanian species will not be included in this study. The access to their distribution range of one (*A. glyphus*) is difficult and/or expensive, and the other is has disappeared (*A. chiriquensis*).

o **Microsatellite marker study** COST - \$15,000  
(estimated by Corrienne L. Richards, PhD Candidate, University of Michigan)

The development of a set of microsatellite markers for genetic analysis would provide parentage and inbreeding analyses. This data would be useful when re-releasing frogs into the wild, as well as managing the captive population, however is a low priority. Previous attempts at this have been unsuccessful, however there are companies that

guarantee marker development, or your money back. Facilities and funding would need to be identified.

- ***In vitro* fertilization (IVF) study** COST - \$5,000  
(estimated by Mike Cranfield, DVM, Maryland Zoo in Baltimore)

As an attempt to salvage and artificially fertilize eggs from dead or dying female golden frogs. This technique has practical conservation applications for other *in situ* amphibian programs as well as to species impacted by chytrid

- **Sperm collection and cryopreservation studies** COST - \$5,000  
(estimated by Katharine M. Pelican, DVM, PhD, Smithsonian's National Zoological Park)

To develop a viable sperm collection and freezing technique for the Panamanian Golden Frog and as a model for other endangered *Atelopus* species.

### Educational Initiatives

Funding opportunities exist for many of these initiatives. If interested in assisting with financial support for any of the programs outlined below, please contact the author for more information. Unless stated, costs were estimated by the author.

- **Website update** COST - \$1,000

The [www.projectgoldenfrog.org](http://www.projectgoldenfrog.org) website, originally created in 2000, has been the primary method for information on the plight of the golden frog. Despite small upgrades over the years, is in need of a complete redesign with updated information. The following materials would also be added to the website:

- Classroom activity materials in Spanish developed by Tracy Stetzinger (Sea World Orlando) – downloadable;
- PowerPoint presentation of Panamanian Golden Frog Exhibits – viewable;
- An *A. zeteki* frog call – audible,
- Information on the EVACC and ARCC efforts in Panama (or at least links).

- **Teacher Workshop and classroom activity materials** COST - \$6,000



The Rana Dorada Workshop offered at the 2003 Panama national educator's annual conference provided primary schoolteachers with information and classroom activities that would help promote the conservation of this unique amphibian. An bi-annual repeat of this program would keep the information current in the curricula, and potentially have more impact on the Panamanian conservationists of tomorrow. If pursued, it would be wise to first contact participants from the 2003 workshop and assess their implementation of the information and materials.

Materials exist and could be distributed freely without instruction via the website (see **Website update** above), however for the workshop, an in-country coordinator,

instructors, and funding would have to be identified. Additional materials from the AZA *Year of the Frog* campaign could also be distributed.

- **Golden frog graphics**

**COST - \$1,500**

Updating exhibit graphic panels provided to zoos and parks in Panama. By utilizing the golden frog as the highlighted culturally-significant species, information of the decline in amphibian population due to the chytrid fungus can also be provided. Facilities and funding would need to be identified.

- **Chytrid warning flyers**

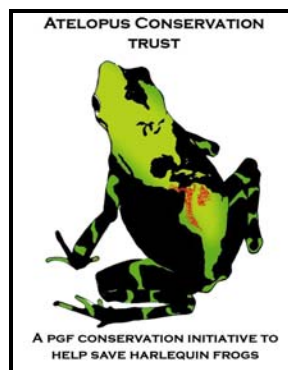
**COST - \$500**



Printing and posting additional bi-lingual warning flyers on waterproof paper warning of the presence of chytrid in the immediate area along with disinfection methods for equipment and field gear could potentially help reduce the spread of the pathogen between sites. Current flyers exist made by PGF/PRD and ANAM, but additional re-printing and distribution costs could be funded (not a huge priority).

### **Atelopus Conservation Trust**

Due to the spread of the chytrid fungus, Panamanian golden frogs (*A. zeteki*) are soon going to be extinct in the wild. Project Golden Frog is beginning to evolve into an umbrella organization called the Atelopus Conservation Trust (PGF/ACT) to continue to support the research and conservation of these and other harlequin frogs. The Panamanian golden frog can continue to be significant as a flagship species since what has happened to them, will happen to other *Atelopus*.



Their first initiative, the PGF/ACT Grant, newly established in 2007, supports conservation programs that seek to increase our basis of knowledge or continue to protect the endangered Panamanian golden frog and other *Atelopus* species throughout their range and in captivity. Field studies and other projects that demonstrate a multidisciplinary approach to *Atelopus zeteki*

receive highest funding priority, although projects involving *ex situ* conservation of other *Atelopus* species will be considered. Research and projects that involve range-country collaborators are encouraged. Environmental education programs, development of techniques that can be used in a natural environment and captive propagation programs that stress an integrative approach to conservation are also appropriate. Projects must directly affect biological conservation. Application deadline will be July 1 annually.

### Fundraising

Although a majority of the necessary field work and golden frog research projects that have been funded by grants and individual institutional commitment for PGF/PRD historically, fundraising efforts to support *Atelopus* projects and golden frog research will continue for the PGF/ACT granting program. Current fundraising opportunities for T-shirt, hat, or plush golden frog sales exist on the PGF website and for institution's gift shops. Contact Tom Weaver at the Denver Zoo ([tweaver@denverzoo.org](mailto:tweaver@denverzoo.org)) or the author for more information.

Plush golden frog and bilingual hang tag text developed by the Cleveland Metroparks Zoo:



| GLOBAL AMPHIBIAN CRISIS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| Did you know that nearly one third of the world's amphibians are threatened with extinction right now? Amphibians are disappearing due to habitat destruction, pollution, ultraviolet radiation, illegal collection for the pet trade, and a rapidly spreading disease called chytrid. Amphibians are living indicators of the planet's health. Their disappearance tells us that there is something going wrong in the ecosystems where they live. The Panamanian golden frog ( <i>Atelopus zeteki</i> ) is one of those amphibian species in real danger of extinction. For centuries, people in Panama have seen the golden frog as a symbol of wealth and good luck. Today, zoos are working together with partners in Panama to help golden frogs survive. You can help give luck back to the golden frog and other amphibians in trouble by adopting this golden frog and supporting amphibian conservation efforts like Project Golden Frog ( <a href="http://www.projectgoldenfrog.org">www.projectgoldenfrog.org</a> ). For more information about amphibian declines and how you can help, see <a href="http://www.globalamphibians.org">www.globalamphibians.org</a> and visit your local AZA accredited zoo or aquarium ( <a href="http://www.aza.org">www.aza.org</a> ). |  |

### Repatriation

The intended, yet *long-term goal* of establishing an *ex situ* population of golden frogs, has been the return of golden frogs to the wild should all *in situ* populations become extinct and the chytrid fungus is no longer a threat to survival.

PGF/PRD has always recommended that tadpoles be reintroduced into the national parks, but only if independent research concludes that the chytrid fungus is no longer a danger. The rationale for releasing tadpoles versus adults is that captive reared adults presumably will have difficulty in properly homing after reintroductions.

This goal is an estimated 5-10 years off, and with the construction of EVACC, there is now a potential site for staging reintroductions prior to release, and/or establishing in-country breeding pairs from which offspring can be utilized. The development of chytrid-resistant strains of golden frogs would become quite valuable for this effort. All animals would be released following strict health guidelines. And finally, a population monitoring program would also have to be in place to assess survivorship of released frogs.

A formal plan as well as funding for these efforts would still have to be developed.

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## **RECOMMENDATIONS**

Order listed does not reflect relevance.

### **AP GOAL 1 \$125,000 (plus \$50,000 annual operating budget)**

Solicit Summit Zoo as an addition in-country ex situ facility.

*Identify an institution to construct, manage the facility, and secure funding including operating budget.*

### **AP GOAL 2 \$18,000**

Encourage funding for resistance studies and development of a chytrid-resistant strain of golden frogs (if possible).

*In process.*

### **AP GOAL 3 \$1,000**

Update and expand PGF/PRD website.

*Solicit content writers.*

### **AP GOAL 4 \$6,000**

Repeat the Rana Dorada teacher workshop and redistribute educational materials within Panama.

*Re-establish relationship with Teacher's conference.*

*Identify instructors and funding.*

### **AP GOAL 5 \$1,500**

Fabricate and distribute new graphic panels to zoos and parks in Panama.

*Update text and identify interested appropriate facilities and funding.*

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| <b>AP GOAL 6</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <b><u>\$10,000</u></b>           |
| Continue with periodic field monitoring of known <i>A. zeteki</i> and <i>A. varius</i> sites.<br><i>Design program, and identify participants and funding source.</i>                                                                                                                                                                                                                                                                                                                                                         |                                  |
| <b>AP GOAL 7</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <b><u>\$21,200</u></b>           |
| Complete outstanding publications on the genetic work of golden <i>Atelopus</i> in Panama and perform the karyotyping study and pursue the microsatellite marker study.<br><i>Identify facilities and funding for the microsatellite marker study.</i>                                                                                                                                                                                                                                                                        |                                  |
| <b>AP GOAL 8</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <b><u>\$11,000</u></b>           |
| Pursue other miscellaneous research projects, including tadpole diet, <i>in vitro</i> fertilization, sperm collection, and cryopreservation studies.<br><i>Identify funding sources.</i>                                                                                                                                                                                                                                                                                                                                      |                                  |
| <b>AP GOAL 9</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <b><u>\$50,000</u> annually</b>  |
| Assist Houston Zoo support annual operating costs of EVACC – through direct or in-kind donations and/or staff time assisting in Panama.                                                                                                                                                                                                                                                                                                                                                                                       |                                  |
| <b>AP GOAL 10</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <b><u>\$To Be Determined</u></b> |
| Repatriation efforts (LONG TERM – 5 to 10 years): <ul style="list-style-type: none"> <li>• Perform a complete presence/absence survey prior to any repatriation.</li> <li>• Return specimens to Panama, staged through breeding efforts at EVACC prior to release of tadpoles, for genetic diversity.</li> <li>• Establish a population monitoring program for repatriated localities.</li> </ul> <i>Develop formal plan and identify funding before implementing except for any necessary genetic exchange of specimens.</i> |                                  |
| <b>AP GOAL 11</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <b><u>\$1,000</u></b>            |
| Translation of this document and all other related materials into Spanish and disseminated to authorities in Panama.<br><i>Identify translator and funding source.</i>                                                                                                                                                                                                                                                                                                                                                        |                                  |



## **Action Plan for the Conservation of the Puerto Rican Crested Toad (*Peltophryne lemur*)**

Prepared by Diane Barber on behalf of the Amphibian Taxon Advisory Group

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### **Background**

The Puerto Rican crested toad, easily distinguished by its distinctive head crest, is the only toad native to Puerto Rico. This toad is biologically significant because of its unique morphological features and its 44-60 million year old lineage, which is much older than other groups of New World toads. Little is known about the ecology of this fossorial amphibian. They are rarely seen throughout the hot, dry months of the year and spend the majority of their lives underground in the moist caverns of karst limestone that defines their habitat. Because of their cryptic lifestyle, inaccessible natural environment, and a lack of adequate monitoring technology, it is nearly impossible to assess population dynamics, except during breeding events triggered by heavy rains.

### **Conservation Status**

Habitat loss, pesticides and competition from introduced species, including the marine toad, (*Rhinella (Bufo) marinus*) are major causes for the toad's decline and have led to a listing as threatened by the United States Fish and Wildlife Service (USFWS) and critically endangered by the IUCN. Two distinct populations of toads once existed in Puerto Rico- one in the north and one in the south. Mitochondrial DNA analyses suggest that these two populations have been separated for up to one million years and

are genetically distinct. Unfortunately, northern toads have not been seen in the wild since 1988. Although a small number of northern toads remain in captivity, biologists consider this population extirpated in the wild. Currently, the only known wild population is the southern form, which resides in Guanica National Forest. When the toads were rediscovered in Tamarindo 25 years ago, the population was estimated at 3,000 adults. Since then, population estimates fluctuated from 200 and 500 adults. Surprisingly, an unprecedented breeding event occurred in October 2005, when over 2,000 toads appeared at the pond to reproduce. Obviously, there are still many questions that need to be answered through research to unfold this unique species ecology and obtain a true grasp of population numbers. Recovery efforts to address threats are important given the small population, single breeding pond and increased potential for a single catastrophic event to cause the extinction of the toad.

### **Conservation Efforts**

The Puerto Rican Crested Toad SSP was the first amphibian SSP created and has been active for over twenty years. Strong partnerships have been formed for the recovery of this species between 21 zoos and aquariums (US, Canada, UK and Puerto Rico), USFWS, Puerto Rico Department of Natural and Ecological Resources (DNER), University of Puerto Rico, Puerto Rican National Park Company at Juan Rivero Zoo, Iniciativa Herpetologica, Inc. and Citizens of the Karst. Recovery priorities for this species are coordinated through the FWS Puerto Rican Crested Toad Recovery Plan, Population and Habitat Viability Analysis Working Group Report (2005). Input from recovery group members and other biologists is obtained via annual meetings held in Puerto Rico.

Creation of new ponds to support six self-sustaining metapopulations (three in the north and three in the south), expansion of ecological research, protection and restoration of existing habitat, and island-wide educational outreach are primary conservation goals. Recovery efforts are directed through a Memorandum of Understanding between the USFWS, Department of Natural and Ecological Resources (DNER) and the AZA.

A reintroduction program is a large component of the recovery plan for this species. Each year, captive toads from zoos and aquariums in the United States and Canada are bred, and tadpoles are sent to Puerto Rico for release. Reintroduction efforts began in 1982 before the SSP was formed, as a small-scaled collaborative effort between the Juan Rivero, Buffalo, and Toronto Zoos. Between 1987 and 2007, more than 100,000 tadpoles have been released into Guanica National Forest through these efforts. The Guanica release site in Manglillo, is an isolated man-made pond, geographically separated from the wild population in Tamarindo. Since 2003, random sightings of adult captive born toads have occurred at the release site. In 2003 and 2005, pairs of toads were observed at the constructed breeding ponds laying eggs. Unfortunately, the breeding ponds (4,000sf and 9,000sf) constructed to increase breeding habitat at this site require repair to stop infiltration by sea water. These sightings and breeding attempts are encouraging, as it is evidence of survivorship of released tadpoles and the possible establishment of a second population. As in Tamarindo, little to no monitoring

of tadpoles and adults has occurred at the release site. The terrain in these areas is difficult to survey. Complicating this situation, marking techniques for tadpoles and technology to efficiently track toads through a labyrinth of subterranean limestone caverns has yet to be developed. However, it is important that recovery group members develop techniques needed to evaluate the success or failures of reintroduction efforts.

Restoration of existing habitat and creation of new ponds has been a continuous process. In 1998, a translocation pond was built in Guanica to serve as an emergency refuge for tadpoles in the event of a large-scale disaster or premature water loss at the last remaining natural breeding pond. In 2006, a release pond was constructed in Gabia, securing a third site in the south for crested toads. In order to achieve three protected areas in the north, three small ponds were built in Arecibo in 2005 on private property (El Tallonal) and northern toad tadpoles were released there in 2006 and 2007. Efforts to secure property in Quebradillas with an existing pond are underway and biologists hope to build a third pond in Cambalache National Forest.

## **Action Plan**

### **Captive Management**

Due to the increased number of release sites in Puerto Rico, and small captive population of northern toads, more institutions are needed to bolster the captive population and release offspring.

If an AZA institution is interested in participating in the PRCT SSP:

1. Institutions must be able to adequately house at least four pair of adult toads, follow breeding, transfer and release recommendations, and temporarily rear and hold offspring as needed.
2. Crested toads are to be kept isolated from all other amphibian species in a quarantine situation and institutions are expected to follow pre-release protocol and screening measures.
3. All participating institutions must be included on the existing endangered species permit and follow all federal laws regarding possession and handling of an endangered species.

For further information about program participation, or a copy of the husbandry manual, contact Diane Barber ([dbarber@fortworthzoo.org](mailto:dbarber@fortworthzoo.org)).

**Funding opportunities exist for the following initiatives. If interested in assisting with financial support for any of the projects outlined below, please contact the author for more information.**

### **Research Initiatives**

Primary research projects include: intensive monitoring of all ponds during and after the breeding season, with a focus on habitat utilization by tadpoles and newly

metamorphosed toadlets, predation and competition from other species, diet studies of wild and captive tadpoles and toads, hydrological assessments of pond sites, continued amphibian call monitoring in the north (with emphasis on Quebradillas), and a diet and health assessment study to characterize health threats prevalent in *R. marinus* and *Lithobates (Rana) catesbeiana* that are cohabitating with *P. lemur*.

1. Tadpole and Toadlet Monitoring at Release Sites: \$5,000- \$10,000

*Currently there are three release sites that need to be monitored on a regular basis (preliminary monitoring has begun at El Tallonal and Gabia sites). Although these sites are basically similar, each area and pond is different, creating many hypotheses that need to be researched in order to determine the properties of a successful release and breeding site. Monitoring protocols also need to be developed and standardized for all areas.*

2. Marking and Tracking Adult Toads in Tamarindo and Manglillo: \$30,000

*In order to assess population dynamics and learn more about the ecology and habitat usage of adult toads, it is necessary to start marking toads with ID transponder chips and to conduct telemetry. Costs include graduate student stipends and equipment.*

3. Auditory Monitoring of Historic and Current Toad Habitat: \$30,000

*This project is in progress. Monitoring historic toad habitat in the north through the use of frog call loggers. Graduate student stipends and equipment are needed to complete study in the north and to begin work in southern locations.*

4. Health Assessment Study: \$5,000- \$10,000

*This project is in progress. Additional samples need to be obtained from all existing toad sites at various times of the year. Funds for continued collection and pathology are needed. Research needs to carry over to the captive population for improvement of general husbandry protocols and development of standardized pre-release screening measures for tadpoles.*

5. Tadpole Diet Study: \$10,000

*This project is in progress. More samples need to be collected during breeding events and at release sites. Research needs to carry over to the captive population for improvement of captive diets and preventative health.*

## **Habitat Initiatives**

The last remaining breeding site for the toads consists of a small complex of three ponds: Tamarindo, Atolladora and Aroma. Tamarindo encompasses most of the area and supports the majority of egg laying females. During heavy rain events, females will sometimes lay eggs in the small adjacent Atolladora and Aroma ponds, but toads are rarely found in the area for unknown reasons. Tamarindo has been losing water in recent years, which is concerning and prompted the hydrology studies. Understanding the hydrology and geology of ponds is crucial to habitat preservation. Construction of breeding ponds is essential at reintroduction sites. Release ponds are small and are

only filled with water to support transferred tadpoles until they metamorphose, which is important to stop mass predation from dragon fly larvae.

1. Hydrology Study: \$20,000

*This study is almost complete in Tamarindo, but needs to continue at Atolladora, Aroma and all current reintroduction sites. In addition, permanent water monitoring stations need to be installed at all breeding ponds.*

2. Toxicology Study: \$10,000

*During the dry season, half of the Tamarindo pond is used as a parking lot for beach goers. The parking lot is closed during the rain season to reduce the risk of chemical contamination of the breeding pond and damage to habitat from vehicles. However, research is needed to determine if the soils in the parking lot are contaminated. If they are, land use policies need to be changed and the soils restored.*

3. Breeding Pond Construction: \$15,000

*A breeding pond needs to be constructed near the release pond at Gabia to aid in habitat restoration and establishment of a third population in the south.*

4. Breeding Pond Repair: \$25,000

*The two breeding ponds (4,000sf and 9,000sf) at Manglillo have had salt water infiltration (most likely from damaged liners). It is important for us to restore the ponds in this area in order to support the released population. Captive born toads have returned to the ponds to breed the past several years and the water is too saline to support life. It is crucial that we repair at least the small pond before the next breeding season. Policy and enforcement needs to be in place to keep all-terrain vehicles off of the largest pond during the dry season and to keep the public out of the area during the breeding season.*

## **Educational Initiatives**

Educational materials and programs are needed to increase awareness and encourage community involvement in crested toad conservation efforts. The survival of the Puerto Rican crested toad on public and private lands in Puerto Rico depends upon the attitudes of the people living there. Posters, activity guides, models, decals, baseballs, and mascots serve as outreach materials to help citizens recognize the crested toad and distinguish it from the marine toad. Proposed teacher workshops will enhance education and conservation efforts by teaching basic crested toad ecology and conservation biology to teachers. By introducing these theories and techniques into the curriculum in community schools, an important education foundation for the next generation of decision-makers will be put in place. By educating teachers, as well as children who visit the Juan Rivero Zoo, USFWS, and DNER, the program gains leverage and can make conservation a fundamental part of the curriculum for all Puerto Rican children and their parents. The Puerto Rican Crested Toad SSP web site will be a great tool for disseminating information.

1. Curriculum Packets: \$1,000

Crested toad teacher curriculums have been developed by Cindi Collins, Fort Worth Zoo ([ccollins@forworthzoo.org](mailto:ccollins@forworthzoo.org)) for grade levels K-2, 3-5 and 6-8. Included with the curriculums are information sheets about the biology of the crested toad, marine toad, general amphibian biology, vocabulary, and a review of conservation efforts for the crested toad. The curriculum will be available to download on the website ([www.crestedtoadssp.org](http://www.crestedtoadssp.org)). The curriculum packets will be distributed to Puerto Rican teachers initially through workshops held by the Juan Rivero Zoo.

## 2. Decals and Baseballs: \$5,000

*In an effort to increase island-wide awareness, decals and baseballs adorned with a cartoon caricature of the toad were produced by the SSP and are distributed by FWS, DNER and Juan Rivero Zoo staff during tours and presentations to visiting students and families. These items are also given to graduate students working in the field so that they can be presented to local land owners and used to talk about the toad and its conservation.*

## 3. Toad Models: \$2,000

*There is a need to produce new life-sized models of the crested toad in various life stages that are accurately colored. The models will serve as a visual aid to share with Puerto Rican schools and DNER/FWS biologists to use when speaking with private land owners to explain the morphological difference between a crested toad and marine toad. Many islanders think they have seen a crested toad and therefore do not believe it is rare, when in actuality they have encountered the common marine toad.*

## 3. Activity Guide and Posters: \$10,000

*The Puerto Rican Crested Toad Activity Guide, created by Toronto Zoo ([bjohnson@torontozoo.ca](mailto:bjohnson@torontozoo.ca)), has been updated and is ready for its second printing. This is an educational guide that also doubles as a coloring book, telling the story of the toad and efforts for its conservation. The guide is in Spanish and English. It is distributed to teachers and children through the FWS, DNER and Juan River Zoo staff. There are two posters in production. One is an identification poster, which is in Spanish and describes habitat and the difference between the crested toad and the marine toad. The primary function of this poster is to use as an identification tool to share with private land owners and teachers. The back of the poster is educational and informs readers about the crested toad, its habitat and its conservation. The poster also identifies resources teachers or children can use to learn more about amphibians and their conservation. The second poster is of a young lady kissing the "Prince of Puerto Rico" (a crested toad) and was developed as a marketing tool for older audiences in public areas such as shopping malls, universities, restaurants and cantinas. It is simplistic and serves to catch the eye of the viewer and entice them to learn more about the toad by visiting the SSP website.*

## 4. Costume Mascot: \$4,000 (2 costumes)

*A popular toad mascot costume was created by the Toronto Zoo and sent to Puerto Rico for use in outreach programs. The FWS, DNER and Juan Rivero Zoo have expressed that this is an extremely valuable outreach tool. However, there is a need to*



*modify the costume design to allow for cooler air exchange and to add crested toad vocalizations so that the children can hear the toad “sing.” There is also a need to send two more costumes to Puerto Rico, so the current one doesn’t have to be shared, as it is logistically difficult to move it around the island.*

5. Website: \$500 per year

*The Puerto Rican Crested Toad Website ([www.crestedtoadssp.org](http://www.crestedtoadssp.org)) is being developed and will include: information on the ecology of the toad and what is being done to study and save it; photos and identification information to distinguish the crested toad from the marine toad; research information and suggestions to encourage graduate students to focus on the toad; a “Biomaterial Resources” link to list available preserved specimens and samples to share among researchers; downloadable resources in English and Spanish for teachers including the activity guide and curriculum; and a “Local Heroes” section to spotlight people in the local community. Start-up costs have been obtained, but monthly update, domain name and hosting fees need to be supported on a yearly basis.*

## **Fundraising**

Currently funds for the SSP are obtained through donations, T-shirt sales and grants. As with other programs, it is difficult to acquire funding needed to support recovery goals. Institutions are asked to consider supporting the conservation of the crested toad through financial gifts to the SSP on a yearly basis, or by funding all, or part of an action item. Another opportunity to support the SSP is by selling crested toad T-shirts in zoo gift shops. General donations are also welcome! Donations of \$10 or more will receive a crested toad baseball. Please join us by committing to support the conservation of a critically endangered toad today!

## **References and Acknowledgements:**

The FWS Recovery Plan, IUCN/CBSG Crested Toad PHVA Briefing Book and Final Report, The Amphibian Tree of Life (Frost, et al.) and unpublished breeding data from Miguel Canals, DNER, were used as references for this document. The Amphibian Taxon Advisory Group Steering Committee Members were kind enough to provide comment and I’d like to thank Gail Ross, Amazing Graduate Student, UPR for the use of her photo and all of the PRCT Recovery Team Members for their dedication and hard work for this incredible toad and the unique habitat in which it lives.

## **Action Plan for the Conservation of the Wyoming toad (*Bufo Baxteri*)**

Prepared by Bruce Foster on behalf of the Amphibian Taxon Advisory Group  
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### **Background**

The Wyoming toad (*Bufo baxteri*) is found only in Albany County, Wyoming. The toad was first reported by George T. Baxter, a graduate student in the Department of Zoology and Physiology at the University of Wyoming, in his M.S. thesis (Baxter 1946). Subsequently, as a member of the faculty, Dr. Baxter observed toads and breeding sites for more than 30 years. Baxter reports that the toad was common within the floodplains and wetlands associated with fresh water ponds and irrigated pastures of Albany County from the 1950s until the early 1970s.

Known historic distribution of the Wyoming toad was restricted to within approximately 50 kilometers of Laramie. The population of toads declined dramatically in the 1970s, and by the 1980s, individuals were extremely rare (Baxter and Stromberg 1980, Stromberg 1981, Vankirk 1980, Baxter et al. 1982, Baxter and Stone 1985, Lewis et al. 1985). In January 1984, the Wyoming toad was listed as endangered by the U.S. Fish and Wildlife Service (Service; 49 F.R. 1991), with a recovery priority of 1 (high degree of threat and high recovery potential). However, in 1985, the Wyoming toad was presumed extinct until 1987, when a population was discovered at Mortenson Lake (Lewis et al. 1987). During 1987 and 1988, surveys at the lake revealed an apparently healthy and viable population with many age classes. The Nature Conservancy purchased this habitat in January 1991, as part of a 751 hectare tract including Mortenson, Garber, and Soda Lakes, but not Meeboer Lake (Garber and Patten, 1991). In 1993, this area was purchased by the Service and subsequently incorporated into the Service's National Wildlife Refuge System as Mortenson Lake National Wildlife Refuge.





Mortenson Lake National Wildlife Refuge (Figure 1) was established in May 1993 to protect the last known breeding population of the Wyoming toad and serve as a reintroduction site. The Refuge encompasses 719 hectares of the Laramie Plains and is located approximately 24 kilometers southwest of Laramie, Wyoming in Albany County.

Wyoming toads from the captive breeding program have been released at

Lake George within the boundaries of the Hutton Lake National Wildlife Refuge. This refuge is located 19.3 kilometers southwest of Laramie and was established in 1932 as a resting and breeding ground for migratory birds and indigenous wildlife. The area encompasses 796 hectares consisting of 570 hectares of greasewood-dominated uplands and 227 hectares of open water and marsh. The refuge contains five small lakes (Hutton, Creighton, George, Rush and Hoge) developed from seepage that depends on water provided primarily from an irrigation ditch.

In September 1987, a recovery group was formed consisting of representatives from the Service, WGFD, University of Wyoming, and The Nature Conservancy. This group coordinated protection, research, and recovery efforts. In 2001, this informal group was replaced by a Wyoming Toad Recovery Team, which was officially appointed by the Service. This team is composed of representatives from WGFD, University of Wyoming, the American Zoo and Aquarium Association (AZA), Laramie Rivers Conservation District, Wyoming Natural Diversity Database, landowner/rancher, and the Service.

In December 1996, AZA approved a Species Survival Plan (SSP) for the Wyoming toad. The SSP is a coordinated effort to save an endangered species through captive breeding, public education, habitat preservation, supportive research and, in some cases, the release of captive-bred animals into the wild. Under the direction of a species coordinator and management group, the SSP recommends how to manage the captive population to maintain as much genetic diversity as possible. Breeding recommendations are made based on genetic and demographic needs, captive holding capacity, and release potential. A studbook keeper who tracks all births, deaths, breedings, and transfers maintains the genealogy of the population. Using this information and specialized computer programs, the management group is able to develop a scientifically sound breeding program. As a member of the Wyoming toad Recovery Team the SSP Species Coordinator reports the activities of the captive reproductive program

### **Conservation Status**

The Wyoming toad was listed as an endangered species under the Endanger Species Act on January 17, 1984. Today the toad is classified as on of the four critically endangered amphibians found in North America.

Currently, the factor that is most limiting to the recovery of the toad is the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). This fungus has been implicated in declines and extinctions of amphibian species worldwide. Retrospective analysis shows that the fungus has been present at Mortenson Lake since at least 1989. Predation, pesticide use, irrigation practices, and lack of genetic diversity may also limit the abundance of Wyoming toads in the Laramie Basin. The cause of the original decline remains unknown but may be associated with the invasion of *B. dendrobatidis* into the area.

## **Action Plan and Goals**

### **Captive Population Management**

Captive husbandry and reproduction of the Wyoming toad is of paramount importance to the recovery of the species. With the wild population in extreme peril, it is the responsibility of the institutions housing the remaining captive population to explore all options in order to not only significantly increase the overall numbers of Wyoming toads, but also to attempt to understand the unique and challenging physiological and environmental demands of this species.

Much of the toad's natural history is unknown and this lack of information leads to gaps in the knowledge of captive husbandry. A more complete understanding of diet, hibernation and reproductive cues would allow the captive program to rapidly improve their husbandry and captive reproductive procedures. Current wild toad management practices have made acquiring these needed data impossible. The "hands off" attitude has been a major impediment to advancing both the wild and captive programs.

The toad is at a critical juncture in its struggle to survive. In order to resolve this crisis, action must be taken rapidly and the resulting information shared quickly between the participating services and facilities. The fate of the toad does not rest solely on any one organization but rather a united cooperative front.

During the Wyoming toad PHVA held in Laramie Wyoming in February 2001 the top five goals were prioritized for captive population management:

1. Increase the long-term survival rate of yearling to 80%.
2. Improve larval rearing successes from 25% to 95%.
3. Increase the number of viable eggs by increasing the hatch rate from 27% to 95%.
4. Develop protocols for the treatment of toads infected with chytrid fungus.
5. Work with the veterinarian advisors to develop protocols to reduce the risk of disease transmission.

