Amphibian Taxon Advisory Group Regional Collection Plan

Fourth Edition

2020

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This document was respectfully submitted to
AZA Animal Population Management Committee on January 1, 2020

Cover photo credits: Steve Reichling, Jeff Bean, and Matt Baronak

ATAG Definition and Scope

Mission Statement

Working globally to address amphibian declines, the ATAG supports AZA members and partners in the conservation of amphibians, both in situ and ex situ, through scientific management of populations, education, capacity-building, and research.

Amphibians are in Trouble!

In 2008, the International Union for Conservation of Nature (IUCN) conducted the Global Amphibian Assessment (GAA), which evaluated the status of 6,285 amphibian species. The GAA reported that the sizes of more than 43% of all measured amphibian populations had declined and less than 1% of populations had increased, indicating a troubling trend. Almost one-third (32%) of amphibians are threatened with extinction globally and 168 amphibian species may have already been lost. The majority of threatened amphibians reside in the New World, with the highest numbers in Columbia, Mexico and Ecuador. Nevertheless, the bulk of endemic species in rapid decline (80-90%) are from the Dominican Republic, Haiti, Cuba and Jamaica (IUCN, 2014).

Now at the beginning of 2020, over 8,311 species of amphibians are described (Amphibiaweb, 2019). New diseases, such as the salamander chytrid fungus, *Batrachochytrium salamandrivorans*, are also identified, posing novel pressures upon amphibians to those that already exist, such as habitat alteration, chemical contaminants, and climate change. The warnings that were sounded when the amphibian crisis was brought to the forefront remain and new threats continue to appear on the horizon. It is important for us to remain diligent and steadfast in our messaging to those that share amphibian habitat and to empower the next generation of amphibian caregivers, program leaders, and researchers to combat declines before they occur.

Addressing the Amphibian Crisis at a Global Level

The IUCN, WAZA (World Association of Zoos & Aquariums), CPSG (Conservation Planning Specialist Group), the ASG (Amphibian Specialist Group) have called on zoos and aquariums to join in the global response to this conservation crisis. Recognizing that the size of the problem far outpaces our ability to respond with *in situ* programs, *ex situ* assurance populations have been recognized as the only hope for survival for many amphibian species. The Amphibian Conservation Action Plan (ACAP) (Gascon, et. al., 2007) was published in response to the 2005 Amphibian Conservation Summit in Washington, D.C. It is a comprehensive global response to amphibian population declines, of which *ex situ* breeding is one component.

The Association of Zoos & Aquariums (AZA) community has been directly active in this global initiative on multiple levels. In addition to trying to build capacity for species in AZA facilities, the ATAG has produced numerous materials to aid in the immediate development of successful amphibian conservation and/or research programs (either *in situ* or *ex situ*; internationally or domestically). These publications include the *Action Plan for Ex Situ Amphibian Conservation in the AZA Community* (2007), a detailed description of current amphibian collections and spaces within the AZA community; the *Conservation Resource Manual* (2007) to aid in the development of successful amphibian conservation programs that fit into institution's collection plans, which are appropriate for different levels of resources, and provides species specific action plans and husbandry manuals; and the *ATAG Amphibian Husbandry Resource Guide* (2012) a user-friendly source for amphibian husbandry and *ex situ* management. The AZA also published *Amphibian Conservation: Highlights and Accomplishments* annually from 2010-2016, which provides excellent examples of *in situ* and *ex situ* amphibian programs/techniques that can be applied to new programs as they arise. All of these resources can be accessed at: www.saveamphibians.org/documents. Additionally, the ATAG awards up to $2,000 per grant cycle for projects that focus on amphibian conservation, contributing over $33,000 since 2006 (download application at www.saveamphibians.org). Furthermore, the ATAG recommends the Amphibian Management School (see **ATAG Resources**) to improve amphibian husbandry techniques and to benefit from interacting with other amphibian herpetologists, as well as participate in networking opportunities at the annual ATAG meetings. The ATAG also requests more involvement in implementation of RCP initiatives through the appointment of Institutional Representatives (IRs) that can effectively communicate and actively contribute.

Metamorphosis of the ATAG Regional Collection Plan (RCP)

Since the completion of the first edition of the 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 can aid in this crisis by developing assurance colonies of at-risk species within a global framework.

Current data indicate that the general trend of amphibian extinctions is accelerating at an unprecedented rate and future catastrophic losses are inevitable. Within this context, 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.

Rana pretiosa Photo: Michael Durham

While using this RCP to develop institutional collection plans and conservation programs, keep in mind that resources are limited, and space is at a premium for managed programs. Never before has the zoological community been tasked with the conservation of so many species at such a rapid pace. The global amphibian crisis has been and will continue to be a challenge for us all, but we must remain optimistic and engaged, as we know AZA institutions are up to the task. Please help conserve these unique and important creatures by following the recommendations of this RCP to expand space and develop new amphibian programs and strategies through collaborative partnerships.

Taxa within ATAG Purview

Due to the quarantine and spatial requirements of amphibian assurance colonies, the ATAG considers exhibit space to be outside of the periphery of usable space for program species with reintroduction potential (except for those deemed surplus to the SSP may be used for display/education). Therefore, while this RCP will provide limited guidance regarding institutional exhibiting of amphibians, the primary focus is centered on building capacity for species of immediate conservation concern.

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 originally engaged by IUCN/ASG to prioritize and help manage 353 Critically Endangered amphibian species found in the Caribbean and North, Meso, and South America. Due to limited space in AZA facilities and the presence of other zoological associations within Meso and South America, the ATAG is asking AZA institutions to focus amphibian conservation efforts towards New World species with a governing mandate for *ex situ* management and commitment of resources to North America and the Caribbean when possible.

The ATAG recognizes that AZA institutions have commitments to other regions of the world and does not discourage continued involvement in those areas and programs. However, the ATAG encourages and will endorse/support programs developed for North America and the Caribbean above all other regions until there is adequate space for program species. Without increased capacity, we cannot plan for large-scale conservation efforts in an indiscriminate manner. As additional species from the Caribbean, Meso America and South America continue to be assessed, new species may be identified for priority action by zoos and aquariums which can be integrated into the RCP if additional space is created and identified in the surveys sent to our participating institutional representatives.

Notophthalmus meridionalis Photo: Patty Scanlan

Throughout the world, there are zoological organizations that have agreed to follow global initiatives set forth by IUCN/ASG. These organizations have been asked to support species in need within their individual regions and to assist adjacent neighbors without resources. ***If AZA institutions fail to care for species within their own backyards, then who will?***

Priority Conservation Activities

The zoological community has endeavored for the past thirty years to gain credibility and recognition as a key conservation leader. We are finally reaching audiences with important conservation messages and have strong programs in place but exhibiting an SSP species is no longer enough to aid amphibians. Zoos must continue to raise the bar and dedicate conservation resources equally to a wide variety of taxa, even those that do not produce revenue. Amphibians are important. They are needed in our ecosystems. They are rapidly disappearing and require our immediate assistance. Simply put, we must increase capacity to save our amphibians!

Priority actions from AZA institutions should include:

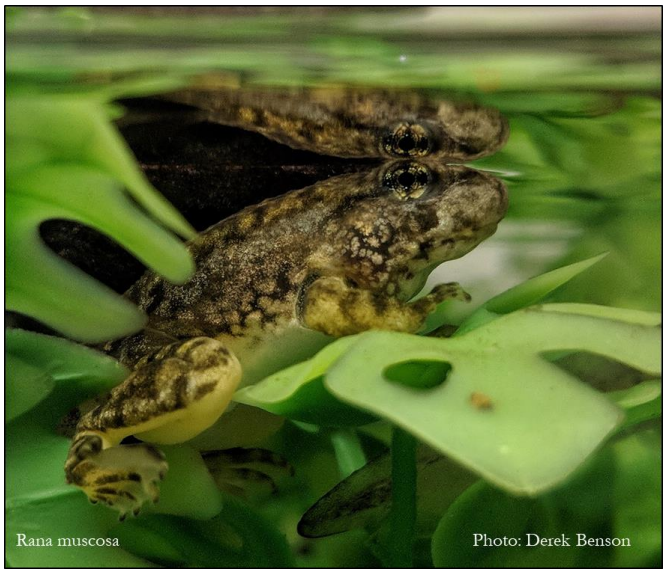

Photo: Dustin Smith

- Addition of amphibians of conservation concern to institutional collection plans.
- Isolation rooms for species with reintroduction programs in order to appropriately manage current programs and/or provide space for future programs (see examples in **Appendix II**).
- Long-term financial, technical, physical and logistical support to programs and projects identified as priorities in the ATAG RCP. It is also encouraged to pool resources with other facilities or consortia formed to address regional amphibian concerns.
- Formation of local or foreign agency partnerships for amphibian conservation.
- Creation and funding of additional *in situ* amphibian conservation centers
- Support of biologists conducting field research.
- Capacity building at home institutions and within range countries and improve amphibian facilities to reflect the needs of conservation-oriented collections.
- Appropriate training opportunities for staff, which can be obtained by attending AZA's *Amphibian Management Course*, or by participating in internships at institutions with existing capacity. The ATAG's *Amphibian Husbandry Resource Guide* (Poole and Grow, 2012) can be used to reinforce lessons learned.
- Active participation within ATAG by assigning an Institutional Representative (IR), serving on or as an advisor to the ATAG steering committee, responding to survey requests in a timely manner, and communicating through meetings and forums in order to promote partnerships between institutions and improve amphibian husbandry and programs across AZA institutions.

Institutional Capacity of AZA Communities

In response to the amphibian crisis, we are encouraged by individuals and institutions that have dedicated resources to aid amphibians at a multitude of levels. A number of institutions are participating in and leading amphibian conservation efforts. We have dedicated buildings for amphibians and are making great strides in translocation programs as a whole, we have increased community awareness, expanded educational graphics and programs, supported *in-situ* recovery efforts across a wide variety of regions, increased monitoring and research efforts, improved husbandry and assisted reproduction techniques, and developed new programs. However, have we accomplished enough, and do we still have the momentum, resources, and time to stop new amphibian extinctions?

Space needed for Amphibians


Rana muscosa Photo: Derek Benson

The first ATAG space survey conducted in 1999 showed the potential for AZA institutions to collectively manage ten SSP populations. However, this estimate was made before isolation space was required for species with reintroduction programs. In the 2000 RCP, ATAG Chair, Dr. Kevin Wright, wrote:

"*…In these same institutions there is enough space allocated for mammals to accommodate at least 57 SSPs and the majority of these mammals have a body mass of more than 10 kg and significant space requirements. If each AZA institution allocated an additional 400 square foot building to amphibian management and provided keeper support for the facility, the number of taxa that could be managed at a PMP or SSP level would easily exceed 100 taxa. If AZA is to "Keep all the Pieces", the theme of its 1996 annual conferences, then a wave of dedicated amphibian facilities must be built. Amphibians need dedicated space and should not be simply incorporated into Reptile Houses or included as a small part of biome or zoogeographically themed facilities. If this dedicated space is lacking, zoos will never play a major role in maintaining amphibian biodiversity.*"

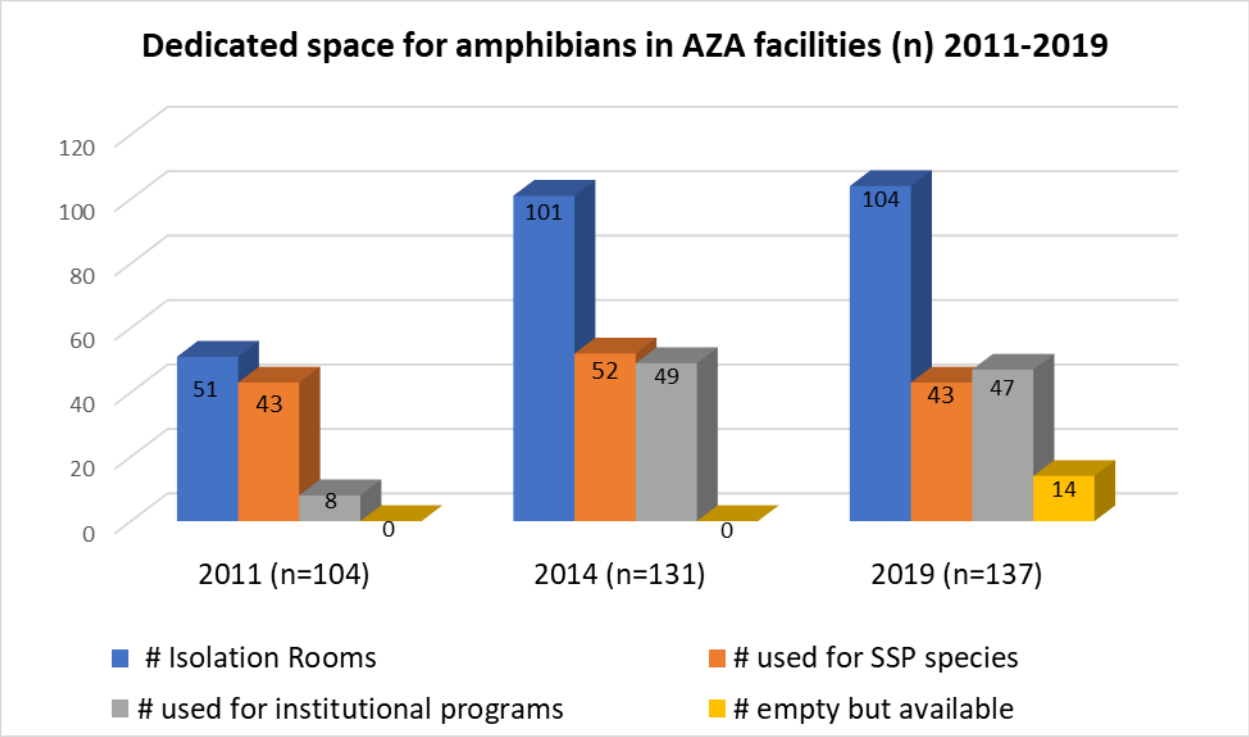
Sadly, Dr. Wright is no longer with us, but it is imagined he would be sorely disillusioned to learn that we are still paddling in the same boat 20 years later, despite the magnitude of the global amphibian crisis and the amount of resources/space needed for an amphibian program compared to a larger vertebrate species.

