Association of Zoos and Aquariums
Amphibian Taxon Advisory Group Regional Collection Plan

2nd Edition, December 2008

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ATAG Definition and Scope

"If we continue on our current course, the accelerating loss of amphibians and other groups can only lead to losses in human comfort and quality of life, not to mention ecosystem function." (McCallum, 2007)

Mission Statement

To support the conservation of amphibians, both *in situ* and *ex situ,* through scientific management of assurance colonies, education and research.

Background

The World Conservation Union's (IUCN) Global Amphibian Assessment (GAA), a comprehensive conservation status and distribution assessment of 5,918 amphibian species, has shown that almost one-third (32% or 1,896 species) of amphibians worldwide are threatened with extinction and that 165 amphibian species may have already been lost to extinction (IUCN, 2006). This represents the highest percent extinction rate of any vertebrate group, including that of birds and mammals (Regan et al., 2001).

Globally and in North America, habitat degradation and destruction, chemical pollutants, invasive species and climate change have been the driving culprits behind species declines. A rapidly dispersing infectious disease chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (*Bd*, the amphibian chytrid fungus) has now been identified as the responsible agent behind many declines, including those found in relatively pristine and protected habitats.

The IUCN is calling on zoos and aquariums to participate in the global response to this conservation crisis. Recognizing that the rate of decline far outpaces the ability to respond to environmental problems *in situ*, captive assurance populations have been recognized as the only hope for survival for many amphibian species and will buy time to respond to threats in the wild. The IUCN, WAZA (World Association of Zoos & Aquariums), CBSG (Conservation Breeding Specialist Group), the ASG (Amphibian Specialist Group), and regional zoological associations have hosted a series of workshops and developed a number of resources to support the zoological community's *ex situ* response to this crisis.

Key among these initiatives and resources are:

• IUCN Technical Guidelines on the Management of *Ex Situ* Populations for Conservation (IUCN, 2002).

• *CBSG/WAZA Amphibian Ex Situ Conservation Planning Workshop - Final Report* which was held in El Valle, Panama and includes best practices recommendations for quarantines and housing (Zippel *et al*., 2006).

• The development of the Amphibian Ark (AArk), a joint initiative of WAZA, the CBSG, and the ASG, to implement the *ex situ* components of the *Amphibian Conservation Action Plan* (ACAP). Amphibian Ark's web site (www.amphibianark.org) is a great resource.

• The ACAP, published in 2007 in response to the 2005 Amphibian Conservation Summit in Washington, D.C. is a comprehensive response to amphibian population declines, of which *ex situ* captive breeding is one component.

•The identification of priorities and action steps by regional zoological associations. Species prioritization workshops have taken place in the United States, Europe, Madagascar, Mexico, Costa

Rica, Malaysia, and South Africa. A regional action plan was published for the Australasian zoological community in June 2007, a Costa Rican Amphibian Conservation Strategy was released in July 2007, and a Regional Collection Plan was completed by the European zoological community in January 2008.

The Association of Zoos & Aquariums (AZA) community has been directly and indirectly active in many of these efforts. AZA and its members have attended and helped sponsor some of these workshops, AZA has partnered with the Amphibian Ark, and AZA's Amphibian Taxon Advisory Group (ATAG) and the AZA-member supported organization Zoo Conservation Outreach Group (ZCOG) have paid for international colleagues to attend workshops and be trained at AZA's *Amphibian Biology and Management* course; ZCOG and Cameron Park Zoo have developed an annual scholarship specifically for this course. The AZA Board of Directors has supported member participation and leadership by declaring 2008: the Year of the Frog, in cooperation with the global community, creating a conservation biologist position dedicated to amphibian work, and developing an Amphibian Fund within the AZA's reputable Conservation Endowment Fund.

Introduction

Since the completion of the first edition of the ATAG Regional Collection Plan (RCP) in August 2000, much of the ATAG's direction has changed due to increased awareness about the extent and causes behind rapid amphibian population declines and the role zoos and aquariums could play in this crisis by developing assurance colonies of at-risk species. Current data indicates that the general trend of amphibian extinctions is accelerating at an unprecedented rate and future catastrophic loses are inevitable. Within this context, the second edition of the ATAG's RCP reflects a more tightly defined scope for suggested amphibian programs in AZA institutions that will enable colleagues to utilize their resources to their fullest potential and respond in chorus with the rest of the global amphibian community as we face the challenges that lie ahead. While using this RCP to develop institutional collection plans and conservation programs, keep in mind that collectively resources are limited and space is at a premium for managed programs. Never before has the zoological community been tasked to conserve so many species at such a rapid pace. It has been and will continue to be a challenge for us all, but we remain optimistic, as we know AZA institutions are up to the task. Please consider following the recommendations within this RCP and help develop new amphibian programs and strategies through partnerships on a global scale to conserve these unique and important creatures.

Due to the quarantine and spatial requirements of amphibian assurance colonies, the ATAG views exhibit space outside of the periphery of usable space for captive programs with reintroduction potential. Therefore, while this RCP will provide limited guide regarding species exhibited in institutions, the RCP focuses on species of conservation concern.

Taxa within ATAG Purview

Priority Species and Regions

In accordance with the global *Amphibian Conservation Action Plan*, critically endangered species in need of immediate conservation concern action should be subject to *ex situ* management, as appropriate, to insure recovery of wild populations. Under this directive, ATAG was directed by IUCN/ASG to prioritize and help manage the 353 Critically Endangered amphibian species found in the Caribbean and North, South, and Meso America. The ATAG has identified priority species for *ex situ* managed

programs within North America, Canada and the Caribbean. The ATAG is asking AZA institutions to focus amphibian conservation efforts on New World species, with an emphasis on North America. The ATAG recognizes that AZA institutions may currently have on-going projects and commitments to other regions of the world and does not discourage those institutions from their continued involvement in those areas. However, the ATAG encourages and will endorse and support programs developed for New World species above those in other regions. The scope of this edition of the RCP covers North America, Canada and Puerto Rico. Species from the Caribbean, Meso America and South America will be analyzed in additional meetings in various range countries with experts in their field. Once this is done the species identified for priority action by zoos and aquariums will be reviewed and integrated into our RCP as addendums and programs for those species will be encouraged. The ATAG anticipates that most of the new programs for animals from range countries without AZA facilities will require in-country action, including both *in situ* and *ex situ* management. The ATAG anticipates that most of these programs will not include import of animals to AZA facilities for additional *ex situ* management, unless it is specifically requested by the governing agencies in each country.

Priority Ecoregions within North America

There are 261 described species of amphibians in the United States, 52 of which are threatened. Of the 261 species 186 are salamanders, constituting 35% of the world's known salamander species. AZA-accredited zoos and aquariums are encouraged to support *in situ* amphibian conservation programs throughout the United States, particularly in regions rich in amphibian diversity and areas under development pressure. Ecoregions of high amphibian diversity and threatened species include: the Appalachian, Ozark, and Southwestern Mountains; the Pacific Northwest; and the West and Southeastern coasts.

Priority Conservation Activities

AZA zoos and aquariums should continue providing long-term financial, technical, physical and logistical support to programs and projects identified as priorities by the ATAG. Resources should be pooled with other facilities, or form regional consortiums to address regional amphibian projects. Every effort should be made to reach out to local agencies and to form partnerships for amphibian conservation. *In situ* amphibian conservation centers, such as El Valle Amphibian Conservation Center (EVACC) in Panama, should be created and funded and biologists conducting field research should be supported. Capacity building at home institutions and in range countries should be expanded and amphibian facilities should be updated to reflect the needs of conservation-oriented collections. Staff should receive appropriate training, which can be obtained by attending AZA's *Amphibian Biology Management* course, or by participating in internships at institutions with existing capacity. The ATAG's *Amphibian Husbandry Resource Guide* (www.aza.org/ConScience/Amphibians_Intro/index.html) can be used to reinforce lessons learned.

Institutional Capacity of AZA Communities

Space Analysis

In January 2008, space surveys were sent to 152 AZA institutions and 125 (82%) responses were received. The survey queried institutions for their current and future capacity to display and hold amphibians in both isolated and non-isolated space.

Based on previous ATAG space surveys, a 5 gallon equivalent was used as the organizing subunit to determine current and future amphibian housing space. Although this method is not ideal in deciphering precise space for all species of amphibians in AZA facilities, it is a simple way to standardize housing units in a comprehensible manner. A 5 or 10 gallon enclosure is adequate for raising many larval amphibians, as well as breeding smaller anurans. Typically these units hold no more than 6 adult specimens. A 20 gallon enclosure is adequate for breeding and housing many larger anuran species and salamanders. Typically this space houses no more than 6 specimens. An enclosure over 20 gallons is required for housing larger amphibians such as caecilians, or those that require complex environments such as stream dwelling salamanders (e.g., Cryptobranchus alleganiensis). Therefore, 40 gallon enclosures were assigned for the larger taxa. However, it is recognized that enclosures used at facilities may be larger than the assigned values.

Survey results revealed that current exhibit (33,300 gallons) and non-isolated holding space (40,730 gallons) has the potential to be expanded by an additional 26,970 and 54,930 gallons, respectively. This nearly doubles exhibit space over the next 1-5 years and more than doubles non-isolated holding space.

Institutions stated that 199 isolated rooms or quarantine units are available for either individual species or species assemblages. In these isolated rooms, the current available space is 14,075 gallons. Within six months, institutions estimated that space could be expanded by 18,305 gallons to 32,380 gallons. An additional 22,335 gallons could be added within one year, and a further 17,920 gallons available within 2 years. Therefore, total space two years from now could total as much as 72,635 gallons. This represents more than a five-fold increase (516%) in isolated holding space within two years.

Regional institutions said they would support capacity building in-country for species in the United States (73 institutions), Canada (34), Mexico (48), the Caribbean (42), Central American (60), and South America (50). Sixty-three (41%) institutions said they would support capacity building in-country even if it were not possible to link their conservation activities directly to their display animals. This in-country support would come from financial donations (58 institutions), donation of supplies and/or equipment (60), donation of staff time and travel expenses (55), participation in and/or support of facility construction (47), and training of local staff (34). Seventy-five institutions said they would prefer to collaborate with one or more institutions to pool funds for a project or capacity building.

Target population numbers for existing managed amphibian programs (Table 1) have been developed by the individual managed groups and their SPMAG advisors. Maximum target population size (N) was based on the number of facilities currently involved in each program, the available appropriate spaces at these institutions, and the genetic and demographic goals for the species. Goals for reintroduction are largely based on maximizing offspring production, limited only by logistics. Program species for current or future reintroduction must be kept in bio-secure isolated rooms to prevent the introduction of diseases into the wild. Specimens used for exhibit purposes only are considered surplus to the managed populations (unless they can be maintained in bio-secure exhibits), but are included in the target population numbers. For example, Puerto Rican crested toads are housed at 21 facilities and the target population remains at 300. However, on average, only 50 pairings are made per breeding cycle due to the limitations of breeding and larval holding tanks.

"Breeding space" varies from institution to institution and throughout the year. If animals that are recommended to breed are not healthy, they may be pulled from the breeding pool and if possible replaced with equivalents. There is also limited breeding success. For example, only ~10-20% of the pairings may produce offspring in the crested or Wyoming toad programs for a single breeding cycle.

The biology of many of the amphibian species requires special consideration in their management. Many anuran species have relatively short reproductive lifespans (usually 5-7years). These animals are also generally very fecund, producing thousands of offspring in a single clutch. This creates great population size variance over short periods of time when population numbers may increase 100+ fold between pre and post breeding numbers. The new IUCN ex-situ population guidelines have been used for guidance in establishing population sizes. The most important consideration is not the total N, but the effective population size (N_e), which is relative to the numbers of animals that actually produce descendants. For amphibian programs, maximum captive population numbers are not as relevant to the success of the program as breeding population. For programs with a reintroduction component, excess offspring are usually released, eliminating the usually limitations of captive carrying capacity that is an essential management criterion for many other programs.