To meet these goals currently there are only six AZA member institutions and two USFWS facilities holding Wyoming toads for breeding and reintroductions as members of Wyoming toad SSP. More zoos and aquariums are needed to expand this program. Any institution that wishes to come on board must meet the following criteria.

1. Commit to providing information to the studbook keeper and SSP coordinator in a timely fashion.

2. Institutions planning on acquiring Wyoming toads will need to apply for a USFWS Endangered Species Permit.
3. Commit to maintaining a minimal captive population made up of four different cohort groups totaling 20- 40 toads.
4. Institutions should follow the guidelines set by WAZA-CBSG of “Best Practices Husbandry “by keeping Wyoming toads in quarantine away from other collection amphibians.
5. During the breeding season your institution should be prepared to breed four pairs of toads. Offspring that are produced will be released at locations in the Laramie basin as part of the USFWS reintroduction program. You will be asked to keep back a small group of 25 toadlets for future breeding.
6. Attend yearly SSP-Master Planning meeting held during the first week of August in Laramie Wyoming. This meeting puts all of the players in one place at one time including the USFWS Recovery Group. A major portion of the meeting involves pairing recommendations for the following year’s breeding season so it is important that all the facilities are represented so we make informed pairings. Often field trips (surveys) to the release sites are scheduled and participants are able to get out and see toads in the wild.
7. Provide an annual report at the end of each calendar year to the SSP Coordinator and to Ecological Services in Cheyenne, Wyoming.
8. Participate in ~ 5 conference calls per year to discuss the program.
9. Send necropsy reports from all toads that have died at your institution to Dr. Allan Pessier at the San Diego Zoo.

### **Health Issues**

In captivity, infectious disease including chytridiomycosis, other types of fungal infection and systemic bacterial infection are among the most common causes of death in Wyoming toads. The reasons for a high incidence of infectious disease are unclear, however, recent investigation into “short-tongue syndrome” (STS) observed in captivity may unexpectedly provide some information in this regard. Animals with short-tongue syndrome develop an inability to capture prey as they mature. Histologic changes in the tongue tissue have been observed in animals diagnosed with this disorder. A primary consideration for these changes is a vitamin A deficiency and preliminary tests indicate that captive animals with short-tongue syndrome have significantly lower levels of liver vitamin A (retinol) compared to three wild-caught Wyoming toads and several wild-caught American toads (*Bufo americanus*) and southern toads (*Bufo terrestris*). If confirmed, these findings are significant because vitamin A deficiency can lead to immuno suppression with subsequent increased susceptibility to a variety of infections. Vitamin A deficiency could also have negative impacts on captive reproductive success (A. Pessier pers. comm.).

### **Research Initiatives**

Funding opportunities exist for many of these initiatives. If interested in assisting with financial support for any of the projects outlined below, please contact the author for more information. Unless stated, costs were estimated by the author.

#### **Adult toad diet study - Cost \$4000.00**

Review all participating institution adult toad diets for nutritional content. Looking at available amount of vitamin A and retinol A levels. Collect diet items and supplements from each facility for analysis.

### **Reproductive hormone study – Cost \$6000**

Continue the work began at Memphis Zoo by Andy Kouba PhD on the successful use of hormones LHRL and hCG and their impact on female Wyoming toad egg production and male sperm cryopreservation.

### **Temperature sampling study – Cost \$10,000**

Continued the work started by Bruce Foster of the Wildlife Conservation Society in 2000 on the seasonal variation of environmental temperatures Wyoming toad face each summer. Water, surface, air temperature and relative humidity levels will be take from each of the four established release sites in the Laramie basin and one new proposed site. Five Onset Computer Weather stations will placed at Mortenson Lake NWR, Lindzey Ranch, Rock River Ranch, Buford Foundation property and University of Wyoming Red Buttes biological lab. TidBit data loggers will set up in all five locations to monitor surface and water temperature

### **Auditory study of release sites – Cost \$8000**

Place a Froglogger Model FL IV automated recording system at each of four release site to monitor the abundance of male toads. This study will be conducted at the same time the temperature/weather sampling study is underway. Important information should learn about the environment cues that set Wyoming toads into wild reproductive cycle.

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February 2001, Laramie Wyoming

### **Wyoming toad SSP Contact Information**

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## **Appendix 5. Available Husbandry Manuals**

The following husbandry manuals are included:

- o Mountain yellow-legged frog (*Rana muscosa*)
- o Panamanian golden frog (*Atelopus zeteki*)
- o Puerto Rican crested toad (*Bufo lemur*)

The following husbandry manuals are available online:

- o Eastern hellbender (*Cryptobranchus alleganiensis*) –  
Taxon Management Account:  
[http://www.caudata.org/cig/taxon\\_management\\_account.html](http://www.caudata.org/cig/taxon_management_account.html)
- o Boreal toad (*Bufo boreas boreas*)  
<http://wildlife.state.co.us/NR/rdonlyres/23912565-3F5F-4026-A4C3-BD34C29BB564/0/FinalHatcheryManual122402.pdf>

## **Husbandry Manual for Rana Muscosa**

### **2007 Final Version**

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#### **Enclosures**

As large an enclosure as possible should be used to provide maximum water capacity. A large water volume with proper filtration maintains more stable water quality parameters than a smaller water volume similarly equipped. (Can be met by use of a sump).

All should be constructed of easily disinfected materials like plastic, glass or fiberglass.

Containers of cement-based products are one alternative, provided they are well aged and no longer leaching alkaline. (Test with pH meter). Unsealed concrete surfaces can be problematic to disinfect between groups of animals. Rough concrete surfaces have been linked to mycobacterial infections in aquatic frogs, an incurable fatal infection.

No metal containers, galvanized or not. These may leach metal ions that are known toxicants to amphibians.

Aquaria, plastic kiddie pools, plastic cattle troughs, and aquaculture tubs work well. The specific enclosure depends on the husbandry plan to be implemented. In most cases as large an enclosure as possible (i.e., mechanical, chemical, biological, UV irradiation) to achieve and maintain stable water quality within appropriate parameters is desired.

Water depth should be at least 5 inches for swimming larvae and no more than 5 inches for metamorphosing larvae and froglets.

Disturbances to the frogs should be minimized by setting up the holding containers in low human/animal activity areas or by covering the outsides of the aquaria with a visual barrier such as black shade cloth.

#### **Equipment**

Each enclosure should have it's own set of designated equipment – nets, gravel siphon etc and should not be used in other enclosures.

Plastic or latex gloves should be used when handling frogs and should be changed between enclosures.

The previous mentioned precautions as well as any additional care should be taken to help reduce the spread of any disease or contaminant from animal to animal and enclosure to enclosure.

### **Lids**

All containers should have screened or solid lids to prevent metamorphosing froglets, or adults from jumping out or escaping. An exception to this can be the tadpole aquaria, however once the larvae begin to metamorphose a lid should be placed on the aquaria or the metamorphosing tadpoles should be moved to a tank that has a lid.

### **Cage furniture**

Hiding spots, basking spots and aquatic perches are essential for frogs to feel comfortable in their enclosures. Visual barriers are important to reduce stress between frogs within the same enclosure and to reduce stress caused by activity outside their enclosure. Examples include plastic plant pots that are placed upside down with a hole cut in the side large enough for the frog or froglets to enter and exit.

Artificial floating plants provide larvae with resting and hiding places.

Live plants or algae may be used if obtained from the same location as the animals or if the plants are thoroughly rinsed and stored in tap water for more than 30 days. More stringent disinfection measures may be appropriate depending on the level of quarantine desired for the population of frogs. Copper sulfate, levamisole, and chlorhexidine baths may be used to eliminate protozoa, helminthes and other pathogens that may find refuge in the plants. Chytrid fungus may survive on aquatic plants but may be eliminated by soaking the plants in water maintained at 99°F for at least 18 hrs.

Plastic window screen mesh can be used as rafts and feeding platforms. Tadpoles often prefer resting above the bottom of the water column.

PVC pipe and fixtures can be used as underwater refuges.

The underwater perches should be stratified so that an animal can seek refuge at a comfortable depth of water. Some of the perches should be placed beneath overhead basking lights.

### **Lighting**

Where practical, access to natural sunlight at levels approximately equal to the wild habitat is beneficial.

Artificial lighting can be provided using compact fluorescent lights, set on an ambient timer, to simulate daylight in an indoor set up. Ambient lighting should consist of fluorescent bulbs in a four-foot fixture set on a timer. Each Fixture should contain 1 Sylvania brand Blacklight bulb and 1 Phillips brand full spectrum Colortone bulb.

Ultraviolet B lighting should be provided by using a 100 watt Flood, Active U.V. Heat Bulb. (T-Rex Products 2391 Boswell Rd., Chula Vista, CA 91914. Tel 619) 482-4424). The need for this is uncertain at present, but may be beneficial to the frogs for metabolizing vitamins and minerals.

Set the basking lights on a timer to come on for 3 hrs/day reducing to 1 hr/day in winter. The Photoperiod should roughly follow seasonal changes, adjusting time clock every few weeks.

Multiple basking sites should be provided on the land and on underwater perches using incandescent lights or ceramic bulb heaters.

Light should also be provided in a patchwork mosaic so an animal can choose between light and dark spaces. Proper placement of lights should be taken into consideration. Enclosure props will also provide light and dark refugia for frog security.

Date the bulbs when installed and replace bi-annually or as needed and when possible, the UVB output of the lights should be monitored regularly (various UVB readers are available commercially).

### **Temperature**

Water temperature should be maintained between 50° and 85° F.

Fall 55° F

Winter 50° F

Spring 58° F

Summer 65° F

Monitor temperatures routinely with a digital probe thermometer.

Basking lights may be suspended over underwater rocks to provide thermal variation that offers larvae the chance to thermo regulate.

Larval growth rates are directly correlated with environmental temperatures. Within the biologically appropriate temperature ranges, higher temperatures typically yield faster growth rates.

### **Stage Specific Considerations**

#### *Housing-Embryos*

In general, the enclosure should be large enough so that the pump produces minimal current to agitate the egg mass or recently hatched larvae.

Gently aerate water in embryo holding tank with a sponge filter and aquarium pump or aquarium power head. If a sponge filter is not available than an air stone may be used. A sponge filter is preferred as it provides biological filtration if it has properly aged. An air stone does not provide any filtration (see water quality section for more information on filter maintenance).

Egg masses and recently hatched larvae should be suspended off the bottom of the holding container. Plastic window screen mesh or rinsed cheese cloth material are useful for building a hammock underneath the eggs to suspend them in the water.



Remove dead hatchlings or eggs covered with fungus from the mass if possible with minimal disturbance. Ammonia levels can quickly rise to toxic level from decomposing eggs or hatchlings even with biological filtration. Send dead bodies up to Pathology, following their protocol for disposition of bodies.

*Stocking density:*

1 egg mass (up to 1000 eggs) per 10 gallons of filtered aerated water.

*Housing larvae*

Mechanical, chemical and biological filtration is essential to maintain water quality. UV sterilizers should be used.

External canister filters are best for maintaining high volumes of water and moderate to high stocking densities. Under gravel filters and sponge filters are best for low water volumes and low stocking densities.

Even with filtration, water changes are important to reduce buildup of organic waste product. Approximately 10 percent of the water volume should be changed weekly.

Systems that include algae growth and living plants are encouraged as it provides additional buffering of water quality parameters. Additionally, algae is excellent food for larvae.

*Stocking density:*

Sizes can be mixed; with *R. muscosa* there is no evidence that large tadpoles harm small individuals. Stocking capacity declines as tadpoles grow larger, so it is important to monitor water quality closely and check for signs of overcrowding.

*For maximum growth:*

25-30 larvae per 10 gallons of filtered aerated water.

*Housing Metamorphosing Larvae*

Water depth should be decreased to no more than 5 inches for larvae showing hind limbs only.

Edges of the enclosure should have haul out areas and underwater perches. Some larvae may drown swimming the perimeter of the enclosure looking for haul out areas if none have been provided.

Cover should be provided on dry land and underwater.

Some haul out areas should be beneath basking light.

Wattage of light should be adjusted to provide a hotspot of 85-90° F.

Larvae that have developed 4 legs but retain a tail should be maintained in a separate tank from the 2-legged larvae. The water level can be decreased to 3 inches or less to reduce the risk of drowning and placed on a slant so at least one quarter to one half of enclosure is land based.

Newly metamorphosed froglets should be separated by size to keep cannibalism to a minimum. Although larvae are not cannibalistic, juvenile and adult frogs are. Feeding the frogs is also easier if separated into groups based on size.

*Stocking density:*

No more than 10 metamorphs or froglets per 10 gallon half tank enclosure.

**Diet**

Many of the problems with metamorphosis are due to poor plane of nutrition as a tadpole. Mistakes during tadpole development may result in dying tadpoles, stunted metamorphs or froglets that are unthrifty.

*Rana muscosa* tadpoles typically graze off the bottom of the water column or on the surface of objects. Food should be placed on bottom of the water column to ensure the tadpole find it easily. Some food items are buoyant, such as zucchini, and may need to be weighted with stones so they don't float.

Type of food for larvae:

Live algae and aquatic plants are excellent food sources for tadpoles.

Where possible, enclosures should be heavily planted so that tadpoles can graze on live food plants.

Duckweed (Lemma) is easy to raise and a good food source. It may need to be harvested and crushed to sink to the bottom of the enclosure where it is easily found by tadpoles.

Other aquatic plants are useful food sources (e.g., Elodea).

If it is not practical to maintain algae and live plants in the rearing enclosures, algae cultures can be started in other enclosures and used as a food source.

Firm plastic sheets, pieces of tile or nonporous stone may be placed into an algae-rich environment and seeded with algae. Once a layer of algae is growing, the "plot" of algae can be removed and placed in with the tadpoles for grazing.

If multiple plots are maintained, fresh algae is available for harvesting continuously.

Larvae feed well on dark green leafy produce.

Dark green leafy produce should not exceed 50 percent of the total diet offered.

Romaine lettuce

Use fresh romaine lettuce that has been frozen overnight. Freezing breaks down the cell walls of the lettuce and makes it more digestible by the tadpoles.

Romaine lettuce should comprise no more than 15 percent of the diet offered.

#### Mustard greens

Should be frozen overnight to break the cell walls and increase its digestibility.

Mustard greens should comprise no more than 15 percent of the diet offered.

#### Turnip greens

Should be frozen overnight to break the cell walls and increase its digestibility.

Turnip greens should comprise no more than 15 percent of the diet offered.

#### Other produce

##### Cucumbers or zucchini slices

Should be frozen overnight to break the cell walls and increase its digestibility.

Cucumber or zucchini slices should comprise no more than 15 percent of the diet offered.

#### Processed fish foods

Spirulina-based fish foods and algae wafers designed for herbivorous cichlids work well.

They may comprise up to 50 percent of the diet.

Sinking wafers or pellets are preferred to floating wafers or pellets.

(“Aquarian” brand Cichlid flakes, “Kent Marine” brand Platinum Cichlid Extreme pellets, “New Life” brand Spectrum Premium Discus pellets are all good commercially available foods).

Powdered variety can be mixed with a small amount of water and then poured onto shallow, non floating, plates that are then left to dry. Once dry these plates can be placed in the tadpole enclosures where they will graze off of them.

High protein fish foods should comprise at least 25 percent of the diet offered.

Dehydrated or frozen bloodworms, tubifex worms and earthworms are excellent sources of protein.

Sinking foods are preferred to floating foods.

Frozen Bloodworms, daphnia and rotifers are excellent protein sources and should comprise at least 5 percent of the diet offered.

A complete tadpole diet will vary from species to species and depends on water quality in part. Variations of the previously listed food items can be mixed together with unflavored gelatin and set in the refrigerator, this can then be cut into small pieces that will sink to the bottom of the water table.

This can be kept in the refrigerator and used later – up to 5 days.

Food should be offered *ad libitum*. This means that fresh food is constantly available for feeding throughout the entire day and night.

Uneaten decomposing food should be removed daily.

#### *Types of food for juveniles and adults*

Juvenile and adult frogs feed well on domestic crickets, mealworm larvae, mealworm adult beetles, flightless houseflies, wax worm larvae, earthworms, phoenix worms (black soldier fly larvae) small fish and small roaches. Insect prey needs to be of an appropriate size to feed to different sized frogs.

Food must be offered alive.

Insects should be dusted with calcium carbonate powder, every other feeding, prior to feeding to increase the calcium content ingested by the frog.

Feeder crickets should be gut loaded with a high calcium cricket diet (at least 8% Ca, DM basis available from Zeigler Bros., Marion Zoological, or Mazuri).

Insects should be dusted with a multivitamin powder every other feeding just prior to feeding out to the frogs (alternating between the calcium powder and the multivitamin powder each feeding).

#### **Air Quality**

If the air smells bad to you for any reason, it may contain chemicals that are harmful for amphibians.

Avoid the use of strong smelling chemicals in the airspace around an enclosure.

Make sure the ventilation leading to the amphibian enclosure does not communicate with any space that has dangerous chemicals in the air.

Water in enclosures should have sufficient aeration so that the larvae are not gasping for air at the top of the tank or looking distressed.

### **Water Quality and Changing Schedule**

Water quality parameters for the frogs in the wild are slightly basic with a pH range of 7.5-8.3 (7.9 avg and 8.0 at City Creek, CA) with a high dissolved oxygen reading that can be met by providing adequate filtration and water movement.

Monitor pH and ammonia levels as well as nitrate and nitrite levels frequently – daily to weekly depending on stocking levels and age of system set up. Use simple aquarium test kits for this process.

The importance of appropriate water for raising young amphibians cannot be overstated. Larval development and metamorphosis are incredibly complex and demanding life stages for amphibians. In addition to diet, some dissolved substances in the water provide nutrients for growth of the larval amphibian. Conversely, some dissolved substances are toxic and create metabolic demands that can interfere with normal growth and metamorphosis.

Water samples from natural breeding sites should be analyzed for various parameters and efforts made to reproduce those parameters in the captive setting.

#### *Changing schedule*

All holding containers should ideally be cleaned daily by siphoning off a minimum of 10 percent and a maximum of 50 percent of the water in the larvae holding containers, and then replacing it with one of the water types under water quality.

Chiller units and parts should be checked monthly or as needed and maintained according to the manufacturers guidelines.

Sponge filters should be rinsed out using either fresh de-chlorinated clean water or siphoned out tank water as this will help maintain the beneficial bacteria growing within them. This should be done as needed or at least weekly.

The frequency of water changes will depend on the stocking density of larvae and the type of filtration system used.

Clean water to be used in tadpole and frog tanks, should be made with either de-chlorinated city water mixed with reverse osmosis (R/O) water at a ratio to create the appropriate water hardness levels, or R/O water mixed with Kent R/O Right (Kent Marine Inc., Acworth Georgia 30102, [www.kentmarine.com](http://www.kentmarine.com)), added to create the appropriate water hardness levels. (GH = 160-230 ppm or matching to that of City Creek, CA levels). Follow directions on Kent R/O Right product for mixing ratio.

Kh and Gh (carbonate hardness and general hardness) of water can be tested with simple aquarium grade test kits or with a conductivity meter (Follow directions for specific brand used).

Never use just straight, un-mixed R/O water for frogs or tadpoles.

Charcoal in filters should be changed at least every 6 months or more frequently as needed. (If medications are used then charcoal filters may need to be temporarily removed from system – check with medication instructions prior to use).

Water for the froglets and adult frogs can be changed once a week to minimize stress as long as dead prey items and feces are being skimmed out daily.

Siphon out all old, uneaten food and feces every day.

### *Water Issues*

Tap water that is aged or allowed to sit for 24 hours is not recommended as the San Diego Co water supply contains Ammonia and Chloramines that are used as disinfectants. If tap water is to be used it must first be treated with dechlorinating and ammonia removing chemicals prior to use in any frog enclosure. Kordon brand NovAqua and Amquel should be used (available at most pet stores and [www.drsfosterandsmith.com](http://www.drsfosterandsmith.com) for example).

Carbon filters can be placed in-line to assist with chlorine and chloramines removal.

All filters need to be changed regularly, either according to manufacturers guidelines or more frequently depending on stocking densities (1x per week to 1x per month).

All nets, siphons and other equipment should be disinfected with a Chlorine Bleach and water mix. (1 part bleach to 10 parts water).

Water should be tested daily – weekly, with test kits to make sure the filters are functioning properly (chlorine, pH, Kh, Gh, ammonia, nitrite, nitrate, etc).

During a water change, replacement water should be the same temperature as the water in the holding tanks to minimize stress on tadpoles and frogs.

Should a tadpole or frog die please see Appendix I for guidelines on how to handle and submit body to pathology for necropsy.

## **Appendix I**

### **Pathology Submission Protocol for Dead Mountain Yellow-Legged Frog Tadpoles and Postmetamorphic Frogs**

All MYLF tadpoles and postmetamorphic frogs that die in the CRES facility will be submitted to the CRES Wildlife Disease Laboratories (formerly, Department of Pathology) located in the San Diego Zoo veterinary hospital for complete necropsy examination and final carcass disposition. Complete necropsies that include histopathology of all major organ systems are essential for detection and control of important infectious disease agents or other disease problems that could be related to captive husbandry or nutrition. Necropsy surveillance for diseases important for species reintroduction efforts (e.g. chytridiomycosis or *Ranavirus* infection) is also essential. After necropsy examination, remains of MYLF carcasses will be retained for return to government partners. The priority of the Wildlife Disease Laboratories will be on disease diagnosis necessary for captive management of this species.

Submission procedures will differ slightly between tadpoles and postmetamorphic animals as follows:

#### **Tadpoles**

1. All dead tadpoles from mountain yellow-legged frogs (regardless of state of preservation or completeness of the carcass) should be submitted to the Wildlife Disease Laboratories for examination. An animal history form (“pink sheet”) available on the outside of the WAP Harter Hospital carcass cooler (“square boma”) should be filled out for all submissions.
2. **If less than or equal to 3 tadpoles are found dead** they should be placed into formalin fixative (jars of formalin available from veterinary staff in WAP Harter Hospital) prior to shipment to pathology on the next Boma run. **Prior to placement in formalin**, the genetics division should be contacted and if staff is available, they will remove the tail tip from tadpoles for establishment of cell lines. There **should not** be a significant delay between finding animals dead and formalin fixation (greater than half hour at room temperature or two hours under refrigeration). If genetics staff are not available, tadpoles should be immediately placed in formalin.
3. **If greater than 3 tadpoles are found dead**, approximately 25% of the dead tadpoles should be submitted fresh (refrigerated) to pathology on the next Boma run; the remainder of the dead tadpoles should be fixed in formalin as described in # 2 and submitted to pathology on the next Boma run. The opportunity to collect fresh samples should be offered to the genetics division as for # 2, but should not affect timely delivery of samples to pathology (if greater than 3 tadpoles die, prompt diagnostic evaluation will be essential).
4. **If greater than 3 tadpoles are found dead** samples should be immediately collected for water quality evaluation to include pH, ammonia, nitrite and nitrate.

### **Postmetamorphic Animals**

1. A pink sheet should be filled out as completely as possible as described for Step # 1 for tadpoles. Information for the pink sheet should include accession number (if available, do not delay shipment if not available), any medical history (if available) and circumstances surrounding death ("found dead" is acceptable if unexpected death).
2. Animals should be placed in a labeled plastic bag and placed in the WAP Harter Hospital carcass refrigerator ("square boma") for delivery to the Wildlife Disease Laboratories on the next boma run. Carcasses should NOT be placed into formalin.
3. Samples for Molecular Genetics will be collected at necropsy and should not be collected at the WAP.



**Husbandry Manual**  
**Panamanian Golden Frog**  
*Atelopus zeteki*

Second Edition by Vicky Poole, National Aquarium in Baltimore

## **BACKGROUND HISTORY**

A pathogenic fungus capable of causing sporadic deaths in some amphibian populations and 100% mortality in others, *Batrachochytrium dendrobatidis*, has been isolated from the skin of dead and dying amphibians on every amphibian-inhabited continent. Chytrid fungi typically live in water or soil, although some are parasites of plants and insects. They reproduce asexually and have spores that 'swim' through the water. Only the amphibian chytrid fungus is known to infect vertebrate species. Individual frogs are thought to contract the disease when their skin comes into contact with water that contains spores from infected animals. Chytrid causes death in post-metamorphic frogs by causing focal hyperkeratosis (thickening) and erosion of epidermis in frogs (excessive skin shedding and/or red areas, generally the first clinical sign) and infects the keratinized mouthparts of tadpoles causing erosion (potential starvation). Some amphibians, including two commercially important and widely traded species, American bullfrogs (*Rana catesbeiana*) and African clawed frogs (*Xenopus laevis*), have proven to be carriers of the *B. dendrobatidis* without becoming susceptible to the disease chytridiomycosis.

In Central America, chytridiomycosis (or “chytrid”) was first observed in montane central Costa Rica, where it may have been the cause for extinction of the golden toad, *Bufo periglenes*. It has since become evident that this epizootic is advancing southeast through the mid- to high-elevation mountain forests of Central America, decimating entire populations of amphibians, including *Atelopus senex*, and *A. chiriquensis*. As of 2005, the disease front is documented as far east as eastern El Cope (K. Zippel, pers. comm.). The current species being affected by the disease is the Panamanian Golden Frog (PGF), *Atelopus zeteki*. Other factors impacting PGFs include collecting for the local zoos and hotels as well as the illegal international pet trade [listings include CITES Appendix I status since 1975; and USFWS Endangered Species Act (ESA) - Endangered species since 1976], deforestation, and habitat alteration by logging and farming that also leads to sedimentation of their pristine waters.

In response to this crisis, a group of concerned biologists convened and formed Project Golden Frog/Proyecto Rana Dorada (PGF/PRD), a conservation consortium involving numerous Panamanian and US institutions. Specific initiatives of PGF/PRD have included field studies, captive management, education, and financial support (See Appendix I for current contact information).

As a precautionary measure against extinction, *ex situ* populations of golden frogs are being maintained. Over two dozen founder pairs have successfully spawned producing thousands of offspring. To ensure genetic viability, permits were obtained to export specimens collected from unprotected remnant populations outside of two national parks where they occur. The numbers collected are actually less than those removed yearly to replace dying animals at the hotels and a local zoo that display golden frogs for tourism.

Should the chytrid epizootic decimate *in situ* populations of PGFs, PGF/PRD would propose that tadpoles be reintroduced into the national parks, but only if independent research concludes that the chytrid fungus is no longer a threat. The rationale for releasing tadpoles versus adults is that captive reared adults presumably will not possess the skin toxins necessary for survival against endemic predators and or/may have difficulty in properly homing after reintroductions.

The PGF husbandry and captive program is modeled after the 1992 *Atelopus varius* (Fortuna, Panama) breeding at National Aquarium in Baltimore (NAIB). A female of a collected pair went into amplexus and spawned in the shipping bag during transport back to the US. Robin Saunders successfully reared approximately two dozen *A. varius* froglets at NAIB.

## SPECIES DESCRIPTION

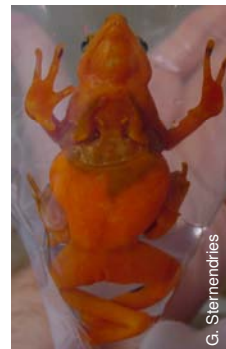
*Rana dorada* or the “golden frog” is culturally significant to the people of Panama. They are as revered as the bald eagle is in the US, with a history dating back to the Mayan civilization. Golden or pottery replications, called huacas (“wa-cas”) were symbols of good fortune and the frogs are still considered lucky to Panamanians. PGFs are used to promote hotels and restaurants, and are such a part of the culture that they show up on their lottery tickets.

Atelopid frogs are unique members of the family Bufonidae (toads). Endemic to the cloud forests, the Panamanian golden frog, *Atelopus zeteki*, was originally described by Dunn in 1933 as a subspecies of *Atelopus varius*. It has been recognized as a distinct species based on a unique skin toxin, zetekitoxin, and bioacoustical differences. In addition to vocalizing, PGFs communicate by semaphoring, a hand-waving phenomenon that is theorized to have developed so that the frogs could locate conspecifics for breeding near the deafening sounds of waterfalls, where their gentle vocalizations are inaudible. Male frogs will perch on rocks in or along the banks of streams and waterfalls, defending their territory by semaphoring as a warning or wrestling other males that come too close while awaiting the females’ return to the breeding areas.

Mature golden frogs can be sexed easily as the females are significantly larger in size for most populations (wild Locality A females range 55-63 mm while males range 39-48 mm, for example). Females full of eggs are easily identified by examining their ventral surface for light-colored abdomen. Males also have obvious nuptial pads (darkened and enlarged during breeding season) on their hands that they used to increase pressure on the female during amplexus.



The female's eggs appear light in color.



Male amplexing gravid female.

Depending on their locality, PGFs will differ in appearance. Their colors range from brilliant gold, egg-yolk or pale yellow, to a greenish-yellow. Their individual black patterns may vary from large solid chevrons, large random blotches, lots of small spots, few markings at all somewhat isolated to sides and limbs, to no markings whatsoever. Compared to other atelopids, PGFs have smooth skin.

There are two general types of habitats that golden frogs occupy in Panama, and unique sizes that vary with locality:

- There are the wet forest PGFs that are larger and more dispersed in and along the streams (up to 3m above the ground). The habitat typically includes waterfalls and large boulders covered with moss that they utilize as visible territories. These animals have been found sleeping on big leaves at night.
- There are also frogs that inhabit dry forest streams and are more likely to be seen on the forest floor (no higher than 1.5 m). They are smaller, ~2/3 as large as the other frogs, but they exist in much greater densities.

For more explanation on this see \*genetic conversation in the section below.

In 2002, PGF skin samples were collected in the field for toxicology analysis in order to compare to that of captive-bred offspring, performed by Dr. John Daly of the National Institutes of Health (NIH). It appears that all Atelopids secrete digitalis-like substances, bufadienolides, synthesized even when raised in captivity. These steroids, which are also found in plants, are potent inhibitors of the Na<sup>+</sup> pump. The frogs also possess the unique zetekitoxin. Dr. Daly believes that zetekiton, like all other tetrotoxins requires a symbiotic microorganism and

will not be present in captive raised PGFs, similar to his findings with captive bred *A. varius*. Samples have been sent to Dra. Yotsu-Yamashita in Japan for further analysis.

NOTE: Type Locality: El Valle de Anton, Provincia Cocle, Panama  
Holotype: MCZ 16018, Harvard University, MA.

## COLLECTION NUMBERS, DATES, AND SITES

Prior to any involvement from US zoos, the only known captive specimens were a few wild-caught golden frogs exhibited at two hotels and one small local zoo in Panama. These animals have never reproduced, mortality rates are extremely high, and their legality is highly questionable. Although the local Panamanians were concerned about the future of golden frogs in the wild, none of the facilities in Panama at that time were equipped or skilled to handle the challenge of a captive breeding effort.