Three years after the 2008 *Year of the Frog* campaign, a second space survey was sent out to ATAG IRs (n=104) to gauge if we had increased capacity within AZA institutions for amphibians. Only twenty-four percent of respondents (n=25) reported creating new isolation space for amphibians in 2009 and 2010. Fifty-seven percent of respondents reported having no dedicated isolated space for amphibians, and out of those institutions, 75% (n=51) expressed that they did not anticipate creating new isolated space within the next five years. Thirty-two institutions reported having dedicated isolation space, totaling 51 individual rooms. Over 85% of the isolation rooms were already occupied by the four SSP species and every program manager needed additional institutions to participate. The remaining occupied rooms were being used for local species of concern or for institutional initiatives. The last question on the 2011 survey asked participants what types of resources were needed from the ATAG to help with future conservation initiatives. Thirty-eight of the respondents answered the question. Out of those, the most common response was "Provide more information to sway or apply pressure to Directors to become more involved". The second most common request was for funding.

A third survey was distributed in 2014 and represented data from 80% of ATAG IRs (n=131). Thirty-eight percent of respondents (n=50) reported having dedicated isolation space for amphibians. Of those institutions, 101 individual rooms were currently in use for amphibians. Although, only 50% (52) were actually being used for recommended SSP program species. Collectively, it was noted that 19 rooms could possibly be made available for amphibians if needed. However, 69% of IRs (n=80) indicated that there was no intent to create space for amphibians at their institution within the next five years. This represented a total of 71 rooms that were available for ATAG SSP programs for the

next five years. Since 73% of those rooms were already in use, and four of the five SSP Coordinators were pleading for more participating institutions to expand and accomplish current reintroduction/recovery efforts, there was little to no space available for new SSP programs. Furthermore, there were eight additional species identified within the 2014 RCP for *ex situ* program establishment.

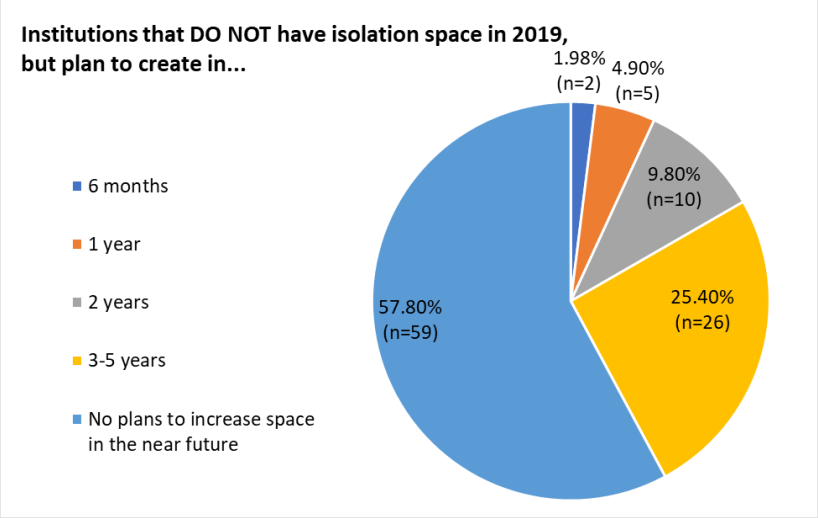
In 2019, the fourth space survey was completed by ATAG IRs (n=170) with an 80% response rate (n=137). Sixty of the respondents (44%) indicated that they had at least one dedicated isolation space for amphibians at their facility and 14 of those rooms were described as empty. There are currently 104 isolation rooms in use (43 for SSP species and 47 for institutional initiatives) holding roughly 24 species, of which 6 are SSPs.



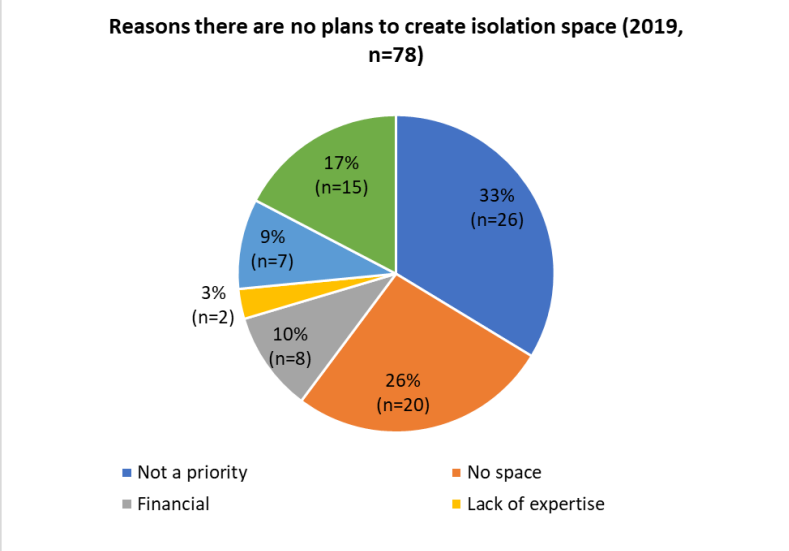
It is evident from the combined data shown in the chart above that participating institutions doubled isolation space for amphibians during the first five-year period between surveys (2011-2014). However, by 2014, most of those spaces were dedicated to institutional initiatives, rather than SSP programs, limiting our ability to increase capacity for reintroductions and stalling our ability to add new collectively managed programs (conveyed as concerning in the 2014 ATAG RCP). Five years later, 2019 survey data shows that occupied dedicated space for amphibian programs has slightly declined overall (10% for SSP program species and 3% for institutional initiatives), with an increase in empty spaces. There are currently 14 empty rooms that are not being utilized for various provided reasons (i.e., 9 have been "closed" due to exodus from a program, renovations, lack of staffing and/or resources; 5 are new rooms currently available to unspecified species). Although it appears there has been a slight waiver of institutional support for SSP programs since 2014, there is a potential of additional participation if directors continue to provide the necessary staff and resources that are needed. Overall, it was envisioned we would be able to create more isolation space and new programs in response to continued global amphibian declines, but progress has been slow during this decades-long push. However, we now have adequate isolation space available to fulfil our current SSP's needs

and some flexibility to consider formation of another collectively managed program. Although space is currently available, there are many species identified within this document as candidates for program development (e.g., Candidate and PIP), and requests for new programs will be carefully considered by the ATAG Steering Committee based upon species global population, ranking within the RCP, regional scope, and urgency.

Despite being in a slightly better position than we were in 2014, most of the IRs recently surveyed conveyed that there are no plans to increase space in the future, imparted that amphibians are not a priority at their facility, and that space is still a major limiting factor for conservation efforts.



What does it mean?



If we do not commit to creating more space and support for amphibians and we don't have an "out" for programs that are not effective, we will NOT be able to manage more than our current five SSP programs.

The horrid truth is that some species programs will not succeed despite our best efforts. Some species will never have an effective champion or enough resources to reverse threats in wild in time to save them from extinction. Other programs will be successful, and species will be recovered in the wild only expending decades of effort and millions of dollars. Species recovery plans include definitions of successful recovery for individual species in order to delist, or deem a species *recovered*, but what about those species that are most likely <u>not</u> going to recover? Due to space and resource constraints, and an increasing number of threatened species identified each year, it is imperative that program leaders address exit strategies BEFORE initiating or joining new programs. Plans should be developed to evaluate and quantify "success" or "failure" of short and long-term goals. There are many examples of *ex situ* programs that are floundering due to the participants' inability to "concede

defeat". There is typically no exit strategy for species programs and extinction may be the outcome for many that we try to save. It is our responsibility to try to reverse declining population trends, particularly if human activities are to blame, but the reality is that there are not enough resources and time for every amphibian in need. Being savvy and efficient is vital to do more with less. It is essential that stakeholders streamline projects by being clear and up-front regarding resource limitations, the expected level of participation from partners, and the duration of projects.

In the 2014 edition of the ATAG RCP, it was predicted that we would most likely still have 75% of the priority species listed without a champion, with little to no increased capacity for our program species, and another 20-30 new amphibians recommended for *ex situ* management in the wings. Unfortunately, we were able to predict the future. There have been few new champions, or programs developed, and support for established programs has begun to waver. It is evident that few people lack the initiative and support to champion new conservation programs and those that possess the gumption to act are not backed by management or government. This has forced us to take a harder look at the ATAG recommended species lists, not only at the species' global status and ranking, but to be realistic concerning the human factor - that not all species will be able to be saved due to absence of champions, scarcity or availability of specimens, political and economic environments, and shortage of resources. Ultimately, resulting in the removal of species such as the California tiger salamander, Crawfish frog and some *Eurycea* species.

The ATAG has endeavored to support global initiatives by increasing support for prioritized amphibians within our assigned region and providing institutions with multiple resources and a "road map" with which to navigate. Individuals are encouraged to actively participate in and develop new programs on a regional and global scale along the way. The ATAG has hosted two AArk amphibian prioritization meetings for North American species and has used the results from those workshops and updated species assessments as the basis for a sound RCP. This process has enabled the ATAG steering committee to be more selective regarding the formation and maintenance of priority amphibian programs for extremely limited regional space and resources. However, there has been a less than expected response for increasing capacity within the zoological community the past twenty years; there remains a major disconnect between our global initiatives and implementation of recovery efforts, particularly regarding the species selection process at the local and federal levels.

Despite the ATAG's ability to demonstrate the need and offer options for institutions to create space and resources (**Appendix II**), state and federal agencies have a different process of choosing species and allocating resources that do not always align with our current focal species selection criteria. Increasingly, these agencies are reaching out directly to local zoos and aquariums, as well as the ATAG, asking for assistance with the development of and participation in new *ex situ* amphibian programs. Although participation in local recovery efforts for amphibians is encouraged, those species identified for recovery efforts at the state level, may actually be considered stable throughout the majority of the population's range, taking up valuable space in zoos for species that may not be in dire straits compared to others.

To be successful on a global scale, there must be a cohesive species prioritization method aligned with governing agencies that is realistic in terms of number of species zoos and aquariums have the capacity to recover long-term. If capacity cannot expand and the way species are identified for *ex situ* management is transformed, new programs will continue to be developed at the local level, based on personal interests, or by lobbyists. Ultimately, this will deflect resources from more imperiled or truly unique species, undermining global species prioritization efforts. Amphibians favored by individuals

will continue to be the species that are driven to program status in zoos and aquariums rather than those that have been identified as most important.