Some species, such as the Panamanian golden frog currently have a large target population due to their inability to be able to import potential founders and the responsibility of managing 35-40 bloodlines from three unique wild populations. These animals are also in great demand as amphibian ambassadors in education/conservation exhibits. Several of the SSP/PMP programs are scheduled for a PMC session in the near future. Therefore, target populations may change at that time.

Table 1: Space Analysis Summary of Existing Managed (SSP/PMP) Programs

Common Name	Scientific Name	Current Population Size (per program head)	Target Population Size (per program head)	Gallons per Adult Individual	Total Program Space Needs	Total Space Available (per 2008 Survey)	PMC Approved Plan	Isolated Space Needed (if so why?)
Wyoming Toad	*Anaxyrus (Bufo) baxteri*	176.165.78	300.300 (600) SPMAG Advisor R.A. Odum	3-5 gallons per adult individual.	1,800 - 3,000 gallons	3,000 gallons	No.	Yes. Reintroducing / headstarting.
Puerto Rican Crested Toad	*Peltophryne lemur*	northern-88 southern-402 (490 Total)	350.350 (700) (northern and southern) SPMAG Advisor R. Wiese	3-5 gallons per adult individual.	2,100 - 3,500 gallons	3,500 gallons	Yes	Yes. Reintroduction program
Panamanian Golden Frog	*Atelopus zeteki* and *Atelopus varius*	*A. zeteki* - 1076 *A. varius* - 158	750.750 (1500) SPMAG Advisor A. Snider	Males; 5 -10 gallon ea. Females; 3-5 gallon ea.	6,000 - 11,250 gallons	11,250 gallons	No	Preferred for health reasons in breeding groups, but not necessary
Mississippi Gopher Frog	*Lithobates (Rana) sevosa*	0.0.75 (from ISIS)	n/a	n/a	n/a	n/a	No. Studbook training pending.	Yes, due to disease issues.
Houston Toad	*Anaxyrus (Bufo) houstonensis*	42.45	175.175 (350) P. Crump	3 - 5 gallons per adult individual	1,050 - 1,750 gallons	1,750 gallons	No. Studbook training pending	Yes. Reintroducing / headstarting.
Texas Blind Salamander	*Eurycea rathbuni*	0.0.30	n/a	n/a	n/a	n/a	PMP manager position vacant.	Unknown
				Total Regional Space Needs	**10,950 - 19,500 gallons**			

Collection Management

Management of populations for *ex situ* conservation purposes over long periods of time has its challenges (Snyder *et al*., 1996; Lynch and O'Hely, 2001). The IUCN Policy Statement on Captive Breeding (IUCN, 1987) suggests establishing a captive colony while the total population size of the focal vertebrate taxa number in the thousands. However, consent or resources for *ex situ* conservation are rarely provided with this much forethought and planning. A more typical event is crisis response, and during such an event significant components of the natural genetic diversity of a species can be lost, potentially resulting in a genetic drift-effect during the founding process. Genetic

management of the population and the insurance of adequate founder representation in subsequent generations can be an arduous tasks, as is balancing the need to minimize the spatial demands of the program with genetic objectives (Earnhart *et al*., 2001).

New captive husbandry and reproductive research protocols should emphasize data collection and fine tuning of efficient systems with the ultimate goal, at least for reintroduction programs, being the ability to produce large quantities of healthy amphibians in as short amount of time as possible. Data such as egg laying events, egg size, time until hatching and associated water parameters at which these events took place, size at hatching, time until metamorphosis is complete, size at metamorphosis, age and size at first reproduction, and number of eggs should all be collected. For direct developing and neotenic species, similar but appropriate life history data should be recorded. Maximum advantage should be taken from the *in situ* research links, as increasing knowledge of the species in the wild as it applies to *ex situ* management will ultimately yield some rewards. Conversely, data obtained from *ex situ* research should be made readily available to *in situ* researchers.

In summary, genetic management and data collection is essential to ensure the success of the *ex situ* program.

Quarantine Constraints

North America has a relatively low number of threatened amphibian taxa that desperately require *ex situ* amphibian conservation action when compared with other regions of the New World and given AZA's history of amphibian conservation it is optimistically estimated that the isolated space already exists to fulfill the identified managed programs. It is likely that as time passes isolated space will continue to be created and new and/or existing North American programs can be initiated or expanded as the need arises. It should be noted that the space expansions are based on optimistic projected numbers from the space survey. The constraints that quarantining amphibians subjected to *ex situ* conservation tactics places on a facility obviously go beyond that of just the physical space and include a perceptual shift of management tactics and disease awareness.

Species Selection Criteria

Species Selection

The captive rearing of amphibians in Canada and the United States is not new. In 1982, six wild Puerto Rican crested toads (*Peltophryne lemur*) were captured, brought into AZA-accredited zoos and aquariums, and managed under AZA's first amphibian Species Survival Plan® (SSP). U.S. Fish and Wildlife Service-developed Recovery Plans dating back to 1991 include captive rearing components, and captive populations are also housed at universities, local and national government facilities, and in private collections. However, until now there has been no strategic approach to the use of *ex situ* captive rearing for conservation management purposes. Some communities have developed captive rearing programs to respond to local concerns and the participation of AZA-accredited zoos and aquariums in captive rearing programs has frequently reflected the interests of individual facilities and the needs of state wildlife agencies. This RCP is a strategic blueprint for the creation of new and the expansion of existing captive rearing programs at AZA facilities in order to address North American conservation objectives.

In July 2007, twenty-one participants came together in Fort Worth, TX for an Amphibian Species Prioritization for *Ex Situ* Conservation Workshop. The purpose of this workshop was to utilize a

prioritization tool developed by the Amphibian Ark to examine which of the critically endangered, endangered, data deficient, and phylogenetically unique amphibian species in Canada, the U.S. and Puerto Rico could benefit from captive rearing programs. This list has been refined further and incorporated into the RCP.

Based on the list of species generated from this process, members of the ATAG and regional experts drafted preliminary taxon management actions plans for species that received high listings and had existing *ex situ* mandates. These draft plans describe the goals, objectives, and necessary actions for each program, as well as identify a coordinator responsible for ensuring implementation of the plans.

As described in the IUCN's *Amphibian Conservation Action Plan* (ACAP), *ex situ* conservation is only one component of amphibian conservation. All captive rearing programs must be developed in coordination with appropriate government agencies and partners and must be integrated with *in situ* research and management activities that specifically address and mitigate the threat(s) that caused the population's original decline. Some AZA-accredited zoos and aquariums are already actively engaged in these *in situ* efforts; the partnership building associated with *ex situ* conservation will bring even more opportunities to participate in these efforts. Participation in activities such as population monitoring, research, and habitat management will help integrate the *ex situ* and *in situ* conservation activities, as well as improve and expand on-ground education and public awareness efforts.

Selection Tool: Amphibian Ark's Prioritization Tool for *Ex Situ* Conservation

Amphibian Ark's *Prioritization and Implementation Process for* Ex Situ *Conservation of Amphibians* (Appendix 1) was based on a draft initially developed at the February 2006 CBSG/WAZA Amphibian *Ex situ* Conservation Planning Workshop in El Valle, Panama, and was further refined through the widespread solicitation of comments and finalization by Amphibian Ark staff. The Fort Worth meeting was the first time this tool was used in its final form for prioritization purposes.[1] The tool asks a set of questions about each species and assigns points to each answer. These points form the prioritization rankings at the end of the process. Questions cover new and emerging conservation threats not incorporated in the GAA listing, threat mitigation possibilities, socio/economic importance, phylogenetic uniqueness, scientific and biological importance, and other factors.

The tool differentiates among captive rearing programs and their primary conservation purpose:
• *Ark* programs are few and far between, requiring that the target species be already extinct (or functionally extinct) in the wild. Under this scenario, any survivors discovered would be candidates for an emergency captive rearing program.
• R*escue/supplementation* captive programs support populations who are in imminent danger of going extinct and whose wild population would benefit from *ex situ* management as part of its recommended conservation action.
• *Farming* programs provide individuals to replace a demand for wild harvested specimens. This situation has not been identified for any Canadian or U.S. species.

[1] Based on this meeting, Amphibian Ark made one more change to the tool, eliminating and disregarding one question at subsequent global prioritization workshops. The prioritization rankings changed slightly when this question was eliminated, so rankings provided here may vary slightly from the ATAG's 2007 publication of the Action Plan for Ex Situ Conservation in the AZA Community, available at:
http://www.aza.org/ConScience/Amphibians_Intro/index.html.

• *Conservation research* programs are meant for species whose conservation could benefit from specific applied research or that could provide needed insight for the conservation and management of a related species.
• *Conservation education* captive programs are for species selected and bred specifically for their educational impact.

Many benefits accrue from using this tool to identify appropriate species for captive rearing programs. One benefit is that the ranking is based on discrete scores that can be discussed among experts and can change as new information (i.e., the presence of *Bd* or the initiation of conservation management) arises. This transparency is helpful for discussions with conservation partners that may be skeptical about a facility's interest in a particular species. A second benefit is that the process can be replicated; the subjectivity of answers is minimized through the use of strictly and clearly defined terms. A third benefit is the potential to compare rankings across regions. The urgency of a captive program has the potential to be shown in relation to the needs of other regions, making resource allocation more efficient.

After analyzing biological and socio/economic criteria, the tool states that further action is contingent on the existence of both an official captive rearing mandate and range state approval. If either of these items is lacking, the first step is to develop the relationships necessary for these requirements to be met or for the zoo and aquarium community to understand the reasons for why a captive program is not an appropriate conservation measure. Additional questions evaluate program feasibility and readiness for program implementation.

Management Categories

SSP Taxa (Species Survival Plan): Studbook required, intense management to maintain captive population, compliance by participating institutions required, breeding and transfer recommendations communicated through a Master Plan, program managed by a Species Coordinator, non-member participants must be approved, conservation of the species a consideration, institutional input through IRs.

PMP Taxa (Population Management Plan): Studbook required, moderate management to maintain captive population, institutional compliance encouraged, breeding and transfer recommendations communicated through a Population Management Plan, program managed by a PMP Manager, institutional input through TAG IRs, non-member participation through AZA and institutional Acquisition/Disposition policies.

DERP Taxa (Display, Education or Research Populations): DERPs are not managed under the auspices of AZA or its programs and are not guaranteed population management advice or support from SPMAG/PMC. No studbook or long-term genetic or demographic management is required for these species, but TAGs may choose to identify species champions who may track DERPs through registries.

POP Taxa (Phase-out Populations) Not viewed as a managed program. Currently in AZA institutions but should be phased out through a breeding moratorium; phase-out may be monitored through a registry and a species champion may be assigned to oversee this process; they have no studbooks and are not guaranteed population advice or support from SPMAG/PMC.

PIP Taxa (Phase-in Populations): Taxon not currently in AZA institutions but for which the TAG plans or hopes to initiate a captive population; they have no studbooks and are not guaranteed population management advice or support from SPMAG/PMC. Once in captivity, the taxon will be reassigned to another category as appropriate.

TMAP Taxa (Taxon Management Action Plan Populations): Taxon not currently in AZA institutions, but for which the TAG has identified as a priority taxon for *in situ* conservation. A Taxon Champion will be assigned by the TAG to complete a TMAP (see example in Appendix 2) and identify conservation needs and coordinate efforts for species recovery, which may or may not include *ex situ* management. A mandate for *ex situ* management must be given by governing agencies prior to reassigning the taxa to PIP category. A Taxon Champion Application Form can be found in Appendix 3.

Results of Species Selection Process

Recommendations Table: Priority 1 Taxa for *Ex Situ* Management

Priority 1 taxa include current SSP and PMP programs, as well as species identified through the species selection process from the United States, Canada and Puerto Rico, that are currently listed in DERP and PIP categories **and possess a mandate from a government agency for *ex situ* management**.