In 1999, the Republic of Panama issued scientific collecting permits for a total of 20.20.100 *Atelopus (varius) zeteki* to PGF/PRD's founding researchers, Erik Lindquist, Roberto Ibáñez, Anthony Wisnieski, and Kevin Zippel, in order to conserve genetic variability and maintain viable captive populations of *Atelopus zeteki* due to the impending chytrid crisis. CITES/ESA Importation permits for those specimens and all of their offspring were sought and have been maintained through the Baltimore Zoo since 2000 (original No. 00US027256-9). Intentionally, the ownership of the animals belongs to the permit holder (in this case to The Baltimore Zoo, now called The Maryland Zoo in Baltimore) and not to the Republic of Panama, as has happened with other species of animals. The imported animals and their offspring are then placed on loan by the Maryland Zoo in Baltimore **to other AZA institutions only** (per the USFWS) with a Memorandum of Understanding (MOU). This restriction is intended to prevent the protected species from entering the pet trade via captive zoo breeding, potentially creating a situation in which wild-caught illegal specimens could be "laundered" under the guise of coming from legal "zoo stock," as has happened with other species of amphibians. Once this situation begins, it is impossible to stop and the remaining wild populations become even more at risk of being collected and traded illegally.

As of January 2006, the following collecting trips and importations have resulted in the only known legal wild-caught golden frogs in captivity in the world (*AZA facilities can contact the author or studbook holder to decipher the following population locations in order to protect the localities from illegal collection*):

- January 17, 2001: 7.7.0 *Atelopus zeteki* from Locality **A**, Panama are collected/imported as amplexant pairs and sent to the Baltimore Zoo (3.3) and Detroit Zoo(4.4).
- January 25, 2002: 3.3.12 *Atelopus zeteki* from Locality **A**, Panama and 1.1.25 golden frogs\*\* from Locality **C**, Panama are collected/imported and sent to the Baltimore Zoo (2.2.12 from Locality **A**) and Detroit Zoo (1.1 from Locality **A** & 1.1.25 from Locality **C**).
- December 19, 2003: 9.9.0 *Atelopus zeteki* from Locality **B**, Panama and 0.0.22 golden frogs\*\* from Locality **C**, Panama are collected/imported and the two groups are sent to the Baltimore Zoo and Detroit Zoo, respectively.

Renewals of the permits will allow for the balance of 0.0.41 *Atelopus zeteki* to be collected/imported from Locality **A** at this point to minimize chytrid risk.

\*In 2002, as part of a grant from the St. Louis Zoo, genetic material was collected from several Panamanian localities with golden frogs to determine the relatedness of various PGF populations and the prioritization of our conservation efforts (in prep.). Rough analysis of the three populations were are currently maintaining in captivity has shown the following:

- Locality **A** animals' phenotype is a large golden frog with the genotype of *A. zeteki*
- Locality **B** animals' phenotype is a small yellow/green frog with the genotype of *A. zeteki*
- Locality **C** animals' phenotype is a large golden frog with the genotype of *A. varius*.

In light of this, the Cleveland Zoo solicited permits to collect and import *A. varius* from Locality C to increase the potential founder numbers for this population as *A. varius (varius)* is not listed on CITES or the USFWS ESA. In April 2005, 6.6.0 golden frogs from Locality C, Panama were collected/imported by the Cleveland Zoo.

Since January of 2001, the Baltimore Zoo, Detroit Zoo, and Cleveland Metroparks Zoo have been successful in the captive hatching of eggs laid by amplexant pairs from Panama, and tadpoles were reared through metamorphosis. Other captive population statistics are listed in Appendix II.

NOTE: Since it is a priority for long-term maintenance of our captive program to maximize the gene pool, **it is important for institutions not to mix populations of frogs**. In order to control this we recommend that each AZA institution only work with one population of frogs at a time. This will limit the chance for mistakes. All of these frogs should be reported and maintained in the PGF studbook and managed together due to limited space and resources for golden frogs of either species. Institutions participating in the program will be prohibited from producing hybrids.

## SHIPPING PGFs

Use disposable plastic containers (i.e., deli cups, Gladware®/shareware, Rubbermaid®, or Tupperware®) so that frogs are not crushed during transport. Line containers with damp toilet paper or paper towels (avoid natural materials that could cause delays in customs or with airlines due to agricultural restrictions). Pack securely within an International Air Transport Association (IATA) approved cardboard box/Styrofoam container, with small perforations for air exchange. Avoid shipping during summer and winter, when the temperatures can be severe.

During our initial importations, PGF/PRD was fortunate to obtain written permission from the airline in advance to carry-on our specimens in the airplane cabin along with us to avoid any potentially dangerous cargo-shipping issues.

For international shipments, be sure to notify federal wildlife agencies due to their legal status and preschedule inspections at customs to minimize delays. Make sure that copies of all permits and MOUs relating to the PGFs accompany all shipments.

Specimens should be taken immediately to isolated quarantine facilities upon arrival at the final destination.

## QUARANTINE

Accessioning frogs: It is preferred that adult PGFs be accessioned as individuals, especially if only a few are received. If they are accessioned as a group, it is preferred that each group number represent a single bloodline (sibling group), so that there is minimal confusion for genetic pairing recommendations.

In order to identify frogs as individuals, it is ideal to photograph and record patterns digitally. It is recommended that juvenile frogs be photographed periodically as they grow since their patterns may change as they mature. Adult frogs' pattern are fairly stable and patterns are generally unique. As a final measure, elastomer marking imbedded on a frog's inner thigh may work as an identification method, and was utilized by the Detroit Zoo.

Receiving from the wild: Due to the potential of transferring chytrid into an existing healthy collection, it is important that wild-caught PGFs undergo a minimum 30-day quarantine in an area isolated from rest of the exhibit collection, preferably in another building. Animals should be screened for ecto- and endoparasites. As wild-caught *Atelopus* have been shown to die when housed in sterile enclosures and over-medicated, it is preferred that the animals be placed in a naturalistic enclosure (see the HOUSING section below for details),

parasite loads be monitored, and not medicated unless it becomes a health issue. Be aware that some specimens may appear thin with protruding pelvic girdles, but that may be normal for the individual. Specimens that are considered ‘poor-doers’ should be isolated and medicated as necessary.

It is also important during the first few days in quarantine that the frogs coming from the wild undergo chytrid treatment prophylactically. Safe treatment for post-metamorphic frogs exposed to chytrid or coming from a known chytrid (+) environment involves using recommended itraconazole baths (Nichols and Lamirande, 2000). Animals should be soaked for 5 minutes at a depth of 1cm in a 0.01% suspension of itraconazole (prepared from a 1% suspension of itraconazole using 0.6% saline as the dilutant) for 11 consecutive days (Sporanox®, Janssen Pharmaceutica, Inc, Titusville, NJ, USA).

Good husbandry practices are necessary when dealing with animals housed in a quarantine situation. Every attempt should be made to minimize the potential for contamination between enclosures. Keepers should wash their hands or utilize a different pair of latex gloves between cages; equipment should not be shared between cages and should be disinfected using chemical or thermal sterilization (see Appendix III for recommended protocols). As the amphibian chytrid fungus is extremely sensitive to temperatures above 29 C (84.2°F), and *B. dendrobatidis* is killed at 32 C (89.6°F), there is no zoonotic risk since it cannot survive on human skin. Also, complete drying will kill the amphibian chytrid fungus.

Transfer between AZA facilities: As quarantine protocols may vary between institutions, it is recommended that all captive-bred PGFs undergo a minimum 30-day quarantine with three successive negative fecals (float and direct) in an area isolated from the rest of the exhibit collection. This will allow time for the PGFs to manifest any disease issues due to post-shipping stress and minimize disease transfers to your permanent collection. If the animals have come from an institution with chytrid is in the collection, the procedure for itraconazole baths and equipment disinfection protocols should be employed (see section above).

## FACILITIES & CARE

### ENVIRONMENTAL PARAMETERS

Wild golden frogs reside in high elevation cloud and rain forest habitat with clear waterfalls and streams.



*In situ A. zeteki stream – our ideal!*

Our recommendations for air and water parameters are based upon PGF/PRD environmental assessments of PGF habitat in Panama (see Appendix IV & V for original data):

- Air Temperature: 68-73° F daily, without seasonal variation
- Water Temperature: 69-72° F, without seasonal variation
- Humidity: 75-100% with little seasonal variation
- Water Quality
  - Dissolved Oxygen: High range 8.3 – 9.0 mg/L (influenced by water turbulence)
  - pH: Neutral range 6.5-7.5
  - General Hardness: Soft water 0-1 degrees
  - Ammonia, Nitrite, & Nitrate: 0 ppm

## **WATER AND FILTRATION**

Biofiltration is necessary to provide water quality required for PGFs (i.e., no detectable nitrogenous wastes and high oxygen levels) in captivity. External canister filters work well and should flow at between 250-350 gph. A robust biological filterbed, either through an under-gravel or external canister, will reduce ammonia from animal wastes. Live plants and water changes several times per week (up to 100% preferred for tanks without tadpoles) are needed to help reduce nitrates.

As some institutions have experienced health issues associated with elevated phosphate levels, consider using phosphate sponges or absorbing resins as part of the filtration system.

Water chemistry analyses showed golden frog stream water is surprisingly soft and pure. General Hardness (GH, the measure of calcium and magnesium) of golden frog stream water was very low, only 0-1 degrees. By comparison, hard tap water can be 1-20 degrees. Conductivity, the measure of electrically charged particles in the water in golden frog habitat ranges from 44-86 microsiemens, compared to near 0 for Reverse Osmosis (RO) water and 200-400 microsiemens for tap water.

Depending on source water at each location, aging tap water, adding an in-line carbon filter, using reconstituted reverse osmosis (RO) water, or a dechlorinated tap water RO system may be necessary to be able to house and breed PGFs. A recipe for reconstituting RO water with a pH buffer created by Kevin Zippel is listed in Appendix VI, although commercial additives are available. It is strongly recommended that institutions monitor water quality parameters as part of routine husbandry.

Water temperature should be maintained between 69-72°F. Cool water temperatures plus high turbulence help maintain high dissolved oxygen levels, which decrease as water temperature warms. A thermostatically-controlled chiller or submersible heater may be necessary to maintain desired water temperatures depending on ambient air temperatures.

## **TEMPERATURE, LIGHT, AND HUMIDITY**

Ambient air temperatures should be kept cool, varying between 68-75°F on a daily basis. Some institutions have been able to maintain PGFs successfully at slightly warmer ambient temperatures, ranging from 78-82°F, but it is recommended that enclosures in these environments be misted more frequently.

As PGFs are fond of basking directly in rays of sunlight penetrating the canopy, a full-spectrum or plain incandescent basking spot light (60-100W) works well and can provide isolated thermoclines (up to 100°F). In addition, provide full-spectrum lighting (UV A & UV B) as you would for basking lizards (<sup>1</sup>Vita-lights®, <sup>1</sup>Powertwist®, <sup>2</sup>Verilux®, <sup>3</sup>Reptisun™, <sup>3</sup>PowerSun UV™, <sup>4</sup>GE® Chroma 502™, black lights, compact fluorescents, etc.) on a 12:12 lighting cycle year round

(<sup>1</sup>Duro-Test® Lighting, Philadelphia, PA; <sup>2</sup>Verilux®, Inc., Stamford, CT; <sup>3</sup>Zoo Med Laboratories, San Luis Obispo, CA; <sup>4</sup>General Electric Co.®, Cleveland, OH).

Relative humidity stays around 100% annually in the cloud and rainforests of Panama. Humidity levels can be maintained with automated misting systems. Wide angle, high flow nozzles will aid in simulating the rainy season.

Automated equipment such as light timers and programmable temperature control valves for both air and water make husbandry easier. Back-up generators to power filtration and temperature monitoring systems in case of power failures help with peace of mind, especially for such an environmentally restricted, valuable species. We also recommend logging environmental data, if possible, especially during breeding attempts as one parameter may be the difference between failure and success.

## HOUSING

The housing recommended for golden frogs is large, naturalistic enclosures modeled after rocky streambeds of the frogs' native habitat, but the exact specifications will obviously vary depending on the space and resources of each institution. Minimum space allotted for a pair of adult *Atelopus* should be no less than a 15 gallon aquarium to provide sufficient microhabitats, although up to a total of 6 adult frogs could be housed in a 15 gallon tank.

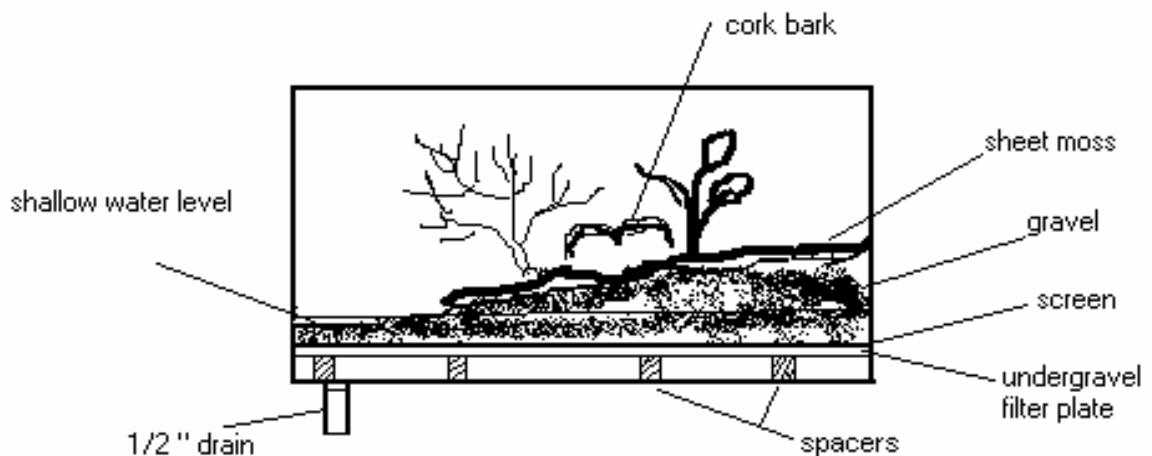
Custom glass enclosures with front-opening access work well for housing PGFs and screen windows/tops allow for ventilation. As golden frogs climb and newly metamorphosed froglets are quite tiny, make sure that all openings are secure and have a tolerance of less than 1/16" (2mm). If lids/doors are loose fitting, consider adding self-adhesive foam weather-stripping to tighten any gaps (available in various thicknesses).

Standard "breeding tanks" should include rocky streambeds flowing down to a large pool (2-3" deep, as more than that may be too deep and tired amplexant females could drown) with lots of large rocks or artificial structures to provide dark egg-laying sites during breeding season and provide territory sites. An inexpensive dark glass plate was used at the Detroit Zoo as an alternative to using large heavy boulders to provide egg laying sites. Use a basic flow-through tank set-up with an elevated undergravel filter plate and a bed of gravel including raised "land areas" covered with moss where animals can get away from flowing water and rest. Be sure to seal the screen mesh to the tank at the edges to prevent tadpoles from getting underneath the filter plate and into the filter.

Natural plants help create microclimates, refugia, and visual barriers for frogs. There was little to no submerged vegetation observed at golden frogs streams, but its addition to captive enclosures will help maintain good water quality. Emergent and terrestrial vegetation are also important for maintaining high humidity and providing sleeping sites. We found one male golden frog sleeping 2 feet above the ground on an arrow-leaf next to the stream, and this behavior is apparently not uncommon for certain species in the genus.

Filters should be used to recirculate water (exterior canister filters work well) and provide biofiltration. Using a 1" hole-saw diamond bit, a drain can be drilled into the bottom, front corner of the tank, fitted with a bulkhead fitting and 1/2" tubing going to a canister filter, and another hole drilled at the opposite back wall corner for returning. Water returned to the cage can be directed over descending rock-piles to create small waterfalls and splash pools.

For juveniles and adults that are not paired for breeding, an enclosure without a flowing stream can be used, although a shallow pool of clean water should be provided, not unlike a standard dendrobatid enclosure (see image below). Aquariums are plumbed with floor drains and include false bottoms, (i.e., undergravel filter plates). Gravel is placed on top of a false bottom (undergravel filter plate) on a sloped grade, which is then covered with sheet moss. Enclosures are then equipped with cork bark, hiding huts, live plants, and a shallow pool (just above surface of gravel).





## SOCIAL GROUPINGS

Males are highly territorial and shouldn't be housed together long term in order to minimize stress. There are reported cases of captive males defending territories in cages by pinning down passing males (wrestling) and semaphoring. Males have been observed to displace other males and begin calling from the new location.

Females can be housed in groups up to 8 animals in a 20 gallon aquaria without stress. When PGFs are paired for breeding, it works well to house females together in groups during the off-season, and introduce them to the males within the "breeding tanks." After oviposition, separate the pair and return the adults to their respective tanks.

Juveniles can be housed in dense groups during rearing (up to 50 froglets in 20 gallon aquaria) and separated by sex and size as they mature. Take into consideration space required for potentially separating out territorial males into individual enclosures when considering the number of PGFs each institution can hold long-term.

NOTE: Several institutions have attempted housing PGFs in mixed species exhibits with no interactions or related problems reported to date. The most diverse attempt is the Houston Zoo in which 3 *Dendrobates auratus*, 3 *D. azureus*, and 1 *Gastrotheca marsupiata* all share the 72"Lx30"Wx18"H exhibit with 8 PGFs. 1.1 *Abronia graminea* lizards have also been housed successfully with 17.0 PGFs at The Philadelphia Zoo in a 3'x 2'x 4' exhibit.

## ROUTINE MAINTENANCE

The enclosures described above reduce the labor involved in maintenance as substrate can be easily rinsed through with fresh water. A keeper's routine should involve the following:

- **Misting:** 1-2 times daily with fresh water; either an automated misting system or manual misting is sufficient.
- **Cleaning cages:**
  - Rinse enclosure thoroughly and change water completely 2-3 times per week to remove nitrogenous wastes.
  - Rinse filter media weekly (don't change media completely unless too clogged to filter due to the beneficial bioload).
  - Dismantling entire cage, disinfecting, and replacing moss substrate and organic decorations every 4-6 months.

## FOOD

Stomach contents of wild adults indicate an assorted diet, including a wide variety of arthropods. Even wild-caught captive adult frogs readily feed on standard captive insects: they readily take flightless fruit flies (*Drosophila melanogaster* or the larger *D. hydei*), gut-loaded 2-week crickets (*Acheta domesticus*), larval and adult flour beetles (*Tribolium* sp.), and they relish termites (Isoptera). Springtails (*Collembola* sp.) or porch/field sweepings can be collected as alternative supplements to the standard fare. Some institutions have also reported offering waxworms (*Galleria mellonella*) every few weeks. Variety may be important for providing all necessary nutrients and producing animals robust enough to handle breeding. Food items offered should never exceed the size of a 2 week cricket (1/2" in length). Although the offered food can be broadcast throughout the enclosure, the volume offered per frog should be roughly 1/4 tsp. per frog 3-5 times per week.

As a standard practice, every feeding should be dusted with a vitamin-mineral supplement as it can be brushed off most food items in a short amount of time once placed into an enclosure and all food is not consumed immediately. It is also important to gut-load crickets for at least 24 hours prior to feeding with commercially available cricket diet (Zeigler®, etc.) and fresh vegetables high in calcium (sweet potatoes



and endive, for example) [Zeigler Brothers Inc.®, Gardners, PA]. Examples of various vitamin-mineral supplements used by facilities breeding PGFs:

- Mix of <sup>1</sup>Nekton® Rep™, <sup>1</sup>Nekton® MSA™, and <sup>2</sup>Rep-Cal® D™ each 1/3 by weight (Baltimore Zoo)
- <sup>3</sup>Walkabout Farms Insectivore and Pinhead Supplement (NAIB)
- <sup>2</sup>Herptivite™ and ground calcium carbonate (Denver Zoo)
- <sup>4</sup>Reptimin® (Cleveland Metroparks Zoo).

(<sup>1</sup>Gunter-Enderle Enterprises Inc. NEKTON®-PRODUKTE, Clearwater, FL; <sup>2</sup>Rep-Cal® Research Labs, Los Gatos, CA; <sup>3</sup>Rock Solid Herpetoculture; <sup>4</sup>Tetra/Spectrum Brands, Inc., Blacksburg, VA)

## HEALTH CARE

### MEDICAL CARE

Beyond the health assessments performed when new animals arrive into quarantine, a proactive health care plan is important in all captive animal collections. Routine health care performed by veterinary specialists and husbandry staff should include the following:

- Weighing valuable specimens periodically for baseline information and health monitoring. Baseline *Atelopus* weights-

Avg. adult male:

8-12g for Locality A & C populations

3-5g for Locality B population

Avg. adult female:

10-15g for Locality A & C populations

4-7g for Locality B population

- Bi-annual testing to monitor parasite loads.
- Worming: We recommend worming wild individuals only to reduce excessive loads in order to maintain natural gut flora species in the event that reintroduction becomes necessary or a symbiotic relationship exists with gut flora. Captive-produced offspring will not have the same parasites as wild specimens, so worming can be performed with the approach that neither animal nor enclosure will ever remain parasite free, however load levels can be reduced.
- Isolation and treatment of thin, ill, or injured specimens
- Any animals that dies in captivity should be sent for thorough histopathology from a qualified pathology lab as part of a necropsy (if you need a suggestion, Northwest ZooPath Laboratory of Monroe, WA is proficient with amphibian pathology).

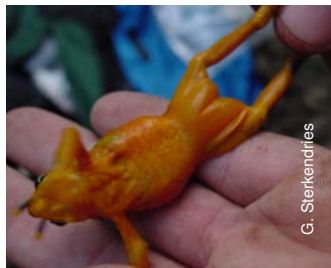
### REPORTED MEDICAL ISSUES IN CAPTIVITY

Since this is the first opportunity for most AZA institutions to work with an *Atelopus* sp., it is expected that there would be many losses at holding institutions as they work out the husbandry of these unusual frogs. There have been various degrees of success and failure with F1s at the holding institutions. Some have lost all of their frogs, while others have lost only a few, and some have lost none. There have been some common medical issues at multiple institutions housing golden frogs over the past years and the brief presentation of these issues is just a starting point for conversations with your facilities' health care and husbandry staff.

- **Chytrid** has been reported in three collections containing PGFs. In all cases it has come from other species in same area of building. It has been an issue in captive collections, especially in walk-through public exhibits since disease control is practically impossible. With the development of rapid-testing techniques, chytrid testing can be done in most diagnostic labs these days. If a specimen in your collection is chytrid (+), isolate the animal (or entire enclosure) and begin the procedure for itraconazole baths on the animal and disinfection protocols on all equipment, enclosures, and cage materials (see Quarantine section and Appendix III for details on treatment and disinfection).
- **Trauma** is a risk with any species. In addition to typical nose rubs cleared with topical medicated ointment, there was an incident of a bleeding eye possibly due to predation from a cockroach

(Houston Zoo) in a PGF exhibit. One case of hyphema (blood under the lens of one eye) from an unknown trauma was also reported. The vet was able to seal the eye shut with liquid surgical glue for a few weeks while the eye healed fine. The glue was shed off with the skin every few days, and more glue was reapplied.

- **Vitamin A deficiencies** may have been the cause for some captive offspring being unable to catch and hold onto their food items. Commonly referred to as “short-tongue syndrome,” it is a squamous metaplasia (loss of mucous glands) of the tongue that was first identified in Wyoming toads. Treatment involves increasing the Vitamin A supplement levels (hepatic retinol).
- **Cutaneous nematodiasis** was diagnosed from dorsal skin lesions in six specimens in separate enclosures at NAIB in 2004-2005. The nematode has not yet been speciated nor origin identified. The disease has only been seen in *A. zeteki* within the collection. Animals were diagnosed using cytology and/or histology. The initial three cases were found dead or died rapidly. However, antibiotics, deworming, and wound treatment cleared three other cases. The lesions initially presented as a pale blanching on the dorsal aspect of the frogs and the skin was easily removed demonstrating an open ulceration. Preventative husbandry management includes more frequent tank break-downs and routine deworming. Animals with skin nematodes did not necessarily have evidence of intestinal nematodes.
- Skin sensitivity to an irritating substance, toxin, or virus that seems to cause **excessive skin sloughing & redness in animals that test chytrid (-)**. These golden frogs have had necrosis in the glands on their skin (interstitial cells). Recommended treatment regimes should include isolating the infected frogs, keeping the environment as clean as possible to minimize secondary bacterial infections, and soaking with Amphibian Ringers Solution (ARS).
- **“Baggy pants” syndrome** refers to the fluid filled area around thighs and vent of frogs, and is common in both male and female PGFs housed in stream systems and high humidity environments. It is not actually a medical concern as it occurs in wild specimens found in breeding streams, as well as captive *Atelopus*. When PGFs were first brought into captive situations, it was treated by draining the excess fluid off of the vent area, but reoccurred quickly. In collections where the frogs were moved to a drier environment, the condition improved, which may be important to maintain kidney health.



“Baggy Pants” edema in a wild specimen.

- A few specimens were slightly lethargic and legitimately had **edema** associated with kidney failure (full body edema, not localized in vent area), but this was not as common as just having baggy pants. A possible cause of this in otherwise apparently healthy animals is the use of improperly reconstituted RO water or other poor water quality issues, causing the pure or low-level ionized water to move into and dilute the highly ionized tissues within the animal’s body via osmosis. The body’s attempts to remove the excess water in the tissues puts a lot of strain on the kidneys causing failure and potentially death if the water quality issue isn’t corrected.
- Several institutions have reported cases of paralysis in golden frogs. Typically, frogs are found with **full hind limb extension and whole body rigidity (tetany)**. The following list of potential factors are probable:
  - Upper thermal limit exceeded: The animals were possibly stressed when an air conditioning unit failed at one facility. All recovered when cooled appropriately.
  - Dietary Imbalance – Calcium or Vitamin A? Confirm that the Ca:P balance in the diet and supplements are adequate, or else kidney failure may occur over time. Central Park Zoo had

reported calcified kidneys from necropsied PGFs (cases associated with tetany and edema) as the calcium had precipitated into the kidneys due to an imbalance. Dietary or environmental causes have not been concluded. See other issues with calcium below.

- Toxicity (chemical or pathogenic):
  - *Case 1:* 24 golden frog offspring awaiting shipping were temporarily maintained overnight in a crowded 5-gallon isolette due to an unexpected delay with the airlines. All animals appeared fine at 24 hours, but 5 PGFs were found in a rigid state, with the hind limbs fully extended at 28 hours. The animals were immediately placed under running filtered fresh water from a hose and rinsed well over a 1-2 hour time period, in which all recovered fully. Potentially toxic steroids, bufadienolides (and possibly, but doubtfully zetekitoxin) secreted from the skin of captive raised *Atelopus* caused a reaction (Daly, pers. comm.). Ingestion of plants containing bufadienolides, including *Kalanchoe* sp., foxglove, oleander, lily-of-the-valley, and various species of milkweed has been responsible for cardiac and neurological symptoms, including tetany in canines.
  - *Case 2:* Overdosing PGFs with a 10x the recommended dose of itraconazole due to a dilution mistake caused frogs to go into a catatonic state within 3 minutes. The animals recovered a few hours later.
  - *Case 3:* Exposure to unexpected chemicals or toxins produced from fungal/viral pathogens on cage materials and/or skin may also be a potential cause of paralysis. The Baltimore Zoo experienced an epidemic of skin sloughing, accompanied by tetany in some cases. Fungal dermatitis was cultured from sacrificed specimens, and it is believed that toxins released by the fungus were responsible for the tetany. Recommended supportive care for the frogs along with complete steam sterilization of cages and enclosure materials stopped the outbreak.
- Water quality issues – In collections where these episodes of tetany occur, many factors, including water quality, have been investigated. The Detroit Zoo noticed an increase in phosphorus in their city source water (1-2ppm) which is used for corrosion control to bind lead in cities with old pipes. Detroit Zoo switched water source to reconstituted RO water, began adding commercially available phosphate binding crystals/resins to the water, and ceased gut loading crickets with phosphorus-rich Zeigler's cricket diet, which stopped the cases from occurring, and when these procedures were discontinued, the symptoms returned (DZI staff is further studying these effects to identify the causative agent and degrees of impact). It is believed that these animals are experiencing tetany due to a phosphate replacing calcium in the metabolic pathway, as can occur in captive fish. It is recommended that phosphate sponges or resins are used to eliminate excess.

Staff at the Detroit Zoo noticed a **pre-tetany posturing**, characterized by animals that *hold their back legs closer to the body than normal with their hind legs, feet, and knees slightly elevated often resulting in hind feet off the ground, ankles that overlap and rest on the lower back* as opposed to ankles just touching and resting on the ground in healthy animals. The toes may be curled a bit, but not always. Pre-tetanous animals also have difficulty self-righting when flipped onto their backs, however healthy animals have been shown to have difficulty with this when repeated multiple times or stressed (M. Whitney and E. Sonntag, pers. comm).



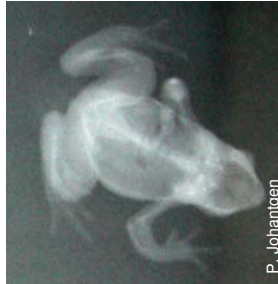
*Curly toe and unnatural hind leg position (resting on lower back)*



*Full hind limb extension associated with tetany.*

Recommended care for cases of paralysis includes isolating the animal, immediately soaking with a buffered Amphibian Ringers Solution (ARS), and having a veterinarian treat with an oral calcium supplement. Also using antibiotics if it is due to pathogenic toxicity. To prevent outbreaks, be careful to sterilize cage materials prior to use; keep full-spectrum lighting levels high; and test water quality regularly, including phosphorus and bacteria.

- **Metabolic Bone Disease** was evident in the curved long bones and rubbery limbs documented in juvenile or recent metamorphs at three facilities. It is due to insufficient calcium absorption, which can be improved by increasing full-spectrum lighting and improving the volume and frequency of vitamin dusting/gut loading food items to increase calcium absorbed in the diet.



Radiograph – curved leg bones.



Deformity from MBD (knees straight out).

## REPRODUCTION

Captive PGF breedings are based on their natural *in situ* reproductive cycles and behavior. From field research, it appears that eggless female frogs move into the forests in the late dry or early rainy season (February-March). Females full of eggs typically return to the streams in the late rainy season and early dry season (November-December). Males tend to stay near the streams year-round awaiting the females return, hence the need for established territories. Male PGFs can be observed challenging intruding males for prime sites along breeding streams. Some males may amplex returning females in the forest, but all females encountered at the stream are amplexed. There is a gender bias in favor of males and the majority of males are therefore single.

Amplexus can last from a few days up to 2 months, with males probably not feeding during this time and females can become exhausted carrying around the extra weight of the male. The amplexant pair search for a suitable underwater, darkened rock crevice to lay eggs. Preferred sites have good water circulation to aerate the eggs that are arranged in strings. The 200-900 white eggs are light-sensitive, thus the need to lay them underneath a darkened rock. Hatching begins in 2-6 days and the tadpoles graze on diatoms (golden-brown algae) on the surface of rocks while adhering to the rocks in the swiftly moving water by utilizing their suctorial ventral disc. They have been observed climbing up rock surfaces into elevated pools while feeding.