Although the AZA amphibian community has made great strides in terms of coming together to follow a global plan and is a model for other communities, the current path remains daunting and time is of the essence. For example, the Ainsworth's Salamander, *Plethodon ainsworthi*, is excluded from our Priority I species list since none are in *ex situ* populations and is categorized as *Extinct in the Wild* (last seen in Mississippi in 1964). Three species of coqui from Puerto Rico (elegant coqui, *Eleutherodactylus eneida*; golden coqui, *Eleutherodactylus jasper*; and stream coqui, *E. karlschmidti*) have been removed since the last RCP revision, as they are now believed extinct.

Species Selection Criteria

The Global Prioritization Process

Ex situ 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 included *ex situ* rearing options using zoos/aquariums, universities, local and national government facilities, and private collections. However, until the second version of the ATAG RCP in 2008, there had been no strategic approach to the use of *ex situ* propagation for conservation management purposes and the majority of programs grew from the interests of individual facilities and the needs of state wildlife agencies.


Peltophryne lemur Photo: Dustin Smith

The ATAG hosted two Amphibian Conservation Needs Assessment Workshops in July 2007 and March 2012 to determine which of the amphibian species in the United States, Canada and Puerto Rico were in need of assistance. AZA ATAG Steering Committee, advisors, program leaders, taxa-specific biologists from primary regions, amphibian decline scientists, and AArk staff came together to assess over 300 regional species. Goals were to assess all species for their conservation requirements, prioritizing those that require immediate *ex situ* actions and to include those species that could benefit from *in situ* efforts. The results from these workshops, and additional assessments that were conducted later in range countries such as Mexico, as well as updated species assessments using Amphibian AArk's prioritization tool (Johnson, et. al. 2020) (**Appendix I**), were used to select species for the current RCP. Any new species included in this revision were run through the assessment tool process by the steering committee and then categorized into the appropriate priority ranking by comparing to our current species lists and recommended *ex situ* role/conservation actions.

Ex situ conservation is only one component of amphibian recovery efforts, for which zoo and aquariums are particularly adept. All *ex situ* rearing programs should 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 cause 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 brings opportunities to contribute to an integrated approach of conservation. 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* (Johnson et al., 2020) (**Appendix I**) 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 the current tool was finalized by Amphibian Ark staff in 2012. 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 original GAA listing; threat mitigation possibilities; socio/economic importance; phylogenetic uniqueness; scientific and biological importance; and other factors. Additional questions evaluate program feasibility and readiness for program implementation. The prioritization tool is included in the RCP (**Appendix I**), which includes an example assessment. All assessments can be viewed in a searchable database at the AArk website and are downloadable at the following link - http://www.amphibianark.org/resources/assessment-results/

Plethlodon yonahlossee Photo: Dustin Smith

Management Categories

SSP Taxa (Species Survival Plan): Studbook required, intense management to maintain *ex situ* 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.

Green SSP: Greater than 50 individuals, ≥90% projected gene diversity at 100 years or 10 generations, studbook and full participation in AZA SSP Policy required.
Yellow SSP: Greater than 50 individuals, <90% projected gene diversity at 100 years or 10 generations, studbook required, full participation in AZA SSP Policy is NOT required.
Red SSP: Greater than 20 Individuals, maintained at ≥3 facilities; OR listed as EX, CR or EN by IUCN, studbook required, full participation in AZA SSP Policy is NOT required, ATAG has developed 3 goals.

Candidate: Taxon is listed as an IUCN species of concern (but not LC), is currently in at least one AZA institution, is not collectively managed at this time, however, the population may become an SSP in the future.

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.

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

ISE Taxa (Populations in need of *In situ* Effort): Taxon for which mitigation of threats in the wild may still bring about their successful conservation and that further research in the wild is required as part of the conservation action for these taxa. Educational outreach and/or biobanking may also benefit.

NC (No Category): Taxon is no longer placed in a managed category.

Results of Species Selection Process

The majority of the species included on Tables 1-3 were initially evaluated during the 2012 prioritizations for North America and Puerto Rico. For this RCP revision, ATAG IRs were solicited for input and all current species were reviewed. New assessments were performed by the steering committee for attainable species that were proposed for inclusion in the new RCP based on available space. All assessments can be viewed in a searchable database at the AArk website and are downloadable at the following link - http://www.amphibianark.org/resources/assessment-results/

Key to Categorical Designations:

Ark/Rescue Role: Species that are extinct in the wild or in imminent danger of extinction (locally or globally) and require *ex situ* management as part of an integrated program, to ensure their survival.
***In situ* Conservation Role:** Species for which mitigation of threats in the wild may still bring about their successful conservation.
***In situ* Research Role:** Species that for one or more reasons require further *in situ* research to be carried out as part of the conservation action for the species. One or more critical pieces of information is not known at this time.
***Ex situ* Research Role:** Species currently undergoing or proposed for specific applied research that directly contributes to the conservation of the species, or a related species, in the wild (this would include clearly defined 'model' or 'surrogate' species).
Conservation Education Role: Species that are 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).
Biobanking Role: Species for which the long-term storage of sperm or cells to perpetuate their genetic variation is urgently recommended, due the serious threat of extinction of the species.

Key to IUCN Red List and USFWS Endangered Species Listing Codes:

IUCN Status
NE – Not Evaluated
DD – Data Deficient
LC – Least Concern
NT – Near Threatened
VU – Vulnerable
EN – Endangered
CR – Critically Endangered
EW – Extinct in the Wild
EX – Extinct

FWS Status
EN – Endangered
TH – Threatened
UR – Under Review
P-(level) – Proposed Listing
C-(level) – Candidate Listing
NL – Not Listed

Priority I Taxa Recommended for *Ex situ* Management

Priority 1 taxa include current SSP programs, as well as species identified through the species selection process that are collectively managed or listed in PIP categories. Each species requires *ex situ* management for survival and should be kept in permanent isolation.

Table 1: Priority I Taxa - Species with mandates for *ex situ* conservation listed alphabetically by scientific name [Ark/Rescue Role (A) recommended because threats CANNOT be mitigated in time].

Common Name	Scientific Name	Category	Species Contact	IUCN Status	FWS Status	A/B	C	D	E	F
Reticulated flatwoods salamander	*Ambystoma bishopi*	DERP	Dante Fenolio	VU	EN	**A**	C	D		
Flatwoods Salamander	*Ambystoma cingulatum*	PIP	Mark Mandica	VU	TH	**A**	C	D		
Wyoming Toad	*Anaxyrus baxteri*	SSP-Yellow	Coordinator: Val Hornyak Studbook Keeper: Sarah Armstrong	EW	EN	**A**	C		E	F
Houston Toad	*Anaxyrus houstonensis*	SSP-Yellow	Coordinator/Studbook: Stan Mays	EN	EN	**A&B**	C		E	F
Panamanian Golden Frogs[1]	*Atelopus zeteki & A. varius*	SSP-Yellow	Coordinator: Vicky Poole Studbook Keeper: Kevin Barrett	CR	EN	**A&B**	C	D	E	F
Ozark Hellbender	*Cryptobranchus bishopi*	Candidate	Mark Wanner	NL[2]	EN	**A[3]& B**	C	**D**	E	
Cricket Coqui	*Eleutherodactylus gryllus*	PIP	Raphael Joglar	EN	NL	**A**	C		E	F
Upland Coqui	*Eleutherodactylus portoricensis*	PIP	Sondra Vega	EN	NL	**A**	C		E	F
Richmond's Coqui	*Eleutherodactylus richmondi*	PIP	Sondra Vega	CR	NL	**A**	C	D	E	F
Dusky Gopher Frog	*Lithobates sevosus*	SSP-Yellow	Coordinator: Steve Reichling Studbook Keeper: Ruth Marcec-Greaves	CR	EN	**A**	C		E	F
Puerto Rican Crested Toad	*Peltophryne lemur*	ISE	Diane Barber	CR	TH	**A**	C	D	E	F

[1] The only exception to the geographical/political region of the RCP, two Panamanian golden frog species (PGFs) are included on the list of ATAG RCP species, list as the program, founded in 1997, predates the global delineation for species conservation focus by IUCN/ASG/Amphibian Ark established in 2007, and the population was >50 specimens that required elevation to an SSP by WCMC (now AMG) standards at the time.

[2] *Cryptobranchus bishopi* was not a species during either IUCN or AArk Prioritization assessment, so assessed under *C. allegeniensis*.

[3] Since the 2012 AArk Prioritization assessment, *C. bishopi* has been elevated to full specie status, and has now has a government mandate for ex situ management (Category A added respectively).

Priority II Taxa Recommended for *In situ* and *Ex situ* Management

The ATAG is seeking additional Species Contacts to monitor conservation needs of taxa and advise the steering committee on any changes in status or needed support. At this time, it is only advised to work with *ex situ* groups of Priority II taxa for recommended research purposes (see **Appendix I**) and to aid with necessary *in situ* actions. *Ex situ* populations may require isolation if a reintroduction program is implemented.

Table 2: Priority II Taxa listed alphabetically by scientific name (B & D – *In situ* conservation needed along with a recommendation for *ex situ* research since threats CAN be mitigated in time).

Common Name	Scientific Name	Category	Species Contact	IUCN Status	FWS Status	A/B	C	D	E	F
One-Toed Amphiuma	*Amphiuma pholeter*	DERP		NT	NL	**B**		**D**		
Boreal Toad	*Anaxyrus boreas*	DERP	Tom Weaver	LC	UR	**B**		**D**		
Eastern Hellbender	*Cryptobranchus alleganiensis*	DERP	Dale McGinnity	NT	P-EN	**B**		**D**	E	
Pygmy Salamander	*Desmognathus wrighti*	PIP		LC	NL	**B**	C[4]	**D**		
Cook's Robber Frog	*Eleutherodactylus cooki*	DERP	Raphael Joglar	VU	TH	**B**		**D**	E	
Chisholm Trail Salamander	*Eurycea chisholmensis*	DERP		VU	TH	**B**		**D**		
San Marcos Salamander	*Eurycea nana*	DERP	DeAnn Chamberlain	VU	TH	**B**		**D**		
Texas Blind Salamander	*Eurycea rathbuni*	DERP	Nick Hanna	VU	NL	**B**		**D**		
Barton Springs Salamander	*Eurycea sosorum*	DERP	DeAnn Chamberlain	VU	EN	**B**		**D**		
Grotto Salamander	*Eurycea spelaea*	PIP		LC	NL	**B**		**D**		
Georgia Blind Salamander	*Eurycea wallacei*	PIP		VU	NL	**B**		**D**		
Carolina Gopher Frog	*Lithobates capito*	DERP	Robert Hill	NT	NL	**B**		**D**		
Chiricahua Leopard Frog	*Lithobates chiricahuensis*	DERP	Diane Barber	VU	TH	**B**		**D**		
Alabama Waterdog	*Necturus alabamensis*	DERP		EN	EN	**B**		**D**		
Neuse River Waterdog	*Necturus lewisi*	DERP		NT	P-TH	**B**		**D**		
Red Hills Salamander	*Phaeognathus hubrichti*	DERP		EN	TH	**B**		**D**		
Illinois Chorus Frog	*Pseudacris illinoensis*	PIP		NE	UR	**B**		**D**		
Mountain Yellow-Legged Frog	*Rana muscosa*	DERP	Ian Recchio	EN	EN	**B**		**D**		
Sierra Nevada Yellow-legged Frog	*Rana sierrae*	DERP	Jessie Bushell	EN	EN	**B**		**D**		

[4] The **C** role comes from *D. organi* which may be broken out genetically as the northern population of *D. wrighti*.

Western Spadefoot	*Spea hammondii*	PIP		NT	UR	**B**		**D**		
Patch-nosed Salamander	*Urspelerpes brucei*	PIP		LC	NL	**B**	C	**D**		

Priority III Taxa Recommended for *In situ* Focus

The ATAG is seeking additional Species Contacts to monitor conservation needs of taxa and advise the steering committee on any changes in status or needed support. These species DO NOT require collaborative *ex situ* management at this time.

Table 3: Priority III Taxa listed alphabetically by scientific name [B – *In situ* conservation recommended without a recommendation for *ex situ* research (D), or C - *in situ* research recommended along with and ex situ research (D) role; other recommended roles may also apply.]