Table 2: Recommended Priority 1 Taxa with Mandate for *Ex Situ* Conservation

Common Name	**Scientific Name**	**Category**	**Program Leader**
Puerto Rican Crested Toad	*Peltophryne lemur*	SSP	Coordinator: Diane Barber Studbook Keepers: Elaine Gabura and Debbie Martin
Wyoming Toad	*Anaxyrus (Bufo) baxteri*	SSP	Coordinator: Bruce Foster Studbook Keeper: Sara Armstrong
Panamanian Golden Frog	*Atelopus zeteki and A. varius*	SSP	Coordinator: Vicky Poole Studbook Keeper: Vacant
Mississippi Gopher Frog	*Lithobates (Rana) sevosa*	PMP	Taxon Champion: Steve Reichling
Texas Blind Salamander	*Eurycea rathbuni*	PMP	Population Manager: Nick Hanna
Houston Toad	*Anaxyrus (Bufo) houstonensis*	PMP	Population Manager: Paul Crump
Ozark hellbender	*Cryptobranchus bishopi*	DERP	Taxon Champion: Mark Wanner
Mountain Yellow-Legged Frog	*Rana muscosa*	DERP	Taxon Champion: Andy Sinder
Barton Springs Salamander	*Eurycea sosorum*	DERP	Taxon Champion: DeAnn Chamberlain
Austin Blind Salamander	*Eurycea waterlooensis*	DERP	Taxon Champion: DeAnn Chamberlain
San Marcos Salamander	*Eurycea nana*	DERP	Taxon Champion: DeAnn Chamberlain
Chiricahua Leopard Frog	*Lithobates (Rana) chiricahuensis*	DERP	Taxon Champion: Tara Sprankle
Oregon Spotted Frog	*Rana pretiosa*	DERP	Taxon Champion: Karen Goodrowe
Locust Coqui	*Eleutherodactylus locustus*	PIP	Taxon Champion: Dr. Raphael Joglar
Richmond's Coqui	*Eleutherodactylus richmondi*	PIP	Taxon Champion: Dr. Raphael Joglar
Dwarf Coqui	*Eleutherodactylus unicolor*	PIP	Taxon Champion: Vacant
Golden Coqui	*Eleutherodactylus jasperi*	PIP	Taxon Champion: Vacant
Elegant Coqui	*Eleutherodactylus eneidae*	PIP	Taxon Champion: Vacant
Stream Coqui	*Eleutherodactylus karlschmidti*	PIP	Taxon Champion: Vacant
Relict Leopard Frog	*Lithobates (Rana) onca*	PIP	Taxon Champion: Vacant
California Red-Legged Frog	*Rana draytonii*	PIP	Taxon Champion: Vacant

Recommendations Table: Priority 2 Taxa for *In Situ* Management

Priority 2 taxa include United States, Canada and Puerto Rican species that have been identified through the species selection process and are currently categorized as TMAP species in need of *in situ* support. **Currently these species do not have a mandate from a government agency for *ex situ* management**. However, many of these species are listed on a state or federal level, listed through IUCN as species of concern, or are phylogenetically unique. The ATAG is recommending that a Taxon Champion be assigned to each of these taxa to assess their *in situ* conservation needs and advise the ATAG on any necessary future support. At this time, it is not advised to form *ex situ* colonies of Priority 2 taxa until assessments are completed by Taxon Champions and mandates are created by government agencies for *ex situ* programs.

Table 3: Recommended Priority 2 Taxa Without Existing Mandate for *Ex Situ* Conservation

Common Name	**Genus**	**Category**	**Taxon Champions**
Chisholm Trail Salamander	*Eurycea chisholmensis*	DERP	Jessica Crowley
One-Toed Amphiuma	*Amphiuma pholeter*	DERP	Jennifer Pramuk
Arroyo Toad	*Anaxyrus (Bufo) californicus*	DERP	Kim Lovich
Eastern Hellbender	*Cryptobranchus alleganiensis allegaiensis*	DERP	Joe Greathouse
California Tiger Salamander	*Ambystoma californiense*	DERP	Andy Snider
Narrow-Striped Dwarf Siren	*Pseudobranchus axanthus*	DERP	Vacant
Dwarf Siren	*Pseudobranchus striatus*	DERP	Scott Pfaff
Black-Spotted Newt	*Notophthalmus meridionalis*	DERP	Bradley Lawrence
Rivero's Coqui	*Eleutherodactylus juanriveroi*	TMAP	Vacant
Alabama Waterdog	*Necturus alabamensis*	TMAP	Eric Keyster
Melodious Coqui	*Eleutherodactylus wightmanae*	TMAP	Raphael Joglar
Sierra Nevada Yellow-legged Frog	*Rana sierrae*	TMAP	Vacant
Jollyville Plateau Salamander	*Eurycea tonkawae*	TMAP	Vacant
San Gabriel Springs Salamander	*Eurycea naufragia*	TMAP	Jessica Crowley
Olympic Torrent Salamander	*Rhyacotriton olympicus*	TMAP	Vacant
Yosemite Toad	*Anaxyrus (Bufo) carnorus*	TMAP	Vacant
Desert slender salamander	*Batrachoseps aridus*	TMAP	Andy Snider
California Giant Salamander	*Dicamptodon ensatus*	TMAP	Vacant
Cascade Torrent Salamander	*Rhyacotriton cascadae*	TMAP	Vacant
Columbia Torrent Salamander	*Rhyacotriton kezeri*	TMAP	Vacant
Flatwoods Salamander	*Ambystoma cingulatum*	TMAP	Dino Ferri
Coastal Tailed Frog	*Ascaphus truei*	TMAP	Nate Nelson
Idaho Giant Salamander	*Dicamptodon aterrimus*	TMAP	Vacant
Cope's Giant Salamander	*Dicamptodon copei*	TMAP	Vacant
Red Hills Salamander	*Phaeognathus hubrichti*	TMAP	Vacant
Neuse River Waterdog	*Necturus lewisi*	TMAP	Vacant
Cook's Robber Frog	*Eleutherodactylus cooki*	TMAP	Vacant
Frosted flatwoods salamander	*Ambystoma bishopi*	TMAP	Dino Ferri
Berry Cave Salamander	*Gyrinophilus gulolineatus*	TMAP	Vacant
West Virginia Spring Salamander	*Gyrinophilus subterraneus*	TMAP	Vacant
Siskiyou Mountains Salamander	*Plethodon stormi*	TMAP	Vacant
Sonora tiger salamander	*Ambystoma tigrinum stebbin*	TMAP	Vacant
Hell Hollow Slender Salamander	*Batrachoseps diabolicus*	TMAP	Vacant
San Gabriel Slender Salamander	*Batrachoseps gabrieli*	TMAP	Vacant

Common Name	**Genus**	**Category**	**Taxon Champions**
San Simeon Slender Salamander	*Batrachoseps incognitus*	TMAP	Vacant
Sequoia Slender Salamander	*Batrachoseps kawia*	TMAP	Vacant
Lesser Slender Salamander	*Batrachoseps minor*	TMAP	Vacant
Relictual Slender Salamander	*Batrachoseps relictus*	TMAP	Vacant
Chamberlain's Dwarf Salamander	*Eurycea chamberlaini*	TMAP	Vacant
Blanco River Springs Salamander	*Eurycea pterophila*	TMAP	Vacant
Blanco Blind Salamander	*Eurycea robusta*	TMAP	Vacant
Eurycea Troglodytes Complex	*Eurycea troglodytes*	TMAP	Vacant
Tellico Salamander	*Plethodon aureolus*	TMAP	Vacant
Shenandoah Salamander	*Plethodon shenandoah*	TMAP	Vacant
Amargosa toad	*Anaxyrus (Bufo) nelsoni*	TMAP	Vacant
Inyo Mountains Salamander	*Batrachoseps campi*	TMAP	Vacant
Weller's Salamander	*Plethodon welleri*	TMAP	Vacant
Santa Cruz Long-Toed Salamander	*Ambystoma macrodactylum*	TMAP	Vacant
Dwarf Black-Bellied Salamander	*Desmognathus folkertsi*	TMAP	Vacant
Columbia Spotted Frog	*Rana luteiventris*	TMAP	Vacant
Cheat Mountain Salamander	*Plethodon nettingi*	TMAP	Vacant

Program Status of All Current SSP and PMP programs

Table 4: Program Status Table of Priority 1 Taxa

Program	Date Program Initiated	Current Program Leader	Date Leadership Assumed	Date of Last Studbook Update	Studbook Keeper	Date of last PMP Publication	Date of Last Master Plan
Puerto Rican Crested Toad SSP	1984	Diane Barber	Jan-05	Jan-08	Elaine Gabura/ Debbie Martin	N/A	Nov-06
Wyoming Toad SSP	Dec-96	Bruce Foster	Apr-07	Nov-07	Sara Armstrong	N/A	Jul-07
Panamanian Golden Frog SSP	Mar-08	Vicky Poole	Mar-08	Feb-08	Vacant	N/A	Not published. Meeting scheduled Fall 08
Houston Toad PMP	Mar-07	Paul Crump	Mar-07	N/A	Paul Crump	Not published- New Program	N/A
Texas Blind Cave Salamander PMP	1998	Vacant	N/A	N/A	Vacant	N/A	N/A
Mississippi Gopher Frog PMP	Mar-08	Vacant	Mar-08	N/A	Deanna Lance	Not published- New Program	N/A

Recommendation Update Table

The Recommendations Update Table is included to provide an overview of ATAG progress from the previous RCP in 2000. The ATAG has included many recommended changes to focus on amphibian species of conservation concern. Some species that were previously ranked as DERP have remained in that category, but are no longer viewed as candidates for captive management within AZA institutions. Rather than removing them from the population they are left in the DERP category as display or education recommended species.

Table 5: Recommendation Updates from Species Listed in 2000 RCP

Common Name	Scientific Name	Previous Program Status (2000 RCP)	Current Recommendation	Program Leader Change?	New Program Leader/Species Contact	Comments
Puerto Rican crested toad	*Peltophryne lemur*	SSP	SSP	Yes	Diane Barber, Fort Worth Zoo	Captive Program
Wyoming toad	*Anaxyrus (Bufo) baxteri*	SSP	SSP	Yes	Bruce Foster, Central Park Zoo,	Captive Program
Panamanian golden frog	*Atelopus zeteki* and *A. varius*	PIP	SSP	Yes	Vicky Poole, National Aq in Baltimore	Captive Program

Common Name	**Scientific Name**	**Previous Program Status (2000 RCP)**	**Current Recommendation**	**Program Leader Change?**	**New Program Leader/Species Contact**	**Comments**
Texas blind cave salamander	*Eurycea rathbuni*	PMP	PMP	Yes	Nick Hanna, Audubon Zoo	Need known founders to start new population
Mandarin newt	*Tylotoriton shanjing*	PMP	DERP	Yes	N/A	Display/Education
Blue dart frog	*Dendrobates (azureus) tinctorious*	PMP	DERP	Yes	N/A	Display/Education
Golden mantella	*Mantella aurantiaca*	PMP	DERP	Yes	N/A	Display/Education
Central American caecilian	*Dermophis mexicanus*	DERP	DERP	No	N/A	Display/Education
Aquatic caecilian	*Typhlonectes sp.*	DERP	DERP	No	N/A	Display/Education
Mexican Axolotl	*Ambystoma mexicanum*	DERP	DERP	No	N/A	Display/Education
Mexican lake salamander	*Ambystoma sp.*	DERP	Not recommended	No	N/A	*In situ* support
Hellbender	*Cryptobranchus alleganiensis alleganiensis*	DERP	DERP	Yes	Joe Greathouse, Oglebay's Good Zoo	Display/Education
Ozark Hellbender	*Cryptobranchus alleganiensis bishopi*	DERP	DERP	Yes	Mark Wanner, Saint Louis Zoo	Captive Program-upgrade to PMP once bred
Mudpuppy	*Necturus spp.*	DERP	DERP	No	N/A	Display/Education
Dwarf siren	*Pseudobranchus spp.*	DERP	DERP	No	N/A	Display/Education
Red-eyed tree frog	*Agalychnis callidryas*	DERP	DERP	No	N/A	Display/Education
North American tree frog	*Hyla sp.*	DERP	DERP	No	N/A	Display/Education
Waxy tree frog	*Phyllomedusa sauvagii*	DERP	DERP	No	N/A	Display/Education
Surinam horned frog	*Ceratophrys cornuta*	DERP	DERP	No	N/A	Display/Education
Ornate horned frog	*Ceratophyrs ornata*	DERP	DERP	No	N/A	Display/Education
Coqui	*Eleuthrodactylus coqui*	DERP	DERP	No	N/A	Display/Education
Mountain chicken	*Leptodactylus fallax*	DERP	DERP	No	N/A	Display/Education
Northern tomato frog	*Dyscophus antongilii*	DERP	Not recommended	No	N/A	EAZA managed captive program, not within New World scope of ATAG
Tomato frog	*Dyscophus spp.*	DERP	DERP	No	N/A	Display/Education
Malayan leaf frog	*Megophrys montana*	DERP	DERP	No	N/A	Display/Education
Surinam toad	*Pipa pipa*	DERP	DERP	No	N/A	Display/Education