The tadpoles metamorphose into green and black froglets within 120-240 days. Although a few have been found associated with the streams, it is believed that the juveniles also move into the forests for foraging.



A recent captive-bred *Atelopus zeteki* metamorph.

## POPULATION MANAGEMENT

The primary goal of our breeding endeavors has been to breed unrepresented potential founders at our **Primary Breeding Facilities** (facilities that received wild-caught animals). Per our Small Population

Management (SPMag) Advisor, it is recommended to breed unrepresented wild caught specimens only to each other to maximize genetic potential in the overall captive population, minimizing overall gene loss. Further, each F1 bloodline will be bred to another F1 bloodline of the same population (Locality **A**, **B**, or **C**) and not bred back to a wild-caught animal for the same reason.

The next step was to place offspring from one unique bloodline at other AZA institutions for housing (**Phase I Facility**). Once an institution had become proficient at PGF husbandry and was able to successfully breed and raise metamorphs, that facility would be designated to receive a second (or more) bloodline(s) from the same population for designated valuable breedings (**Phase II Facility**).

Long-term captive management plan is to maintain 30-50 frogs from each bloodline. The Population Management Plan (PMP) is still in process as of this writing.

If you have questions about which animals should be bred, contact the Studbook/Population Manager (see Appendix I). Basic recommendations are as follows:

- Phase I Facilities: If all of your animals are from the same bloodline (sibling group), you should practice breeding PGFs in order to become more proficient at cycling and rearing PGFs. It is not as easy as some other anuran species, so do not become discouraged if the first attempt is not successful. These offspring will be undesirables and may displace other more valuable offspring from a desirable breeding, so euthanasia will be necessary to eliminate, or at least reduce their numbers. Be prepared to house offspring indefinitely if allowed to survive.
- Phase II Facilities: If you have two different bloodlines, then it is recommended that pairs represent each bloodline to maximize genetic diversity. Offspring from desired pairings may be sent on to other facilities, but be prepared to maintain desired numbers indefinitely.
- Over-represented females or egg-heavy females can be paired randomly and allowed to dump eggs, which should be discarded. As the female F1s began reaching sexual maturity (2-3 years), we lost a few animals that were not able to spawn successfully. It is recommending that institutions set these animals up in breeding situations and dispose of any eggs that are laid or practice rearing techniques and cull as a metamorph.

## **CAPTIVE REPRODUCTION METHODS**

### **PREPARATIONS**

- Preparation for the breeding season begins 1-2 months in advance by setting up clean “breeding tanks” with suitable underwater egg-laying sites and allowing the tank to condition (i.e., establishing healthy water quality and natural algae growth). You will need one tank per recommended breeding pair for ease of assigning sire/dam and since these are the enclosures in which the eggs and tadpoles are to be raised.
- In preparation for rearing offspring, establish 5-20 springtail (*Collembola* sp.) colonies several months in advance for feeding new metamorphs since fruit flies and pinheads are too large. Also consider increasing the number of fruit fly jars (flightless *Drosophila melanogaster*) your facility maintains by 6-12 jars weekly per breeding pair for when the offspring are large enough.
- Find out your institution’s policies and procedures on culling (euthanasia for population management purposes). Euthanasia as a management tool has been approved by the USFWS. Due to the number of eggs laid and froglets produced, it may be necessary to eliminate individuals at the tadpole or metamorph stage. Consult your medical department in advance, if needed. An overdose of the anesthesia tricaine methanesulfonate (Finquel MS222) has been used successfully on PGFs for this purpose (Argent Chemical Laboratories, Inc., Redmond, WA)
- Acquire food for the tadpoles - Sera-micron® and/or ground Hikari Algae Wafers® and/or consider setting up a diatom culture (methods described by Edi Sonntag in Appendix VII).



## CYCLING

- In captivity, pairs may go into amplexus at anytime of the year, however it is recommended to **target November-December for pair introductions**. For ease, rotate introduced pairs into stream breeding tanks from smaller holding enclosures.
- Pairs tend to go into amplexus almost immediately (1-2 days) after being introduced. Pairs that fail to amplex immediately tend to never spawn and should be re-paired if possible, to take advantage of their breeding condition.
- Monitor the pairs closely during amplexus for healthy body condition. Separate and feed heavily for a few days if it appears that either is wasting.
- It has been shown that it is not necessary to manipulate any environmental parameters (temperature, lighting, humidity, etc.) for PGFs to breed in captivity.

## SPAWNING/EGGS

- Spawning tends to take place overnight. Successfully introduced pairs spawn typically within a 15-30 days of pairing. On average, wild-amplexed frogs took 2-17 days to spawn after being placed into the breeding tanks (if the pair spawned at all), however it is unknown how long they were amplexed prior to collection.
- If you find the pair separated, it is most likely that they have spawned under a rock. Do not move rocks to locate eggs or you risk crushing them. Be patient!
- **Do not remove the eggs to another tank for rearing.** It is preferred to leave them in the well-cycled breeding tank.
- Once the adults have spawned, remove them from the reproduction enclosure so that they do not disturb eggs or eat metamorphs once they develop.
- Typically, a single strand of 200-400 white (or off-white) eggs are laid that take 3-7days to hatch. Infertile eggs show no signs of development and may appear fuzzy (dispose of them after all other eggs hatch).

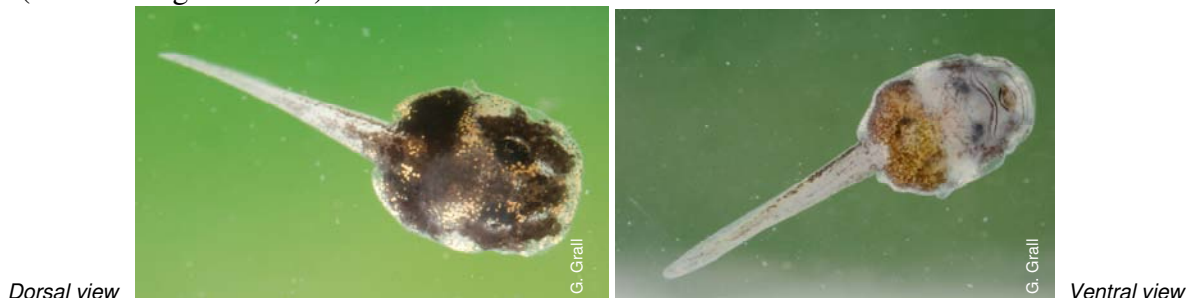


*Strings of white Atelopus eggs at the Detroit Zoo.*

- The eggs are light sensitive, so shield from direct light if exposed and avoid photography!
- To provide a larger volume to stabilize water quality and to increase the surface area for tadpoles to graze upon, consider raising the water level, if possible, once the adults have been removed from the breeding tank.
- During routine maintenance (water changes, etc...), be very careful not to disturb eggs.
- Even if you are not prepared to rear all of the offspring or if the breeding is just for the experience, avoid disposing of a portion of the eggs at this point. Hatch success varies, especially for novice breeding efforts, so it is recommended that you wait to dispose tadpoles if a portion of the breeding is to be culled. As your confidence in successfully rearing multiple clutches of PGFs increases, it may be easier to dispose eggs rather than tadpoles or froglets (consult the studbook holder or Vicky Poole before making this step). [Be aware that you may be risking your overall chance of breeding success when disposing eggs, as you may inadvertently select infertile eggs or eggs that are not as healthy as others. Eggs may be removed and placed into a strong bleach solution for disposal.]

## LARVAL HUSBANDRY/METAMORPHOSIS

- Tadpoles are entirely white for the first few days after hatching, and then they develop pigmentation (black with gold flecks).



- They graze on rock surfaces and tank walls, consuming diatoms (golden-brown algae) if available. They should also be offered supplemental foods daily since the diatoms in most tanks are not sufficient. As they are grazers, it is necessary to adhere offered food to the surface of rocks, Petri dishes, tank walls, etc., placed in a location that the tadpoles will encounter it. A paste of the preferred tadpole foods, <sup>1</sup>Sera-micron® and/or ground <sup>2</sup>Hikari® Algae Wafers™, can be made and smeared onto tank rocks or the tank surface directly using a syringe, or onto a Petri dish, rock, or other solid surface, left to dry, and placed with the tank once dry (overnight works well). [<sup>1</sup>Sera Laboratory, Heinsberg, Germany; <sup>2</sup>Hikari® Sales USA, Hayward, CA]
- In our initial year we even collected rocks from a clean, local stream to provide adequate food diversity. This proved to be unnecessary, yet can be a source for diatoms if needed.
- Maintain water temperatures between 72-74°F as tadpoles housed below 70°F do not feed well and waste away.
- Good water quality is very important for tadpoles, and water changes/filter rinses should be maintained. Be careful of the small tadpoles and try to remove as much uneaten food as possible. If there is uneaten food left in the gravel on a regular basis, reduce the amount offered to encourage the tadpoles to forage within the gravel and improve water quality.
- If the Dissolved Oxygen (DO) is lower than 5mg/L, it may be necessary to add an airstone or two to increase the oxygen level in the water.
- If there is an unexpected loss of tadpoles, check water quality, especially phosphates. A phosphate binding resin may be necessary to prevent the loss of additional tadpoles.
- If you are not prepared to rear all of the offspring or if the breeding is just for the experience, it is a good idea to dispose of a portion of the tadpoles at this point (up to 50%). Do be aware that you may be risking your overall chance of breeding success as you may inadvertently select individuals that are not as healthy as others. Selected tadpoles should be removed from the breeding tank and euthanized by an overdose of tricaine methanesulfonate (Finquel MS222) [Argent Chemical Laboratories, Inc., Redmond, WA].
- It will take roughly 75-100 days to see the first metamorph and as long as 150-265 days until the final tadpole metamorphs.
- SVL upon metamorphosis: ¼" (6mm)

## JUVENILES

- Juvenile care can be difficult based on the need for successful springtail colonies or alternate small food items. It is helpful to heavily seed the moss substrate within the breeding tank with springtail colonies in advance (while there are still tadpoles in the tank) as a constant source of food.
- Offer *ad libitum* springtails, fruit flies, or pinheads daily (volume offered increasing with population density and as the size of the frogs increase). Dust all pinheads and fruit flies with the same vitamin supplements as offered to adult PGFs in order to help minimize MBD issues.
- As the frogs grow, they can be offered larger food items.

- Enclosures housing juveniles should be misted more often than the adults to prevent desiccation of small froglets. It is recommended at least twice daily with fresh dechlorinated water. Relative Humidity should be between 80-100% and air temperatures should be maintained between 72-75°F.
- Mist and rinse more frequently to keep cages more humid and cleaner.
- Caution should be used when opening tanks as froglets climb glass sides of tanks and may congregate in the upper corners of tanks.
- NOTE: It is acceptable to accession juveniles into ISIS as a group, as long as each breeding is designated by its own group number in order to track parentage.
- If animals are to be euthanized, it is recommended to wait until the offspring are at least 6 months post metamorphosis due to the difficulties of getting PGF froglets to feed sufficiently and survive this problematic time period. Euthanizing frogs immediately after metamorphosis may instill a false confidence in an institution's abilities to rear PGFs through to adulthood.
- Juvenile and subadult animals can be housed together in mixed sex groups for the first one or two years, however be aware that they may need to be separated by sex as they grow and become sexually mature.

## **SURPLUS SPECIMENS, CAPACITY BUILDING, AND REINTRODUCTIONS**

PGF/PRD's primary goal is to produce genetically valuable specimens and place them at other AZA institutions, however there will always be a surplus of offspring. It will be necessary to maintain a certain number of every PGF bloodline in order to have as great a genetic diversity as possible so that valuable genes are not lost, especially once the species goes extinct in the wild.

The USFWS has granted the permission to use euthanasia as a population management tool, so excessive numbers of offspring do not need to be produced once a facility is comfortable with their PGF reproduction methods.

Some animals' offspring may be placed at other institutions in accordance with recommendations made by the Studbook Keeper/Population Manager (see Appendix I for contact information).

One of PGF/PRD's original goals was to relieve collection pressure for Panamanian "zoos" and hotel displays by providing over-represented specimens to captive situations in Panama. Creating breeding and holding centers within range countries is referred to as "capacity building," and is a valuable conservation tool due to the decreased costs and limiting health risks. The ideal situation is to empower the range country to be able to sustainably manage their own species. However, providing long-term stable and skilled husbandry staff and population management within range country will be the challenge.

Our current efforts to establish one or more captive centers and possibly a preserve in Panama will soon reach fruition. The Houston Zoo has partnered with the El Nispero Zoo in El Valle to build, staff, train, and support the El Valle Amphibian Conservation Center (EVACC), and is also soliciting a similar partnership with the Summit Zoo in Panama City. Other initiatives are being pursued by the Denver Zoo and the Cleveland Metroparks Zoo. **Institutions can help by offering financial support, construction assistance, equipment, and/or staff training and funding to the EVACC (through PGF/PRD) or for another center in Panama.**

There is also the development of another amphibian initiative within Panama. The Amphibian Recovery and Conservation Coalition (ARCC), initiated by Zoo Atlanta and the Atlanta Botanical Gardens, focuses on Panamanian amphibian species *other than PGFs* at risk to extinction due to the coming chytrid epizootic. Comparable to PGF/PRD, they are also working with the EVACC to house animals from their rescue efforts.

One last, yet long-term possibility for surplus PGFs, would be the return of golden frogs to the wild should all *in situ* populations become extinct and the chytrid fungus no longer a threat to survival. Animals would



be released following strict health guidelines, of course. The likelihood of this occurring is low, yet always a possibility when a species is brought into captivity as a last measure for survival.

## MISCELLANEOUS

- How do I get frogs for my institution?



We are always looking for facilities to house and exhibit golden frogs; however, **if you are not affiliated with an institution accredited by the Association of Zoos and Aquariums (AZA), then you will not be able to receive frogs** due to their conservation status and the restrictions on our permits. This is to protect the frogs by not providing a conduit for them to make it into the pet trade, thereby increasing collection pressure on wild animals.

If you are an AZA facility, please consider how much space you can allot for the frogs and contact the PGF studbook/PMP holder or Vicky Poole ([vpooles@aqua.org](mailto:vpooles@aqua.org)) for other husbandry questions. Contact information is available in Appendix I.

- ATAG Recommendations  
Prior to PGF/PRD's efforts, the Amphibian Taxon Advisory Group (ATAG) 1999 Regional Collection Plan lists *Atelopus zeteki* as a Priority 4 – Phase-in Population. Now that golden frogs have been imported into AZA institutions, the taxon has been upgraded to a Priority 1 – PMP. PGF has been a priority project on the ATAG's action plan.
- PGF/PRD's Field Recommendations to Minimize the Spread of Chytrid  
As pathogens can last a long time on field clothing and equipment, we are quite diligent about scrubbing off all soil and disinfecting all field equipment used in this project with 10% bleach or 10% ammonia solution, and completely drying equipment between uses. In addition, we always work in an east to west pattern to prevent the spread of chytrid. For more field disinfection options, see Appendix III.

For more information on chytrid and other amphibian disease links, we recommend the following:

[http://dendroworld.co.uk/BDGarchive/chytrid\\_fungus.html](http://dendroworld.co.uk/BDGarchive/chytrid_fungus.html) - treatment of chytrid fungus  
<http://coloherp.org/cb-news/Vol-28/cbn-0111/Chytridiomycosis.html> - another article on chytrid treatment  
<http://www.jcu.edu.au/school/phtm/PHTM/frogs/adms/scope.htm> - strategies to control amphibian diseases  
<http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm> - up to date global distribution of chytrid  
<http://www.jcu.edu.au/school/phtm/PHTM/frogs/chpr1/longcore.htm> - description of *B.d.*  
<http://www.cdc.gov/ncidod/EID/vol10no12/03-0804.htm> - origin of the amphibian chytrid fungus

- Website and PGF/PRD Listserve  
Like all modern organizations, we have developed a website, which is maintained through the Denver Zoo. The site is bilingual (English/Spanish) in order to share information with the range countries of *Atelopus* frogs and the addresses are as follows:

[www.projectgoldenfrog.org](http://www.projectgoldenfrog.org)  
[www.proyectoranadorada.org](http://www.proyectoranadorada.org)  
[www.ranadorada.org](http://www.ranadorada.org)

Also, it is recommended that supervisors, staff working with golden frogs, and appropriate researchers join the AZA *zeteki* listserve in order to stay current with husbandry information and field efforts. Please contact Pete Johantgen at the Columbus Zoo to join ([Pete.Johantgen@columbuszoo.org](mailto:Pete.Johantgen@columbuszoo.org)).

- Field Opportunities  
If you or a staff member at your institution is interested in participating in PGF/PRD field efforts, please contact Kevin Zippel or Rick Haeffner for upcoming trip information.

- Fundraising

Fundraising efforts for PGF/PRD's activities have been in place since it's inception as Financial Support was one of our four main goals, along with Field Studies, Captive Management, and Education. Most of the efforts to date have been funded by grants. The balance of personnel costs and time has been covered by individuals or their institutions. Current fundraising efforts include grants applied for by PGF/PRD directors or coordinators, donations, and t-shirt/cap sales. Donations can be made on-line at the [www.projectgoldenfrog.org](http://www.projectgoldenfrog.org) website or made payable to "Project Golden Frog" and sent to the Denver Zoological Gardens (address listed in Appendix I).

- Museum specimens

As captive-bred, surplus PGFs were culled, we preserved specimens (entire or skinned) by various methods including 10% buffered formalin, ethanol, or methanol to make them available to researchers and museum collections. To date, PGF/PRD has provided preserved specimens to the following institutions: American Museum of Natural History (NY), The Field Museum (Chicago, IL), Museum of Zoology (Ann Arbor, MI), Museum of Comparative Zoology (Cambridge, MA), and The National Museum of Natural History (DC). Surplus preserved specimens with permits can be made available to accredited museum collections only by contacting Erik Lindquist (address listed in Appendix I).

Second Edition by Vicky Poole, National Aquarium in Baltimore

First edition by Kevin Zippel, PhD, National Amphibian Conservation Center, Detroit Zoological Institution

Thanks to the folks at 11 institutions that took time in their busy schedules to fill out and return the husbandry surveys I sent out in 2005, and those who've answered my inquiries and given input since. My appreciation also goes out to Eli Bryant-Cavazos, Meredith Whitney, Bill Flannagan, Edi Sonntag, Kathy Duffy, Chris Tabaka, Leigh Clayton, and John Daly for answering all of the last minute questions that needed their valuable input and to the PGF photographers for the use of their pictures. Thanks to Karen St. John and Holli Friedland for editing advice. My undying gratitude belongs to Rick W. A. Haeffner for stepping up and helping out with PGF/PRD when all the pieces were falling apart. And finally, special thanks to Kevin Zippel for all of his help, advice, data, general comments, and friendship while allowing me to bastardize his original edition of this document. ☺

-V

## APPENDIX I – Project Golden Frog/Proyecto Rana Dorada Contacts



### DIRECTORS:

### MANAGERS:

|                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                             |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
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| Vicky Poole, <b>PGF Coordinator</b><br>National Aquarium in Baltimore<br>Pier 3/501 E. Pratt St.<br>Baltimore, MD 21202<br>410-576-1193<br><a href="mailto:vpooles@aqua.org">vpooles@aqua.org</a>                                                             | Pete Johantgen – <b>Zeteki Listserve Manager</b><br>Columbus Zoo and Aquarium<br>PO Box 400<br>Powell, OH 43065-0400<br>614-645-3446<br><a href="mailto:Pete.Johantgen@columbuszoo.org">Pete.Johantgen@columbuszoo.org</a>                  |
| Roberto Ibáñez, <b>Field Research – Panama Coordinator</b><br>Smithsonian Tropical Research Institute<br>Attn: Roberto Ibáñez/Tupper<br>Unit 0948<br>APO AA, 34002-0948<br>011-507-212-8302<br><a href="mailto:ibanezr@tivolisi.edu">ibanezr@tivolisi.edu</a> | Paul Crump, <b>Liaison for EVACC</b><br>Houston Zoo, Inc.<br>15134 N. MacGregor Dr.<br>Houston, TX 77030<br>713-533-6500<br><a href="mailto:pcrump@houstonzoo.org">pcrump@houstonzoo.org</a>                                                |
| Erik Lindquist <b>Field Research – US Coordinator</b><br>Messiah College<br>One College Ave.<br>Grantham, PA 17027<br>717-766-2511 x2044<br><a href="mailto:quist@messiah.edu">quist@messiah.edu</a>                                                          | Alex Saunders – <b>Webmaster</b><br>Denver Zoological Gardens<br>2300 Steele St.<br>Denver, CO 80205-4899<br><a href="mailto:asaunders@denverzoo.org">asaunders@denverzoo.org</a>                                                           |
|                                                                                                                                                                                                                                                               | Corinne Richards, <b>In situ Population Researcher</b><br>University of Michigan/Museum of Zoology<br><a href="mailto:clrichar@umich.edu">clrichar@umich.edu</a>                                                                            |
|                                                                                                                                                                                                                                                               | César Jaramillo, <b>Population Geneticist</b><br>University of Panama<br>011-507-212-8729                                                                                                                                                   |
|                                                                                                                                                                                                                                                               | VACANT - <b>Veterinary Advisor</b>                                                                                                                                                                                                          |
|                                                                                                                                                                                                                                                               | VACANT - <b>Education Liaison</b>                                                                                                                                                                                                           |

## **APPENDIX II – Captive Population statistics as of March 2006.**

For a rough idea of the number of specimens...

- Number of living wild-caught in US institutions –
  - 9.8 Locality **B** at MZB and National Zoo
  - 3.4 Locality **A** at DZI (2.0) and MZB (1.4)
  - 10.6 Locality **C** at DZI and Cleveland Zoo
- Number of total founders/potential founders collected to date:
  - 12.15.5 collected at Locality **A**
  - 9.9 collected at Locality **B**
  - 14.15.27 collected at Locality **C**
- Number of bloodlines represented: 2121 living offspring representing 28 bloodlines
- Number of F1 offspring currently –
  - 417 Locality **B** F1s from 6 bloodlines at 2 institutions
  - 889 Locality **A** F1s from 19 bloodlines at 27 institutions
  - 815 Locality **C** F1s from 3 bloodlines at 2+ institutions
- Number of institutions holding PGFs (of any population) – 27+ (2161 specimens total) per MZB
- List of institutions producing offspring as of 2006 (inbred or not):
  - MZB
  - Detroit Zoo
  - Denver Zoo
  - San Diego
  - Cleveland Zoo
  - NAIB
  - National Zoo
- Number of new institutions awaiting offspring – 11 (8 new to PGF/PRD)

## APPENDIX III – Recommended Protocols for Disinfection of Chytrid on Surfaces

Table 1: Disinfection strategies suitable for killing *Batrachochytrium dendrobatidis* and ranaviruses in field studies. Where concentrations and time are given, these are minimum shown to be effective. For *B. dendrobatidis* based on Berger (2001) and Johnson et al (2003) and for ranaviruses on Langdon (1989) and Miocevic et al (1993).

| Purpose                                                             | Disinfection                       | Concentration   | Time               | Pathogen killed                        |
|---------------------------------------------------------------------|------------------------------------|-----------------|--------------------|----------------------------------------|
| Disinfecting surgical equipment and other instruments (e.g. scales) | Ethanol                            | 70%             | 1 min              | <i>B. dendrobatidis</i><br>Ranaviruses |
|                                                                     | Vircon                             | 1 mg/ml         | 1 min              | <i>B. dendrobatidis</i><br>Ranaviruses |
|                                                                     | Benzalkonium chloride              | 1 mg/ml         | 1 min              | <i>B. dendrobatidis</i>                |
| Disinfecting collection equipment and containers                    | Sodium hypochlorite (bleach)       | 1%              | 1 min              | <i>B. dendrobatidis</i>                |
|                                                                     | Sodium hypochlorite (bleach)       | 4%              | 15 min             | Ranaviruses                            |
|                                                                     | Didecyl dimethyl ammonium chloride | 1:1000 dilution | 0.5 min            | <i>B. dendrobatidis</i>                |
|                                                                     | Complete drying                    |                 | 3 hours or greater | <i>B. dendrobatidis</i>                |
|                                                                     | Heat                               | 60 C            | 5 min              | <i>B. dendrobatidis</i>                |
|                                                                     |                                    |                 | 15 min             | Ranaviruses                            |
|                                                                     | Heat                               | 37 C            | 4 hours            | <i>B. dendrobatidis</i>                |
| Disinfecting footwear                                               | Sterilizing UV light               |                 | 1 min              | Ranaviruses only                       |
|                                                                     | Sodium hypochlorite (bleach)       | 1%              | 1 min              | <i>B. dendrobatidis</i>                |
|                                                                     | Sodium hypochlorite (bleach)       | 4%              | 15 min             | Ranaviruses                            |
|                                                                     | Didecyl dimethyl ammonium chloride | 1:1000 dilution | 1 min              | <i>B. dendrobatidis</i>                |
| Disinfecting cloth (e.g. bags, clothes)                             | Complete drying                    |                 | 3 hours or greater | <i>B. dendrobatidis</i>                |
|                                                                     | Hot wash                           | 60 C or greater | 5 min              | <i>B. dendrobatidis</i>                |
|                                                                     |                                    |                 | 15 min             | Ranaviruses                            |

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### HYGIENE PROTOCOL FOR HANDLING AMPHIBIANS IN FIELD STUDIES

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8 October 2004

## APPENDIX IV – Water Quality Data Collected in PGF Habitat

|                              |               |               |               |               |               |               |
|------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Locality                     | <b>A</b>      | <b>D</b>      | <b>D</b>      | <b>E</b>      | <b>E</b>      | <b>E</b>      |
| Elevation (above sea level)  | 2550'         | 2200'         | 2200'         | 2000'         | 2000'         | 2000'         |
| Site Number                  | <b>Site 1</b> | <b>Site 1</b> | <b>Site 2</b> | <b>Site 1</b> | <b>Site 2</b> | <b>Site 3</b> |
| Date                         | 10/20/99      | 10/23/99      | 10/24/99      | 10/19/06      | 10/19/06      | 10/19/06      |
| Time                         | 12:00 PM      | 10:30 AM      | 11:30 AM      | 1:00 PM       | 2:30 PM       | 4:30 PM       |
|                              |               |               |               |               |               |               |
| Temp                         | 22.5 C        | 21.1 C        | 21.1 C        | 21.6 C        | 21.8 C        | 21.7 C        |
| pH                           | 7.6           | 7.5           | 8.5           | 6.8           | 6.5           | 6.9           |
| Dissolved Oxygen - DO (mg/L) | 9.06          | 8.6           | 8.6           | 8.41          | 8.37-8.68     | 8.79          |
| Ammonia                      | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         |
| Nitrite                      | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         |
| Nitrate                      | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         |
| Conductivity (microsiemens)  | 59            | 86            | 62            | 49            | 47            |               |
| General Hardness - GH        | 0-1 degree    | 1 degree      | 2 degree      | 0-1 degree    | 0-1 degree    | 0-1 degree    |
| Carbonate Hardness - KH      | 1 degree      | 0-2 degree    | 0-2 degree    | 0-1 degree    | 0-1 degree    | 0-1 degree    |
| Iron                         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         |
| Phosphate                    | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         | 0 ppm         |

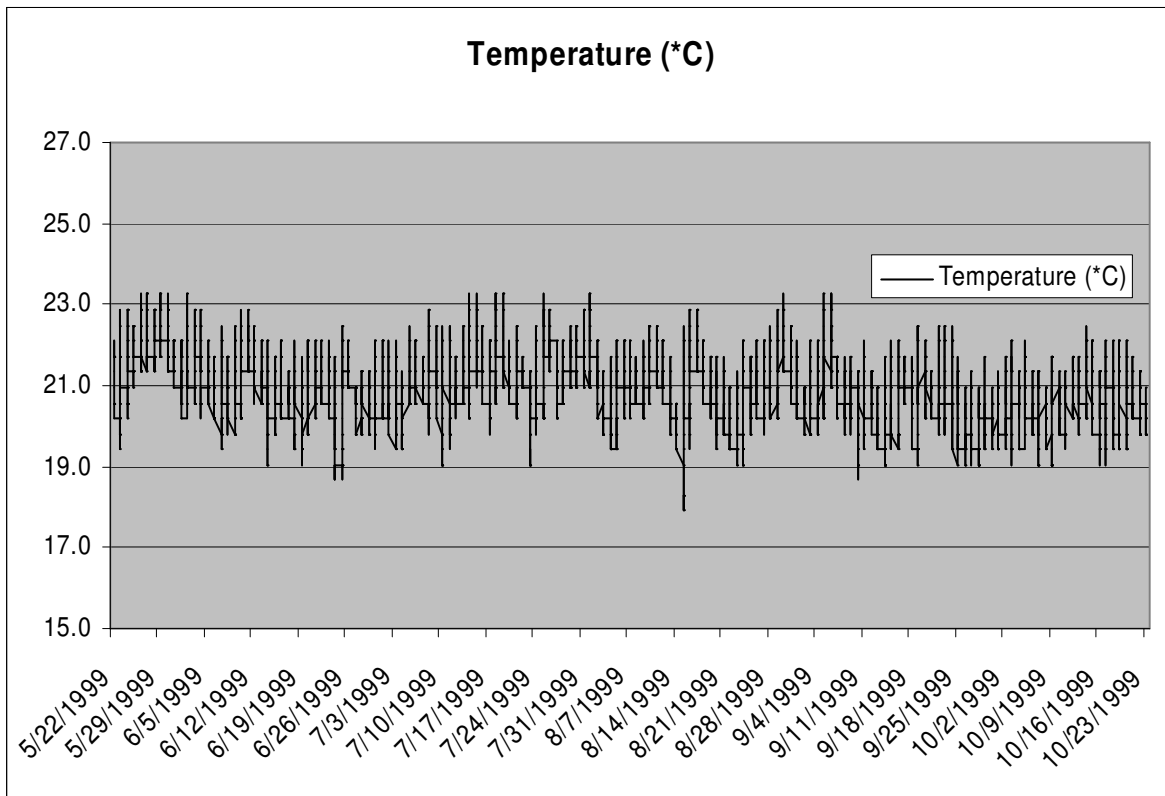
*AZA facilities can contact the author or studbook holder to decipher the following population locations in order to protect the localities from illegal collection*