Common Name	Scientific Name	Category	Species Contact	IUCN Status	FWS Status	A/B	C	D	E	F
Taylor's salamander	*Ambystoma taylori*	DERP	José Alfredo Hernández Díaz	CR	NL	**B**	**C**		**E**	
Arroyo Toad	*Anaxyrus californicus*	ISE	Kim Lovich	EN	EN	**B**				
Yosemite Toad	*Anaxyrus canorus*	ISE	Jessi Bushell	EN	TH	**B**				
Black Toad	*Anaxyrus exsul*	ISE		VU	NL	**B**				
Amargosa toads	*Anaxyrus nelsoni*	ISE		EN	NL	**B**	**C**			
Oak Toad	*Anaxyrus quercicus*	ISE		LC	NL	**B**				
Green salamander	*Aneides aeneus*	DERP	Robert Hill	NT	UR		**C**	**D**	**E**	
Clouded Salamander	*Aneides ferreus*	ISE		NT	NL	**B**				
Black Salamander	*Aneides flavipunctatus*	ISE		NT	NL	**B**				
Sacramento Mountain Salamander	*Aneides hardii*	ISE		LC	NL	**B**	**C**			
Wandering Salamander	*Aneides vagrans*	ISE		NT	NL	**B**				
Giant Palm Salamander	*Bolitoglossa dofleini*	PIP	Ruth Marcec-Greaves	NT	NL		**C**	**D**	**E**	
Seepage Salamander	*Desmognathus aeneus*	ISE		NT	UR	**B**				
Hedrick's Coqui	*Eleutherodactylus hedricki*	ISE	Raphael Joglar	EN	NL	**B**			**E**	
Locust Coqui	*Eleutherodactylus locustus*	ISE	Raphael Joglar	CR	NL	**B**			**E**	
Plains Coqui	*Eleutherodactylus juanariveroi*	ISE	Raphael Joglar	CR	EN	**B**			**E**	
Melodious Coqui	*Eleutherodactylus wightmanae*	ISE	Raphael Joglar	EN	NL	**B**			**E**	
Junaluska Salamander	*Eurycea junaluska*	ISE		VU	NL	**B**				

San Gabriel Springs Salamander	*Eurycea naufragia*	ISE		EN	TH	**B**				
Blanco River Springs Salamander	*Eurycea pterophila*	ISE		DD	NL	**B**				
Comal Blind Salamander	*Eurycea tridentifera*	ISE		VU	UR	**B**				
Berry Cave Salamander	*Gyrinophilus gulolineatus*	ISE		EN	C-EN	**B**				
Tennessee Cave Salamander	*Gyrinophilus palleucus*	ISE		VU	UR	**B**				
Relict Leopard Frog	*Lithobates onca*	ISE		EN	NL	**B**				
Tarahumara Frog	*Lithobates tarahumarae*	ISE		VU	NL	**B**				
Black-Spotted Newt	*Notophthalmus meridionalis*	ISE	Clint Guadiana	EN	UR	**B**				
Striped Newt	*Notophthalmus perstriatus*	Candidate	Cayle Pearson	NT	NL	**B**				
Big Levels Salamander	*Plethodon sherando*	ISE		VU	NL	**B**				
Southern Zigzag Salamander	*Plethodon ventralis*	ISE		LC	NL	**B**				
Webster's Salamander	*Plethodon websteri*	ISE		LC	NL	**B**				
Wehrle's Salamander	*Plethodon wehrlei*	ISE		LC	NL	**B**	**C**			
Weller's Salamander	*Plethodon welleri*	ISE		EN	NL	**B**				
Rana Pata Amarilla (Spanish)	*Rana boylii*	ISE		NT	UR	**B**				
Cascades Frog	*Rana cascadae*	ISE		NT	UR	**B**				

Program Status and Data Summary Tables of Current SSP Programs

Table 4: Program Status Table of Current SSP Species

Program	Date Program Initiated	Current Program Leader	Date Leadership Assumed	Date of Last Studbook Update	Studbook Keeper	Date of last Master Plan Publication
Wyoming Toad SSP	Dec 1996	Val Hornyak	Sept. 2009	Apr 2017	Sarah Armstrong	Jul 2018
Panamanian Golden Frog SSP	Mar 2008	Vicky Poole	Mar 2008	Jan 2019	Kevin Barrett	Jun 2019 (Sora) & Jun 2018 (Ahogado)
Harlequin Golden Frog SSP	Mar 2008	Vicky Poole	Mar 2008	Jan 2017	Kevin Barrett	Jun 2017
Dusky Gopher Frog SSP	Mar 2008	Steve Reichling	Mar 2008	Mar 2017	Ruth Marcec-Greaves	Jan 2018
Houston Toad SSP	Mar 2007	Stan Mays	2016	Jan 2018	Stan Mays	Apr 2019

Table 5: Program Summary Data of Current SSP Species

Common Name *Scientific Name*	Date of Last Breeding & Transfer Plan	Current Population Size (N)	Current Number of Participating Institutions	Sustainability Score (retained %GD at 100 years or 10 generation)	Animal Program Designation	5- year Target Population Size (N)	Space IN USE/ Space NEEDED (*Isolation Rooms**)	Recent 5-year population trend (increasing, decreasing, or stable)	USFWS IUCN CITES
Wyoming Toad *Anaxyrus baxteri*	Jul 2018	454 adults	9	78.8% for 10 generations	Yellow SSP	600	10/2	Stable	EN EW N/A
Panamanian Golden Frog *Atelopus zeteki* (population)	Jun 2019 (Sora) & Jun 2018 (Ahogado)	594 (Sora) & 619 (Ahogado) = 1213	46 (Sora) & 16 (Ahogado)	84.6 (Sora) & 76.0 (Ahogado) for 10 generations	Yellow SSP	900 (Sora) & 600 (Ahogado) = 1500	6/0 (space managed collectively)	Stable	EN CR Apdx I
Harlequin Golden Frog *Atelopus varius*	March 2020 (in draft)	254	9	75.6 for 10 generations	Yellow SSP	400		Increasing	EN CR Apdx I
Dusky Gopher Frog *Lithobates sevosus*	Jan 2018	584	12	71.3% for 10 generations	Yellow SSP	1000	12/0	Decreasing	EN CR N/A
Houston Toad *Anaxyrus houstonensis*	Apr 2019	437	3	76.3% for 10 generations	Yellow SSP	1000	3/1	Increasing	EN EN N/A

*__ISOLATION ROOMS__: Isolation levels are defined and may vary based on each program need but implies isolation from main institutional collection; space needed is estimated for within a 5-year period (goal) to meet current needs for management and is defined as needed to maintain both SSP and release programs.

TABLE 6: Animal Program Summary Table with Primary Roles, Goals, and Essential Action Items *for the Next 1-5 years.* (see *TAG Handbook* - Table 4, p. 47-51 for more information). Please provide the action steps the TAG/SSP is taking, or intends to take, in order to achieve each identified goal, and indicate an approximate timeline for these actions.

Common name (Taxon)	Designation	Primary Role	Goal #1 Essential Action	Goal #2 Essential Action	Goal #3 Essential Action
Wyoming Toad (*Anaxyrus baxteri*)	Yellow SSP	Assurance Population; Breed for Reintroduction	Increase production for release by continuing to improve diets, health and breeding success of managed population; bring 1-2 new facilities on board.	Identify genetics of managed population and look for unrepresented (wild) genes	Become more effective SSP/USFWS Recovery Partners by improving communication and assessing roles of facilities.
Panamanian Golden Frogs (*Atelopus zeteki* & *A. varius*)	Yellow SSP	Assurance	Provide manpower and financial support to EVACC; assist with advisory, grant writing and equipment procurement needs during construction.	SSP will identify specimens and begin the permitting processes to send unrepresented bloodlines to EVACC.	Participate in and financially support the annual Golden Frog Day festivals and expanding EVACC's educational mission regionally in Panama.
Dusky Gopher Frog (*Lithobates sevosus*)	Yellow SSP	Support *In Situ* Recovery	Document natural reproduction in our introduced in situ population.	Expand zoo-based reintroductions to second site.	Secure federal funding to assist with growing the reintroduction and monitoring program.
Houston Toad (*Anaxyrus houstonensis*)	Yellow SSP	Recovery Program/Egg Propagation & Release	Increase egg production for release to 1.5 million eggs per season by 2021.	Increase managed breeding population to at least 700 adult toads; establish new breeding groups at Dallas and USFWS San Marcos Fish Hatchery by 2020.	Secure additional release sites in Bastrop, Leon, Montgomery, and Robertson counties by 2023.

YELLOW SSP Population

Wyoming Toad

Anaxyrus baxteri

Species Summary: The Wyoming toad is a Federally Endangered Species that currently exists in the wild only within a small range in the Laramie Basin of Wyoming. All known populations were created by the reintroduction of produced toads and tadpoles after the species was thought to be extinct, making this the first amphibian conservation program to re-establish a species within historic range through managed breeding efforts. Causes of its decline are uncertain but include habitat change/usage, aerial insecticides, predation, and amphibian chytrid fungus. Created populations are augmented by releases using progeny from the SSP breeding program. Gene diversity in the managed population will never increase unless new wild populations are discovered and collected, as all currently known *wild* populations were created with progeny from the *ex situ* population.

Program Goals & Objectives: The primary role of the SSP in the USFWS Recovery effort is to maintain a physically healthy and genetically diverse assurance population and to produce numbers of offspring for release back into the wild. The SSP also assists the USFWS in Wyoming with field work and research. Recovery partners work together to monitor the reintroduced populations and conduct studies of behavior and habitat use. Insights gained help to develop the most effective husbandry practices for this toad. Spring and summer field surveys are conducted by the USFWS and volunteers, to monitor and assess the wild populations. The annual SSP Master Plan and Husbandry meeting is held in Wyoming. This allows Wyoming Toad IRs and other zoo staff to assist with field work. Participating in surveys is also a great opportunity for Zoos that wish to make a contribution to an important amphibian conservation effort but do not hold Wyoming toads in their collections. For more information please contact Wy toad SSP Coordinator.

Exhibit & Educational Qualities: Due to the small *ex situ* population size and the requirement for ES permit, this program does not offer exhibit toads to non-participating facilities. At breeding facilities, biosecure holding rooms may be designed with viewing windows or display toads requested from the studbook keeper. At any Zoo, videos of field surveys and breeding events may be offered as examples of native species amphibian conservation—contact SSP managers for information.

Interpretive Messages:
Global and regional amphibian declines
Amphibian Diseases
Endangered Species Act protection and coverage
Egg-laying, larval development, and metamorphosis
Zoo and native range conservation

Husbandry: Prior experience breeding and raising similar Bufonids is highly-recommended for facilities wishing to work with Wyoming toads. Within the biosecure room, adult toads may be maintained in aquariums or similar enclosures that are approximately 3:1 land area/water. Flow through or circulating systems may be used, with a carbon filter recommended. A 20 long tank can house 5 adult toads. Breeding tanks, tadpole rearing, and metamorph set-ups are similar to those for other Bufonids. Lights for basking and UV must be provided. A dedicated refrigeration unit for hibernation within the biosecure holding room is necessary for cycling toads recommended for reproduction. Scheduled breeding is done during a specified window of time, using hormone injections according to SSP protocol. Offspring are shipped to Wyoming for release or held back for the *ex situ* population. Nutritional needs for this species are still being determined. Commonly available feeder insects such as crickets, earthworms, wax worms, roaches and others are offered but do not replicate wild diet adequately; extra supplementation is necessary, especially with Vitamin A.

Other Notes: All toads are the property of the USFWS. More zoos and aquariums with amphibian management and breeding experience are needed to expand the Wyoming toad assurance population. Per the USFWS Recovery Plan, production needs to be increased as more Safe Harbor release sites are now available to establish additional populations. Criteria for SSP participation: 1.) DEDICATED BIOSECURE HOUSING AND QUARANTINE AREAS – Isolation from all other amphibians in both quarantine and permanent holding is required in this breeding-for-release program; 2.) SPACE COMMITMENT – Participants must be able to hold at least four separate cohort groups (20 – 40 toads), be able to breed several pairs of toads annually and provide adequate space for breeding transfers and tadpole holding prior to release; 3.) USFWS ENDANGERED SPECIES PERMIT is required to hold the federally endangered Wyoming Toad, and permits are only issued to breeding facilities; 4.) ACTIVE PARTICIPATION in conference calls/listserv discussions and IR representation at annual Masterplan and Husbandry sessions is important to make informed pairings and group decisions. This is a very interactive program that requires involvement at the keeper level to address husbandry issues. Participants must follow guidelines and protocols of the USFWS and the SSP developed for this program, answer requests for information, and supply updates to Studbook Keeper, Species Coordinator, SSP Pathologist and USFWS in a timely way.

Program Leader Contacts:

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Primary Roles, Goals, and Essential Action items for the next year.