Common Name	Scientific Name	Previous Program Status (2000 RCP)	Current Recommendation	Program Leader Change?	New Program Leader/Species Contact	Comments
Solomon Island leaf frog	*Ceratobatrachus guentheri*	DERP	DERP	No	N/A	Display/Education
Flatwoods salamander	*Ambystoma cingulatum*	PIP	TMAP	Yes	Dino Ferri, Jacksonville Zoo	*In situ* support
Kihansi spray toad	*Nectophrynoides asperigus*	PIP	DERP	Yes	Jennifer Pramuk, Bronx Zoo	Captive program-2 institutions (Bronx and Toledo Zoos)
California red-legged frog	*Rana (aurora) draytoni*	DERP	DERP	Yes	Vacant Species Champion	Captive Program-upgrade to PMP once bred
Foothill yellow-legged frog	*Rana boylii*	DERP	Not recommended	No	N/A	
Cascades leopard frog	*Rana cascadea*	DERP	Not recommended	No	N/A	
Bullfrog	*Lithobates (Rana) catesbeianus*	DERP	DERP	No	N/A	Display/Education
Chiricahua leopard frog	*Lithobates (Rana) chiricahuensis*	DERP	DERP	Yes	Tara Sprankle, Phoenix Zoo	Upgrade to PMP once bred
Columbian spotted frog	*Rana luteiventris*	DERP	TMAP	No	Vacant Species Champion	*In situ* support
Mountain yellow-legged frog	*Rana muscosa*	DERP	DERP	Yes	Andy Zinder, Chaffee Fresno Zoo	Upgrade to PMP once bred
Relict leopard frog	*Lithobates (Rana) onca*	DERP	DERP	No	Vacant Species Champion	Develop captive program
Northern leopard frog	*Lithobates (Rana) pipiens*	DERP	DERP	No	N/A	Display/Education
Oregon spotted frog	*Rana pretiosa*	DERP	DERP	Yes	Karen Goodrowe, Point Defiance Aquarium	Display/Education
Ramsey Canyon leopard frog	*Lithobates (Rana) subaquavocalis*	DERP	N/A subsumed to *L. Chiricahuensis*	No	N/A	
Tarahumara frog	*Lithobates (Rana) tarahumarae*	DERP	Not recommended	No	N/A	

Suggested Taxa for Exhibit

ATAG recognizes there is a need to exhibit amphibians from all regions of the globe for various reasons specific to each institution. Due to the quarantine requirements of assurance colonies of amphibians, the ATAG views exhibit space outside of the periphery of usable space for captive programs with reintroduction potential, therefore is not concerned about the species exhibited in institutions aside from their educational value. Species that are surplus to Species Survival Plan (SSP)

or Population Management Plan (PMP) programs should be exhibited with informative graphics about the recovery efforts for the species. Obviously out of nearly 6,000 species of amphibians, there are a wide variety of animals that can be recommended for exhibit. Included below is a modest list of species that are broad examples of taxa commonly used for exhibit and that are relatively easy to acquire reasonably (i.e., from fellow AZA facilities or through reputable breeders) and/or maintain. For further assistance in choosing species for exhibit to reflect individual institution messaging needs, contact steering committee members directly, or use the amphibian tag or amphibian discussion listservs to inquire which species would be suitable for exhibit under specified requirements.

Table 6: Suggested Taxa for Exhibit

Common Name	**Scientific Name**	**Category**	**Definition**
Mandarin newt	*Tylototriton shanjing*	DERP	Display/Education
Blue dart frog	*Dendrobates (azureus) tinctorious*	DERP	Display/Education
Mantella	*Mantella spp.*	DERP	Display/Education
Central American caecilian	*Dermophis mexicanus*	DERP	Display/Education
Aquatic caecilian	*Typhlonectes spp.*	DERP	Display/Education
Mexican Axolotl	*Ambystoma mexicanum*	DERP	Display/Education
Eastern Hellbender	*Cryptobranchus alleganiensis alleganiensis*	DERP	Display/Education
Mudpuppy	*Necturus spp.*	DERP	Display/Education
Dwarf siren	*Pseudobranchus spp.*	DERP	Display/Education
Red-eyed tree frog	*Agalychnis callidryas*	DERP	Display/Education
North American tree frog	*Hyla spp.*	DERP	Display/Education
Waxy tree frog	*Phyllomedusa sauvagii*	DERP	Display/Education
Surinam horned frog	*Ceratophrys cornuta*	DERP	Display/Education
Ornate horned frog	*Ceratophyrs ornata*	DERP	Display/Education
Coqui	*Eleuthrodactylus coqui*	DERP	Display/Education
Mountain chicken	*Leptodactylus fallax*	DERP	Display/Education
Tomato frog	*Dyscophus spp.*	DERP	Display/Education
Malayan leaf frog	*Megophrys montana*	DERP	Display/Education
Surinam toad	*Pipa pipa*	DERP	Display/Education
Solomon Island leaf frog	*Ceratobatrachus guentheri*	DERP	Display/Education
Bullfrog	*Lithobates (Rana) catesbeianus*	DERP	Display/Education
Northern leopard frog	*Lithobates (Rana) pipiens*	DERP	Display/Education

Suggested Taxa for Outreach

ATAG appreciates the value in using amphibians in educational outreach programs. This list of species would be appropriate for community outreach and Appendix 4 includes information for safe handling and transportation of amphibians. It is not the intent of the ATAG to produce an all-inclusive or restrictive list of species to be used in outreach. Rather, the list is intended for use as a

resource and includes some of the more common species that have been safely used in outreach programs.

Table 7: Suggested Taxa for Outreach

Common Name	**Scientific Name**	**Husbandry Level**	**Experience Level**
Central American caecilian	*Dermophis mexicanus*	Moderate	Moderate
Tiger salamander	*Ambystoma tigrinum*	Hardy	Novice
Red-eyed tree frog	*Agalychnis callidryas*	Moderate	Moderate
White's tree frog	*Litoria caerulea*	Moderate	Novice
Tomato frog	*Dyscophus spp.*	Moderate	Moderate
Ornate horned frog	*Ceratophrys ornata*	Hardy	Novice
Fire-bellied toad	*Bombina orientalis*	Hardy	Novice
North American tree frog	*Hyla spp.*	Moderate	Moderate
African bullfrog	*Pyxicephalus adspersus*	Hardy	Novice
Red-legged walking frog	*Kassina maculata*	Moderate	Moderate
Marine toad	*Rhinella (Bufo) marina*	Hardy	Novice
North American toad species	*Anaxyrus (Bufo) spp. and Ollotis (Bufo) spp.*	Hardy	Moderate
Couch's spadefoot	*Scaphiopus couchi*	Hardy	Moderate
Bullfrog	*Lithobates (Rana) catesbeianus*	Hardy	Novice
Poison dart frog	*Dendrobates spp.*	Moderate	Moderate
Leopard frog	*Lithobates (Rana) pipiens*	Hardy	Novice
Wood frog	*Lithobates (Rana) sylvaticus*	Moderate	Moderate

Husbandry Level Definitions:
Hardy - basic diet, lighting and housing needs, easy to handle
Moderate - could require more space, more cleaning, specialized diet, complex environment, will tolerate handling in brief intervals.
Difficult - requires large space or complex environment, UV lighting imperative, intense heating, specialized feeding strategies, will tolerate handling in brief intervals, could potentially be difficult to handle, unpredictable or deliver a potentially dangerous bite.

Animal Caregiver/Handler Experience Level Needed:
Novice- very little animal husbandry and handling experience.
Moderate- some reptile experience for at least one year.
Experienced- diverse reptile experience for more than two years.

ATAG Guidelines

Quarantine Guidelines

Newly acquired amphibians should be quarantined from the established collection for a period of 30-90 days to reduce the potential spread of pathogens and parasites (Wright and Whitaker, 2001). It is recommended that all incoming amphibians be tested for the fungus *Batrachochytrium dendrobatidis* (*Bd*; the amphibian chytrid fungus). Amphibian species involved in reintroduction programs and those that are highly likely to be reintroduced in the future should be permanently quarantined from metropolitan collections. The recovery group and governing agencies for each species and situation should determine what level of quarantine and pre-screening measures are acceptable for individual programs. All reintroduced amphibians should be pre-screened prior to release for foreign disease and parasites. Any affected amphibians should be held back from release and treated. Treated amphibians that have been re-tested can be released to the wild (IUCN, 1998). For additional information regarding quarantine protocols in the face of emerging diseases and the creation of new amphibian facilities and exhibits, see Chapter 3 of the *AZA Amphibian Husbandry Resource Guide* (Appendix 5).

Culling/Euthanasia Guidelines

Many amphibians are explosive breeders and some females (e.g. *Lithobates* (*Rana) catesbeianus and Rhinella (Bufo) marina)* can lay 20,000-35,000 eggs during one spawning event. The ATAG recognizes that the humane culling of eggs or tadpoles may be necessary during the captive management of amphibians, particularly species that are regulated by federal or international laws and cannot be surplused to the general public, and where there is no potential for reintroduction into the wild. Due to their relatively short life span and brief period of reproductive fitness, SSP and PMP managed amphibian species are bred frequently, relative to programs for long-lived species, in order to maintain genetically viable populations. Hundreds to thousands of offspring may be produced from a single breeding and typically only 10-50 offspring per breeding are kept for genetic management. In order to avoid flooding the captive population with overrepresented lineages and to insure adequate space for genetically important animals, removing excess offspring from the group is necessary. If there is a reintroduction component to the captive program, every effort should be made to breed animals according to SSP and PMP recommendations and release all surplus offspring. However, when release is not an option, humane culling is acceptable. When program animals are owned by other agencies, participants are bound by loan agreement and permit stipulations and should follow the culling/euthanasia policies outlined within individual contracts. Euthanasia must be done in accordance with the established policy of the individual institution and the *American Veterinary Medical Association's Guidelines on Euthanasia (*formerly *The Report of the AVMA Panel on Euthanasia)* (AVMA, 2007) as directed by AZA's Acquisition and Disposition policy.

Amphibians and Outreach Statement

Live amphibians in demonstrations can be powerful ambassadors for conservation messaging. They are loved by many and are seen as harmless creatures by most. Audiences gain a lasting memory of events when they are able to experience animals up close. Using amphibians in outreach is an important tool to create bonds between humans and animals that cannot be created through media and images in books. When choosing amphibians for outreach, important considerations such as staff expertise, husbandry requirements, medical and nutritional requirements, length and types of programs, environmental needs, restraint and transportation methods, species temperament, safety issues, and educational messaging should all factor into sound collection planning. Native species are all too often overlooked in zoo programs. Through the use of locally occurring species in outreach,

audiences can learn about conservation in their own backyard. Using amphibians for outreach is also a good opportunity to teach audiences about: state protected species, cohabitation, the affects of urban sprawl, pollution, global warming, biomedical applications, amphibians as bioindicators, and of course, the global amphibian crisis. A suggested outreach species list and handling and transportation guidelines are included in the ATAG RCP.