## APPENDIX V – Environmental Data Collected in PGF Habitat

### WATER

SITE: Locality D, Panama

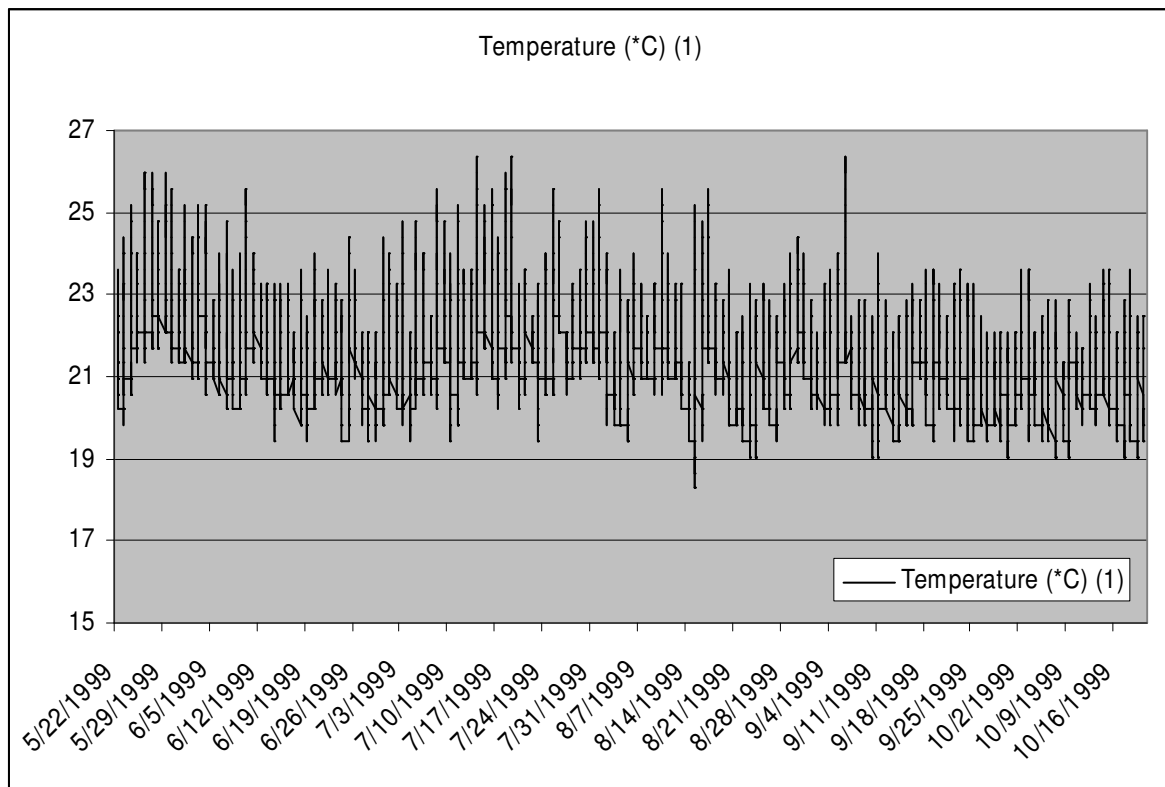
(5/22/99 – 10/23/99)

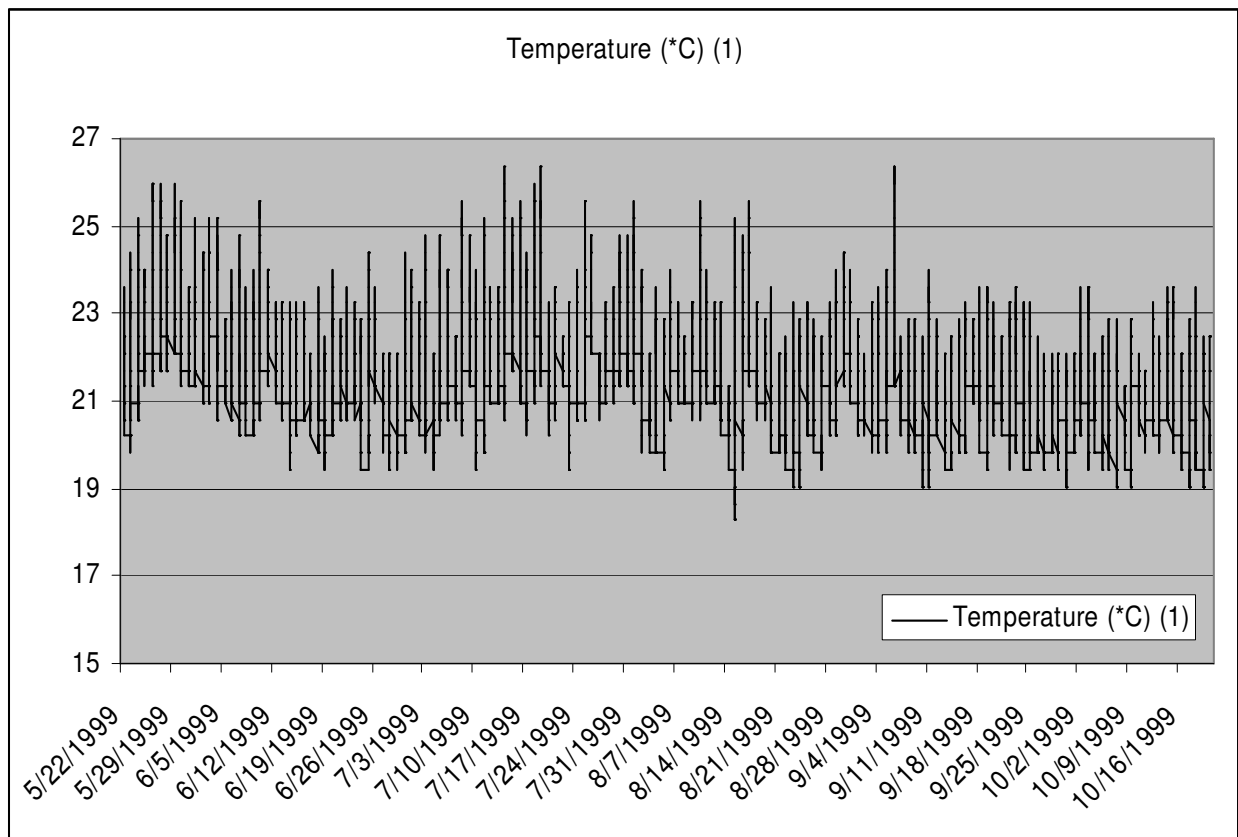
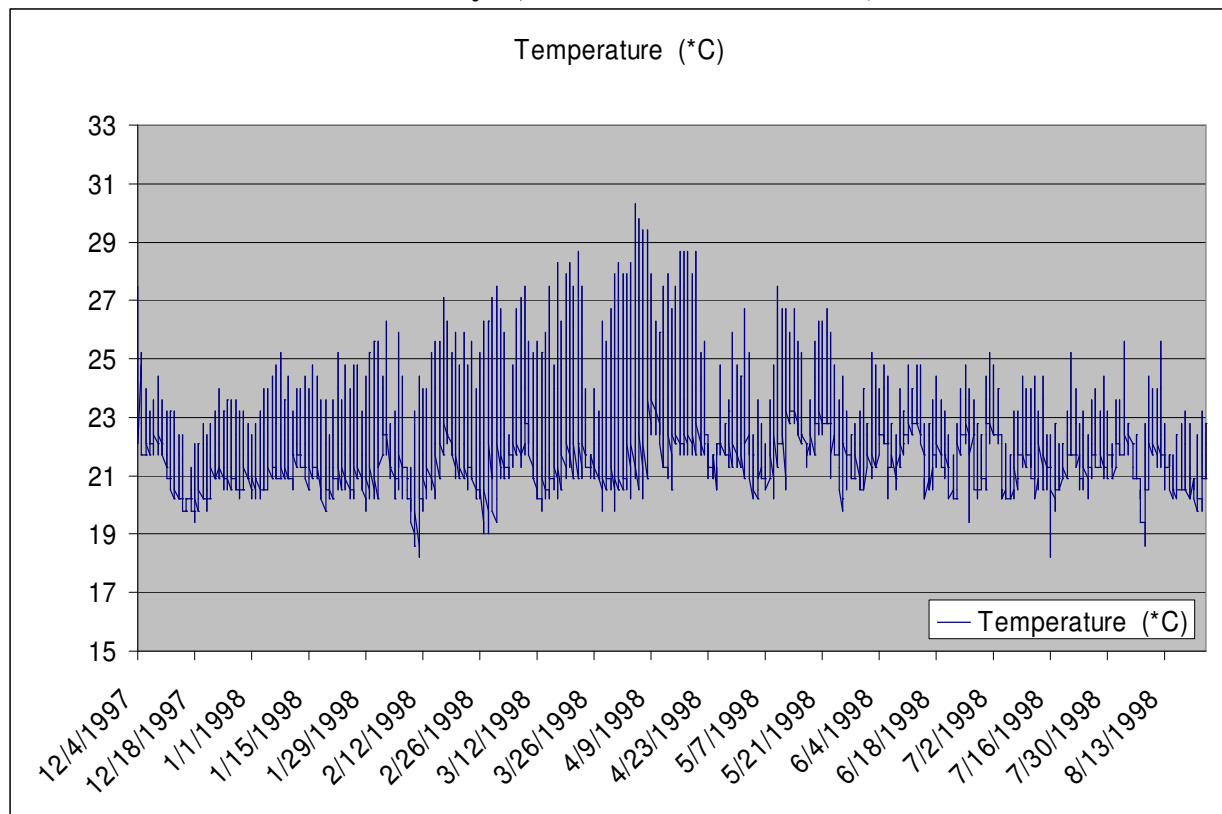


### AIR

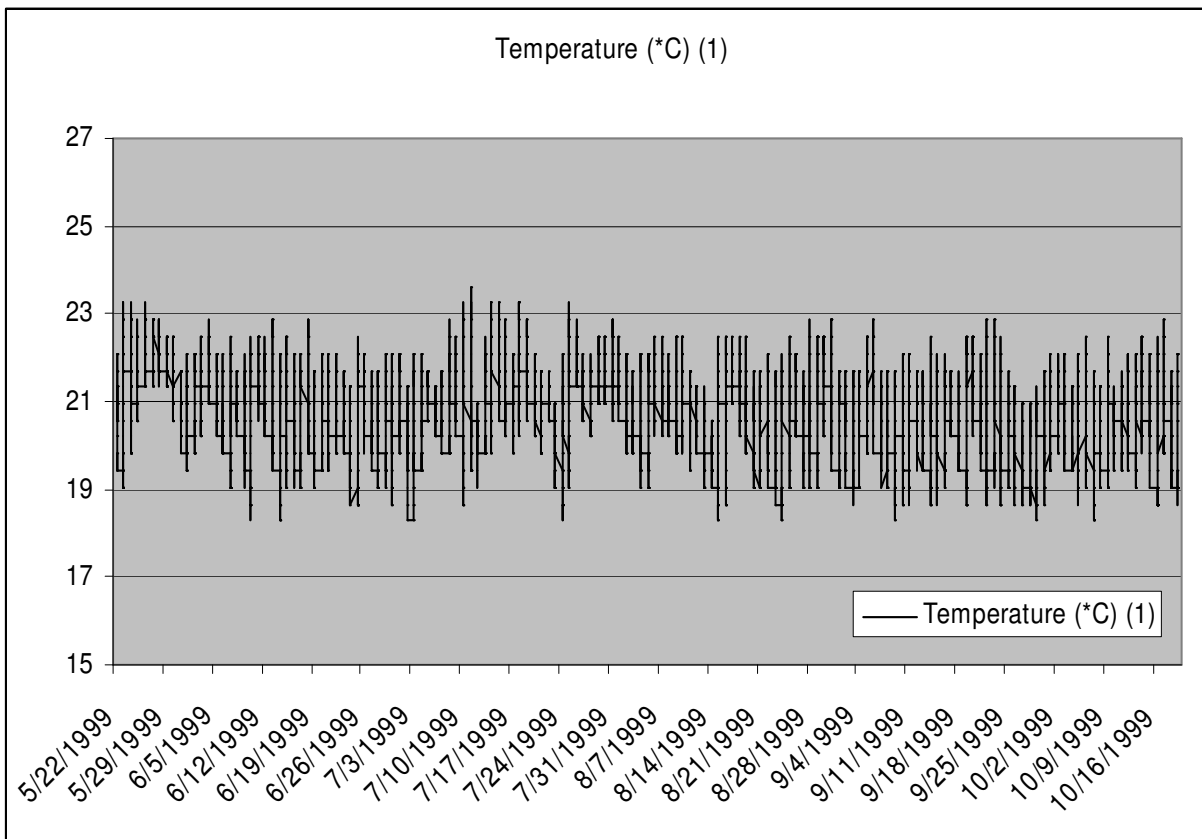
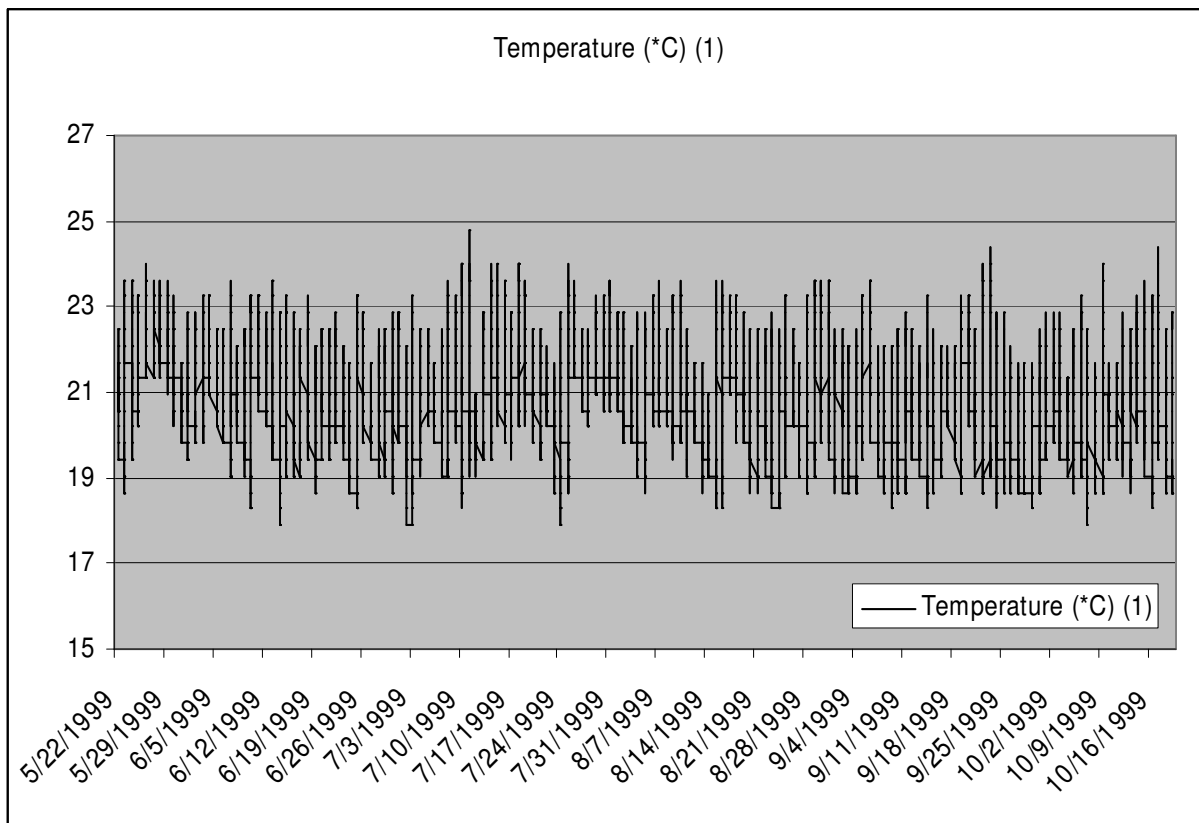
SITE: Locality D, Panama

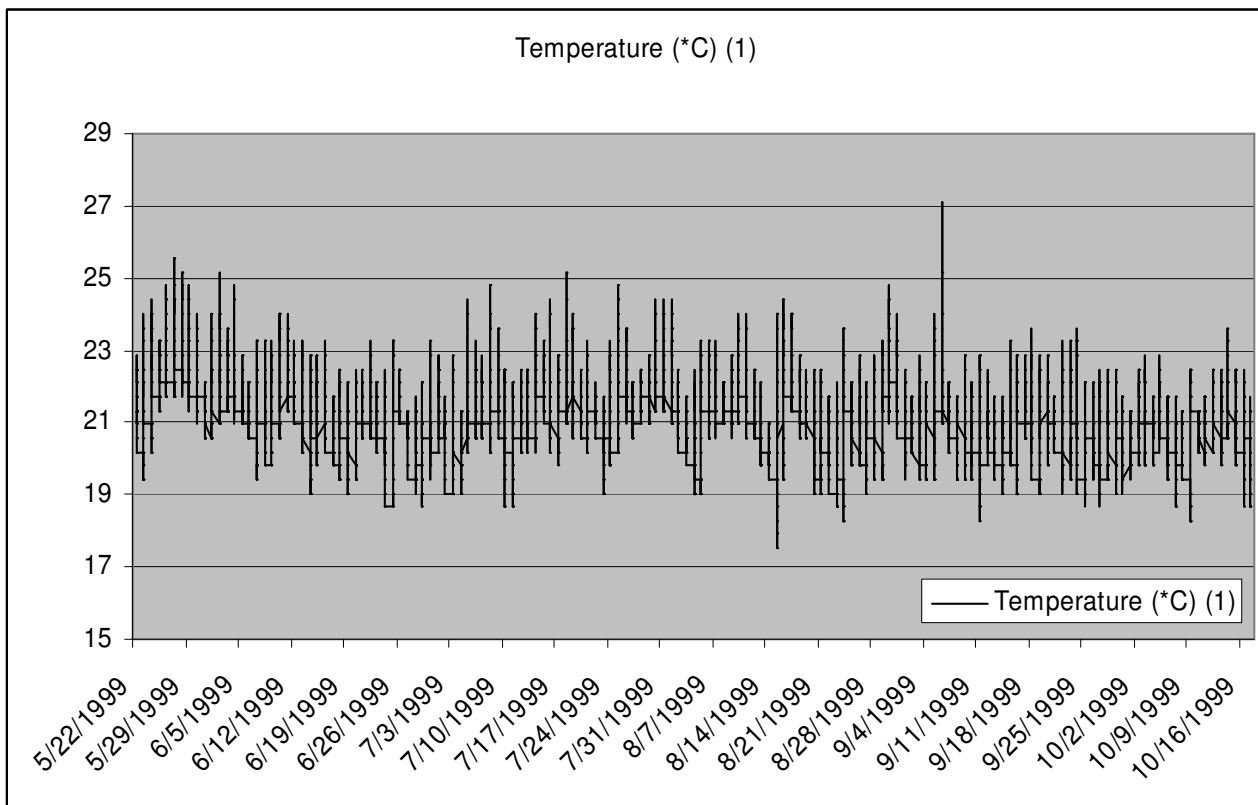
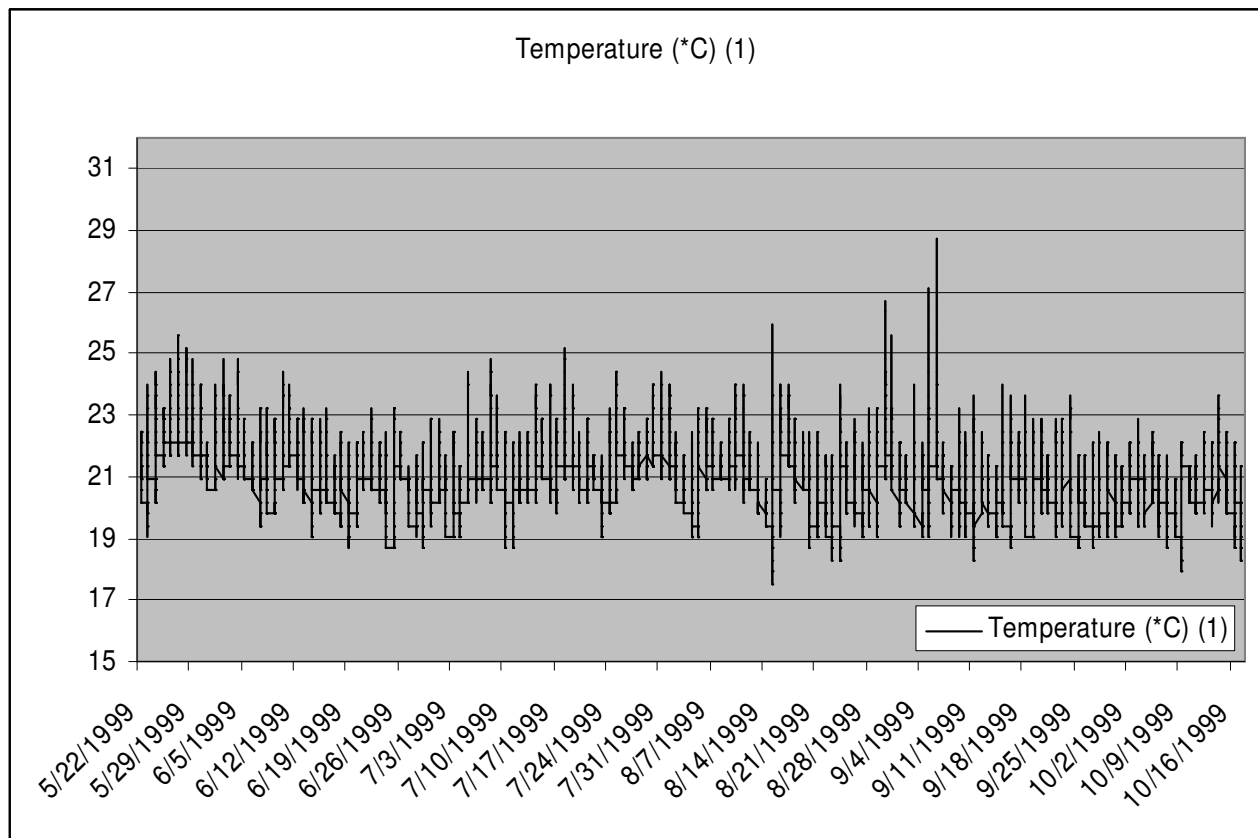
(5/22/99 – 10/20/99)





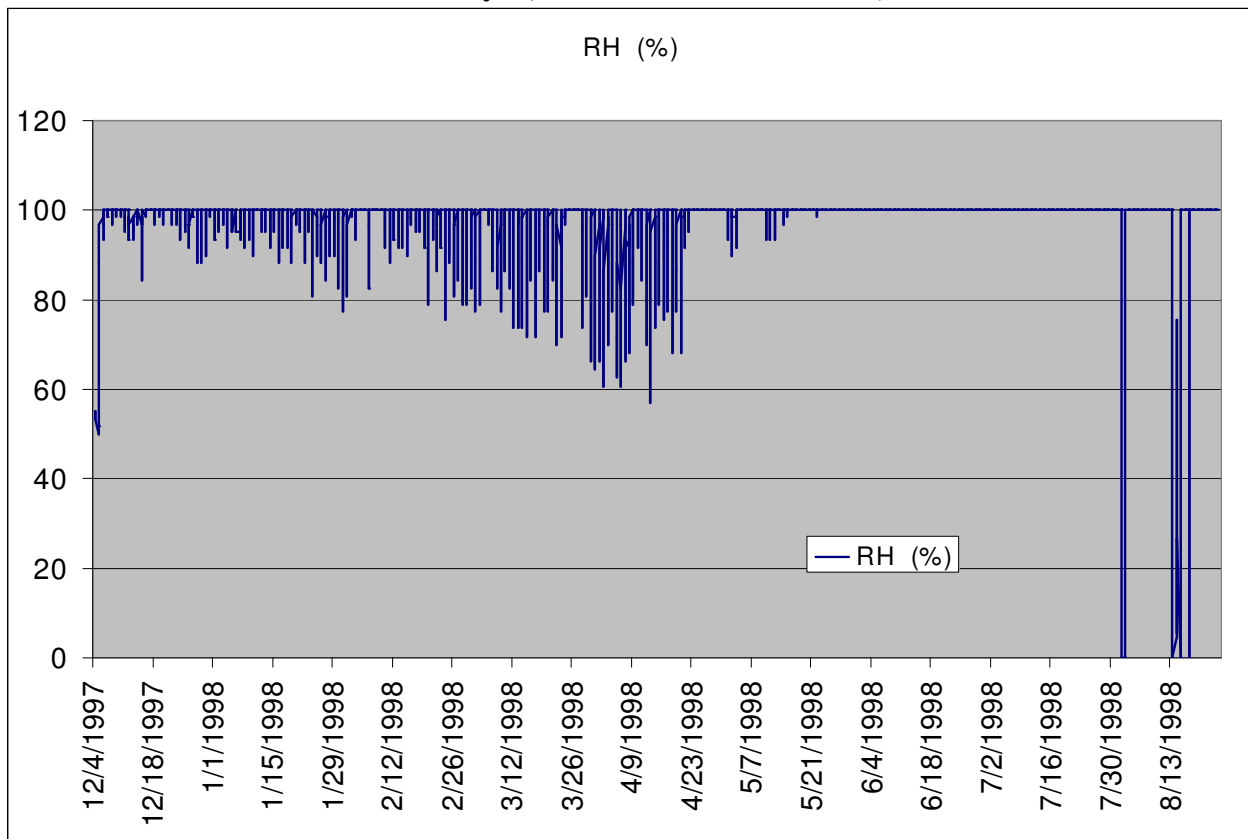






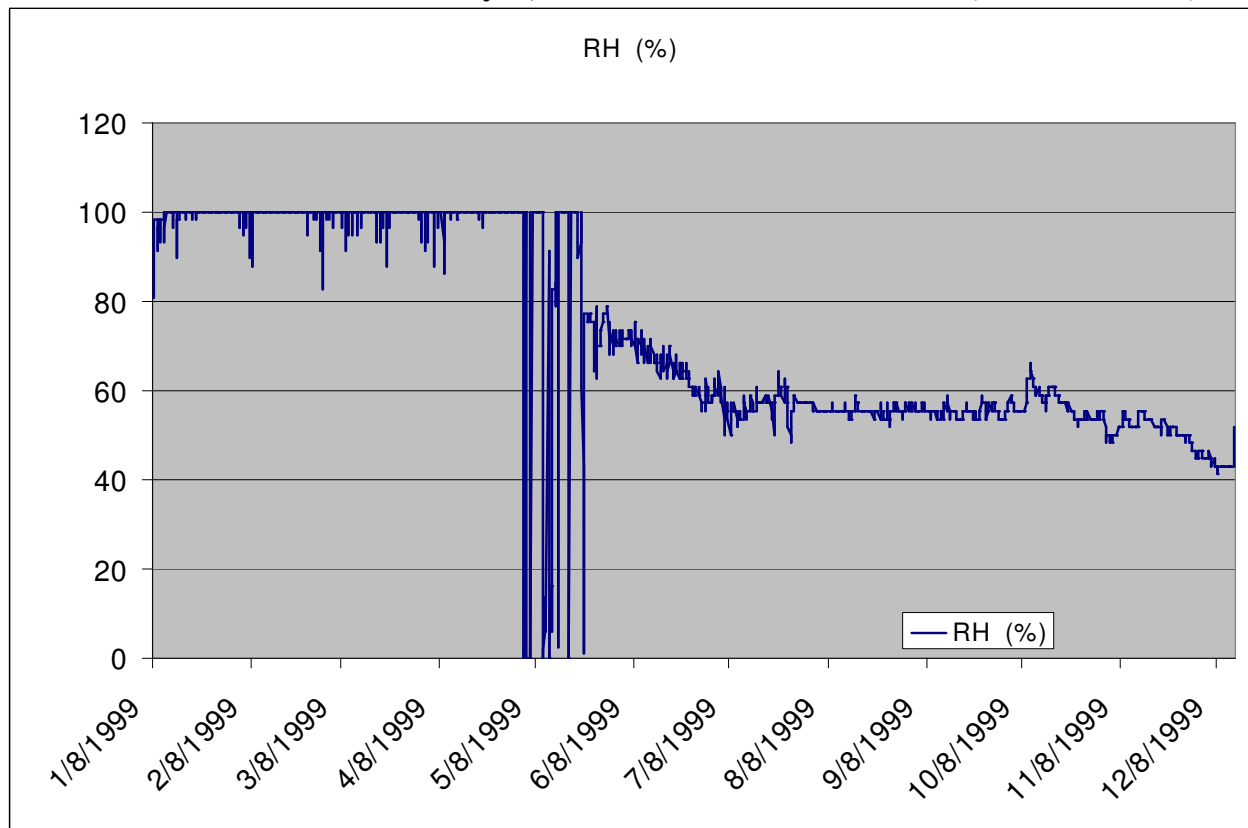
**RELATIVE HUMIDITY SITE: Locality D, Panama**

(12/4/97-8/31/98 El Nino Year)



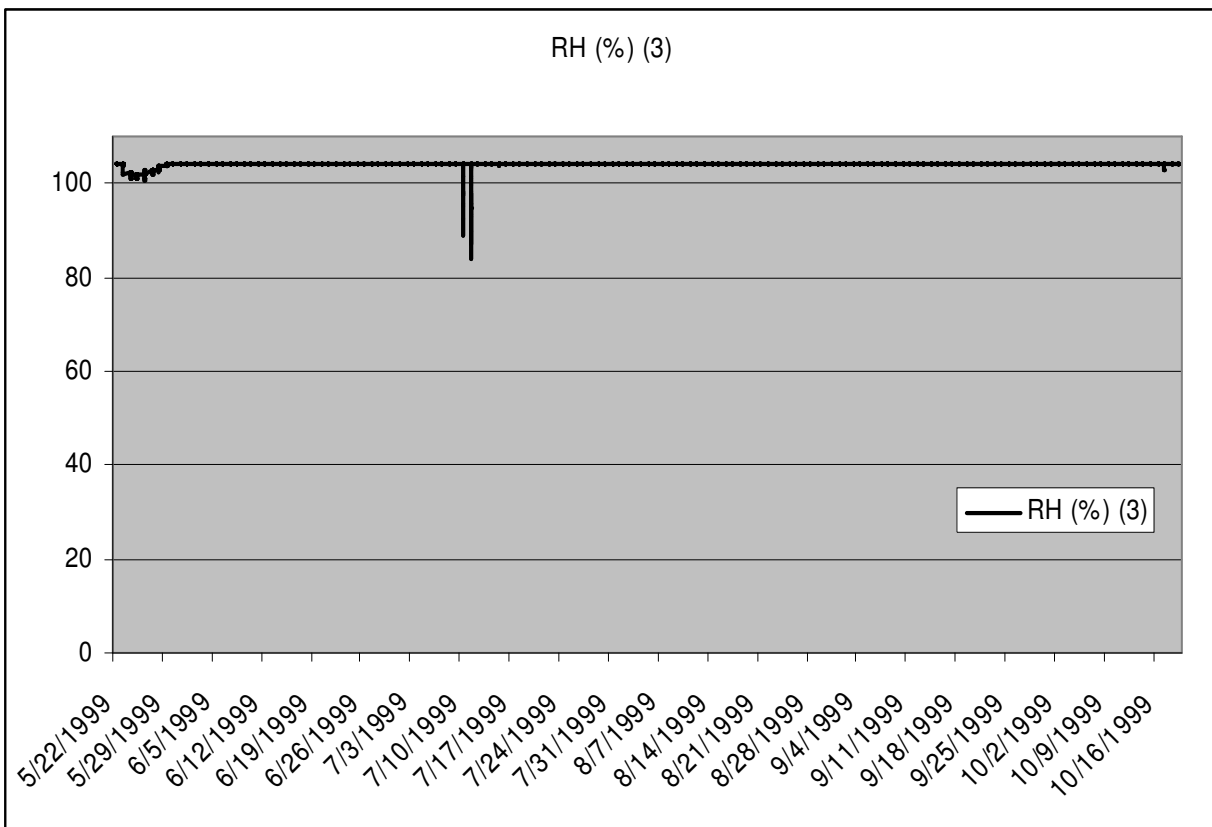
**RELATIVE HUMIDITY SITE: Locality D, Panama**