Common name (Taxon)	Designation	Primary Role	Goal #1 Essential Action	Goal #2 Essential Action	Goal #3 Essential Action
Wyoming Toad (*Anaxyrus baxteri*)	Yellow SSP	Conservation	All breeding partners reach an agreement concerning compliance of existing SOPs by June 2020 breeding season in order to participate.	Follow up tracking of released toads to investigate effectiveness of current release strategies and life stages	Change breeding timing to better match releases to wild toads' timetable.

YELLOW SSP Population

Panamanian Golden Frogs

Atelopus zeteki & Atelopus varius

Species Summary: Brilliantly-colored Panamanian golden frogs (PGF) are striking exhibit specimens, serving as a flagship species for global amphibian declines due to the impact on populations from amphibian chytridiomycosis (*Batrachochytrium dendrobatidis; Bd*). PGFs originate from tropical montane cloud and drier, lowland forests, with breeding and larval development taking place in forest streams. True Panamanian golden frog, *Atelopus zeteki*, was recognized as a distinct species from the very similar-looking golden harlequin frog, *Atelopus varius*, based on a unique skin toxin, zetekitoxin, and bioacoustical differences. In addition to vocalizing, PGFs communicate by semaphoring, a limb-waving phenomenon that is hypothesized to have arisen so that the frogs could locate mates for breeding near the deafening sounds of waterfalls, where their gentle vocalizations are inaudible.

Program Goals & Objectives: *A. zeteki*, and *A. varius*, are both listed as Critically Endangered (CR) by IUCN, CITES Appendix I, and FWS Endangered since the mid-1970s. Unfortunately, *A. zeteki* has not been observed since 2009 and is most likely Extinct in the Wild (EW), while *A. varius* still persists in small isolated populations. The SSP population collected in advance of the *Bd* front in the early 2000's and brought to AZA institutions serve as assurance colonies and flagship exhibit species. In 2018, *Foundation EVACC*, the SSP partner institution within Panama, relocated the El Valle Amphibian Conservation Center (EVACC) which serves as the in-country breeding facility for PGFs as well as other critically endangered Panamanian amphibians. The SSP is now beginning permitting to repatriate unique PGF bloodlines back to Panama to expand upon the genetics of common populations. Research projects on amphibian disease mitigation and general PGF husbandry and health continues to be actively supported by the SSP. Raising awareness and supporting stewardship of this culturally significant yet highly coveted species through expanded educational outreach within Panama will be necessary to safeguard their survival post-release and remains a focus of the SSP and in-country partners.

Exhibit Qualities: These diurnal species are highly attractive, although will hide among available micro-habitats created by live plants, small boulders, waterfalls, and perching so a group of specimens are recommended for exhibit to increase opportunities for visitor visibility. PGFs will utilize the entire available exhibit space. Compatible species include other Panamanian dart frog, tree frog, and toad species.

Educational Qualities: PGFs will *not* tolerate handling to make them suitable for outreach programs unless only temporarily displayed within small terrariums. This is a good species to illustrate warning coloration, skin toxins, and semaphoring behaviors. PGFs also fit well into programs about tropical rain/cloud forests, biodiversity, wildlife diseases, amphibian declines, and deforestation/habitat loss. An annual *Golden Frog Day* celebration and festival occurs in El Valle de Anton, Panama on August 14, offering opportunities for events within AZA facilities; suggested activities available from the SSP Educational Advisor.

Interpretive Messages:
Global amphibian declines
Extinction
Skin toxins & semaphoring
Egg-laying, larval development, and metamorphosis
Zoo and range-country conservation
Culturally significant species

Care & Facilities: Golden frogs prefer naturalistic exhibits modeled after rocky streambeds of the frogs' native habitat. Minimum space allotted for a pair of adult *Atelopus* should be no less than a 15-gallon aquarium to provide sufficient micro-habitats, although up to a total of 6 non-breeding adult frogs could be housed in a 15-gallon tank. Mature males may need to be separated due to territorial behaviors during breeding seasons, and off-exhibit housing should be available should the need arise to separate out exhibit animals. Ambient air temperatures should be cool, varying between 68-75°F; water temperature should be between 69-72°F. Provide full-spectrum lighting (UV A & B) on a 12:12 lighting cycle year-round, and offer a basking spot light (60-100W) as PGFs bask naturally. Humidity levels of 85-100% can be maintained with automated misting or fogger systems, which also stimulated breeding activity. PGFs require very good water quality, so institutions may have to consider filtration of source and enclosure water. These frogs are insectivores, readily consuming flightless fruit flies, small crickets, flour beetles, termites, and springtails, and every feeding should have a vitamin-mineral supplement. A detailed husbandry manual is available for downloading from the *Project Golden Frog/Proyecto Rana Dorada* website: **www.proyectoranadorada.org**; additionally, a PowerPoint slideshow of example exhibits is available from the Program Leader.

Other Notes: PGFs within AZA facilities are owned by the *Maryland Zoo in Baltimore* (MZB), and arrangements for transfers must go through the Population Manager at MZB. The managed population of *A. zeteki* within AZA institutions represents two distinct localities (Sora & Ahogado). Along with the single golden *A. varius* population, all three populations of PGFs are managed uniquely (i.e. not housed, nor cross bred). The SSP is seeking facilities willing to maintain these species in biosecure facilities and to maintain clean bloodlines for eventual repatriation to Panama partners. Institutions new to the SSP are given a non-valuable bloodline initially to become acquainted with the husbandry of Atelopus before breeding recommendations are considered. Humane euthanasia at all life stages is a necessary and approved method of population management, cleared by the USFWS for this federally endangered species.

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Primary Roles, Goals, and Essential Action items for the next year.

Common name (Taxon)	Designation	Primary Role	Goal #1 Essential Action	Goal #2 Essential Action	Goal #3 Essential Action
Panamanian Golden Frogs (*Atelopus zeteki* & *A. varius*)	Yellow SSP	Conservation	Provide manpower and financial support to EVACC; assist with advisory, grant writing and equipment procurement needs during construction.	SSP will identify specimens and begin the permitting processes to send unrepresented bloodlines to EVACC.	Participate in and financially support the annual Golden Frog Day festivals and have SSP Education Advisor visit El Valle to plan and implement classroom programs within region

YELLOW SSP Population

Dusky Gopher Frog

Lithobates sevosus

Dusky Gopher Frog

Species Summary: As the only endemic anuran of the longleaf pine ecosystem, the dusky gopher frog (DGF), *Lithobates sevosus,* is uniquely capable of advocating for this vanishing and strikingly unique American landscape. As such, it is a species that should not be absent from any southeastern zoo. As the most critically endangered anuran in the eastern U.S., AZA facilities that seek to conserve amphibians should consider SSP participation.

Program Purposes: 100-200 DGFs exist in the wild. Their remaining, highly-degraded, habitat is threatened by a surge in residential development, and the relict *in situ* population has been infected with a new and poorly understood disease, (*Dermomycoides*) for which there is no cure. The overriding purpose of this SSP is to serve as a tool in the recovery of the species under the direction of the U.S. Fish & Wildlife Service. Secondary to this is to maintain an assurance colony as a guarantee against complete extinction; long-term survival of the single wild population is quite uncertain. The Dusky Gopher Frog SSP is a formal partner with the US Fish and Wildlife Service, with representatives serving on the Recovery Group. The Recovery Plan for DGFs includes the reintroduction of *ex situ*-propagated frogs into the historical range, and this activity was initiated in 2017 with the release of ~100 zoo-bred froglets onto Ward Bayou WMA in southern Mississippi. To date (2019) over 1100 froglets have been released. Our partners (USFWS, U.S. Forest Service, Army Corps of Engineers, Mississippi Dept of Wildlife & Fisheries, and The Nature Conservancy) have designated Ward Bayou as a dedicated site to the establishment of a new, self-sustaining population of dusky gopher frogs using zoo-bred specimens as the propagules. Additionally, managed specimens are used to raise public awareness of the status of this species and the plight of the longleaf piney woods.

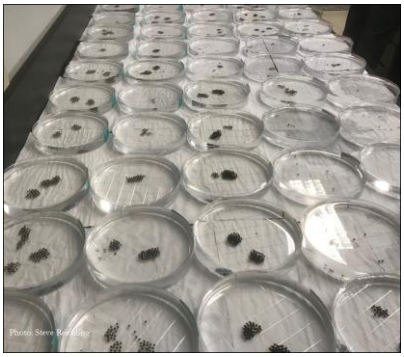
Photo: Steve Reichling

Exhibit Qualities: Successful exhibits are those that provide for the species' fossorial nature while exploiting its propensity to bask. DGFs will spend most of the daylight hours outside but near the entrance to their burrow, or just inside the entrance where they can be readily seen. Their burrow fidelity provides an advantage in that the location of the frogs can be reliably predicted and the exhibit designed around these areas. Ultraviolet basking spots are essential, as is careful monitoring of substrate moisture, keeping it neither too moist nor too dry. For those amphibian specialists who are ready for a species that requires more than a beginner level terrarium environment – one that responds to the work of an advanced keeper – *L. sevosus* is an ideal subject.

Educational Qualities: The species is especially valuable to southeastern zoos within the historic footprint of the longleaf pine belt, serving as an alarming example of forest ecosystem mismanagement.

Interpretive Messages:
Ecology of the longleaf piney woods
Management of longleaf pine ecosystems (prescribed burning, eco-friendly silviculture)
Amphibian diseases
Meta-population dynamics and its role in amphibian conservation

Care & Facilities: The DGF is to be housed in permanent isolation. A variety of enclosures can be used; key elements are size, substrate, drainage, ventilation, and furnishings. Roughly 2 X 2.5 feet floor space for 4-8 adults and 1 X 1 foot area for up to a dozen newly-metamorphed froglets provides general species' space requirements. Mosses and soils is recommended as substrate to a depth greater than 6 cm to afford the opportunity to bury; gravel and sand should be avoided due to potential health risks from ingestion. Substrate concerns can be avoided by using rubber mats, with clumps of damp sphagnum provided. Cage floors should be perforated to allow for thorough drainage of substrate. To provide good ventilation, enclosure top should be screened to permit the daily drying of substrate as these frogs live in relatively open and generally xeric habitat Gopher frogs need artificial burrows which can be created out of cork bark or broken clay pots. Since these frogs naturally bask just outside their burrows, UV light is beneficial. Standard temperate-zone photoperiod seasonal adjustments keep gopher frogs in normal reproductive cycles. Temperatures of 80-85F are ideal, though frogs remaining active and feed as low as the mid-60Fs. They have been artificially hibernated successfully at temperatures ranging from 50-60F. Enclosures should be sprayed with water once daily to saturate the substrate and allowed to surface-dry, inhibiting bacteria and mimicking the natural xeric microenvironment of this species. A shallow water dish should always be available. Frogs do well on a diet of crickets, however they will take wax worms, mealworms, chopped earthworms, and wild-caught insects; frogs at all sizes are fed 2-3 times per week. Nutritional supplementation is critical; crickets should be gut-loaded with and all prey items should be dusted with vitamins just prior to being offered.



Other Notes: Managed reproduction is accomplished solely through species-specific IVF techniques. A highly detailed manual for conducting this activity has been prepared and is available to facilities requesting guidance. The situation for *L. sevosus* in the wild is critical, with a high probability of extinction with one event such as a wildfire, hurricane, reproductive hiatus due to drought, or a recurrence of widespread disease due to the *Dermomycoides* organism. AZA facilities must steward SSP holdings as a demographically and genetically robust population, and this can only be accomplished with enthusiastic support from many institutions.

Program Leader Contacts:

Steve Reichling, SSP Coordinator, Memphis Zoo
sreichling@memphiszoo.org
(901) 333-6711

Ruth Marcec-Greaves, DVM, PhD, Vice Coordinator
Detroit Zoological Society rmarcec@dzs.org; (248) 336-5824

Primary Roles, Goals, and Essential Action items for the next year.

Common name (Taxon)	Designation	Primary Role	Goal #1 Essential Action	Goal #2 Essential Action	Goal #3 Essential Action
Dusky Gopher Frog (*Lithobates sevosus*)	Yellow SSP	Conservation	Obtain funding for monitoring of reintroduced population at Ward Bayou WMA (MS)	Resume the SSP genotyping study once USFWS permit questions are resolved.	Accomplish the first natural breeding of managed frogs

YELLOW SSP Population

Houston Toad

Anaxyrus houstonensis

Species Summary: This small brownish, fossorial toad species may seem unremarkable as a display animal for many zoos, yet this toad is a flagship species for U.S. amphibian conservation due to climactic and anthropogenic causes. The Houston toad is geographically restricted to eight counties in Texas, and is further restricted by habitat type, favoring deep sandy soils with prevalent canopy cover. The Houston toad was the first amphibian listed as an Endangered Species by the USFWS in 1970. The main threats to extinction are habitat destruction and fragmentation by humans and natural events such as periodic severe droughts and wildfires.