Amphibians in Classroom Settings Statement

Live amphibians in classrooms can stimulate students' interest in wildlife and promote respect for animals and their ecosystems. However, it is important that teachers plan for disposition of classroom pets prior to obtaining them. Obviously, it is ideal for teachers to keep the amphibians from semester to semester until their natural death. However, as this is not always possible, it is imperative that teachers seek alternatives to releasing unwanted amphibians into the wild. Releasing larval (e.g. tadpoles and newts) forms and metamorphosed (juvenile and adult) amphibians can have serious impacts on local species and their ecosystems. Released amphibians can introduce harmful pathogens and parasites into the wild. They can also out-compete native species for food and shelter, or act as predators, eating indigenous amphibians. Teachers should act responsibly and plan to keep the pet for its lifetime. If the amphibian can no longer be housed and a suitable home cannot be found, euthanasia is a better alternative than releasing it to the wild.

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Appendix 1: AArk Prioritization and Implementation process for *Ex situ* Conservation of Amphibians

Based upon draft prepared by: Taxon selection and prioritization working group – CBSG/WAZA Amphibian *Ex situ* Conservation Planning Workshop, El Vale, February 2006.

DRAFT December 2007

Rationale

Ex situ conservation of a threatened amphibian species should be considered a necessity when the imperative of *in situ* conservation cannot by itself ensure the survival of a species and its ecosystem.

When *ex situ* management of an amphibian species is considered necessary and appropriate, the priority should be to establish the initiative within the range State of ecological origin. Emphasis should therefore be placed on developing appropriate capacity within the range State where this does not exist. However, if the perceived urgency of the situation requires it, *ex situ* programs will be set up outside of range State wherever expertise and other resources are forthcoming.
Data derived from *ex situ* management of amphibians should be made openly available to workers involved in the *in situ* conservation of the species (or similar species) and *vice versa*.

An *ex situ* initiative should be viewed as just one of the tools that can help in the overall conservation of a species. It therefore follows that strong links between *ex situ* and *in situ* components are fundamental to the long-term success of species conservation. Full integration between *ex situ* and *in situ* conservation approaches should be sought wherever possible. This is normally best highlighted through the establishment of a formal Taxon Management Plan that explicitly states the short, medium and long term goals of each component of the conservation initiative. In cases where an *ex situ* conservation initiative has been established prior to, or in the absence of, a concurrent *in situ* initiative (e.g. where a political situation currently prohibits *in situ* conservation measures, or where a disease problem currently invalidates measures to protect wild populations), emphasis should be placed on establishing the appropriate *in situ* links as soon as it becomes possible to do so.

This prioritization tool has been structured in three sections:

The first section concerns **Prioritization** of species for *ex situ* conservation initiative - i.e. with limited resources (space, staff, money etc.) which species should have *ex situ* programs established ahead of others. It takes the form of a series of questions with weighted scores. The total score for a species indicates how 'important' an *ex situ* program for the species is in relation to others. Some questions may not be straightforward to answer and will require consultation with colleagues, taxonomic experts and other individuals/groups working with the species.

The second section includes just two questions ensuring that there is **Authorization** for the proposed *ex situ* conservation program.

The third and final section represents **Implementation** of a program and considers the practical feasibility of initiating and maintaining a priority *ex situ* program – a sort of check list of essential elements prior to initiation.

This prioritization tool should be an evolving protocol. The criteria and their rankings will be adjusted as we gain experience with the process and continue to work with the broader amphibian conservation community to identify goals, threats, and conservation options. In addition, the selection and prioritization of individual species will be revised as we gain knowledge and as the threats to the species change. Thus, there will be a need to constantly assess species status and monitor threats, so that emerging critical situations are responded to sufficiently quickly.

SECTION ONE – TAXON PRIORITIZATION

1. **Extinction risk:** What is the current IUCN Red List category for the taxon? (modified accordingly if new/additional information is available)

Extinct in wild/Critically endangered	**Score 20**
Endangered	**Score 16**
Vulnerable	**Score 12**
Data deficient*	**Score 8**
Near threatened	**Score 4**
Least concern	**Score 0**

(*taxon has been regionally or nationally recognized as 'at risk' despite data deficiency)

2. **Phylogenetic significance**: What is the taxon's Evolutionary Distinctiveness (ED) score, as generated by the ZSL EDGE program?

ED value > 100	**Score 10**
ED value 50-100	**Score 7**
ED value 20 - 50	**Score 3**
ED value <20	**Score 0**

3. **Threat mitigation:** Are the threats facing the taxon, including any new and emerging threats not considered in the GAA, potentially reversible?

Threats cannot/will not be reversed in time to prevent likely species extinction	**Score 20**
Threats are being managed - conservation dependant	**Score 10**
Threats unknown	**Score 8**
Threats cannot be reversed	**Score 5**
Threats are potentially reversible in time frame that will prevent further decline/extinction	**Score 2**
Species is effectively protected	**Score 0**
Species does not require conservation action at this time	**Score 0**

4. **Primary conservation role:** What is the primary intended conservation role (see Appendix 1) of the target taxon?
   NB – taxon may have other secondary roles which should not be scored

Ark	**Score 20**
Rescue/Supplementation	**Score 16**
Conservation Research:	**Score 8**
Mass production in captivity:	**Score 4**
Conservation Education:	**Score 0**

5. **Biological distinctiveness:** Does the taxon exhibit, for example, a distinctive reproductive mode, behavior, aspect of morphology or physiology, among the Class Amphibia?

Aspect of biology identified that is unique to species	**Score 10**
Aspect of biology shared with <6 other species	**Score 5**
No aspect of biology known to be exceptional	**Score 0**

6. **Cultural/socio-economic importance:** Does the taxon have a special human cultural value (e.g. as a national or regional symbol, in a historic context, featuring in traditional stories) or economic value (e.g. food, traditional medicine, tourism) within its natural range or in a wider global context?

Yes	**Score 5**
No	**Score 0**

7. **Scientific importance:** Is the species vital to current or planned research other than species-specific ecology/biology/conservation? (e.g. human medicine, climate change, environmental pollutants and conservation-science)

Research dependent upon species	**Score 5**
Research dependant upon <6 species (incl. this taxon)	**Score 3**
Research not dependant upon species	**Score 0**

SCORE = ____ (HIGHER SCORE INDICATES HIGHER PRIORITY)

SECTION TWO – PROGRAM AUTHORIZATION

8. **Mandate:** Is there an existing conservation mandate (see Appendix 2) recommending the *ex situ* conservation of this taxon?
   Yes: Go to question 10.
   No: Insufficient authorization for an *ex situ* initiative at this time.
   SEEK MANDATE FROM ASG/AARK OR OTHER AUTHORITY

9. **Range State approval:** Is the proposed *ex situ* initiative supported by the range State (either within the range State or out-of-country *ex situ*)?
   Yes: **Go to Section 3, question 11.**
   No: Insufficient authorization for an *ex situ* initiative at this time.
   SEEK APPROVAL FROM RANGE COUNTRY (WITH HELP FROM AARK/ASG AS REQUIRED) BEFORE PROCEEDING

SECTION THREE – PROGRAM IMPLEMENTATION

Section Three considers the feasibility of undertaking an ex situ *program for priority, authorized species. It functions as a form of evaluation/planning with respect to readiness to implement a program.*

POPULATION ESTABLISHMENT

10. **Founder specimens:** Are sufficient animals of the taxon available or potentially available (from wild or captive sources) to initiate the **specified** *ex situ* program?
    Yes: **Go to question 12**
    No: Insufficient potential founder specimens to initiate the *ex situ* program.
    EVALUATE OPTIONS FOR ALTERNATIVE CONSERVATION STRATEGY INCLUDING GAMETE CRYOPRESERVATION

PROGRAM STABILITY

11. **Financial security:** Is there sufficient financial support for the anticipated life of the *ex situ* initiative? Or is there good reason to believe that further financial support is realistically achievable?
    Yes: **Go to question 13**
    No: Inadequate resources.
    EXPLORE POSSIBILITIES FOR FINANCIAL SUPPORT BEFORE INITIATING PROGRAMME (WITH HELP FROM AARK AS APPROPRIATE)

12. **Organizational and political security:** Is the stability of the institution/region/State etc. sufficient to ensure a continued commitment to the *ex situ* program over its anticipated lifespan?
    Yes: **Go to question 14**
    No: Insufficient stability.
    CONSIDER ALTERNATIVE LOCATION/INSTITUTIONS AND PARTNERSHIPS

TAXON KNOWLEDGE

13. **Background *ex situ* species knowledge:** Is there a history of keeping and breeding this taxon successfully in captivity?
    Yes: **Go to question 17**
    No: **Go to question 15**

14. **Background *in situ* species knowledge:** Is there sufficient understanding of the ecology, behavior and reproductive mode of the taxon to infer the likely *ex situ* requirements?
    Yes: Go to question 17
    No: Go to question 16

15. **Analogous species:** Despite a lack of direct knowledge of the *ex situ* requirements of the target taxon, can they be inferred with a reasonable degree of confidence from similar/related taxa?
    Yes: Go to question 17
    No: Insufficient knowledge of the taxon and its requirements at this time.
    EX SITU PROGRAMME SHOULD BE DELAYED, WHERE POSSIBLE, WHILE RELEVANT EXPERIENCE/INFORMATION IS GATHERED – E.G. BY WORKING WITH ANALOGUE SPECIES

ACCOMMODATION

16. **Current facilities:** Is the appropriate quality and quantity of facilities (in country or out of country) currently available? Not just for founder animals, but also for captive bred offspring of all life-stages/sizes (consider space; heating & cooling; water supply, quality, treatment & disposal; lighting; ventilation etc).
    Yes: Go to question 19
    No: Go to question 18

17. **Planned facilities:** Are there confirmed plans – within a specified timeframe - to develop the appropriate quality and quantity of facilities to permit the full development of the planned *ex situ* program (in country or out of country)?
    Yes: Go to question 19
    No: Insufficient infrastructure available or planned to permit the likely successful development of the *ex situ* program for this taxon.
    CONTACT AARK WITH RESPECT TO POSSIBLE IMPLEMENTATION OF RAPID RESPONSE WHERE URGENT ACTION IS NECESSARY AND/OR WITH RESPECT TO IDENTIFYING SUITABLE PARTNERS/SUPPORTERS

HUSBANDRY & MANAGEMENT

18. **Personnel**: Are adequate numbers of skilled staff available with the appropriate *ex situ* amphibian experience?
    Yes: Go to question 21
    No: Go to question 20

19. Training: Can adequate numbers of skilled staff be made available for training in the appropriate *ex situ* amphibian skills?

Yes: Go to Question 21
CONTACT AARK TRAINING OR PROGRAM OFFICER FOR HELP

No: Suitable personnel not available.
CONTACT AARK WITH RESPECT TO POSSIBLE IMPLEMENTATION OF RAPID RESPONSE WHERE URGENT ACTION IS NECESSARY AND/OR WITH RESPECT TO IDENTIFYING SUITABLE PARTNERS/SUPPORTERS

20. Food supply: Is there a reliable food supply – in both quality and quantity – for adult, immature and larval stages of the taxon?

Yes: Go to question 22

No: Inadequate resources.
EXPLORE FOOD SUPPLY OPPORTUNITIES BEFORE PROCEEDING

21. Management: Is the appropriate standard of record-keeping and knowledge of small population management available to help minimize the risk of potential deleterious effects such as loss of genetic diversity, artificial selection, pathogen transfer, hybridization etc. (This expertise does not necessarily have to be held at the facility itself, but access to these skills is essential).