(1/8/99 – 12/8/99)



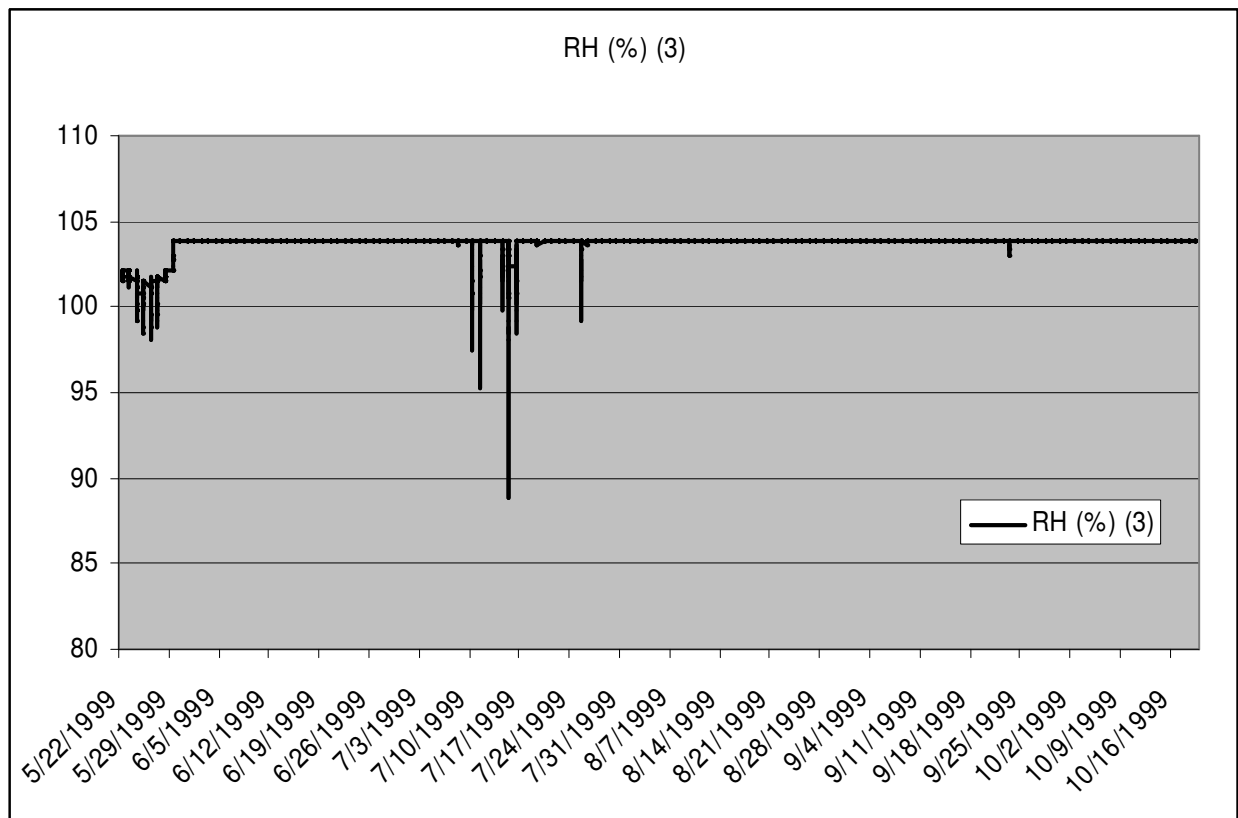
**RELATIVE HUMIDITY SITE: #1 Locality E, Panama**

(5/22/99 – 10/20/99)



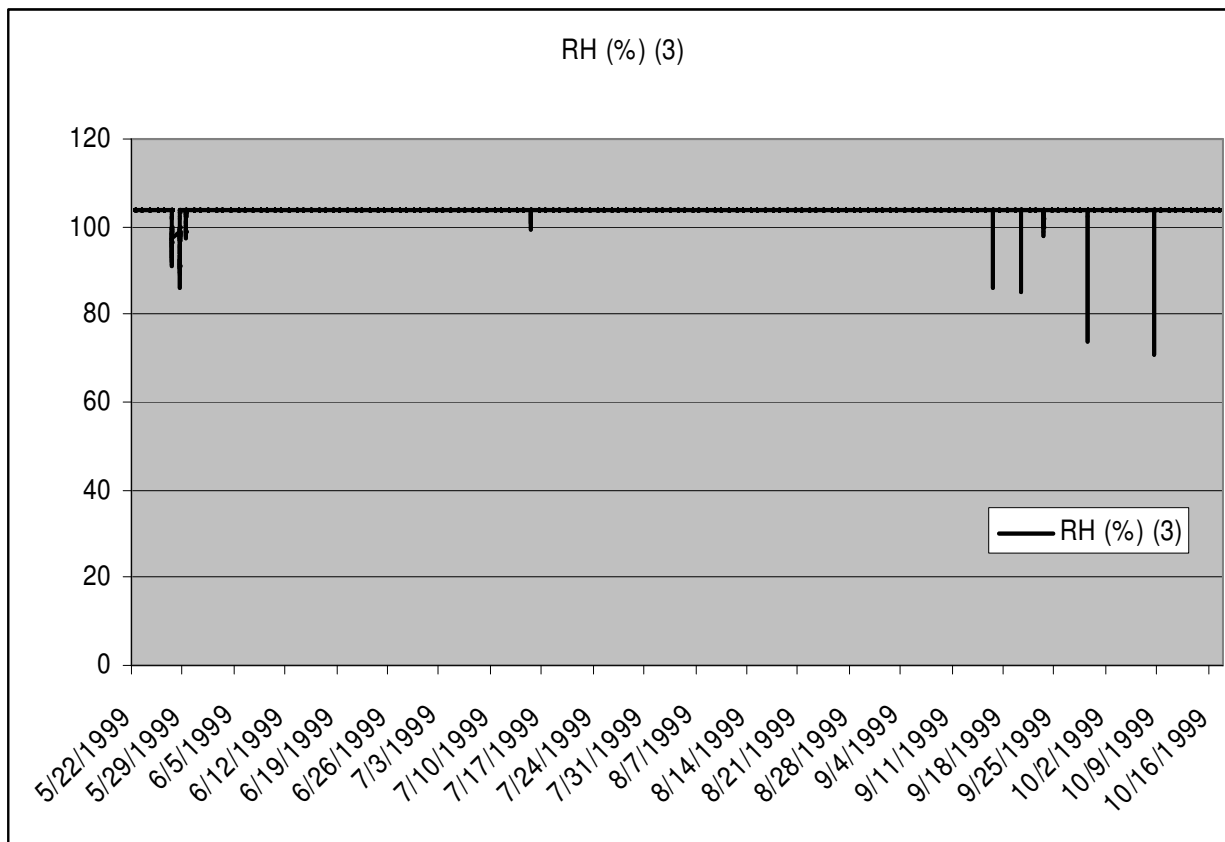
**RELATIVE HUMIDITY SITE: #2 Locality E, Panama**

(5/22/99 – 10/20/99)



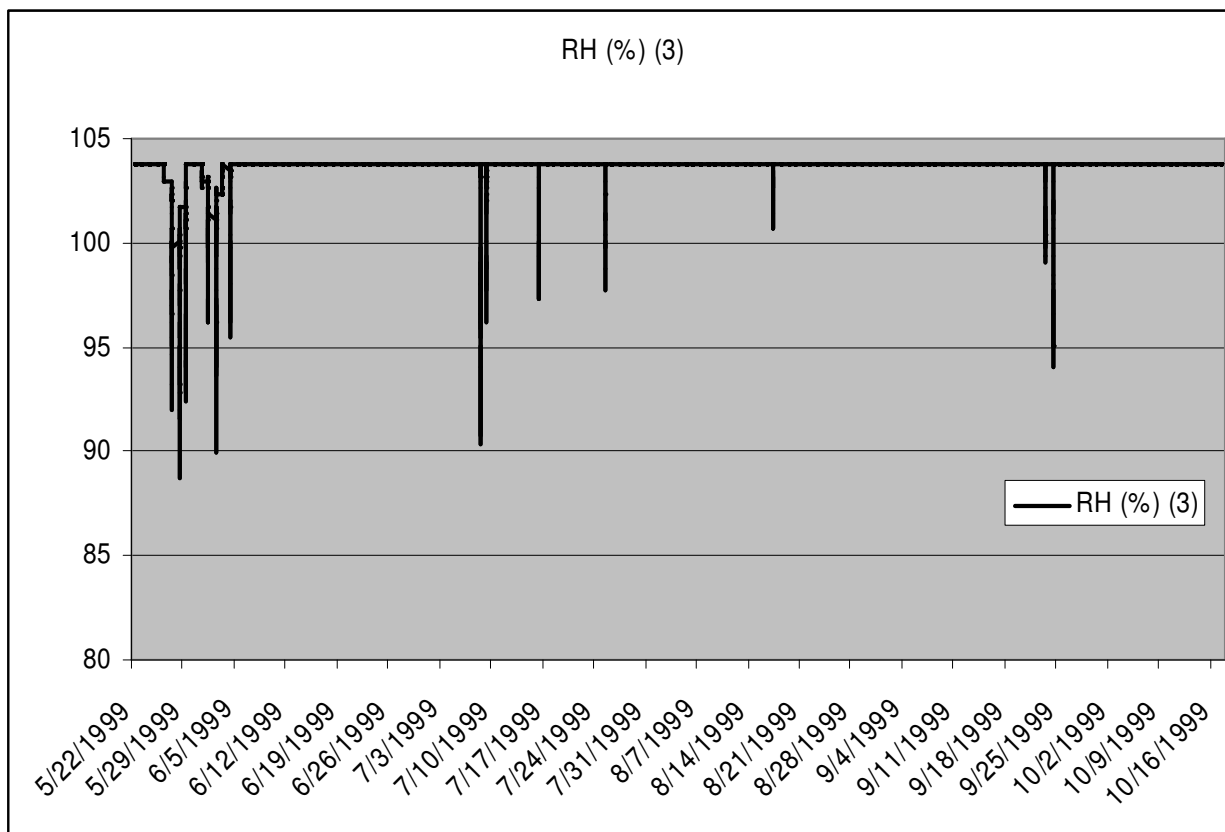
**RELATIVE HUMIDITY SITE: #1 Locality F, Panama**

(5/22/99 – 10/20/99)



**RELATIVE HUMIDITY SITE: #2 Locality F, Panama**

(5/22/99 – 10/20/99)



## **APPENDIX VI – Recipe for Reconstitution Reverse Osmosis (RO) Water**

Kevin Zippel, PhD

100 gallons of RO water  
15.0 g calcium chloride  $\text{CaCl}_2$   
17.6 g magnesium sulfate  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$   
13.6 g potassium bicarbonate  $\text{KHCO}_3$   
11.3 g sodium bicarbonate  $\text{NaHCO}_3$   
0.5 g commercial trace element mix (available through hydroponics suppliers, i.e., #6 chelate trace element, Homegrown Hydroponics)

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

Final composition:

General Hardness: 3 degrees

Carbonate Hardness: 2 degrees

Ca:Mg (3:1)

Na:Ca<sup>+</sup>Mg<sup>+</sup>K (1:4)

pH: ~ 7.4 depending on aeration

For further information on this topic, please see Dr. Zippel's website:

<http://home.att.net/~kczippe/waterqual.html>

## APPENDIX VII – Diatom Culturing as a Tadpole Food

### A SUGGESTED METHOD FOR CULTURING DIATOMS:

At Detroit Zoo's NACC we generally have some diatoms (golden-brown algae) growing in tanks throughout the building, but I know you can purchase cultures as well. We will set 10 or 15 gallon tanks (or plastic tubs), cover the bottom with 6-8" sections of PVC pipe (1 ½" is my preference), add some culture algae and nutrients (available from Aquatic Eco-Systems: Micro Algae Grow Mass Packs™, WITH SILICATES). You need to have a UV light above the tank (the closer the better from what I can tell). Once the pipes are nice and brown they can be transferred directly into the tank and they graze every last little bit off - often by the end of the day. [Aquatic Eco-Systems, Inc, Apopka, FL]

I aim to have at least 8 tanks going at all times for each tad clutch. I will feed out an entire tank for a good size clutch or when they get big and pudgy. It can take 8-12 days for the culture to get going, so plan ahead. Bleach the pipes between uses to keep the cooties down, dechlorinate them well, then restart them. If the tank they came from has some nice brown algae and little to no green algae, you can just wipe off the walls to restart the pipe again. The nutrients can cause a sediment that slows growth if you added too much. I try to change the water on each culture tank once a week and re-feed it. As long as the pipes don't dry out, they do well this way.

Once the green algae start invading - feed out what pipes you can and bleach the rest. It takes very little time for the green to crowd out the browns. Also, we get little midge fly larva on our algae tubes as they sit in the culture. These haven't proven a problem if we feed the tank out and bleach before restarting. We tried covering all tanks with plastic wrap with some improvement if it was tight, but its a big pain and I tend not to do it anymore.

Our best source for starting cultures is usually a chilled setup that has a decent amount of light but not direct light. This year we had a chilled crayfish tank that was a great source and the crayfish had been health tested prior to being cleared to use as food items, so we know the tank is clear. Once a couple tanks are started, you can just reserve a tube to use to start new cultures as needed.

I've trained a couple docents to clean and restart the cultures to knock down the time I spend. It is easy to spend an hour a day dealing with cultures alone. I've had 5 clutches going at once this year, and every free space in the building has cultures. I have about 45 culture tanks running right now and have just about enough for what tads I have going (2 of the clutches are small).

Then end result - lots of work, but the tads that result seem bigger and stronger and they seem to emerge faster than when we had them on sera mix alone. Maybe its just me - haven't compared any of the data.

Hope this helps out - happy culturing!

Edi

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## **HUSBANDRY MANUAL**

### **PUERTO RICAN CRESTED TOAD (*Peltophryne lemur*)**

#### **2002 UPDATE**

Keeper and Curator Edition

Includes Veterinary Care Section and Shipping Instructions



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This manual borrows extensively from "Captive Management of the Puerto Rican Crested Toad" (1995) by Dianne Devison of the Toronto Zoo. It is intended as a guide for those working with this species and will be updated periodically as circumstances warrant.

## 1. INTRODUCTION

### 1.1. DESCRIPTION

Puerto Rican Crested Toads, *Peltophryne lemur* are the only native species of toad found in Puerto Rico and the Virgin Islands. This is a medium sized toad (64 to 120 mm snout-vent length) with distinctive supraorbital crests and a prominent upturned nose. Females are larger than males and have more prominent crests. Their colour ranges from brown to yellow brown with black or brown patches. The ventral surface is a creamy white with some dark mottling. Sexual dichromatism (males are yellower than females) is most obvious when males are in breeding condition. Males also possess prominent nuptial pads on the first and second digits of the front feet.

### 1.2. HABITAT

La Reserva Forestal de Guanica, the national forest in which Puerto Rican crested toads are still found, is a rugged dry sub-tropical forest. There are no permanent bodies of fresh water, and significant rainfall usually comes once a year. It is located on the south-west coast of Puerto Rico. The toads are found at low elevations (not exceeding 200m) in arid or semi-arid rocky areas with well drained soils. The habitat found in Guanica has an abundance of limestone ridges and boulders with dissolution holes and fissures, which are used by the toads as daytime retreats from the oppressive heat.



Daytime highs can reach 40 °C, and the surface temperature of the rocks can climb to 50 °C. A 1990 radiotracking inquiry into post reproductive movements showed that toads moved up to 1.5 km (0.9 miles) in 3 nights before settling down in an area where average nightly movements were 10 meters (33 feet). Toads were able to climb up vertical rock faces to seek shelter in holes in the limestone at least 45 cm (18 inches) above the ground.

### **1.3. WILD POPULATION**

Native to Puerto Rico and Virgin Gorda, this species is listed as threatened by the U.S. Fish and Wildlife Service. The Virgin Gorda population is thought to be extinct. Its range is currently limited to two known sites in Puerto Rico. These remaining populations, one found in the north at Quebradillas, and the other in the south at Guanica are known to be genetically distinct and must be managed as such.

### **1.4. CAPTIVE POPULATION**

As of 99.12.31 the captive population consisted of 84,501,26 animals (260) at 21 institutions. This includes 16 animals at University of Puerto Rico and ZooRico, Mayaguez. Wild caught founders number 20.5 at 2 Institutions. Of these, all are southern animals except the following: 3.1 Northern toads at Cincinnati (no founders), 3.0 Northern toads at Fort Worth (no founders.)

## 2. CAPTIVE MANAGEMENT ISSUES

### 2.1. RELEASES

Captive-bred animals have been designated for release in Puerto Rico, to supply the SSP with breeding stock, and for use in related research. Through the 1980s captive-bred offspring were held in captivity and returned to Puerto Rico post metamorphosis as toadlets or toads. Rearing animals post metamorphosis requires considerable resources (space, time, and food) and increases the shipping cost for a given number of animals. Now, captive-bred animals are sent to Puerto Rico at 7 to 10 days of age as tadpoles. This allows zoos to rear and return large numbers of animals for release without depleting often-scarce resources. Captive bred toads surplus to the SSP have been provided to the University of Puerto Rico at Rio Pedras and ZooRico, the zoo in Mayaguez. Staff and students at the university, and zoo staff have been trained and provided with the equipment necessary to hold and eventually breed the toads in Puerto Rico. The following is a history of the release of captive-bred Puerto Rican crested toads in Puerto Rico.

- 1982: First release of northern toads in Quebradillas 1982/06/19
- 1983: Buffalo releases: 75 toadlets released 83/5/23 Quebradillas (Northern)
- 1984: Buffalo releases: 500 toadlets released 84/4/29 Cambalache (Northern).  
306 toadlets released 84/6/21 Cambalache (Northern)
- 1985: Toronto releases: 370 toadlets released 85/12/2 Cambalache (Northern)
- 1987: Toronto releases: 829 toadlets released 87/6/23 Guanica (Southern)
- 1989: Toronto releases: 6.3 toads and 707 tadpoles (via Buffalo) released 89/02 Guanica
- 1991: Cincinnati releases: 352 toadlets (via Ft Worth) released Cambalache (Northern)
- 1992: Toronto releases: 1026 toads released in 92/12 Guanica (Southern)
- 1993: Toronto releases: 10,000 tadpoles released 1993 Guanica (Southern)  
Buffalo releases: 1,548 tadpoles released 1993 Guanica (Southern)
- 1996: Toronto releases: 1100 tadpoles released 96/05/07 Guanica (Southern)  
1000 tadpoles released 96/12/18 Guanica (Southern)
- 1999: Toronto releases: 1650 tadpoles released 99/04/18 Guanica (Southern).  
980 tadpoles released 99/11/23 Guanica (Southern).  
Sedgwick County releases: 2000 tadpoles released 99/04/18 Guanica (Southern).  
5000 tadpoles released 99/11/23 Guanica (Southern).  
Toledo releases: 4000-5000 tadpoles released 99/11/23 Guanica (Southern).

## **2.2. RESEARCH**

Current research includes:

- The effects of temperature on tadpole growth and development.
- The effects of different diets on tadpole growth and development.
- The effects of different vitamin and mineral supplements on toadlet growth and development.
- The effects of different diets on the growth and development of toadlets.
- Substrate temperature selection by toads.
- Water temperature selection by tadpoles.

## **2.3. PIT TAGS**

At the Toronto Zoo, adult toads are implanted with passive integrated transponders (PIT). The Trovan PIT tags are used. Emla cream <sup>TM</sup> is used as a local anaesthetic, and is applied to the left shoulder area. The needle with the ID chip is inserted over the left thorax placing the implant subcutaneously between the left shoulder and the parotoid gland. The opening in the skin is closed with 3M Vetbond <sup>TM</sup> tissue glue. The area is then rinsed with water to remove traces of the anaesthetic, which is capable of causing general anaesthesia in these toads. Implanting with PIT tags means that all the toads can be housed together rather than in pairs, cutting down on holding space requirements and keeper time.

## **2.4. SHIPPING**

### **2.4.1. TOAD AND TOADLET SHIPPING PROTOCOL**

Toronto Zoo uses 1 or 2-litre wax coated cardboard milk cartons or large plastic deli/margarine containers for housing toads and toadlets for shipping. The containers must be thoroughly washed and rinsed and allowed to air dry before being used. Small holes (1/8" to 1/4") are punched or drilled in the sides and tops of the containers to provide needed air.

In order to provide moisture and a secure shock-absorbing environment in the shipping containers, slightly dampened sphagnum or sheet moss, teased or pulled apart to create air spaces and refuges for the toads is used as a packing medium. Each container is filled with this "airy" substrate. The moss must **not** be saturated with water. During shipping, wet moss will settle and the weight can crush, trap or drown small toadlets. Buffalo Zoo uses dampened paper towel to

provide the necessary moisture during shipping. Moistened sponge pieces or chips may also be suitable.

A one-litre container can hold twelve 1.25-cm (1/2") toadlets. The tops of the cartons are folded and taped or stapled closed. The square milk containers pack easily in Styrofoam shipping boxes. These are the same boxes that are used to ship tropical fish (a Styrofoam inner box placed in a cardboard outer box). To prevent jarring during transport crushed newspaper or Styrofoam packing chips are used to support the containers once in the boxes.

#### **2.4.2. TADPOLE SHIPPING PROTOCOL**

Tadpoles are packed and shipped in the same way that tropical fish are. Plastic bags (10" x 18") are used. These should be square bottomed or have the corners taped up on the outside to prevent tadpoles from being trapped in collapsed corners of bags. The bags are first filled with the tadpoles' original water to approximately 20% volume of the bag. Then an equal amount (20%) of aged or dechlorinated water (same temperature) is added to the bags for a total of 1.2 – 1.4 litres per bag. Approximately 300 tadpoles are put in each bag. To ensure adequate oxygen supply during shipping, the bags are then filled with oxygen from a medical cylinder to approximately 2/3 by volume of the bags. Bags are sealed using heavy rubber bands. The sealed bags are then placed into a second bag and tied again with heavy rubber bands. The shipping containers are the same ones used to ship tropical fish (a Styrofoam inner box placed in a cardboard outer box). The Styrofoam boxes are lined with several sheets of newspaper. To prevent jarring during transport, crushed newspaper or Styrofoam packing chips are used to support the bags once in the boxes. Another layer of newspaper is then placed over the bags. A can of Sera viformo tablet food (270 tablets per can) and a Spanish instruction sheet for releasing the tadpoles are included in each box. The Styrofoam boxes are taped up and placed in their cardboard outer shells, which are then also taped up.

The following shipping labels are then affixed to each box:

**-This way up**

**- Live animals**

**-Keep out of sun (in English and Spanish)**

Air transportation and shipping information can be found in Appendix D.



### **2.4.3. RELEASE INSTRUCTIONS**

#### **CORDIALES SALUDOS DESDE EL ZOOLOGICO DE TORONTO**

Antes de soltar los renacuajos en la laguna, es importante asegurarse que la temperatura del agua en la bolsa que contiene los renacuajos sea identica a la temperatura del agua en la laguna. Para ello proceda de la siguiente manera:

1) Desate la bolsa con los renacuajos y proceda lentamente a agregar agua de la laguna a la bolsa hasta que esta se llene de agua. Esta es necesario para aclimatar a los renacuajos a la temperatura y composicion quimica del agua del nuevo medio ambiente, evitando asi un posible "shock".

2) Una vez que los renacuajos se hayan aclimatado, se puede proceder a soltarlos en el agua.

Aqui les incluyo alimento para los primeros dias. Se puede alimentar entre 30 a 40 "pellets" por dia, dispersandolos en distintos puntos de la laguna. Tambien puede ofrecer algun vegetal verde como lechuga una vez al dia.

**BUENA SUERTE!!**

Greetings from the Toronto Zoo. Before releasing the tadpoles into the pond, ensure that the water temperature in the bags is the same as the pond. To accomplish this, undo the bags and slowly add the pond water to the tadpole bag until the bag is full of water. This is to acclimatise the tadpoles to the temperature and water chemistry of the pond. The tadpoles can then be released in the pond.

We have provided some tadpole food. Feed 30 to 40 pellets daily. Disperse throughout the pond. Lettuce or greens can also be fed once a day.

**GOOD LUCK!**

### **3. EXHIBITRY**

Some zoos have exhibited Puerto Rican crested toads using rock piles, or rock and cemented caves to simulate the limestone of the Puerto Rican habitat. These toads are a challenge to exhibit for they take every opportunity to hide and tuck-up in any crevice available. Substrates that have been used include sand, soil, moss, gunite, fibreglass, gravel, bark, river stone and limestone rocks. Shallow rock crevices and plants provide an environment in which the toads are usually visible, active and enjoyed by the public. A survey of AZA institution exhibits has been compiled by Eli Bryant-Cavazos and is attached as Appendix C. This survey describes exhibit set-ups and graphics.

#### 4. ADULT HUSBANDRY

##### 4.1. HOUSING (Off Exhibit)

At Toronto Zoo, Puerto Rican crested toads are housed off exhibit in 125 litre (35 gallon) aquaria, at a density of 6-10 adult toads per aquarium. If toads are not PIT tagged, animals that have bred are housed in pairs. The room the toads are housed in, is well lit with natural lighting. To supplement natural photoperiod, a Vita-lite™ and Osram Sylvania Blacklight™ are suspended directly over the tanks on a 12:12 light: dark photoperiod. For ease of cleaning, the toads are kept on thin rubber matting in 125 litre (35 gallon) aquarium measuring 45cm wide x30cm high x90cm long (18" wide x 12" high x 36" long) with a plexi-glass and screen lid. Rocks with crevices, PVC tubes, coconut shells, and large leaves serve as hides. Toads commonly use 2" to 3" diameter PVC tubes (cut in 8" lengths) which can be linked with elbows and T's to form a network of tubes that can extend vertically as well as horizontally. The tubes are used as tunnels and daytime retreats. The network of PVC tubes can easily be disconnected for cleaning purposes and to locate a toad's whereabouts.

Water is provided in shallow dishes (plastic plant saucers) or by tilting the tanks and offering a small amount of water at one end (1-2 cm deep). Water sources are regularly utilised for defecation. Water dishes and tanks are cleaned daily by scrubbing soiled are and then rinsing and refilling with dechlorinated water.



Off exhibit holding tanks at  
Toronto Zoo

#### **4.2. DISINFECTION**

Disinfection is not routinely done but if required, a 1.0% sodium hypochlorite solution can be used. Household chlorine bleach ranges from 3% to 5% sodium hypochlorite and commercially available bulk solutions can be of much higher concentration (up to 12%). It is therefore vital to confirm the concentration on the container label or with the manufacturer or supplier before preparing a dilute working solution for use in empty tanks. After disinfection, tanks are rinsed thoroughly with hot tap water and left to air-dry overnight before being used to house toads again. Porous materials such as bark or driftwood are best replaced if excessively soiled. If these materials must be disinfected with bleach, they must then be rinsed with water and then sprayed with a solution of 16 ppm sodium thiosulphate (8mg per 500 ml of water). This solution has a neutralising effect on chlorine.

Iodine toxicity has been seen in amphibians from iodine reversibly bound to plastic holding containers. Because of this potential binding with plastics, it is recommended that iodine not be used to disinfect any material that will come in contact with the toads. Other disinfectants are not recommended for use on amphibian tanks or furniture.

### 4.3. NUTRITION

The adults are each fed one mouse pinkie every second week, and 3-4 large crickets (1 gram per toad) twice per week. All food is dusted with vitamin/mineral supplement. Occasionally mealworms, wax worms and dew worms are offered instead of the crickets. The toads are checked on feeding days and removed from their hides. This also ensures each toad gets its share of the food items. Some toads quickly adjust to the feeding regime and readily emerge from their hides on their own, anxious to feed. All the toads are weighed monthly in order to assess general health. They are not fed two weeks prior to and during the pre-breeding conditioning period (up to six weeks). After each breeding attempt, the toads are offered small amounts of food daily for about one week to increase lipid stores and then cut back to the two day per week feeding schedule.



Toads emerging from hides to feed.

## 5. REPRODUCTION

### 5.1. SEXING OF ADULT TOADS

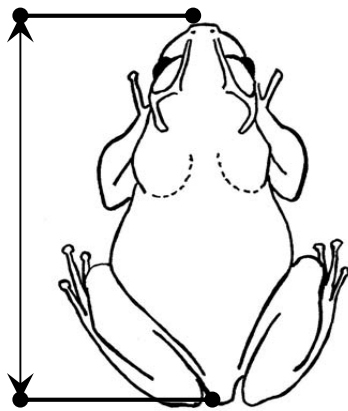
There are several prominent features that allow toads to be sexed as they approach adult size. The most reliable indicator is size. Measurements of adult toads are usually significant indicators of sex. Female toads are larger in size with a broader, more distinct headcrest (see table and diagram below).

**Table 1: Adult toad measurements**

|                     | MALE      | FEMALE    |
|---------------------|-----------|-----------|
| Weight range        | 27 to 54g | 43-140g   |
| Body length average | 6.6cm     | 8.3cm     |
| Body length range   | 6.1-7.5cm | 8.1-8.4cm |
| Head length average | 1.8cm     | 2.7cm     |
| Head length range   | 1.8-1.9cm | 2.2-2.9cm |
| Head width average  | 1.8cm     | 2.5cm     |
| Head width range    | 1.8-2.0cm | 2.5-2.6cm |

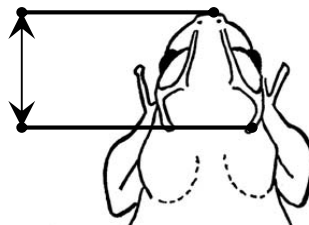
#### TOTAL LENGTH

Snout



Vent

#### HEAD LENGTH



Snout to middle of head at the end of cranial crest

#### HEAD WIDTH



Between the posterior edge of the cranial crests

When the males are in prime breeding condition, sexual dichromatism is particularly evident. Their colour changes to lemon yellow, particularly on the sides of the body, and the throat area. Males also possess prominent nuptial pads on the first and second digits of the front feet. Males will produce a male release call. By clasping the toad just behind its front legs, with thumb and

forefinger, it is possible to elicit a chirping release call from a male. This chirp may be barely audible or quite loud, depending on the toad, but in any case it is often possible to feel the jerking of the male's body as he exhales air to produce the call. This method is not infallible as some males do not readily produce the release call, and some females will attempt to do so.