Program Goals & Objectives: Labeled as Endangered (EN) by both the IUCN and the U.S. Fish and Wildlife Service, Houston toads have experienced significant range reduction. Although once common in the Houston area, urbanization and habitat destruction eliminated the toad throughout Houston in 1975. Populations are now thought to be in only eight counties in Texas with the largest population in Bastrop County. The Houston Zoo first became involved in the recovery program for this animal in 1981. and partners with the USFWS, Texas Parks and Wildlife, Texas State University, as well as Fort Worth and Dallas Zoos. Severe drought and wildfires in 2010-2011 destroyed much of the toad's remaining habitat and almost entirely wiped out the species. The Houston Zoo's assurance colony produces as many eggs as possible for release into native habitat with a goal of producing up to 1,000,000 eggs/year for release. Egg release efforts have concentrated on restoring the population on the Griffith League Ranch, which is owned by the Boy Scouts of America and have resulted in the re-establishment of wild toad populations. Houston toads are no longer managed as three distinct geographic sub populations, but as one range-wide population. Continued efforts in field surveying have resulted in the confirmation of toad populations in Robertson, Leon, and Austin Counties. In addition to the goals listed below, there are other objectives that have been established for Houston toads. Plans are to continue research into *Chlamydophila* and *Mycobacteria* infections occurring in *ex situ* populations as well as sampling of wild amphibian populations for these pathogens, and to develop effective screening methods for managed populations.

Exhibit Qualities: Due to the fossorial and cryptic nature of the Houston toad, this species can be challenging, but not impossible, to exhibit. However, it is a great outreach and educational animal due to its ability to be mildly handled or displayed in a temporary terrarium.

Educational Qualities: Tolerate mild, moderate handling which makes them suitable for outreach programs, though only with the use of small terrariums. This is a good species to illustrate cryptic coloration and camouflage, fossorial adaptation, and a conservation message which focuses on habitat destruction and fragmentation.

Photo: Houston Zoo

Interpretive Messages:
Global and regional amphibian declines
Extinction
Endangered Species Act protection and coverage
Egg-laying, larval development, and metamorphosis
Zoo and native range conservation

Care & Facilities: Although the Houston toad can be a temperamental species to house due to its specific microhabitat needs, most AZA institutions have the facilities and expertise necessary to meet the husbandry requirements of this species. They do well when housed in either a naturalistic exhibit kept on a sand or sandy/loamy soil mix, or within a reserve enclosure with a false bottom that allows for substrate to be added to one end and water to be held and utilized at the other. Native range substrates and plants can also be used. Animals should be housed in no less than a 10-gallon tank per 1 adult individual toad and no more than 2 to 3 similar sized adult individuals per 20-gallon long tank. Toads should be given access to water every day or sufficiently misted to mitigate dehydration. Temperatures for toads housed indoors should be kept between mid-60's to upper 70's. Lighting should consist of full spectrum fluorescent light bulbs along with UVA and UVB halogen light bulbs. Reconstituted reverse osmosis water should be utilized. Houston toads will readily take crickets, along with mealworm and flour beetles, wax worms, and isopods dusted with a vitamin-mineral supplement.

Other Notes: All Houston toads are owned by the U.S. Fish and Wildlife Service; consequently, all transfers and releases must be approved by USFWS prior to the transaction. All *ex situ A. houstonensis* are managed as one single population, so breeding crosses from strands originating from different locations are recommended by the PMC. Humane euthanasia at all life stages is an approved method of population management, with prior approval by the USFWS. All toads used for the reintroduction program are to be housed in isolation.

Program Leader Contacts:

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713-533-6527

Diane Barber, Vice Coordinator, Fort Worth Zoo
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817-759-7160

Primary Roles, Goals, and Essential Action items for the next year.

Common name (Taxon)	Designation	Primary Role	Goal #1 Essential Action	Goal #2 Essential Action	Goal #3 Essential Action
Houston Toad (*Anaxyrus houstonensis*)	Yellow SSP	Conservation	Grow assurance colony to include Dallas Zoo	Continue reintroductions of *ex situ*-produced eggs into Bastrop State Park and Griffith League Ranch; goal is 1 million eggs/season.	Establish new area for egg release at Cooks Branch Conservancy, Montgomery County, TX

Recommendation Update Table

The Recommendations Update Table is included to provide an overview of ATAG progress from the previous RCP (2014).

Table 7: Changes from the Last RCP listed in order of former ranking[5]

Taxa listed alphabetically by scientific name.

Common Name	Scientific Name	Former Category	New Category	Species Contact	Reason for change from last RCP
Reticulated Flatwoods salamander	*Ambystoma bishopi*	PIP	DERP	Dante Fenolio	Currently managed at one AZA institution for USFWS rescue effort
California Tiger Salamander	*Ambystoma californiense*	PIP	NC		Lack of government support for *ex situ* efforts
Taylor's salamander	*Ambystoma taylori*	NC (new)	DERP	José Alfredo Hernández Díaz	Attainable salamander with conservation value evaluated for inclusion to expand husbandry knowledge. Currently managed at one AZA institution.
Houston Toad	*Anaxyrus houstonensis*	SSP-Green	SSP-Yellow	Coordinator/Studbook: Stan Mays	Gene diversity dropped below 90% over 10 generations.
Green salamander	*Aneides aeneus*	NC (new)	DERP	Robert Hill	Attainable salamander with conservation value evaluated for inclusion to expand husbandry knowledge. Currently managed at two AZA institutions
Giant Palm Salamander	*Bolitoglossa dofleini*	NC (new)	PIP	Ruth Marcec-Greaves	Attainable salamander with conservation value evaluated for inclusion to expand husbandry knowledge.
Ozark Hellbender	*Cryptobranchus bishopi*	DERP	Candidate	Mark Wanner	Due to taxonomy change, federal listing, and government mandate or *ex situ* management, elevated to Priority 1 species
Elegant Coqui	*Eleutherodactylus eneidae*	PIP	NC	Raphael Joglar	Believed to be EW (FWS Caribbean Field Office)
Golden Coqui	*Eleutherodactylus jasperi*	PIP	NC	Raphael Joglar	Believed to be EW (FWS Caribbean Field Office)
Stream Coqui	*Eleutherodactylus karlschmidti*	PIP	NC	Raphael Joglar	Believed to be EW (FWS Caribbean Field Office)
Cascade Caverns Salamander	*Eurycea latitans*	ISE	NC		Genetics are still in debate
Texas Salamander	*Eurycea neotenes*	PIP	NC		Genetics are still in debate
Blanco Blind Salamander	*Eurycea robusta*	DERP	NC		*Ex situ* population not likely
Jollyville Plateau Salamander	*Eurycea tonkawae*	DERP	NC	DeAnn Chamberlain	*Ex situ* population not likely
Eurycea Troglodytes complex	*Eurycea troglodytes*	ISE	NC		Genetics are still in debate
Oklahoma Salamander	*Eurycea tynerensis*	ISE	NC		Genetics are still in debate
Austin Blind Salamander	*Eurycea waterlooensis*	ISE	NC	DeAnn Chamberlain	*Ex situ* population not likely
Crawfish Frog	*Lithobates areolatus*	PIP	NC	Michael Lannoo	*Ex situ* head-starting attempts were made, but program lacked agency support to protect habitat thus no reintroduction possible
Carolina Gopher Frog	*Lithobates capito*	PIP	DERP	Robert Hill	Currently managed at two AZA institutions head-starting for reintroduction

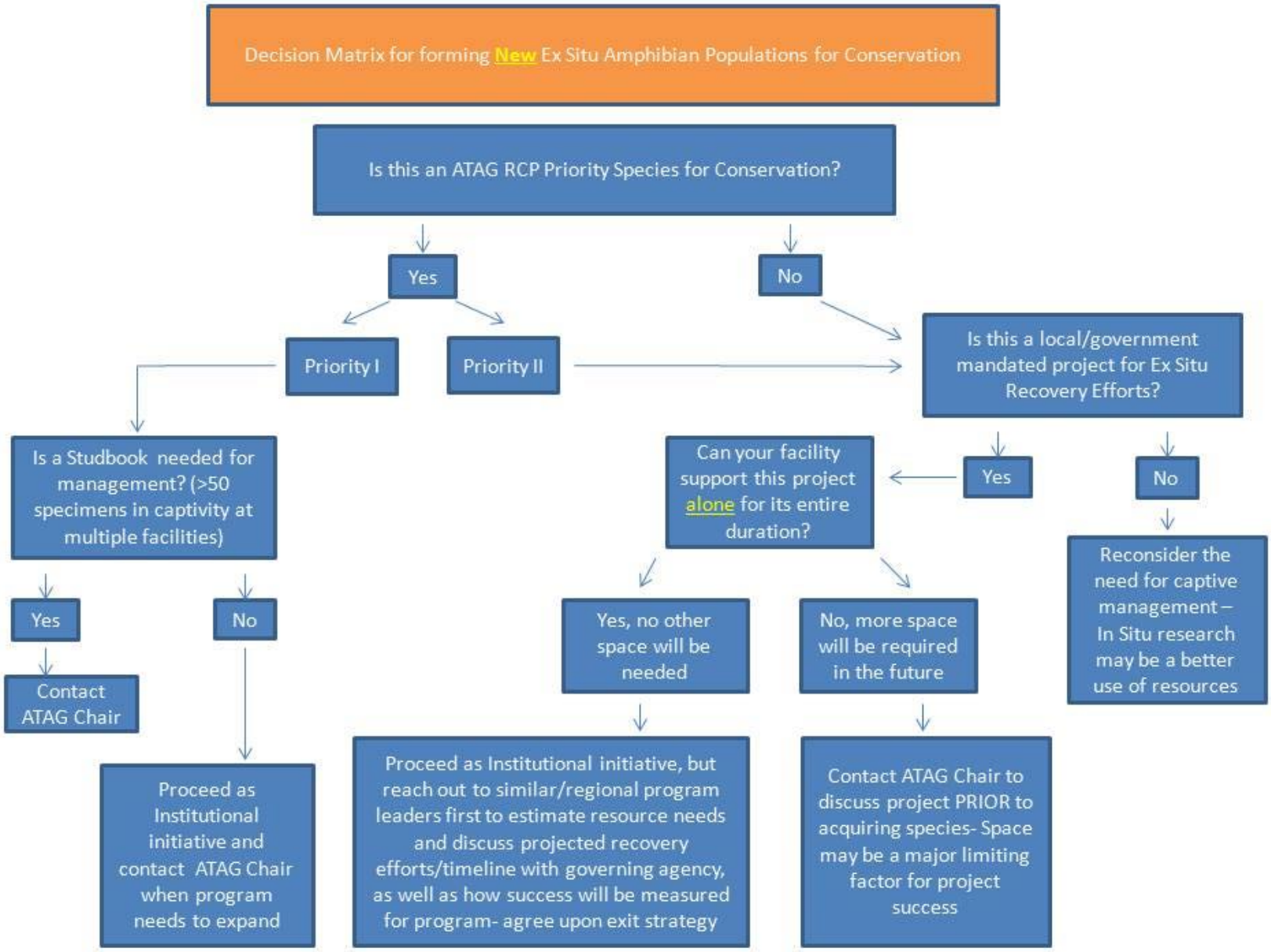
[5] For additional information regarding specific species assessments referenced herein, please see the SUMMARY TABLES listed in **Appendix I.**

Striped Newt	*Notophthalmus perstriatus*	ISE	Candidate	Cayle Pearson	Currently managed at two AZA institutions for reintroduction
Puerto Rican Crested Toad	*Peltophryne lemur*	SSP - Green	ISE	Diane Barber	SSP status is no longer needed for this successful program. Although continued participation from primary AZA partners for reintroduction efforts is imperative, transition to a Puerto Rican-based program with NGO partners enables greater flexibility for recovery efforts.