Yes: Go to question 23

No: Shortage of skills to support the *ex situ* program.
SEEK SUPPORT FROM AARK AND/OR REGIONAL ZOO &AQUARIUM ASSOCIATIONS BEFORE, OR SOON AFTER, PROCEEDING

22. Veterinary care and health screening: Has provision been made for the routine health monitoring of the population and is the appropriate level of veterinary care available?

Yes: Go to question 24

No: Inadequate resources.
SEEK VETERINARY SUPPORT THROUGH UNIVERSITIES, ZOOS &AQUARIUMS OR OTHERS BEFORE PROCEEDING

QUARANTINE & BIOSECURITY

23. Escapes: Are measures in place to minimize the risk of animal escapes and introduction of an invasive species?

Yes: Go to question 25

No: Animal security measures not sufficient.
REVIEW AND IMPROVE BIOSECURITY – REFER TO AARK GUIDELINES – BEFORE PROCEEDING

24. Species isolation: Are appropriate measures in place at the proposed *ex situ* facility to minimize the risk of possible disease transfer to or from other *ex situ* or wild amphibian populations?

Yes: Go to question 26

No: Insufficient measures currently in place to prevent disease transfer. **REVIEW AND IMPROVE BIOSECURITY – REFER TO AARK GUIDELINES – BEFORE PROCEEDING**

25. **Water treatment:** Are the appropriate waste water treatment regimes in place to eliminate the possibility of disease transfer from the *ex situ* population to the external environment?
    Yes: ***Ex situ conservation program currently feasible and ready for implementation***
    No: Bio-security measures inadequate.
    REVIEW AND IMPROVE BIOSECURITY – REFER TO AARK GUIDELINES – BEFORE PROCEEDING

If you have answered 'Yes' to questions 9 through 26 you are ready to implement your AARK *ex situ* conservation breeding program. Good luck.

Appendix One – Conservation Role

Defined Conservation Role

Simply keeping and breeding threatened amphibian species in captivity does not in itself equate to *conservation.* As part of a genuine amphibian conservation initiative, the *ex situ* captive management should not only form part of the recommended conservation action for the species but must also have a clearly defined role in the conservation of the species or its habitat:

a) **Ark** – An amphibian species that is extinct in the wild (locally or globally) and which would become completely extinct without *ex situ* management.

b) **Rescue** – An amphibian species that is in imminent danger of extinction (locally or globally) and requires *ex situ* management as part of the *recommended* conservation action.

c) **Supplementation** – An amphibian species for which *ex situ* management benefits the wild population through breeding for release as part of the *recommended* conservation action.

d) **Mass production in captivity** – An amphibian species threatened through wild collection (e.g. as a food resource), which is being bred in captivity – normally in-country, *ex situ* - to replace a demand for specimens collected from the wild. *This category generally excludes the captive-breeding of pet and hobbyist species, except in exceptional circumstances where coordinated, managed breeding programs can demonstrably reduce wild collection of a threatened species.*

e) **Conservation Research** – An amphibian species undergoing specific applied research that directly contributes to the conservation of that species, or a related species, in the wild (this includes clearly defined 'model' or 'surrogate' species).

f) **Conservation Education** – An amphibian species that is specifically selected for management – primarily in zoos and aquariums - to inspire and increase knowledge in visitors, in order to promote positive behavioral change. For example, when a species is used to raise financial or other support for field conservation projects (this would include clearly defined 'flagship' or 'ambassador' species).

Appendix Two – *Ex situ* Mandate

Mandate for Ex situ Conservation

The decision about which species should be protected in *ex situ* conservation programs should not be made by the AARK community alone because such programs must be part of broader plans for species conservation. The AARK community needs to respond to needs identified by appropriate

conservation authorities, especially since the decision to safeguard species in *ex situ* programs needs to follow from a careful assessment of which species cannot currently be assured of adequate protection *in situ*. A recommendation for an *ex situ* population of a threatened amphibian species can come from a number of recognized sources, such as:

- The IUCN/SSC Amphibian Specialist Group (ASG).
- The Global Amphibian Assessment (**www.globalamphibians.org**) - the authority on IUCN Red List status for all amphibian species and which recommends *ex si*tu conservation action for at least 240 species.
- The IUCN - the IUCN Technical Guidelines for the Management of *Ex situ* Populations recommends *ex situ* populations for all Critically Endangered species.
- An IUCN/SSC Conservation Breeding Specialist Group (CBSG) Population and Habitat Viability Assessment (PHVA) workshop process. (**www.cbsg.org/toolkit/phvas.scd**)
- An IUCN/SSC Conservation Breeding Specialist Group (CBSG) Conservation Assessment and Management Plan (CAMP) process. (**http://www.cbsg.org/toolkit/camps.scd**)
- An IUCN/SSC regional amphibian (and reptile) specialist group recommendation (Madagascar & Mascarene, Europe or China).
- A published Species Action Plan.
- A local, regional or national government request.

Appendix 2: Sample Taxon Management Plan

AZA Amphibian Taxon Management Plan

Date of completion: 08/02/07

Author(s): Paul Crump

BACKGROUND

Species (common/scientific names):

Houston Toad (*Bufo houstonensis*)

Distribution:

Central Texas

Conservation status (IUCN, CITES other):

IUCN – Endangered
USFWS – Endangered

Threats:

Habitat modification and fragmentation, urbanization, regional drought, predation from introduced fire ants.

Proposed *ex situ* roles (Ark; Rescue; Supplementation; Research; Education):

Rescue; Supplementation; Education; Research

Husbandry guidelines (Y/N, if yes give details and/or hyperlink):

N, but being developed by PC (09/01/08)

Co-ordinator and contact details:

Paul Crump

Programme Goal:

To provide an self sustaining *ex situ* colony to 1) prevent the extinction of the toad, 2) to use the colony as a source to specimens to reintroduce the toad into historical localities, and 3) to use the colony as a source for specimens to increase the number of individuals within each population.

ACTION PLAN

Ex situ population management

Current population (no. of individuals and/or institutions):

0.1.800

Target population (no. of individuals and/or institutions):

100.200 at 5 institutions, Houston, potentially Dallas Zoo, Ft. Worth, Omaha, Oklahoma City, Audubon.

Objectives (clearly defined and measurable):

Self sustaining ex situ population exists that is genetically and demographically stable

Proposed actions and respective time frames:

- Collect additional egg strands (if less than 15 were collected in 2007). January – May 2008
- Release the remaining juvenile Houston Toads after 30 (10.20) Toads are kept back from each cohort for inclusion into ex situ assurance colony. September – October 2008
- Solicit other regional and Texas zoological and academic institutions to participate in program to hold, research and/or breed Toads for release (the timeframe will allow addition of Bufo houstonensis to holding permits in before receiving them). September – October 2008
- Collect additional egg strands (if less than 15 were collected in 2007 and 2008) January – May 2009
- Pair up first round of founders for breeding as soon as wild breedings are documented in Bastrop January – May 2009.

***Ex situ* Research**

Current research objectives and expected time frames:

Husbandry and nutritional requirements of the larvae and juveniles – 07/01/08
Meiotic sex ratio vs. operational sex ratio – 01/01/08
Disease monitoring and parasite treatments – 01/01/09

Proposed research objectives and expected time frames:

Study if differential growth rates exist between sexes (females grow quicker and both mature in 1 year or males mature in 1 year and females mature in 2 years) – PC 01/01/09
Investigate tadpole "spinning" issues – PC 09/01/08

Actions necessary to meet research objectives:

Expand ex situ capacity to allow for tadpole and juvenile experimental replication – PC Houston Zoo is providing additional amphibian quarantine space due to be ready in early 08 01/01/08
Solicit amphibian pathologist/disease expert to examine tadpoles with "spinning" issues – PC to contact Allan Pessier about this 10/01/07
Assess health status of captive toads rigorously once data from wild populations is available – PC 01/01/09

***Ex situ* Education**

Educational message:

Example of an amphibian that suffered dramatically due to the synergistic efforts of man and nature. Rapid urbanisation and prolonged drought (as a result of climate change?)

Objectives (clearly defined and measurable):

Teach Texans about the story behind the population decline and range contraction of the Toad and how despite intentions, the conservation tactics have failed.

Use the Toad as an organism to illustrate the process of glacial expansion and contraction and how this process can lead to relict species and speciation.

Proposed actions to meet above Educational Objectives with time frame:

- Look for and solicit regional zoos and educational facilities to display the Houston Toad – PC 06/01/08
- Develop supplementary educational material for institutions wishing to display toads – PC to work with an educator to develop this material 06/01/08

Any other information:

In-country/field initiatives

Current activities:

Population surveys and searches,
Metapopulation genetics,
Larval and juvenile recruitment rates and other demographic and life history parameters,
All in situ research is being conducted by the lab of Dr. Mike Forstner at TX State Univ., San Marcos.

Objectives (clearly defined and measurable):

The main goal is to reintroduce the Houston Toad into at least one historical locality and evidence of reproduction and population viability is acquired over multiple years to confirm success. Subsequent initial primary objectives then become
1) Restore Toad habitat by brush reduction,
2) monitor for emerging infectious disease,
3) headstart Toads to bypass tadpole and juvenile mortality,
4) Population searches and monitoring

Other in situ research needed is a wild population health assessment to compare incidence and prevalence of significant, known and unknown diseases with ex situ colony specimens.

Proposed actions and respective time frames:

- The main core of in situ research requires little assistance from AZA institutions. However, PC will maintain regular contact with TSU and provide information as needed.
- Assistance can be provided with the health assessment and a Morris-Animal Foundation Grant is currently being prepared. PC needs to finish this and work with Allan Pessier to see if he can perform disease diagnostics for the project – 06/01/08

Long term goal (exit strategy):

In situ - brush reduction to restore Toad habitat, disease monitoring, ex situ, expand the range of the Toad back into its historical localities; increase the current population size and number of populations of the Toad; establish a self sustaining ex situ colony of toads to serve as a assurance colony and as a source of animals for reintroductions into historical localities. Down list the Houston Toad to threatened by the USFWS.

References

Publications/reports:

1984 USFWS Recovery Plan
2006 Report by Mike Forstner to USFWS for active Toad recovery.

Bibliography:

Appendix 3: ATAG Taxon Champion Job Description

Description

The taxon champion is responsible for developing and implementing a Taxon Management Plan, tracking the status of the *in- and ex- situ* population, and serving as an information resource among Taxon Management Plan participants. The coordinator should have a demonstrated interest and/or expertise in the taxonomic species and should have institutional approval and support for the position.

The taxon champion position differs from traditional PMP or SSP coordinator positions. While PMP and SSP coordinators are often involved with *in situ* conservation, the primary role of these coordinators is management of *ex situ* conservation population. This is not necessarily the case with the taxa being covered by a champion, the position is a more holistic and an evaluation of the conservation needs of the taxa and the feasibility of implementation is likely the first action of the new champion.

Criteria

- Should have husbandry and/or *in situ* conservation experience and expertise that will facilitate conservation success.

- Demonstrated or inferred behavior consistent with guidelines and reporting requirements of the Amphibian TAG and the AZA.

- Must possess excellent communication skills, an enthusiasm for interacting with a diversity of amphibian conservation partners, and a willingness to present progress of the Taxon Management Plan.

- Possess basic computer skills and record keeping abilities.

Application Process

Taxon champions may be solicited by a member of the steering or special committee of the Amphibian TAG for the task because of their specific skills and experience with the taxon in question, or else may apply to positions posted in the monthly AZA Amphibian Newsletter. All candidates will be required to fill out the application form and submit a CV. The steering and special committee of the Amphibian TAG will vote to approve positional taxon champions

Specific Responsibilities

- Develop a Taxon Management Plan for approval of the AZA Amphibian TAG Steering Committee within three months of accepting the position/appointment.

• Develop a listserv or analogous forum for Taxon Management Plan participants to exchange information.