## **5.2. PRE-BREEDING CONDITIONING PERIOD**

During the dry months in Puerto Rico, the toad becomes dormant and aestivates. This rest period may act as a stimulus for egg maturation in the ovaries of the females. The conditioning period prior to breeding Puerto Rican crested toads appears to be about 30-45 days. In the past, the pre-breeding conditioning involved aestivating toads in a moistened substrate of sphagnum and peat moss at a maintenance room temperature of 30°C. The substrate was allowed to slowly dry out, but it was found that the toads dehydrated as well. This method was discontinued due to toad deaths during and post aestivation.

Egg production and maturation in captive amphibians has often been unpredictable, possibly due to abnormalities in the vitellogenic cycle. Keeping amphibians at lower temperatures for 4 to 6 weeks has been shown to partially correct abnormalities in the vitellogenic cycle of captive amphibians. These animals produce larger numbers of mature ova that can support development. Through the limbic system, environmental cues such as temperature and light may affect the hypothalamus which secretes gonadotropin-releasing hormone (GnRH). GnRH causes the pituitary to release gonadotropin, which in turn stimulates steroid hormone production. The oestrogen steroid hormones play an important role in stimulating yolk protein synthesis in most of the vertebrates. The length of time and the degree of cooling, and the need to cool males, are currently under review.

Toads are maintained at a temperature range of 26°C-30°C (78°F- 86°F). As pre-breeding conditioning, Toronto Zoo now recommends that toads be cooled for 21-45 days. Before this conditioning period, toads are weighed and a general health examination is performed. If fat stores appear good and weight has remained stable, the selected toads are moved to a cool room, or incubator. The toads are not fed two weeks prior to and during the cooling period, but always have access to water. When a large number of toads are to be bred, the Toronto Zoo cools the room in which the toads are housed. For smaller numbers, the animals in their existing tanks, as is (i.e. no substrate other than the rubber matting and hides), are moved into an environmental chamber or incubator for cooling. The temperature is slowly dropped to 18°C(66°F) over the first 7 days of the cooling period. The open mesh of the aquarium lid is partly covered to raise the

humidity to over 80% in the aquarium. As little disturbance as possible is encouraged during the cooling period.

In the past Buffalo Zoo has had successful breeding using the following pre-breeding conditioning. The toads are cooled in aquaria filled with wet sphagnum. The aquaria are moved to a cool room where temperatures range from 13-18°C(56-66°F), slightly cooler than at Toronto Zoo. The moss is kept generally wet throughout the cooling period, although the top layers are allowed to dry out somewhat. Temperature is either lowered or raised 5°F at a time, keeping the toads at a new temperature for 4-6 days until reaching the desired temperatures.

### **5.3. BREEDING**

Captive breeding events are scheduled for the late fall (Oct-Nov) to coincide with the likelihood of rain in Puerto Rico. If an important fall breeding fails, then an early spring (March-April) breeding may be attempted. It is important to conduct a faecal examination after each breeding attempt. Despite normal loads, the stress of conditioning may affect the immune system response, and bacteria/parasite loads may be elevated.

Breeding tanks are set up with aged tap water a minimum of 14 days before the pre-breeding conditioning period begins. This allows for adequate preparation of filters and allows sufficient algae growth for tadpoles to feed on. These tanks are either 140L(35gal) aquaria or larger 400L(100gal) fibreglass tubs. The breeding tanks are set up on a slant with approximately 8cm(3") of aged or dechlorinated water at one end and a deeper end with 15cm(6") of water. This allows the adult toads the choice of water depth. The tanks are seeded with an algae culture (future tadpole food). The water temperature is maintained at 30°C(86°F). Large tubs have sponge filters and a canister filter circulating the water and the return is pumped through a perforated wand in an effort to simulate rain. The smaller aquaria have a sponge filter and a corner filter with charcoal.

A calcium block, used in home aquariums, is also placed in the tanks. Plastic plants, rocks and large half clay pots, some submerged, others half submerged to form out of water perching spots, are placed throughout the tank. Algae grows well on these and adults string their eggs around the clay shards and the plastic plants. In the wild, emergent plants are important as egg laying sites.

At the end of the cooling period the toads are warmed back up to 28°C(82 °F) over three days (i.e. approx. 3 °C per day). Day 1 marks the first increase in temperature. The toads are not otherwise



disturbed on this day. On day 2 the tanks are filled with 1" of dechlorinated aged water. Taped toad calls\* are played throughout the day and into the night. As early as possible on the morning of day 3 the males are placed into the large breeding tank with simulated rain and the taped calls are played throughout the day. Approximately 6 hours later in the afternoon of day 3, the females are added to the breeding tanks. Wrestling of competing males appears to be very stimulating to male toads. As toads amplex they are moved to a separate breeding tank to keep track of parentage. If toads do not amplex, hormones are used (see section 5.4 **Hormonal Reproduction Protocol**). Eggs are usually laid overnight within 24 hours of amplexus or after hormonal injections. Once eggs are laid, the adults are removed to holding tanks and the eggs are allowed to hatch in situ. The water level is then gradually increased in order to provide a larger volume of water for the developing eggs.

\*A tape of Puerto Rican toad calls is available from the Toronto Zoo.

#### **5.4. HORMONAL REPRODUCTION PROTOCOL**

The following protocol used for the induction of reproduction in Puerto Rican crested toads at Toronto Zoo has proven successful on most occasions. The most success has occurred in young toads in their first breeding season, (males 1-2 years old and females 2-3 years old) however, hormone injections have stimulated egg laying in 7 year old toads at the Cincinnati Zoo, and 9 year old toads at the Toronto Zoo. The use of hormones for breeding amphibians does not negate proper pre-breeding conditioning. Hormone treatment will not be effective in the absence of mature ova in the ovary. In other anurans, ova maturation is dependent upon an extended maturation time and a good nutritional status. Stress is a powerful inhibitor of amphibian reproduction. It seems that it is not possible to induce ovulation in Puerto Rican crested toads that have not been cooled. In some cases, pairs of toads will go into amplexus for variable periods but fail to deposit eggs.

The luteinizing hormone releasing hormone analog des-Gly (d-Ala) LHRH ethylamide from Sigma Chemical (catalogue number L4513) is used. Sigma Chemical now requires USDA approval to sell LHRH for use in amphibians. Institutions should contact the USDA Center for Veterinary Medicine, at 301-594-1785 for assistance and submit an application to purchase LHRH for use in amphibians. The product comes as a powder in 1 or 5 mg bottles, needs to be dissolved prior to use, and should be stored in the freezer. At TZ, LHRH is diluted to make a 100 microgram/ml solution and frozen in 1 ml syringes prior to use. The shelf life of the dry product is 2 years. Whenever possible, new product should be obtained for each breeding cycle.

**If toads are in amplexus:** Females are injected using a dose of 0.1 ug/g subcutaneously. Intraperitoneal injection can also be used. These doses are higher than those used by some working with other species, but each species can be slightly different and there has not been enough research to find the minimum or the optimum regime. If no eggs are laid overnight, then females may be injected again the following day, however females seem to be susceptible to fluid overload and death from drowning can occur (see section **7.4.2 Non-infectious disease**). As a general rule, it seems that if hormone injection does not work the first time, it is not likely to work later.

**If pairs are not in amplexus:** Injecting females may cause premature deposition of unfertilised eggs. In these cases, males are injected first (0.1ug/g) which should induce amplexus behaviour. Females in amplexus are subsequently injected once a male has amplexed with them. Further injections are not usually required for males.

## 6. EGGS , TADPOLES & TOADLETS

### 6.1. EGG CARE

After the adult pairs are removed from the breeding tanks, and detritus is siphoned out. Care is taken not to disturb the strings of eggs. Filters and return water are moved away from any eggs. Water depth is slowly increased until it reaches 18-23 cm (7-9"). Egg development is rapid at 30°C, and development is seen within 12 hours. After 24 hours the eggs hatch, and the embryonic tadpoles cling to the egg strings before dropping to the bottom of the tank. It is important not to disturb the embryonic and externally gilled tadpoles at this stage. The tanks are left another 24 hours and then any unhatched eggs are carefully siphoned out of the tank. Aeration by airstone or filter is kept low to avoid a whirlpool effect on the eggs, and newly hatched tadpoles. It is necessary to cover the filters with a fine netting (panty hose works well) to avoid trapping tadpoles inside the filter.

### 6.2. TADPOLES

**Recording hatch dates for toads:** The **hatch date** is the **date the tadpole emerges from the egg**. Metamorphosis dates can be recorded as separate comments. This is in accordance with the AZA "Standards for Data Entry and Maintenance of North American Zoo and Aquarium Animal Records Databases"

To maintain the highest water quality, detritus and uneaten food are siphoned off, and measured water changes with dechlorinated water (up to 40%) are done up to three times per day. Successful metamorphosis is more likely if the tadpoles are left in large volume tubs. When food items are placed in the tanks, a few tadpoles begin to feed, starting what appears to be a swarming around the food items. Tadpoles appear to move en masse throughout the tank.

The tadpoles are fed three times daily so that old food can be removed and fresh food is always available. The morning feeding consists of equal parts Sera-san enhancing flakes, Tetra 4 in 1 FD menu, aquaria herbivore diet, and spirulina flakes. The food is moistened with a few drops of water to form a firm ball and rolled in ascorbic acid powder. For example, 1/4 teaspoon (approx. 0.6 grams) of food plus supplement feeds 50 tadpoles (10 days old) at one feeding. Three to four feeding stations are provided per tank. The small balls of food are stuck to the tank wall just at the water surface, or are allowed to fall to the bottom of the tanks. The amounts and number of

feeding stations are increased as the tadpoles grow. Calcium blocks will slowly dissolve in the water. A new one should be added to the water before the old one has completely dissolved. Food from the morning feeding is removed in the afternoon prior to the second feeding. The afternoon feeding consists of Sera-viformo tablets. Two tablets (approx. 0.5 grams) are given per 50 tadpoles (at 10 days old).

The third feeding consists of pieces of 4" by 4" frozen or microwaved (to rupture the plant cells) spinach or lettuce per tank. Lettuce and spinach are fed on alternate days and the pieces are left in the tanks overnight. This provides an overnight food source with little risk of fouling the water.

Prior to the morning feeding, the remaining food and faecal material from the previous night are removed. The pH, ammonia levels and the water temperatures are checked. Ammonia levels under 0.1 ppm, nitrates below 1ppm, pH between 7 and 8, and temperature of 30 °C(86°F) are recommended. Water changes to adjust water chemistry and to clean the water are performed. Water temperatures have ranged from 21-34°C (72-93°F) over the course of tadpole development. Recent studies at Toronto Zoo suggest that a temperature of 30°C (86°F) is best suited for raising healthy tadpoles. Ammonia and pH levels have ranged from 0.02 to 3.3 ppm and from 6.7 to 8.3 respectively, prior to morning water changes. Daily water changes vary from 10% to 50% depending on pH and ammonia levels and the clarity of the water. Sponge filters are rinsed clean with dechlorinated water once per week; filter wool and activated charcoal are changed three days later. Sudden fouling of the water due to tadpole mortalities or overfeeding can be addressed with massive water changes of up to 75% using fully aged or dechlorinated water.

Buffalo Zoo houses their Puerto Rican crested toad tadpoles in a glass box with a screen bottom. A 100% daily water change is performed by lifting out the tadpoles on the screen and placing them in a clean tank that has aged water at the same temperature. Water chemistry has not been monitored.

From studies done in Puerto Rico, metamorphosis occurs approximately 21 days after hatching. At cooler temperatures, captive metamorphosis can occur 10-14 days later than wild tadpoles. At 30°C, hind legs are obvious after day 9 and some tadpoles are ready to metamorphose by day 14. At this point "beach" areas are created in the tanks, by slowly dropping the water levels and offering more plant material and cork bark. From day 10 to day 21 (some tadpoles are delayed in development and some fail to metamorphose) front legs appear and toadlets start appearing on the "beaches".

### **6.3. CLEANING AND FEEDING PROTOCOL FOR TADPOLES**

#### A.M. Cleaning

1. Take water temperatures and record on individual enclosure sheets. Maintain temperature between 28-30°C.
2. Siphon off old food and faeces from bottom of tank. Remove up to 50% of water and replace with dechlorinated water at the same temperature.
3. Take water samples (prior to feeding) to lab on Monday, Wednesday, and Friday.
4. If ammonia levels are above 0.5 ppm do an additional 40% water change.
5. Every Friday - replace filter wool and activated carbon in all filters.  
Every Monday - rinse off sponge filters with dechlorinated water (a light rinse just to remove detritus on surface of sponge).

#### A.M. Feeding (after water changes)

1. Feed appropriate amounts of flake food mixture (see Appendix A for composition). Mix dry food with a small amount of water (2-3 drops) and roll into a ball. Roll ball in Vitamin C powder to coat and place at side of tank or drop to bottom of tank.

#### Mid-day Feeding

1. Siphon off uneaten food and faeces. Replace water with dechlorinated water at the same temperature. If indicated by water chemistry (ammonia levels above 0.5 ppm) do a 40% water change at this time.
2. Feed appropriate amounts of tablet food.

#### Evening feeding

1. Offer appropriate amount of frozen or microwaved lettuce or spinach.

#### **6.4. FEEDING SCHEDULE EXAMPLE**

The following amounts are averages fed to a tank of fifty tadpoles at 30°C at the specified ages (day after spawning).

##### A.M.: Flake Mixture

Day 2 - Day 5: 1/16 tsp. (0.15g)

Day 5 - Day 8: 1/8 tsp. (0.3g)

Day 8 - Day 14: 3/16 tsp. (0.45g)

Day 14+ - amount reduced as metamorphosis progressed.

##### Mid-day: Tablet Diet

Day 2 - Day 5: 3/4-tablet (0.18g)

Day 5 - Day 8: 1 1/4 tablet (0.30g)

Day 8 - Day 14: 1 1/2 tablets (0.38g)

Day 14+ - amount reduced as metamorphosis progressed.

Evening: Lettuce and Spinach:

Offered in 4" X 4" pieces ad lib (usually 2 to 4 pieces per tank) since it does not foul the water overnight.

#### **6.5. METAMORPHOSIS**

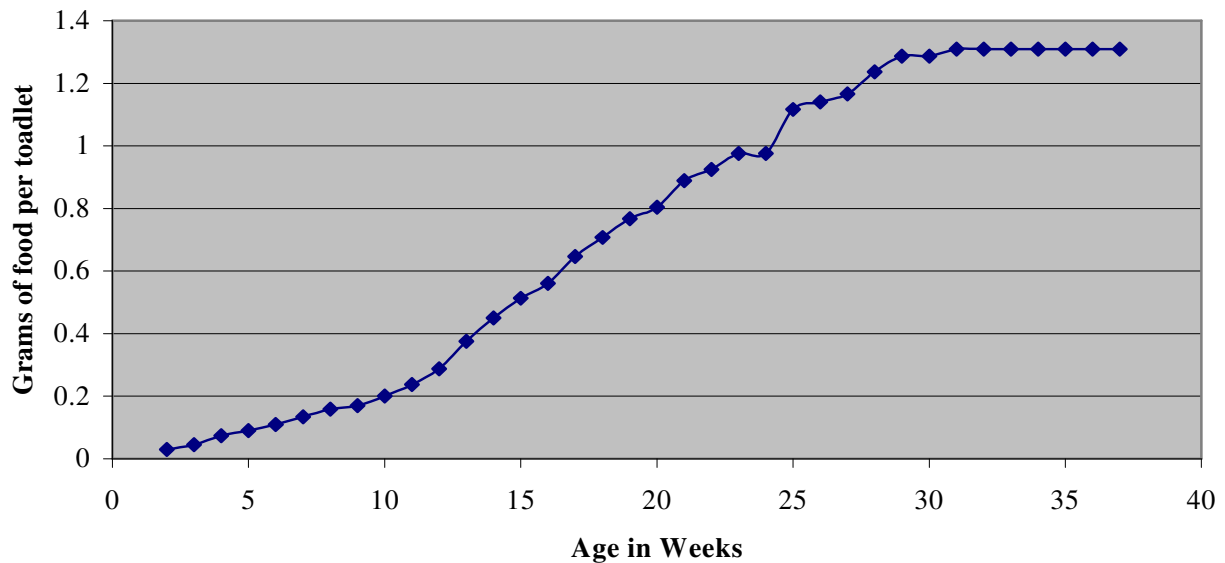
As toadlets start appearing on the "beaches", they are moved to toadlet rearing tanks. The Buffalo Zoo has had success using pea gravel as a substrate. Water is added to increase humidity and to prevent dehydration. At Toronto Zoo, the tank is set up with rubber matting or indoor/outdoor carpeting at one end of the tank and half coconut shells, clay shards, plastic hides, plastic plants and leaves are offered as refuges. One cm (.5 inch) of water is offered at the other end. To avoid desiccation after metamorphosing, the toadlets seek cover as soon as they leave the water. This

occurs even if water is available. Although many hide spots are offered, the toadlets usually mass together in large numbers under one or two hides. This may be an adaptive behaviour for moisture conservation since the surface area to mass ratio is much reduced. This drought reducing behaviour has been observed in both captive and wild situations with captive-bred toadlets.

The Toronto Zoo houses toadlets and toads in tanks with well fitted Plexiglas lids. Newly emerged toadlets are quite capable of scaling the vertical glass walls of these tanks, so secure lids are required. These lids have sections screened for air circulation. This also allows for some control over humidity levels, increasing or decreasing it by covering or exposing the screened areas of the tank lids. Since young toadlets are susceptible to dehydration, humidity in the tanks should be maintained over 80%.

The main food items for toadlets are pinhead crickets. If gravel is used as a substrate, care is required to ensure that the small crickets do not hide or disappear into the substrate. Fruit flies may be fed as well in the first few weeks. From graph 1 below, the appropriate amount of food to offer for a group of toadlets can be determined. For example, a group of 25 toadlets at 5 weeks would require 2.5grams of food.

**Graph 1: Average Weight of food offered per toadlet**



As the toadlets increase in size, so does the cricket size. At eight to ten weeks they are offered 1/4" crickets, at 16 weeks they are offered 1/2" crickets and at 24 weeks they are offered 3/4" adult crickets. At 18 weeks the toads are reduced to four feedings per week. As they approach adult size, other food items such as dew worms or new-born mouse pinkies are introduced. At this time they are put on the same feeding schedule as adults, and the amount of crickets offered is slightly reduced.

## **7. VETERINARY CARE**

**By Dr. Graham Crawshaw, Puerto Rican crested toad SSP Vet Advisor**

### **7.1. CLINICAL PROBLEMS**

When provided with the appropriate living conditions, the Puerto Rican crested toad has proven to be a resilient and long-lived animal in captivity. Disease conditions have been seen in individuals as well as in groups. There is no reason to believe that the Puerto Rican crested toad is in any way unique among amphibians from a disease point of view, but there is still little documented evidence of any specific disease processes. Most deaths have been sporadic, although multiple die-offs have occurred in both adults and juveniles. As with other amphibians, the diagnosis of the extent and cause of a particular problem in the live animal is not easy. A complete necropsy may be required to elucidate the problem and develop a course of treatment for other affected individuals.

### **7.2. QUARANTINE**

A suitable quarantine period in order to evaluate in-coming animals, to allow them to adjust to new conditions, recover from transit and to avoid transfer of disease to an existing collection. At Toronto Zoo, Puerto Rican crested toads are maintained in quarantine rooms for a minimum of 60 days or until animals are considered healthy, free from parasites, eating well and are gaining weight. The quarantine will be extended indefinitely if these conditions are not met. Animals are weighed upon arrival and a brief examination is performed. Later, at some stage during the quarantine period closer examination, identification, and parasite treatment are performed. Antibiotics are not used routinely unless the condition of the animals or medical history dictates. At least three negative faecal samples are required before animals are released from quarantine. Since Puerto Rican crested toads or their offspring may be designated for transfer to other institutions or for release in to the wild, protection of the species from medical conditions which may be present in other amphibians in the collection is highly desirable. The use of a separate room or facility for Puerto Rican crested toads is strongly encouraged.



### 7.3. INFECTIOUS DISEASE

#### 7.3.1. VIRUSES

Viral disease is as of yet undiagnosed in Puerto Rican crested toads. There have been cases of high tank mortality in groups of tadpoles that have generalised oedema reminiscent of lesions caused by the iridovirus, tadpole oedema virus, but no attempts have been made to isolate a virus.

#### 7.3.2. BACTERIA

As with other amphibian species, bacterial disease is not uncommon. Many of the cases of sepsis have been associated with outbreak situations during times of stress, such as aestivation, introduction to breeding tanks, or in young individuals. Post-shipment mortality has also been seen. Under normal circumstances conditioned established adults appear to be very hardy. While every effort should be made to keep the toads' environment free from pathogens, over cleaning, excessive disturbance and the use of disinfectants can be stressful and may render toads more susceptible to infectious disease. Affected animals tend to be lethargic and less responsive than normal, fluid accumulation may be seen in the legs and coelomic cavity, and reddening of the ventral surface and of the toes may be apparent. Occasional skin ulcers may be seen particular on the feet and at the points of contact with the ground. As with all amphibian species, bacterial septicaemia and dermatitis are of concern in Puerto Rican crested toads. Most of the bacterial species of concern are gram-negative opportunistic invaders. Organisms isolated from cases of sepsis and dermatitis include *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Bordetella*, *Alcaligenes*, and *Serratia*. Multiple organ systems are often infected in cases of septicaemia, including liver, kidney, spleen, and occasionally heart. *Acinetobacter* has been isolated from a case of pneumonia.

Thirty eight cases of systemic disease have been seen at Toronto Zoo . Most of these were diagnosed with single or multiple organism septicaemia. *Pseudomonas* spp. were the most commonly isolated bacteria from septic animals. *Flavobacterium*, *Bordetella*, *Alcaligenes*, and *Serratia marcescens* have also been cultured. In only four individuals was *Aeromonas hydrophila* cultured. There has been very little need for the use of antibiotics in Puerto Rican crested toads, since under Toronto Zoo conditions either individual or group outbreaks of bacterial infection are uncommon. Antibiotics of choice include aminoglycosides (Amiglyde, 5mg/kg s.c. q24h) and fluoroquinolones (Baytril-enrofloxacin, 5-10 mg/kg p.o., or s.c. q24h). Tadpoles also get bacterial sepsis, often associated with poor water quality or some other stress. Treatment of tadpoles can be performed with methylene blue baths (3 mg/l), or antibiotics.

Mycobacteriosis (tuberculosis) is a common condition in captive amphibians, and is manifested by either skin lesions (nodules and ulcers) or generalised disease. No records of mycobacteriosis causing death of Puerto Rican crested toads exists, but acid-fast bacteria has been identified in an abscess of a live toad.

### **7.3.3. FUNGI**

Fungal dermatitis and systemic fungal disease are occasionally seen in the clinical setting. *Nigrosporum* and *Trichoderma* have been isolated in fungal dermatitis at Toronto Zoo. Lesions are variably distributed, and typically black, necrotic and ulcerated with occasional white raised areas. In addition, one case of dermatitis has been seen in which the skin was black, necrotic and oedematous. Tadpoles occasionally also get fungal infections manifesting as multiple deaths within a tank with fungal organisms evident grossly or histologically. One case of chromoblastomycosis, a systemic fungal disease that has been well documented in other amphibian species has also been seen. Treatment of fungal disease can be undertaken with topical or systemic therapy. Itraconazole (Sporanox™)(10 mg/kg p.o. q 24h) or ketoconazole (10-20 mg/kg PO q12-24h) has been utilised with some success in amphibians. Topical therapy with enilconazole (Imaverol™) suspension was successful in treating the case of *Trichoderma*. While chytridiomycosis has not been documented in Puerto Rican crested toads it has been diagnosed in other bufonids, and there is no apparent reason the species should not be considered susceptible to infection by chytrids. Lesions are described as having a predominantly ventral distribution with a brown discoloration and granular appearance. Animals are often found dead before gross lesions are noted. No treatment for chytridiomycosis has been described.

### **7.3.4. PARASITES**

Parasitic disease has been relatively uncommon in captive Puerto Rican crested toads. Strongyloid infestation has been noted in a few individuals and has been quite refractory to treatment with various anti-parasitic drugs. A group of individuals at one zoo was diagnosed histopathologically with a *Balantidium*-like enteric parasitism. As there was no gross evidence of enteric disease or histologic evidence of inflammation, the pathogenesis remains unclear. Amoebic enteritis was diagnosed in one individual. *Entamoeba* has been shown to be pathogenic in other amphibian species, and may be diagnosed on direct smears or flotation, although systemic infection of the liver and kidneys can also occur. One zoo reported an infection with a coccidian. These are traditionally hard to eliminate in amphibians and reptiles. Treatment of parasitic diseases has met with variable success, depending on the parasite. Nematodes can be hard to eliminate permanently either due to ineffectiveness of the treatment or reinfestation from the environment. At Toronto Zoo the current treatment for strongyloids is with

moxidectin (Cydectin™) (1mg/kg s.c., q14d). Fenbendazole (Panacur™)(100 mg/kg p.o. q14d), and ivermectin (Ivomec™) (0.2-0.4 mg/kg s.c, q14d) have also been used.

## **7.4. NON-INFECTIOUS DISEASE**

### **7.4.1. NUTRITIONAL DISEASE**

Nutritional requirements of Puerto Rican crested toads, while being specifically undefined, seem to be generally met by the captive diets used at many zoos. Metabolic bone disease either associated with hypocalcemia or hypovitaminosis D has been seen in growing toadlets which have very high growth rates. In addition, adults may develop fibrous osteodystrophy, pathologic fractures and vertebral malformation secondary to long term calcium or vitamin D deficiencies. The use of powdered supplements and high calcium invertebrate diets are essential when feeding insects that often have inverse calcium: phosphorus ratios and are deficient in certain other nutrients.

Spindly leg syndrome has been seen in Puerto Rican crested toads, and as in other species, the cause has not been determined. The condition may affect up to 100% of developing toadlets but it appears to be more common in offspring from older breeding stock. Tadpoles appear to develop normally until the time of limb generation, at which time very small, poorly muscled forelimbs develop. At this stage no treatment is feasible and although affected animals may survive for some time, euthanasia is recommended. The cause of this disease also remains undefined and multiple factors may be involved. With the diet fed at the Toronto Zoo, for which nutritional analysis has been performed, this syndrome is not seen commonly. It is vitally important that the ingredients for the tadpole diet, including vitamins are fresh. Oxidation of some of the nutrients is likely a contributing factor in spindly leg syndrome.

Other developmental deformities have also been seen in juvenile Puerto Rican crested toads including missing eyes and rotated elbows. A cause has not been determined. Gastric impaction secondary to overfeeding has been seen in a few Puerto Rican crested toads, and consideration should be given not to overfeed individuals. Young and small animals may be at higher risk due to the lower potential gastric volume.

Myopathy (muscle pathology) has been seen in a number of individuals. While no aetiology has been defined for this myopathy, nutritional deficiencies (i.e. hypovitaminosis E) are a concern, and vitamin E levels should be quantified in suspect cases. A hind limb paralysis has also been seen in a number of individuals. Whether or not nutritional deficiencies (i.e. vitamin E, A, or B) may play a role in this disease is not known.

#### **7.4.2. PHYSICAL FACTORS**

Drowning is seen periodically in individuals at breeding time. Small females, that cannot support the weight of amplexing males, are particularly at risk. Submerged structures where individuals can sit at the water surface are helpful in preventing drowning, but not totally effective. Metamorphosing toadlets may drown if left in deep water too long without access to shoreline once their forelimbs emerge. Trauma is an occasional and unfortunate cause of death. Mortality has been associated with preparation for breeding both through drying (aestivation) and cooling (hibernation). Accidental excessive cooling has lead to deaths in hibernated breeders. As with any amphibian species, *P. lemur* is susceptible to desiccation particularly in small individuals. Animals aestivated in drying conditions may also succumb to desiccation if the substrate is too dry. Animals aestivated in this manner must be monitored carefully for dehydration. Mortalities have also been seen when cooled or aestivated animals are placed in breeding tanks and rehydrated. It is not uncommon that individuals put into breeding tanks 24-48 hours after being removed from aestivation die from overhydration with lesions of ascites (coelomic fluid) and generalised oedema. Older animals appear to be much more susceptible. It is suggested that these animals be rehydrated more slowly over a period of 2-3 days before being placed into breeding tanks to allow for a more gradual return of normal osmoregulatory function, and that they be monitored closely. It is possible that the hormone used to induce reproduction (luteinizing hormone releasing hormone) may increase susceptibility to osmoregulatory imbalances since the hormones associated with egg-laying oxytocin and arginine vasotocin increase the permeability of skin. To counteract this effect, furosemide (Lasix <sup>TM</sup>) may be given by injection (5mg/kg s.c.).

#### **7.4.3. TOXINS**

As with all amphibians, the Puerto Rican crested toad is susceptible to environmental contamination and toxicity. Tadpoles and toadlets are the highest risk groups. Degradation of environmental conditions and contamination with such things as cleaning chemicals should be considered in cases of high tank mortalities. Regular testing of water for ammonia, nitrates, pH, and temperature is imperative. Ammonia levels under 0.1 ppm, nitrates below 1ppm, pH between 7 and 8, and temperature between 29 °C and 31 °C are recommended. Regular water changes or excellent biological filtration are generally recommended to maintain such conditions. High levels of dissolved gases can cause "gas bubble disease," in tadpoles as is seen in fish.

#### **7.5. OTHER CONDITIONS**

Tumours are generally quite rare in amphibians. One case of myeloproliferative disease was diagnosed at necropsy. Kidney and urinary pathology is common in older animals. The majority

have glomerular disease. A cluster of four cases of cystic nephropathy was noted at one zoo. The causes remain unclear.

## **7.6. MORTALITY PEAKS**

Mortality peaks are associated with particular times in the life cycle of captive Puerto Rican crested toads. Infertility does occur. It is not uncommon that 15-25% of a clutch of eggs is infertile. Higher levels of infertility may also be seen. While tadpole survivability is greatly increased in captivity, the tadpole stage can experience high mortality. Tadpole mortality rates seem to peak within the first few days after hatching and at or near metamorphosis. However, large numbers of tadpole deaths should not be considered normal and diagnostic efforts should be pursued. Tadpoles that are fed adequately grow at variable rates, and a small percentage of individuals never seem to flourish. The exact reasons for this are unclear, and undoubtedly these are individuals that would not survive natural conditions.