Considerations for New Studbooks and Managed *Ex situ* Programs

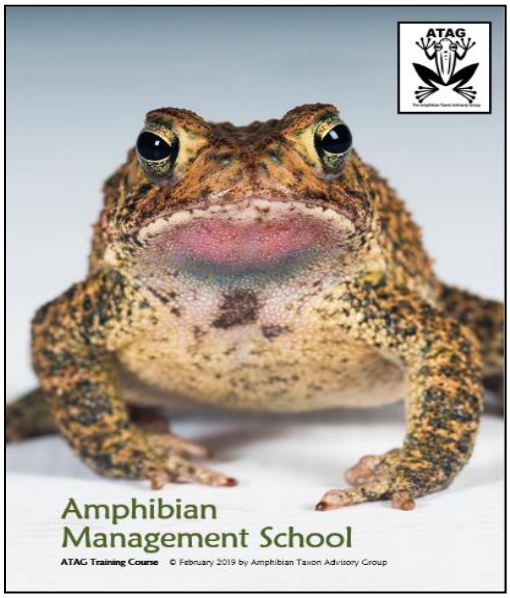
Studbooks are an important tool used to track populations of amphibians for genetic and management purposes. Before starting a studbook, it is recommended to contact the ATAG Chair. The Chair can discuss options and make appropriate suggestions for next steps.

Before collecting new species from the wild for *ex situ* management or joining local/regional non-AZA initiatives for *ex situ* programs, discuss resources required, level of commitment needed, short and long-term goals, and exit strategies with partners. Isolation space is extremely limited for long-term collaborative programs and discussions with the ATAG Steering Committee are highly recommended before proceeding. A decision matrix is included on the following page and may be helpful when making considerations for new amphibian program development.


Decision Matrix for forming New Ex Situ Amphibian Populations for Conservation
Is this an ATAG RCP Priority Species for Conservation?
Yes
No
Priority I
Priority II
Is this a local/government mandated project for Ex Situ Recovery Efforts?
Yes
No
Can your facility support this project alone for its entire duration?
Reconsider the need for captive management – In Situ research may be a better use of resources
Is a Studbook needed for management? (>50 specimens in captivity at multiple facilities)
Yes
No
Contact ATAG Chair
Proceed as Institutional initiative and contact ATAG Chair when program needs to expand
Yes, no other space will be needed
No, more space will be required in the future
Proceed as Institutional initiative, but reach out to similar/regional program leaders first to estimate resource needs and discuss projected recovery efforts/timeline with governing agency, as well as how success will be measured for program- agree upon exit strategy
Contact ATAG Chair to discuss project PRIOR to acquiring species- Space may be a major limiting factor for project success

ATAG RESOURCES

Professional Development for Amphibian Keepers and Researchers



Initially created to expand the overall capacity to successfully house *ex situ* frogs, salamanders, and caecilians within zoos, aquariums, and related facilities, the AZA Board of Regents approved the *Amphibian Biology and Management* professional development program first hosted by the Detroit Zoological Institute in 2003. This entry-level amphibian husbandry, health, interpretation, and field technique class continued to be offered once or twice annually at either Detroit or Toledo zoos through 2012. As global declines of amphibian species became more imperative, the course evolved to include relevant subjects such as amphibian diseases, assurance conservation programs, population management, and data entry to provide for the changing needs of amphibian caregivers.

Beginning in 2013, the ATAG took over the management of the training course, and due to the vastly varied skillsets and interests of participants restructured the annual basic Amphibian Management School course (AMS; now hosted by the Detroit Zoological Society) and began alternating annually with an advanced amphibian subject workshop led by instructors who are experts in their fields. To date, the advances subjects have been as follows:

2014 Hellbender Husbandry Workshop (hosted at the Saint Louis Zoo)

2016 Assisted Reproductive Technologies (ART) Workshop (hosted at Omaha's Henry Doorly Zoo)

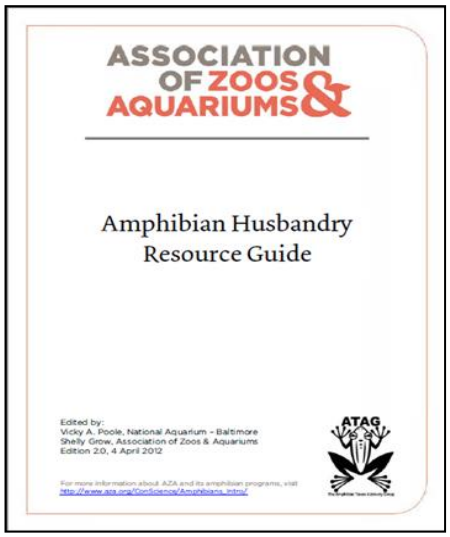
2018 Amphibian Field Research Course (hosted at the North Carolina Zoo)

Attendees of the ATAG 2016 Assisted Reproductive Technologies (ART) Workshop with the fertilized eggs produced from five species of amphibians (Photo: Omaha Zoo)

The ATAG wishes to thank the continued support of the following institutions who financially support dedicated scholarships for amphibian courses. Since 2008, the Cameron Park Zoo has been sponsoring the *Amphibian Conservation Scholarship* through Zoo Conservation Outreach Group (ZCOG) for an employee or associate of Latin American zoological institutions or related facilities to attend AMS. Similarly, the Detroit Zoological Society sponsors a student from the United States or Canada to attend AMS through their *Marcy Sieggreen Professional Development Scholarship*. Due to our continued partnership with AZA, the ATAG courses will also accept the winner of any of the flexible AZA Professional Development Scholarships as well.

Advertisements for future courses and available scholarships are shared via AZA community platforms and professional listservs. Institutions are encouraged to budget to send their amphibian husbandry staff to ATAG courses as they are offered.

Amphibian Husbandry



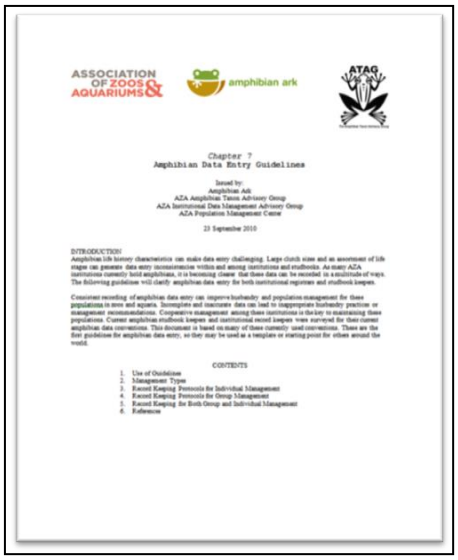
Zoo amphibian collections have evolved from mere filler-specimens within the exhibits of larger reptile houses to dedicated facilities and conservation collections, and the skills and tools to maintain these animals have also expanded dramatically. As basic husbandry of amphibians varies widely based on taxa, natural history, life-stage, and habitat, in 2012 the ATAG produced the second edition of the *Amphibian Husbandry Resource Guide* (Poole and Grow, 2012) to offer basic recommendations and resources for the management of amphibians for purposes including conservation programs, exhibitry, education/outreach, and the field. Information on basic but critically important aspects to keeping amphibians is offered, including enclosures, water (sources and quality), environmental parameters (light, temperature, and humidity), food, natural history and behavior, and veterinary care (Pramuk and Gagliardo, 2012). Where possible, materials and suggested suppliers are listed, and in some cases, alternatives are offered for items that may not be available in all areas. Due to the limited available information on successful reproduction of majority of amphibian taxa, communication with others who have worked with that species (or closely related species or genera) in *ex situ* situations is strongly encouraged, and new methods/experiences should be shared with colleagues, preferably in peer-reviewed literature. With the expansion of amphibian conservation programs primarily for release into the wild, the extensive chapter on Assisted Reproduction Technologies (ART) explains in great detail the theories and practicalities of utilizing hormones to stimulate ovulation and spermiation in amphibians, natural *vs. in-vitro* breeding, and offers resources and contacts to improve overall production of specimens for programs (Kouba, et al., 2012).

The first edition of the husbandry manual, *Guía para el Manejo de Anfibios en Cautiverio* (2009), was also translated into Spanish by Luis Carrillo for use with colleagues in Latin America.

These helpful husbandry manuals and program information are available on the ATAG website: **saveamphibians.org**

Amphibian Disease Management

The need for disease management of amphibians in field and *ex situ* settings began with the onset of local declines and malformations described in the 1990s, and sanitary techniques evolved as new diseases and syndromes were described at a regional level. At present, research efforts into amphibian diseases are global and timely, with standardized field, husbandry, and collection management practices that address prevalent diseases, such as the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; *B.d.*) and *ranavirus*, have become common place by zoo/aquarium personnel worldwide. In a zoo situation with display animals from different geographic locations (i.e., a cosmopolitan collection), biosecurity is applied to prevent pathogens from coming into the collection, transferring among amphibians in the collection, or moving outside the zoo into the native amphibian populations. For reintroduction programs, this concept similarly embraces all directions of disease transfer where pathogens should not move into, among, or out of assurance colonies. Biosecurity levels for each *ex situ* species or species assemblage is dependent on the ultimate goal of the program and the risk that wild-collected animals pose to the existing collection and native wildlife. It is possible to achieve a realistic level of biosecurity in *ex situ* amphibian populations by following some simple and inexpensive protocols, including considerations for housing (e.g., permanent isolation for animals intended for reintroduction), equipment, water treatment, and staff procedures. While caring for amphibians, use of proper equipment is just as important as employing proper housing types when it comes to hygiene and disease management. Equipment such as tools, gloves, footwear, and clothing should be designated for use on a room-by-room or tank-by-tank basis depending on the desired level of biosecurity.


ASSOCIATION OF ZOOS & AQUARIUMS
amphibian ark
ATAG

Chapter 7
Amphibian Data Entry Guidelines

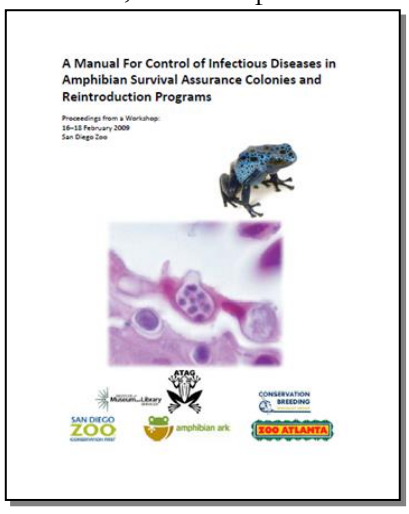
Issued by:
Amphibian Ark
AZA Amphibian Taxon Advisory Group
AZA Institutional Data Management Advisory Group
AZA Population Management Center

23 September 2010

INTRODUCTION

CONTENTS

1. Use of Guidelines
2. Management Types
3. Record Keeping Protocols for Individual Management
4. Record Keeping Protocols for Group Management
5. Record Keeping for Both Group and Individual Management
6. References



Infectious disease management guidelines evaluated by animal health and the scientific community are available and the ATAG recommends the standards established and outlined by Pessier and Mendelson (2010), especially for amphibians utilized in release programs. Additional information on amphibian disease management in the field and zoological facilities is available in the *Amphibian Husbandry Resource Guide* (Poole and Grow, 2012).

Amphibian Population Management & Data Entry Guidelines

With the global AArk efforts, standards were established with the support of the Population Management Center for amphibian population management, along with data entry guidelines to minimize conflicts between programs (Schad, 2008; Schad, 2010). ATAG follows/supports these recommendations.

Amphibian Assisted Reproductive Technologies and ATAG National Amphibian Genome Cryobank Project

Photo: Fort Worth Zoo

Ex situ reproduction of amphibians is often challenging, depending on the species. Amphibians are extremely sensitive to various changes in the environment that trigger, or halt, reproductive cycling. The primary goal of managed breeding is to allow natural reproduction in simulated environments that replicates nature, but with many species of amphibians on the brink of extinction there may not be the luxury of time to determine specific environmental reproductive needs. In these cases, assisted reproductive technologies (ART) may be employed to help increase numbers, promote genetic sustainability of a species and provide animals for reintroduction purposes. ART may involve methods including ultrasound analysis of gonads, hormone use to induce breeding behavior or collect gametes, *in-vitro* fertilization or artificial fertilization and cryopreservation of sperm and eggs. ART has been used with amphibians for decades and is considered a standard operating procedure for assisted breeding of many program species (e.g. Wyoming toads, Houston toads, boreal toads, Panamanian golden frogs, dusky gopher frogs, Puerto Rican crested toads, etc.). Cryopreservation of sperm is of value for sustainability of managed populations, as sperm from geriatric or deceased animals can be stored indefinitely to promote gene diversity. Cryotechnology has been used in multiple amphibian species to produce offspring and is a promising tool for propagation and sustainability of genetics in future planning of amphibian conservation (e.g. genes from a deceased male Puerto Rican crested toad have already been reintroduced to the managed population). Moreover, genetics from wild animals can be collected in the field, frozen and later reintroduced into the *ex situ* population to produce new animals, thereby diversifying the founder base. This technology to link *in-situ* and *ex-situ* populations is valuable for sustainability of the gene pool, without needing to reduce wild populations. The Amphibian TAG, in partnership with Dr. Andy Kouba (Mississippi State University), is collaborating in testing of various ART technologies for the conservation of threatened amphibian species and creation of the ATAG National Amphibian Genome Cryobank.