• Initiate a computer database of Taxon Management Plan participant holdings. This database should include all specimens in captivity (both within and outside of AZA institutions) that are appropriate and consistent with the objectives of the Taxon Management Plan.

• Actively seek new and appropriate Taxon Management Plan participants. Participants should be well aware of the species-specific goals of the program (e.g., ark population for extinct-in-the-wild taxa, necessary biosecurity, and captive research on reproductive biology).

• Active linkages and communication with *in situ* activities are mandatory for all species. This includes but is not limited to any municipal, state or federal programs, university or museum research, NGO, non-profit, or any other organizations or individuals with objectives and behavior consistent with the goals of the Taxon Management Plan. Regional contacts, advice and recommendation(s) can be obtained through the steering or special committee of the Amphibian TAG, through the CBSG/WAZA Amphibian Ark, or from the IUCN's Amphibian Specialist Group.

• Keep the Taxon Management Plan updated and, at least annually, distribute to participants.

Accountability

The taxon champion is responsible for working with the Amphibian TAG and the AZA Conservation Department to ensure that the program is run effectively and efficiently. The TAG can remove the taxon champion if s/he is consistently not responsive to requests for information, is out of communication with the TAG for an extended amount of time, or fails to meet the management and communications responsibilities as outlined in this position description.

Application for an Amphibian TAG Taxon Champion

Individuals interested in coordinating the Taxon Management Plan of a selected amphibian species should complete the following application and return it to the Amphibian Taxon Advisory Group Chair.

1. **Applicant Information**

   Name:
   Institution:
   Institutional Address:
   Phone:
   Fax:
   Email:

2. **Taxa encompassed in plan**

3. **Please submit a curriculum vita, including all relevant experience with the taxon for which the applicant is applying.**

4. **Statement of Individual Commitment**

   As a Taxon Champion, I am willing and able to devote the necessary time and energy to oversee the establishment/progress of the plan. I am willing and able to meet all deadlines and responsibilities as outlined in the job description document. Failure to meet these requirements will result in my removal as taxon champion.

   _____
   Name of Applicant

   _____
   Signature of Applicant

   _____
   Date

5. **Statement of Institutional Support**

The_____ (name of institution) is committed to providing the adequate resources and support for the establishment/progress of the Taxon Management Plan for _____ (name of taxa) as outlined in the job description document.

_____
Name of the Director / Governing Official

_____
Signature of Director / Governing Official

_____
Date

Appendix 4: Recommendations for Amphibians Used for Outreach Programs

Compiled by Diane Barber, Fort Worth Zoo
Updated May 2008

INTRODUCTION

This document has been created by the AZA Reptile and Amphibian Taxon Advisory Groups to be used as a resource to aid in the development of institutional outreach programs and has been updated specifically for amphibians. Within this document are lists of species that are commonly used in amphibian outreach programs. With nearly 6,000 species of amphibians in existence today, there are numerous combinations of species that could be safely used in outreach programs. It is not the intent of the ATAG to produce an all-inclusive or restrictive list of species to be used in outreach. Rather, the list included in the RCP is intended for use as a resource and are some of the more common species that have been safely used in outreach programs.

It is hoped that during the species selection process for outreach programs, educators, collection managers, and other zoo staff work together, using ATAG outreach recommendations, ATAG RCP, and Institutional Collection Plans as tools. It is well understood that space in zoos is limited and it is important that outreach animals are included in institutional collection plans and incorporated into conservation programs when feasible. However, this does not mean that all outreach animals must be part of a conservation program. There are many parallel species that can be used in place of rare or "fragile" animals to convey conservation and education messages.

There are numerous components to consider when choosing appropriate species for use in outreach programs. Some popular species used for exhibit can be categorized as "difficult" from documented experience or by reputation, rendering them unsuitable for outreach programs. However, it is important to remember that individual animals often have individual personalities and can sometimes be evaluated on a case-by-case basis. These considerations as well as staff expertise, husbandry requirements, medical and nutritional requirements, length and types of programs, environmental needs, restraint and transportation methods, temperament, safety issues, and educational messaging should all factor into sound collection planning for outreach program animals.

HUSBANDRY CONSIDERATIONS

It is imperative that adequate housing is provided for all animals. Housing and quarantine requirements for each species should be addressed and approved by qualified staff before animals are acquired. Because of their relatively small size, ease of availability, and seemingly routine housing components, amphibians are oftentimes acquired before enough is learned about their husbandry requirements. Although some amphibians, such as marine toads, can live long and seemingly well-adjusted lives housed in nothing more than a glass terrarium with no substrate and minimal props, this is not the case for every amphibian. Many species require elaborate environments just to survive. Components such as space utilization, ultraviolet light requirements, thermoregulation needs, nutritional and medical requirements, stress factors, water quality issues, adequate record keeping, etc., all factor into maintaining healthy captive amphibians. There is no simple recipe or cookie-cutter approach to choosing the right species for an outreach program. It is imperative that

each animal is researched and experienced personnel are consulted before acquisition in order to evaluate whether or not the animal can be adequately housed and cared for. The *ATAG's Husbandry Resource Guide* (available at: **http://www.aza.org/ConScience/Amphibians_Intro/index.html**) and Steering Committee Members can be used as resources outside of institutions if necessary, but by all means utilize herpetological staff if they are present.

All efforts should be made to acquire animals that can be properly cared for. Some species are suitable for trained keepers or educators who have little to no amphibian husbandry experience. On the other hand, there are many species that require expert care and are labor intensive. Adequate training should be given to all caregivers and expertise levels of staff should mirror the attention needed for the animals in the collection. The animals recommended for outreach programs in the ATAG RCP have been categorized by level of experience needed by the caregiver in terms of meeting husbandry requirements, as well as the animal's relative level of hardiness in captive situations in order to help facilitate the decision-making process.

HANDLING AMPHIBIANS

During the handling or transporting of any animal, safety considerations for the animal, handler, and audience are of utmost importance. These factors include animal transport and handling methods, animal size, temperature limitations, stress levels, audience contact with the animal, appropriate settings and situations, disease and handling protocols, experience and number of handlers.

Each institution should develop its own handling protocols for its outreach programs. This document touches upon some of the more common issues and suggestions for safely handling and transporting amphibians. Once again, this is not an all-inclusive document and is meant to serve as a guideline for developing a handling protocol.

Animal Transport and Restraint

Many amphibians are easily transported from one destination to the other with little to no demonstrable adverse effects. Amphibians can be placed in small plastic containers filled with damp moss or paper towels and transported within styrofoam boxes or coolers to regulate temperature changes. As with any animal, care must be taken to keep them from direct sunlight and extreme temperature changes and to provide adequate ventilation. (For more information read IATA's Live Animal Regulations for standard shipping methods for amphibians by commercial airlines: http://www.iata.org/ps/publications/lar.htm.)

Most of the amphibians recommended in this document require little restraint when handling. When choosing outreach animals, it is important to consider the size and temperament of the animal and the type of restraint necessary for each species. It is imperative that the handler is experienced enough to reasonably anticipate the animal's reactions and behavior in various situations. The handler should also know how to adequately provide support to the animal so that is comfortable and relaxed. It is recommended that powder-free surgical gloves be worn when handling amphibians due to their permeable skin. Many institutions restrict the handling of amphibians entirely, using a clear box. When holding amphibians, it is important to restrain them from jumping and injuring themselves. Large amphibians can be held by both legs at the base of their body, while small species, such as dart frogs, may be carefully grasped by one or both hind limbs. It is not recommended to

allow the audience to hold the amphibians. If the audience is small, it is acceptable to allow a few people to touch the amphibian with one finger. However, the amphibian should be rinsed and rehydrated with dechlorinated water. Many species of amphibians are capable of excreting toxic, irritating secretions through glands in their skin. Therefore, the audience should wash their hands after they come in contact with amphibians.

Evaluating the Situation

Comparing the animal's temperament and anticipated stress levels against the actual type of event will help determine the amount of time each animal should be handled. For example, a dart frog may be appropriate for an intimate 10-minute news interview, but will most likely become stressed during a 30-minute demonstration walking among a group of party guests in someone's hand. Most species of amphibians should be placed in plastic containers if they are going to be used for more than 10 minutes at a time. The actual environment can have a direct effect on the amount of time an animal should be used in a program. Amphibians can stress easily and dehydrate quickly if used outdoors during a hot day. In such cases, it is best to keep the amphibians moist in shaded areas and to keep direct contact at a minimum. It is also important to know the natural history for each species. For example, a Gulf Coast toad would fair well on a warm summer day, but hot temperatures could be dangerous for a cool, forest dwelling species such as marbled salamander for extended periods of time.

Whatever the case, always evaluate the setting and consider stress-related factors such as amount of time handled, potential contact with the audience, and environmental influences such as temperature, as well as the animal's actual role in the program's messaging in order to help choose the most appropriate species.

Minimization of Disease Transfer

Each institution should decide what level of intermixing between "collection animals" and "outreach animals" is allowed to reduce the risk of spreading infectious diseases into the zoo population. This needs to be decided through thoughtful consultation of the reptile staff, veterinary staff and programs staff so that the institutional goals for the outreach program may be obtained without placing unreasonable or unnecessary limits to the use of the animals. As a general guideline, it is best to maintain outreach animals in a separate facility from the core animal collection.

Outreach animals may need to be held to a more rigorous preventive medicine program with routine fecal checks performed quarterly. Any animals that are not behaving normally or are known to be ill should not be used until the issue is resolved.

EDUCATIONAL MESSAGING

The use of threatened species can be effective. It is recommended that all state and federal laws are followed and that the audience is made aware of the laws. Animals that are surplus to SSPs and captive management programs can be important ambassadors for their own conservation.

Native species are all too often overlooked in zoo programs. Through the use of locally occurring species in outreach programs, audiences can learn about conservation in their own backyard. Using amphibians for outreach is also a good opportunity to teach audiences about state-protected species, cohabitation, the affects of urban sprawl, pollution, global warming, biomedical applications, amphibians as bioindicators, and of course, the global amphibian crisis.

ATAG Amphibians in Classroom Settings Statement:

Live amphibians in classrooms can stimulate students' interest in wildlife and promote respect for animals and their ecosystems. However, it is important that teachers think about disposition of classroom pets prior to obtaining them. Obviously, it is ideal for teachers to keep the amphibians from semester to semester until their natural death. However, as this is not always possible, it is imperative that teachers seek alternatives to releasing unwanted amphibians into the wild. Releasing larval (e.g.: tadpoles and newts) forms and metamorphosed (juvenile and adult) amphibians can have serious impacts on native species and their ecosystems. Released amphibians can introduce harmful pathogens and parasites into the wild. They can also out-compete native species for food and shelter, or act as predators, eating indigenous amphibians. Teachers should act responsibly and plan to keep the pet for its lifetime. If the amphibian can no longer be housed and a suitable home cannot be found, euthanasia is a better alternative than releasing it to the wild.

Appendix 5: Amphibian Quarantine Guidelines

Chapter 3 of the *Amphibian Husbandry Resource Guide*, Edition 1.0. A publication of AZA's Amphibian Taxon Advisory Group, 2008

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

TYPES OF QUARANTINE

Quarantine 1 (Q1): *Out-of-range with intent to return to the wild*

These animals are not from the locale where the facility is located. The main concerns are both the entrance and exit of pathogens from this quarantine group, as either direction engages a new host/disease interaction with potentially fatal effects.

Quarantine 2 (Q2): *Range country with intent to return to the wild*

These are wild animals from the general locale where the facility is located. The main concern is entrance of a novel pathogen into this captive group from outside the facility (i.e., a new disease agent has advanced into a geographic range as additional specimens are extracted to a facility, risking exposure to entire captive collection).

Quarantine 3 (Q3): *Out-of-range for display, education, and research; no possibility of return to the wild in range country*

These are animals in the standard collection of the zoo or aquarium designated for education, display, or research. Although they are not to be released, they can be considered to be in a semi-quarantine state, as they are not exposed to animals outside the collection.