## **7.7. ANAESTHESIA**

Anaesthesia in Puerto Rican crested toads is similar to anaesthesia of other toad species. For general anaesthesia toads are placed in a container containing a shallow depth of water containing 2 g/l tricaine methanesulphonate (MS-222). Solutions should be buffered to bring the pH close to neutral using the same concentration (2g/l) of sodium bicarbonate (powder or premixed solution). The toad sits in and is soaked in the solution until the appropriate level of anaesthesia is achieved. This can be judged by increasing muscular relaxation, loss of righting reflex, lack of response to pain, and slowing respiration. Induction usually takes 8-12 minutes at room temperature (smaller animals induce faster).

Blood collection and surgery can be performed following MS-222 anaesthesia. At surgical levels, respiration ceases. Toads have been kept anaesthetised for an hour or more (especially at cool temperatures) without the need for additional anaesthetic. In fact, keeping the animals in anaesthetic solution will result in overdose and death. Additional MS-222 can be applied or injected if needed to maintain surgical plane. Animals should be kept moist during surgery. Following completion of the procedure, toads are rinsed in clean water and allowed to recover. It is recommended that toads are warmed to reduce recovery times – this can be achieved by placing the container on a heating pad.

Local anaesthesia is used for placing transponders and surgery of superficial lesions. Emla cream <sup>TM</sup> is applied to the location with cotton tipped applicator and rubbed in. In toads anaesthesia of the area is achieved within a few minutes. Avoid excessive application of the cream as this can cause general anaesthesia. Excess cream is wiped and rinsed of at the end of the procedure.

## 7.8. SURGERY

Puerto Rican crested toads are quite amenable to surgery if satisfactory anaesthesia can be achieved. Laparotomy, laparoscopy and skin surgery can be performed. Local anaesthesia can be used for superficial biopsies and lacerations. Wounds are sutured with a soft absorbable suture (e.g. Vicryl <sup>TM</sup>) and VetBond <sup>TM</sup> glue is applied to the suture line. For small wounds, VetBond alone is used.

## 7.9. BLOOD SAMPLING AND HAEMATOLOGY

The usefulness of blood samples in the diagnosis of amphibian disease is much less proven than in other taxa. Blood parameters are more labile in amphibians and there has been little work on correlating changes in blood values with disease. However, sampling is encouraged in order to develop knowledge in this area. Obtaining samples can be difficult and cardiac puncture remains the most reliable source for blood. Anaesthesia is recommended, to achieve deflation of the lungs and allow palpation of the heart. Puerto Rican crested toads typically have high basophil counts - a feature common to some amphibians.

A study at the Toronto Zoo in 1998 looked at haematological parameters. Blood samples were taken from anaesthetised toads by cardiac puncture. The heart is located beneath the sternum (it may be necessary to retract the xiphoid process cranially). Blood is collected via a small gauge needle and syringe introduced into the ventricle with gentle aspiration. This procedure appears to be quite safe. Haematological parameters of forty (eight-month-old) anaesthetised toads are presented in Table 2.

**Table 2: Haematological parameters**

| Parameter                                            | Mean  | Standard Deviation |
|------------------------------------------------------|-------|--------------------|
| Erythrocyte count (automated) ( $\times 10^{12}/l$ ) | 0.987 | 0.109              |
| Erythrocyte count (manual) ( $\times 10^{12}/l$ )    | 1.237 | 0.208              |
| Leukocyte count (manual) ( $\times 10^9/l$ )         | 10.16 | 3.071              |

| Parameter                         | Mean  | Standard Deviation |
|-----------------------------------|-------|--------------------|
| Neutrophil (%)                    | 4.400 | 2.458              |
| Lymphocyte (%)                    | 63.76 | 8.224              |
| Basophil (%)                      | 27.73 | 7.225              |
| Eosinophil (%)                    | 3.000 | 3.242              |
| Monocyte (%)                      | 1.325 | 1.421              |
| Hematocrit (%)                    | 32.93 | 3.744              |
| Hemoglobin (g/l)                  | 105.2 | 13.90              |
| Protein (total) (g/l)             | 38.45 | 5.5                |
| Albumin (g/l)                     | 18.00 | 2.699              |
| Albumin/Globulin ratio            | 0.885 | 0.158              |
| Alkaline Phosphatase (IU/l)       | 51.90 | 19.36              |
| Alanine Aminotransferase (IU/l)   | 13.68 | 7.956              |
| Aspartate Aminotransferase (IU/l) | 228.5 | 154.5              |
| Bile Acids (total) (μmol/l)       | 1.780 | 0.645              |
| Bile Acids (Conjugated) (μmol/l)  | 0.480 | 0.304              |
| Amylase (IU/l)                    | 91.73 | 80.98              |
| Lipase (IU/l)                     | 53.73 | 20.63              |
| Calcium (mmol/l)                  | 2.015 | 0.210              |
| Chloride (mmol/l)                 | 76.18 | 6.520              |
| Phosphorus (mmol/l)               | 1.863 | 0.673              |
| Potassium (mmol/l)                | 4.30  | 1.48               |
| Sodium (mmol/l)                   | 109.5 | 5.849              |
| Sodium/Potassium ratio            | 30.43 | 15.94              |
| Creatinine Phosphokinase (IU/l)   | 2389  | 1852               |
| Creatinine (μmol/l)               | 28.23 | 5.568              |
| Urea (mmol/l)                     | 15.04 | 6.437              |
| Glucose (mmol/l)                  | 2.720 | 0.958              |

#### 7.10. EUTHANASIA

Tadpoles and small toadlets (under 5 grams) may be euthanised by placing them in a solution of T-61 or pentobarbitone. Large toads should be injected individually (i.p.).

## 8. PRODUCTS MENTIONED IN THE TEXT

- Amikacin (Amiglyde™) – Ayerst Veterinary Labs, Wyeth-Ayerst Canada Inc. 400 Michener Rd, Guelph, ON N1K 1E4, Canada
- Ascorbic Acid, coated - Hoffman-LaRoche Ltd., Etobicoke, Ontario, M9C 5J4, Canada.
- Aquarian Herbivor Diet –
- Enrofloxacin (Baytril™)—Bayer Inc. Ag. Division- Animal Health, 77 Belfield Rd. Toronto, Ont. M9W 1G6
- Calcium blocks - Turtle Tank Neutralizer: Wordley Corporation, New Jersey, U.S.A.
- Enilconazole (Imaverol™) Janssen Animal Health, 19 Green Belt Dr., Toronto, ON M3C 1L9, Canada
- Fenbendazole (Panacur) – Hoechst Roussel Vet, 240 Henderson Dr., Regina, SK, S4N 5P7, Canada
- Furosemide (Lasix™) - Hoechst Roussel Vet Vet, 240 Henderson Dr., Regina, SK, S4N 5P7, Canada
- Itraconazole (Sporanox™)Janssen-Ortho
- Ivermectin (Ivomec™) – Merial Canada Inc., 500 Morgan Blvd. Suite#1, Baie D’Urfe, PQ H9X 3V1, Canada
- Luteinizing hormone releasing hormone analog des-Gly (d-Ala) LHRH ethylamide: Sigma Chemical (catalog number L4513)
- Moxidectin (Cydectin™), Ayerst Veterinary Labs, Wyeth-Ayerst Canada Inc. 400 Michener Rd, Guelph, ON N1K 1E4, Canada
- MS222™- Sandoz, Basle, Switzerland
- Murex Spirulina Flakes - Murex Aqua Foods Inc., Langley, British Colombia, V3A 5E8, Canada.
- Osram Sylvania Blacklight F40/350BL-40W—Osram Sylvania, 100 Endicott St. Danvers, MA, 01923, USA
- Sera San Colour Enhancing Flakes - Sera Aquarisk GmbH 5138 Heinsberg, Germany.
- Tetra FD Menu, Special Food, 4 in 1 blend: Tetra Werke, Ulrich, Germany
- Trovan™ – InfoPet Identification Systems Inc., 517 W. Travelers Trail, Burnsville, MN 55537-2548, USA
- Vatbond™ – 3M Animal Care Products, St. Paul, MN 55144-1000, USA
- Vita-lite™, Duro-Test Corp. 9 Law Dr., Fairfield, NJ 07004, USA



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## 10. ACKNOWLEDGEMENTS

Dianne Devison, Supervisor Toronto Zoo; Rick Paine, Buffalo Zoo; Cincinnati Zoo; Dr. Graham Crawshaw, Veterinarian, Toronto Zoo; Nubar Dakessian, Keeper, Toronto Zoo; Elaine Gabura, Puerto Rican crested toads Studbook keeper, Toronto Zoo; Bob Johnson, Curator Amphibians and Reptiles Toronto Zoo; Mavis Russell, Animal Care Clerk, Toronto Zoo; Eldon Smith, Curatorial Assistant, Toronto Zoo; Dr. Eduardo Valdes and Nutrition Centre staff, Toronto Zoo; Animal Health Centre staff, Toronto Zoo;

**Appendix A                      TADPOLE DIET NUTRIENT COMPOSITION**

| <b>Component</b> |       | <b>Dry Basis</b> | <b>As Fed</b> |
|------------------|-------|------------------|---------------|
| Dry Matter       | %     | 100.00           | 93.00         |
| Crude Protein    | %     | 51.18            | 47.60         |
| Crude Fat        | %     | 5.81             | 5.40          |
| Ash              | %     | 12.04            | 11.20         |
| Crude Fibre      | %     | 1.83             | 1.70          |
| Gross Energy     | Cal/g | 4.920            | 4.575         |
| ADF              | %     | 2.47             | 2.30          |
| Ca               | %     | 2.47             | 2.30          |
| P                | %     | 1.45             | 1.35          |
| Mg               | %     | 0.17             | 0.16          |
| K                | %     | 0.88             | 0.82          |
| Na               | %     | 0.77             | 0.72          |
| Fe               | Ppm   | 1018.82          | 947.50        |
| Zn               | ppm   | 142.69           | 132.70        |
| Cu               | ppm   | 6.88             | 6.40          |
| Vit C            | mg/g  | 8.67             | 8.11          |
| Vit E            | mg/kg | 396.77           | 369.00        |
| Vit A            | IU/kg | 5836.70          | 5428.13       |
| Vit D            | IU/g  | 3.33             | 3.10          |
| Asp              | %     | 4.15             | 3.86          |
| Glu              | %     | 7.28             | 6.74          |
| Ser              | %     | 2.18             | 2.03          |
| Gly              | %     | 4.37             | 4.06          |
| His              | %     | 1.12             | 1.04          |
| Arg              | %     | 2.90             | 2.70          |
| Thr              | %     | 1.97             | 1.83          |
| Ala              | %     | 3.17             | 2.95          |
| Pro              | %     | 3.22             | 2.99          |
| Tyr              | %     | 1.42             | 1.32          |

**Appendix A (cont.)      TADPOLE DIET NUTRIENT COMPOSITION**

| <b>Component</b> |   | <b>Dry Basis</b> | <b>As Fed</b> |
|------------------|---|------------------|---------------|
| Val              | % | 2.20             | 2.05          |
| Met              | % | 1.22             | 1.13          |
| Ile              | % | 1.81             | 1.68          |
| Leu              | % | 3.40             | 3.16          |
| Phe              | % | 1.95             | 1.81          |
| Cys              | % | 0.44             | 0.41          |
| Lys              | % | 3.12             | 2.90          |

Flake diet Ingredient Composition

|                                 |        |
|---------------------------------|--------|
| Aquarian Herbivore Diet         | 24.84% |
| Tetra FD-Menu 4 In 1 Blend      | 24.84% |
| Sera-San Color Enhancing Flakes | 24.84% |
| Murex Spirulina Flakes          | 24.84% |
| Ascorbic Acid, Coated           | 00.63% |

## TADPOLE REARING RECORD

Month:                  Year:                                                  Enclosure:

Species: Puerto Rican Crested Toad I.D.:          Dam:         

I.D.:      Sire: \_\_\_\_\_

Date Laid:

Notes: Follow posted Protocol

[illegible]

**Appendix C**

**AZA EXHIBIT SURVEY BY ELIZABETH BRYANT-CAVAZOS**

**Part 1: Graphics and Exhibit Technique**

| INSTITUTION          | GRAPHICS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | SECRET TO EXHIBITING TOADS                                                                                                                                                                                                                                                                        |
|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Buffalo              | We currently have a very basic graphics: new graphics would highlight conservation (particularly the release program) and adaptations of semi-fossorial amphibians in harsh environments.                                                                                                                                                                                                                                                                                                                                                                                                                                                            | They are not capable of burrowing into the hard mud (the dried mud looks great to) and the toads hang out in the stone bunkers in view of the public. They get active for feeding and misting.                                                                                                    |
| Calgary              | Habitat loss is the major reason why these distinctive looking toads are endangered. Zoos are trying to help Puerto Rican crested toad populations recover through the Species Survival Plan breeding and release program.                                                                                                                                                                                                                                                                                                                                                                                                                           | Haven't been able to keep visible. Possibly try small potted plants at front of the exhibit. They seem to like sitting in the pots under vegetation                                                                                                                                               |
| Cleveland Metroparks | The sensitive skin of frogs and toads is highly permeable, allowing water and air to pass, much like out lungs. This unique property also makes the animals particularly vulnerable to pollutants and pesticides. Amphibians act as environmental monitors, providing us with early warnings of changes in the Rainforests conditions. At the bottom of the graphic -- SSP The Puerto Rican crested toad is highly endangered due to extensive habitat destruction on its tropical island. Cleveland Metroparks Zoo is an active participant in an international effort called Species Survival Plan (SSP) to help breed and conserve the rare toad. |                                                                                                                                                                                                                                                                                                   |
| Columbus             | Puerto Rican crested toad ( <i>Peltophryne lemur</i> ). Loss of coastal wetlands due to development, conversion to crop land and drainage for mosquito control, threaten this amphibian with extinction. Survival depends upon habitat protection and captive breeding and reintroduction plans. The Columbus Zoo participates in a Species Survival Plan for the Puerto Rico and Virgin Gorda. Habitat: exposed limestone outcroppings.                                                                                                                                                                                                             | They like to squeeze between the rocks, but they can't go back very far. Rocks are stacked to leave cracks that the toads utilise, but are still visible. There is also a glass petri dish that is buried to its rim and filled with sheet moss that the toads sit in if the moss in not too wet. |
| Detroit              | Once thought to be extinct in the wild, this small toad with bony ridges above its eyes is known to have a very small population on the island of                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Put water up front; they like to sit in a water bowl.                                                                                                                                                                                                                                             |

**Appendix C (cont.)                      Part 1: Graphics and Exhibit Technique**

| INSTITUTION     | GRAPHICS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | SECRET TO EXHIBITING TOADS                                                                                                                                                                                                                                   |
|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Detroit (cont.) | Puerto Rico. Captive-bred offspring of this endangered amphibian have been taken back to Puerto Rico for release into their native habitat. Tiny radio transmitters "backpacks" have been placed on some individuals by researchers to track the movements of these animals in the limestone crevices where they reside. Puerto Rican crested toad ( <i>Peltophryne lemur</i> ). Range: Puerto Rico. Also SSP logo on graphic. The exhibit in the amphibian center will have a continuously running loop playing above the exhibit showing habitat, backpacked animals, as well as traditional graphics.                                                                                                                                                                                                   |                                                                                                                                                                                                                                                              |
| Fort Worth      | Puerto Rican crested toad ( <i>Peltophryne lemur</i> ) Limestone karst formations, Puerto Rico and Virgin Gorda. Once believed to be extinct, there have been recorded sightings of this rare toad in the wild. SPECIES SURVIVAL PLAN. Zoos and aquariums throughout the world are currently working together through the AZA to protect and conserve the world's rare and endangered species. Through captive breeding and education, the SSP was established by the AZA to ensure viable gene pools of selected endangered species. The dream of all dedicated zoo and aquarium professionals is to see a time when, through captive propagation, stable populations of animals may be reintroduced to protected natural habitats. The members in this exhibit are members of the Species Survival Plan. | Shallow limestone "caves" are provided. Longer more narrow caves allowed toads to hide too much. When filled in with gravel, the toads started scraping there snouts. Caves were then enlarged and their snouts healed up and the public can still see them. |
| Louisville      | The Puerto Rican Crested Toad was the first amphibian to become part of the American Zoo and Aquarium Associations Species Survival Plan. For more than 30 years it was thought to be extinct. The larger Cane Toad was introduced in the early 1900's and competes with the Puerto Rican crested toad for resources. Due to human development, toad populations have disappeared in many areas. The largest breeding site for this species is a dirt parking lot under constant threat of development. Also a range map, diet info and a toad sticker.                                                                                                                                                                                                                                                    | Opening up crevices in front and closing them off in the back. Also place pool in front of exhibit.                                                                                                                                                          |



**Appendix C (cont.)                      Part 1: Graphics and Exhibit Technique**

| <b>INSTITUTION</b> | <b>GRAPHICS</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | <b>SECRET TO EXHIBITING TOADS</b>                                                                                                                                                                                                                               |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Philadelphia       | It is hard to believe that up until 1967 everyone thought the Puerto Rican crested toad was extinct. Today we released some more tadpoles that were born at the Toronto Zoo. Starting with six toads, zoos have returned 4,000 captive-bred toadlets and 12,000 tadpoles to the wild. The Philadelphia Zoo is building artificial breeding ponds in Puerto Rico to continue this success.                                                                                                                                                                                                                                       | Rocks are stacked securely to provide dimly lit low caves. Caves must be low enough so the toads have to hunker down to fit. Tank is constantly moist using a magnum 350 filter for recirculation of water.                                                     |
| St. Louis          | Focus on conservation. The crested toad is only found in Puerto Rico. This species is endangered of extinction and depends on the limestone habitat along the north and south-western coasts of the island. Heavy seasonal rains trigger the toads to emerge from the limestone and breed in the temporary ponds formed by the rain. There is very little freshwater habitat remaining in these areas and the toads now breed in cement cattle troughs that are filled by rainwater. Freshwater ponds are essential for the survival of this species. Imagine waiting all year to breed, only to discover that the pond is dry! | The rockwork in the exhibit is set up in such a way that the animals feel secure, but are visible by the public.                                                                                                                                                |
| Santa Barbara      | Listed as a threatened species, the Puerto Rican crested toad is part of the SSP. This program attempts to maintain the diversity of each species listed through a strict breeding program. Range....                                                                                                                                                                                                                                                                                                                                                                                                                           | Have tried placing curved bark against glass or positioned so that the only cover was under it and the animals could be viewed. After a day or so they found a way of moving the bark or positioning themselves out of sight. Have also used minimal substrate. |
| Sedgwick County    | SSP - Those initials stand for Species Survival Plan. SSP is a way for co-operating zoos to manage the captive breeding of endangered species on an international scale. The object is to avoid inbreeding and to weed out harmful inherited traits. The Puerto Rican crested toad is one of this zoo's SSP species.                                                                                                                                                                                                                                                                                                            | Rock crevices large enough to squeeze into, but shallow enough so the toad can't completely conceal itself -- a difficult balance to achieve; a "rain" period will bring them out of hiding for a while.                                                        |

## Appendix C (cont.)      Part 1: Graphics and Exhibit Technique

| INSTITUTION        | GRAPHICS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | SECRET TO EXHIBITING TOADS                                                                                                                                                              |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Toronto            | Conservation - how precarious survival is; role of community in conservation and role of zoos (SSP) in assisting survival (release to adjacent constructed ponds).                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Will provide limited rock depressions shallow enough to hide in but remain visible. Plastic tubes will be designed to keep toads in clear part of the tube, but secure in the "tunnel". |
| Vancouver Aquarium | You are looking at some very lucky toads. They were thought to be extinct until 1967. Fortunately there were enough of them left to start a captive-breeding program in 1982. Our Aquarium is proud to be one of the zoos and aquariums breeding these toads under a Species Survival Plan. So far, 16,000 captive-bred tadpoles and toadlets have been released back to Puerto Rico. These toads are now listed as threatened by the U.S. Fish and Wildlife Service – Not bad for toads thought to be extinct just over 30 is the loss of their breeding ponds and limestone-hill homes to residential and tourist developments. | The sandstone slabs are stacked so most of the hiding spots are somewhat visible to the public. Most of the time the toads' heads are visible, and the visitors get a pretty good view. |

## Part 2: Exhibit Detail

| Institution | Dimensions                       | Substrate                                    | Plants                                | Props                                                                    | # of toads | Other Information                                                                                                                                                                                                                                     |
|-------------|----------------------------------|----------------------------------------------|---------------------------------------|--------------------------------------------------------------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Buffalo     | 4' wide<br>3' high<br>2' deep    | Topsoil mixed with water and allowed to dry. | Pear cacti and other species of cacti | Cacti, hawthorn branches (dead), stone bunkers pushed into mud when soft | 3.2 - 4.5  | Tried several other mixes of substrate; if it was loose at all, the toads disappeared. The mud worked great in my opinion; you can even imbed rocks in the mud and make it look like a limestone hillside. Cacti pots were buried in the mud as well. |
| Calgary     | 40" wide<br>65" high<br>38" deep | Rock, moss, gunnite                          | Asparagus fern, pothos, silver king   | moss, slate                                                              | 2.4        |                                                                                                                                                                                                                                                       |

**Appendix C (cont.)**

**Part 2: Exhibit Detail**

| <b>Institution</b>   | <b>Dimensions</b>                | <b>Substrate</b>                                                | <b>Plants</b>                                                                          | <b>Props</b>                                                                                           | <b># of toads</b> | <b>Other Information</b> |
|----------------------|----------------------------------|-----------------------------------------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-------------------|--------------------------|
| Cleveland Metroparks | 3' wide<br>5' high<br>3' deep    | Fibre glass                                                     | Phiodendron, bromeliad                                                                 | 2" - 3" washed river rocks, native wood branches, and sheet moss.                                      | 3.0.0             |                          |
| Columbus             | 13" wide<br>16" high<br>36" deep | Dry topsoil                                                     | Tillandia species (air plants - bromeliads) placed in rock crevices.                   | Back and side walls are covered by tuffa rock stacked to the top. Crevices are filled with sheet moss. | 0.1 - 0.3         |                          |
| Detroit              | 12" wide<br>10" high<br>14" deep | Sharp sand                                                      | Succulents                                                                             | Limestone rock                                                                                         | 2.0.0             |                          |
| Fort Worth           | 38" wide<br>19" high<br>19" deep | Gravel (white limestone gravel mixed with brown and tan gravel) | Asparagus fern                                                                         | Limestone rocks, sand-stone, twig-like branches and a slow waterfall                                   | 1.1               |                          |
| Louisville           | 24" wide<br>14" high<br>18" deep | Soil, gravel, rock, moss, bark                                  | Creeping asparagus                                                                     | Limestone, sheet moss cork bark.                                                                       | 0.3               |                          |
| Philadelphia         | 24" wide<br>14" high<br>36" deep | Pea gravel                                                      | Pothos                                                                                 | Limestone rock                                                                                         | 3.0.0             |                          |
| St. Louis            | 87.6 cm<br>45.7 cm<br>55.8 cm    | Gravel mixture, soil in areas where there are plants            | Various types of ivy, pothos, and ficus. Future exhibit will have arid adapted plants. | Rocks, driftwood.                                                                                      | 0.3               |                          |
| Santa Barbara        | 11" wide<br>12" high<br>12" deep | Bark, moss                                                      | Cut plants from zoo grounds that are changed every 1-2 days.                           | Bark and rocks.                                                                                        | 2.0.0             |                          |

**Appendix C (cont.)**

**Part 2: Exhibit Detail**

| <b>Institution</b> | <b>Dimensions</b>                      | <b>Substrate</b>                                                             | <b>Plants</b>                          | <b>Props</b>                                                 | <b># of toads</b> | <b>Other Information</b>                                                                                                                                                                          |
|--------------------|----------------------------------------|------------------------------------------------------------------------------|----------------------------------------|--------------------------------------------------------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sedgwick County    | 23" wide<br>24" high<br>19" deep       | Medium size gravel in pool area, lime-stone rocks cover terrestrial portion. | Sanseveria, hoyo, pothos, Swedish ivy. | Twigs, dried grass                                           | 4.0.0             | Exhibit has a floor drain to allow pool water changes (about 3 X a week). Animals are rotated on and off exhibit.                                                                                 |
| Toronto            | 18" wide<br>2' high<br>3' deep         | Shallow bark mulch and 1/2" river stone for drainage.                        | 1 plastic cactus                       | Limestone rock, clear plastic tubes as tunnels, cactus wood. | 3.3               |                                                                                                                                                                                                   |
| Vancouver Aquarium | 40 cm wide<br>40 cm high<br>80 cm deep | Soil covered with sandstone slabs (toads cannot get to soil).                | Pothos, spathiphyllum ficus, pumilla   | Rocks and plants                                             | 1.1               | Although the sandstone is visually nothing like the toads natural habitat, I feel it allows me to reproduce the environmental conditions fairly accurately, and is easily cleaned and maintained. |

Appendix D

INSTRUCTIONS FOR DOMESTIC SHIPMENTS

**WITHIN THE UNITED STATES**

Shipping will be relatively simple. Book your shipment with the carrier of your choice, ensure the shipping container is of IATA standard, labels are affixed properly, & emergency instructions are affixed to the outside of the shipping container.

Other documents to include with the shipment are, specimen reports, emergency instructions, IATA declaration.

There are no permits required to move the toads interstate, between participating institutions as long as there is no commerce between exchanging institutions. USF&W retains ownership of the toads. As these toad shipments are for breeding and relocation/reintroduction, and involves no commerce, no permits are needed. It would be a good idea to include with your shipment a copy of the USF&W Puerto Rican Crested Toad permit, to confirm that the institutions involved in the shipment are on the list of approved institutions to hold and relocate toads.

**WITHIN CANADA**

Follow the information give above for the United States. Check with the local Provincial Wildlife or Natural Resources Ministry to confirm there are no import or export permits required by the Province. Some Provinces in Canada will require a permit from them to move the toads into or out of the Province.

## **Appendix E**

## **INSTRUCTIONS FOR INTERNATIONAL SHIPMENTS**

### **DOCUMENTS REQUIRED:**

- a valid copy of the USF&W Puerto Rican Crested Toad Permit (for import/export from or to the USA)
- health certificate
- Airway bill—available from the airline and usually filled out by airline staff
- USFW 3-177 form (import / export declaration )
- IATA declaration
- Commercial invoice also known as Proforma invoice
- Emergency instructions

### **FLIGHT BOOKING:**

The airline of choice is Delta Airlines. Call the Delta Air Cargo office and make you're booking 4-5 days in advance of the proposed shipping date. Ensure your shipment is to go "DELTA DASH" (this is a priority service). You will need to book to enter at one of the designated ports for wildlife to enter the USA. We recommend you use Atlanta, as it most suitable to favourable weather conditions at the time of year shipping occurs.

The shipment will be done over two days, day one the shipment arrives in Atlanta at or about noon or early afternoon to allow for USFW inspection. The shipment will then sit overnight and take the first Delta flight out of Atlanta for San Juan, Puerto Rico, arriving San Juan about noon. This allows USFW staff time to clear customs and pickup and deliver to the release sites before nightfall.

### **NOTIFICATION OF CONSIGNEE AND USFW ATLANTA**

#### **USFW Service:**

Once you have booked the shipment and received an airway bill number, proceed to complete all the required paperwork. Then notify by phone (follow up with fax) the USFW Service at Atlanta for the inspection. Send to them via fax a copy of the permit, airway bill, and a copy (unsigned) of the USFW 3-177 form, and any other information they may require.

US Fish & Wildlife Service, Atlanta Airport: Inspector Kenneth Endriss Ph: 404-763-7959  
Fax: 404-763-7560.

#### **CONSIGNEE:**

Ensure the address for the consignee is as follows:

US Fish & Wildlife Service

Carr.301, Km 5.1

P.O. Box 491

Boqueron, Puerto Rico, USA. 00622

Attn: Susan Silander, ph: 787-851-7297 ext: 30, Fax: 787-851-7440

Send to Susan Silander all the information regarding the shipment. This will include the flight details, time of arrival, etc. Also send with the information a copy of the USFW 3-177 (unsigned copy) and ask Susan to Sign and forward to the inspectors at the Atlanta Airport. US law requires the consignee (in this case the USFW, Puerto Rico) to sign the 3-177 at the port of entry

## **Appendix E (cont.)            INSTRUCTIONS FOR INTERNATIONAL SHIPMENTS**

(Atlanta). Susan will need to liase with the USFW inspectors in Atlanta to receive the signed copy. This signed copy is required for USFW clearance.

### **ATTACHED DOCUMENTS AND HOW TO COMPLETE:**

- **AIRWAYBILL:**

Box 1: (Routing and destination)

Note: the airlines use codes for airports ATLANTA is known as “ATL, SAN JUAN is known as “SJU” fill in the TO box with the letters “ATL”, the next box is “by first carrier” fill in Airline taking he shipment, in this case Delta Airlines. The next box is TO fill in the letters SJU for San Juan, the next box is labelled BY fill in the Airlines Code, Delta is DL, Drop down to the Airport of Destination and enter San Juan Puerto Rico,

Box 2:

This is where you will fill in the address of the USFW Boqueron, Puerto Rico with Susan Silander as the contact and her phone number

Box 3:

Fill in the shippers name and address along with a contact name and phone number and enter your account number with Delta in the box as indicated

Box 4:

Fill in the box only indicated “Airport of Departure” this is the name of the departure airport

Box 5:

Enter the currency of the shipment i.e.: for a value in US Dollars enter “USD, for a value in Canadian Dollars enter “CND”. If the shipment is to be sent prepaid, enter an XX in the box indicated by PPD. Enter the declared value for carriage and declared value for customs in the boxes as indicated. Note: this value must be the same in both cases.

Box 6:

Enter the number of pieces as indicated, move across the column as indicted Nature and quantity of goods and enter “LIVE TOADS/TADPOLES”; under this enter the dimensions of the boxes in the shipment.

Box 9:

Enter any handling information, see example

Sign airway bill at the bottom as indicted by “signature of shipper above” and initial the box indicating this shipment does not contain dangerous goods.

**Appendix E (cont.)            INSTRUCTIONS FOR INTERNATIONAL SHIPMENTS**

- US FISH & WILDLIFE SERVICE 3-177 --DECLARATION FOR IMPORTATION OF EXPORTATION OF FISH OR WILDLIFE

See attached copy for a sample of how to fill in

- COMMERICAL INVOCIE OR PROFORMA INVOICE

See attached copy of how to complete, Note: value on this form must be the same as entered on the airway bill in the boxes indicated “ declared value for carriage and declared value for customs”

- IATA SHIPPER’S CERTIFICATION FOR LIVE ANIMALS

See attached copy

- EMERGENCY INSTRUCTIONS

See attached copy of our emergency instructions; you should include this information with the paperwork and also attach a copy to outside of one of the boxes in the shipment.

**TO TRACK INTERNATIONAL SHIPMENTS:**

To track an international shipment, go to the Delta Air Cargo web site (<http://www.delta-air.com/home/index.jsp>) and find the area indicated “Site map and Search” and click on it. Your will be brought to a screen listing “Programs and Services” click on "Delta Air Cargo", a screen will appear where you can enter your airway bill to enable you find out the status of your shipment.