Quarantine 4 (Q4): *Incoming into facility*

These animals are coming into the collection from the wild or other institutions. They can bring diseases, native or non-native to the range country, into the collection. All specimens entering into the facility should complete a full entry-quarantine regimen (Q4) regardless of ultimate designation (Q1-Q3).

QUARANTINE FACILITIES

Natural history of animal

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

Location

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

Rooms

- Surfaces
  Walls, floors, and ceilings should be impervious to fluids, creating easier cleaning and enhancing sanitation.

- Electrical
  Water is often splashed around during cleaning of aquatic amphibians such that all electrical outlets should have ground fault circuit interrupters (GFCI).

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

Enclosures

Glass, fiberglass, or plastic tanks can be used. Acceptable plastics are those used for human food storage as other industrial plastic sources can leach toxicants into the water. Plastic food storage bins (5-15 gallon/19-57 L) with custom-fabricated, ventilated lids are used frequently. Tank dimensions vary with size and number of animals housed. Tanks can be plumbed for constant water flow and drainage, if needed (see *Water* section below for information on plumbing). Opaque containers and the use of hiding sites (PVC pipe, ceramic tiles, or terra cotta pots, etc.) decrease stress and enhance growth. Cages placed on racks at a tilt promote drainage and hygiene, maximize storage, and improve access through lid on top. As many species can escape by climbing or jumping out of the enclosure, lids should be well-fitted and securable. For more information on enclosures, see Chapter 1.

Water

- Desired types
  - *Disease-free water*: Water acquired from sources determined to be free of amphibian-related diseases
  - *Treated water*: Water treated to safeguard inhabitants against disease transmission
    - Heat sterilized to 160 F (71 C) for 15-20 minutes under pressure is the *preferred* method.
    - Sediment removing mechanical pre-filters with chemical treatments (such as chlorine or chloramines) is the *minimal* method. Improper use of chlorine agents could potentially lead to accidental and catastrophic fatal exposure for resident animals and is also of environmental concern. Aeration of water can be used to remove some chlorine compounds. Other agents (sodium thiosulfate, AmQuel®+, and/or activated charcoal) can be added to chemically-treated water to remove any chlorine compounds. If using sodium thiosulfate to remove chloramines, the water will need further treatment to remove the ammonia (i.e., zeolite or biological filter).
- Sources
  - *City/well*
    Inexpensive and commonly used. Tap water from municipalities contains lethal levels of chlorine or chloramines that should be removed by 24-48 hour aeration, chemical treatments (sodium thiosulfate or AmQuel®+), and/or activated charcoal filtration. Activated charcoal is much more effective at removal of chloramines than aeration. Well-water and tap water might have trace toxic chemicals that could be lethal to amphibians, making the use of activated charcoal preferred for treatment. Both tap water and well-water should have the pH and other water quality levels checked to ensure they are within the parameters for the species maintained. Some water sources will need to have the pH manipulated with chemical additives or buffers to be suitable for use with some amphibian species.
  - *Bottled*: Distilled or reverse-osmosis (RO) treated
    Expensive for a large-scale operation; distilled water and RO water is usually not electrolyte-balanced and can be fatal to amphibians without rebalancing with buffers, electrolytes, and pH adjustments (see Chapter 1 on *Source Water Treatment*).
  - *In-house reverse–osmosis (RO) treated*

Expensive for a large-scale operation, but provides the highest water purity available. Only moderate volumes are generated at any given time, involving daily production by staff. This method also requires rebalancing and buffering with salts and electrolytes for safe, long-term use with amphibians (See Chapter 1 on *Source Water Treatment*).

- Plumbing/flow system types
  - *Static*: Closed systems with standing water (*dump and fill*)
    Works well for large or small groups. Enclosures should be plumbed for convenient draining and refilling purposes. These systems require daily manual labor to clean and maintain adequate water quality in the confined environment.
  - *Recirculating systems*: Closed systems
    Pumps force the water through mechanical (i.e., sand and/or charcoal) and biological filters to remove debris and nitrogenous wastes, respectively, from enclosures. Filters can become overwhelmed by debris and waste if used for large populations. These systems require regular maintenance and monitoring to ensure adequate water quality and flow rate.
  - *Continuous flow systems*: Open systems
    A constant stream of water into and out of enclosure usually by a hose, misting system, or other drip source dilutes waste to a non-toxic level in the enclosure water, and removes wastewater and debris continually. A standpipe can be employed to regulate pool depth as well as to drain water from this system. Influent water temperature and quality needs to be regulated and treated to ensure no chlorine compounds or other toxins. Constant monitoring is necessary to prevent temperature fluctuation into extreme ranges or overflow from a blocked drain.
- Water quality testing
  Testing should be performed weekly in Q4 and at least monthly in Q1, Q2, and Q3. Accurate testing equipment is required and staff should be trained for correct use. Electronic colorimetric equipment[a] is highly accurate and should be considered, but is also expensive. Less expensive chemical titration kits and dip-strip tests are available and suitable for non-routine testing, however they are less accurate and precise than electronic colorimeters.
- Modification
  Water chemistries can be manipulated to enhance tadpole growth, breeding, etc. Formulas are available that detail what additives and amounts to add to tank water as needed (Wright and Whitaker, 2001).
- Disposal
  See *Sanitation* section that follows.

HUSBANDRY

Identification

- Morphological identification
  Includes the use of physical characteristics such as size, coloration patterns, sexual dimorphism (i.e., nuptial pads in the males, toe-pad width, etc.), and/or other distinguishing markings to identify individuals within a collection. Photo-documentation is a very valuable tool, but juveniles of some species change dramatically as they age.
- External identification

[a] Such as the Hach® DR/890 Colorimeter

- *Toe clips*
  This inexpensive option for marking individuals involves surgical amputation of the end of specific digits based on a coding scheme for marking purposes (Donnelly et al., 1994). The tissue removed can be saved for DNA banking, *Batrachochytrium dendrobatidis* (*Bd*; the amphibian chytrid fungus) polymerase chain reaction (PCR), and/or other disease investigations, if stored correctly.
- *Attached tags or beads*
  Loose colored wire or elastic bands have been placed around the waist of frogs. Plastic, colored beads have been sewn to the limbs of amphibians using a non-absorbable suture material that passes through a muscle mass and anchors the beads permanently. Placement on animal, added weight, and potential for catching on enclosure furnishings should be taken into consideration for this method.
- *Ink and branding*
  Traditional tattooing and branding (heat or freeze) have been used to mark amphibians successfully (Kaplan, 1959; Clarke, 1971; Daugherty, 1976). However, application of these methods varies between species and testing should be performed before it is used widely. Select a dye or method that will contrast with skin pigmentation and remain legible over time.
- *Radiofrequency biocompatible ink*
  This special ink tattoo emits an identification-signal specific to that animal and can be read with radiofrequency.[b] This is new technology and is unknown for use in amphibians.

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

[b] Somark Innovations, Inc., Saint Louis, MO – available in visible or invisible dyes.
[c] AVID® (125 kHz); Banfield® (125 and 134.2 kHz); Biomark®/Destron Fearing™ (125 and 134.2 kHz); and Trovan® (128 and 134.2 kHz).
[d] Available from Northwest Marine Technology, Inc.

Nutrition

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

Sanitation

- Cleaning schedule: *Minimal* standard with frequencies increasing as amphibian biomass and feedings increase
  - Water change frequency is dependent on the natural history of the animal and type of system used. A continuous, low-volume flow with overflow drains is preferred over the static (*dump and fill*) method and reduces stress to the animals. If closed systems are to be used, weekly or more frequent water changes are recommended, depending on if a filtration system is employed. It is advisable to perform a water change two hours post-feeding for aquatic amphibians.
  - General cleaning of all cages should be performed at least weekly.
  - Complete cage break down and cleaning should be performed weekly in Q4 and at least biannually in Q1, Q2, and Q3.
  - Attempt to clean cages at same time of day and in the same directional order to control disease spread.
- Clothing, gloves, and uniform standards
  - Quarantine 1, 2, and 4 - *Preferred* standard for working between species or species assemblages:

[e] Natural Balance® Reptile Diet sausages.

Dedicated clothing and footwear should be available for each species or species assemblage and changed before working with a different group. Disposable protective clothing (e.g. Tyvek® jumpsuits) may be useful in this regard. Ideally, keepers would have appropriate amenities to shower between servicing each species or species assemblage housed in the Amphibian Quarantine Facility. Gloves should be worn while accessing amphibian enclosures, and dedicated glove use may be required per individual container, per species, or per faunal group depending on pathogen risk.

- Quarantine 1, 2, and 4 - *Minimum* standard for working between species or species assemblages:
  Dedicated clothing and footwear should be available for each species or species assemblage and changed before working with a different group. Disposable protective clothing (e.g. Tyvek® jumpsuits) may be useful in this regard. Gloves should be worn while accessing amphibian enclosures, and dedicated glove use may be required per individual container, per species, or per faunal group depending on pathogen risk.

- Tools
  Ideally, each species or species assemblage will have its own set of tools (nets; forceps; suction tubing; scrub brushes and sponges; etc.) that will not move between cages/rooms. If tools will be used in multiple cages within a room, it is advisable that the tools be soaked in a disinfecting solution for at least 15 minutes. Tools may need to be soaked in specific or multiple disinfectants prior to use depending upon the pathogens to be eliminated (See Chapter 2 for recommendations). After each disinfectant, all tools need to be thoroughly rinsed with fresh water.
- Substrate change frequency
  For substrates that cannot be disinfected (i.e., organic matter and paper towels), complete replacement should be performed daily or weekly in Q4 and at least biannually in Q1, Q2, and Q3.
- Wastewater disposal
  Facility wastewater should be treated to minimize the risk of exporting foreign pathogens out of the facility and introducing them into the surrounding area (Brown et al., 2007). Heat sterilized to 160 F (71 C) for 15-20 minutes under pressure is the *preferred* method and will kill both *Bd* zoosporangia and ranavirus (Johnson et al., 2003; Langdon, 1989). At minimum, chlorine treatment of wastewater with standard household bleach (recommended dilutions and minimum contact time still to be determined) added to the wastewater should take place in an amphibian-safe manner (e.g., ventilation of chemical fumes and disposal into the sewer system rather than a local watershed). The treatment of wastewater may be incorporated into a keepers' daily schedule such that the wastewater is collected, treated, and kept overnight before discharged.
- Solid waste disposal
  Disposal of solid waste from Q1, Q2, and Q4 (and Q3 in the case of a known pathogen outbreak), including all substrate, props, gloves, etc., should be decontaminated by way of incineration or heating to a minimum of 160 F (71 C) for 20 minutes prior to being discarded. Disposal by a medical waste hauler is an alternative.

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

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

DURATION

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

MEDICAL CARE

Records

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

Parasites

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

Medical diagnostics

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

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

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

Treatments

- *Bd* prophylaxis and treatment
  - Prophylactic treatment is suggested primarily for amphibians that are coming from a known *Bd* positive collection or field site, or if animals positive for *Bd* are identified through testing. Specimens destined for release from Q1 or Q2 require a minimum 5-day course of *Bd* treatment (listed below) to be completed immediately prior to release. Animals that test positive for *Bd* (and their cage-mates) should be treated and retested one week post-treatment. Multiple treatment cycles may be required to completely eliminate *Bd* infection.
  - Treatment: The author recommends itraconazole diluted to 0.01% concentration (in 0.6% saline) bath for 15 to 60 minutes daily for 5 days as a prophylactic regimen for

animals destined for release. For treating those animals that are known positives or exposed to known positives, a 0.01% itraconazole bath for 5 minutes daily for 11 days is recommended (Nichols and Lamirande, 2000). For treatment, animals are placed into a plastic container and allowed to soak with their digits and ventral surface of their abdomen covered with the solution.

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

RELEASE SITE ACTIVITY

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

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