ATAG Grant Program

The ATAG recognizes the importance of seed monies for small conservation and research projects focusing on amphibians. Due to fundraising efforts the ATAG has been able to offer grants up to $1000 for one or more projects since 2006. To date, the ATAG has awarded over $33,000 for a variety of programs worldwide. Winning projects are selected by the ATAG Steering Committee at the annual board meeting and are announced during the annual ATAG meeting. Recent winners include:

2019 Dr. Bryan Windmiller, Zoo New England: "Education and Outreach through Marbled Salamander Headstarting." Awarded $1,000.

Edgardo Griffith, EVACC Foundation: "Outfitting a new captive breeding facility for Critically Endangered Panamanian Amphibians." Awarded $1,000.

Debra Miller, DVM, PhD. & Rebecca Hardman, DVM, PhD., University of Tennessee: "Efficacy of Terbinafine Implants to Provide Antifungal Treatment and Increase Survival in Hellbenders (*Cryptobranchus alleganiensis*)." Awarded $1,000.

2018 Margaret Rousser, Conservation Manager, Conservation Society of California/Oakland Zoo: "Headstarting and Reintroduction of *Rana muscosa* to Sequoia and Kings Canyon National Parks." Awarded $2,000

2017 Kristin Hinkson, Research Technician, Memphis Zoo: "Genetic Comparisons between Captive and Natural Dusky Gopher Frog (*Lithobates sevosus*) Populations." Awarded $1000.00

Lacy Rucker, Graduate Research Assistant (Ph.D. Student), West Virginia University: "The Impacts of Climate Change on the Growth, Survival, and Competition of Terrestrial Salamanders in Central Appalachia." Awarded $1000.00

2016 Susan Lyndaker Lindsey, Ph.D., Mesker Park Zoo and Botanic Garden: "Indiana's first captive breeding program for the Eastern hellbender." Awarded $1000

Stephen Nelson, Zoo Knoxville: "Description of a new species of waterdog (*Necturus* sp.) from Tennessee." Awarded $1000

2015 Kiley Buggeln, Hutchinson Zoo: "Dusky Gopher Frog Initiative." Awarded $2,000

Gustavo Ernesto Quintero Diaz, Universidad Autónoma de Aguascalientes: "Reproduction ex situ of *Smilisca dentata* and *Lithobates neovolcanicus*." Awarded $2,000.

Grant information, application, and cycle is available on the ATAG website: saveamphibians.org

Suggested Taxa for Exhibit

The 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 managed 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) programs should be exhibited with informative graphics about the recovery efforts for the species. Obviously, out of over 8,300 species of amphibians, there are a wide variety of animals that can be recommended for exhibit from specific regions or habitat niches. 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 from fellow AZA facilities or through reputable breeders. 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 8: Suggested Taxa for Exhibit

Common Name	Scientific Name	Category	Definition
Emperor newt	*Tylototriton spp.*	DERP	Display/Education
Kaiser's newt	*Neurergus kaiseri*	DERP	Display/Education
Dart frog	*Dendrobates, Phyllobates, Epipedobates, sp.*	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*	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
Horned frog	*Ceratophrys spp.*	DERP	Display/Education
Coqui	*Eleutherodactylus 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 spp.*	DERP	Display/Education
Solomon Island leaf frog	*Ceratobatrachus guentheri*	DERP	Display/Education

Bullfrog	*Lithobates (Rana) catesbeiana*	DERP	Display/Education
Northern leopard frog	*Lithobates (Rana) pipiens*	DERP	Display/Education
Slimy salamander	*Plethodon glutinosus*	DERP	Display/Education
Giant Mexican leaf frog	*Pachymedusa dacnicolor*	DERP	Display/Education
Lemur leaf frog	*Hylomantis lemur*	DERP	Display/Education
Amazon Milk Frog	*Trachycephalus resinifictrix*	DERP	Display/Education
Vietnamese Mossy Frog	*Theloderma corticale*	DERP	Display/Education

Exhibit Species Examples

A few examples of easy to care for exhibit amphibians follow, including options for mixed species exhibits that are typically available.

Solomon Island Leaf Frog

Ceratobatrachus guentheri

Species Summary: This medium-sized frog's snout and crests give it superior camouflage on the leaf-littered forest floor. Individuals vary in color from light yellows to burnt oranges and browns. This unique amphibian breeds by direct development, skipping the aquatic tadpole stage, and hatching from the egg as a tiny froglet. When housed in mixed sex groups, they will readily reproduce on exhibit making rearing of froglets visible to zoo guests. These frogs have been successfully displayed with the Solomon Island prehensile-tailed skink (*Corucia zebrata).* They have a loud call that sounds like a bark.

Interpretive Messages:
Direct development
Leaf-mimicry and camouflage
Island endemism
Responsible pet ownership
Conservation

Care and Facilities: Provided enough space (approximately 4' high x 4'wide x 4' deep) to set up territories, multiple breeding pairs can be kept together. Smaller spaces should only house pairs together as individuals will become stressed with overcrowding. They will burrow in substrate, hide under plant material, or sometimes sit out in the open. Provide large diameter diagonal and horizontal perches with cover. These tropical forest frogs thrive in high humidity (50-70%) and warm ambient temperatures (70-85F). Under these conditions females will lay several clutches of 10-30 pea-sized eggs per year. Eggs are laid in substrate depressions (sphagnum moss or sandy soil) and then buried. They will hatch 6-8 weeks later as tiny froglets. Start froglets on a varied diet of spring tails, pinhead crickets and fruit flies dusted with multivitamins and calcium. They are not adequate swimmers; if water is available make sure there is a gradient to allow them easy entry and exit. (*Submitted by Penny Felski, Buffalo Zoo*)

Lemur Leaf Frog

Hylomantis lemur

Species Summary: While not especially large, this nocturnal species has striking bright-white eyes when viewed awake during daylight hours but change color to a dark brown or maroon in the evening. As well, during the day the frog is green dorsally and changes to a dark brown or maroon color at night. This species faces a number of threats in the forests of Central America including habitat loss and population declines due to *Batrachochytrium dendrobatidis*. The species does well in mixed species settings with similar-sized and smaller tropical anurans (other hylids, centrolenids, dendrobatids, *Atelopus,* other small bufonids, etc.). Specimens often sleep on exhibit glass and with the use of a reverse cycle lighting system, animals can be observed while active and in the nocturnal colors during the day. Eggs are laid on leaves overhanging water and can be left in the exhibit to develop. Additionally, if a sufficiently-large water feature is present the larvae can also be reared on display for further exhibit and interpretive messaging.

Interpretive Messages:
Tropical rainforest biodiversity
Camouflage
Deforestation
Eggs laid above water, not in water
Global amphibian declines

Care and Facilities:

While needing somewhat specialized care, these frogs are fairly hardy, easy to reproduce, and very easy to rear under *ex situ* conditions. If wanting to breed on exhibit, plan to exhibit two pairs or one male and multiple females in an enclosure approximately 18"L x 18"W x 24"H; if breeding on exhibit is not a goal, single sex groupings may be housed together. Optional off-display housing should be available to separate sexes reducing breeding stress. Ambient temperatures of 68 – 78F are ideal, although they can tolerate short periods of temperatures into the low-80Fs. Frequent misting (manual or timed misting system) helps maintain humidity and stimulates animal activity. Good drainage and ventilation are essential to prevent stagnant air and mold growth. Live, broad-leaved plants should be provided along with small branches (½ – 2" diameter) as pathways and perching opportunities. The inclusion of a large water feature or pool can help encourage breeding. Lemur leaf frogs are insectivores and will eat a wide variety of insect prey supplemented with multivitamins and calcium. House flies or other soft-bodied flying prey items can provide good enrichment for this species. The necessity of UVB light has not been well documented in this species but limited exposure is likely beneficial. Larvae are easily reared in aquaria with filtration and/or regular water changes. A diet of powdered flake fish food and/or spirulina-based fish food works well for tadpoles. Larvae metamorphose after 45-60 days and will begin feeding on small insect prey (small crickets, fruit flies, etc.) within 7–10 days post-metamorphosis.

Other Notes: There is evidence that the two populations occurring in US collections at this time (Costa Rica and central Panama) may be genetically distinct. As such, it is important that institutions wishing to work with this species know the pedigree of their specimens and work to keep the Costa Rica and Panama lines as separate populations. (*Submitted by Robert Hill, ZooAtlanta*)

Bumblebee Poison Dart Frog

Dendrobates leucomelas

Species Summary: Brilliantly-colored poison dart frogs (PDFs) make for great exhibit taxa. Native to South America, the small bumblebee PDFs occurs at elevations between 50-800 meters in low-land forest habitat. This bold diurnal species is very active for visitors to see and is a good representative dart frog (Dendrobatidae) which boast some truly beautiful color variations and patterns. In addition to habitat loss/alteration and the amphibian chytrid fungus, dart frogs of all species are under threat from collection for the international pet trade; bumblebee and many other PDF species can be obtained legally thorough zoos, aquariums, and responsible hobbyists.

Interpretive Messages:

Skin toxins, warning coloration, & mimicry
Use by native persons for hunting
Amphibian chytrid fungus
Rainforest deforestation

Care and Facilities: If utilizing a 2'L x 2'W x 3'H enclosure with good ventilation, two to three pairs of this semi-arboreal species may be housed together thus creating large community groups encouraging unique social dynamics and behaviors. Some institutions have successfully exhibited this species with groups of other dendrobatids. Being that *Dendrobates leucomelas* lives in moist tropical forests and requires warm temperatures and periods of high relative humidity. Off-display housing should be available for less-dominant individuals. Ambient temperatures for tropical PDFs should be around 78-85F with a night time low of 68-70F. Regular misting (manual or timed misting system) helps maintain humidity and stimulates activity. Although recirculating waterfalls to a pool and/or drip walls work well in PDF exhibits, good drainage is necessary, so the substrate does not become saturated. Naturalistic branches, broad-leaf plants, and bromeliads should be provided as perching sites used as refugia or by territorial males. These frogs are insectivorous and require a varied diet of springtails, fruit flies, pinhead crickets, and termites supplemented with multivitamins and calcium. Seasonal light and dry/wet season cycles should be provided and should mimic that found in the tropics. (*Submitted by Dan Madigan*)

Lake Titicaca Frog

Telmatobius culeus

Species Summary: This unique, fully-aquatic frog from South America is critically endangered and endemic to one of the world's highest, navigable lakes (elevation 3,812m). Its color is variable but typically light grey, green or brown. Lake Titicaca frogs (LTFs) have interesting physiological and behavioral adaptations to help them survive in their extremely cold environment, including possessing extra folds of skin to absorb oxygen from the water (eliminating the need to swim to the surface to breathe) and regularly preforming *pushups* to pulse

oxygen-rich water across their skin surface. Population numbers are experiencing precipitous declines due to water pollution/habitat degradation, overharvest, and chytrid fungal disease. Local fishermen retain frogs caught as by-catch to satisfy market demands where frogs are consumed because they are believed to possess many healing properties.

Interpretive Messages:
Adaptations for high altitude and cold water
Amphibian chytrid fungus
Water quality/pollution
Conservation of endangered species

Care and Facilities: Up to seven adult LTFs can be maintained within an aquarium measuring 48"L x 24"W x 24"H with a sump, chiller, and recirculating pump; 5-20 gallon tanks can be utilized for holding off-display specimens. Mechanical and biological filtration can be provided through the addition of flow-through mesh or bag filters and bio-balls, respectively. Physical and chemical parameters are manipulated to mimic Lake Titicaca (i.e., 62F and 8.1 pH), and water used for aquatic changes should be match temperature and chemical parameters of the display or holding tank. These frogs require a varied diet of blackworms, red wigglers, earthworms, and small pieces of fish, with preference for moving prey items. Tadpoles are primarily grazers on brown or green algae, and commercially-available products (e.g., Sera Micron plates, fish flakes, gel diet) or diced fish can also be offered as they grow. (*Submitted by Tom Weaver, Denver Zoo*)

Crowned Tree Frog
Anotheca spinosa

Species Summary: This large, robust tree frog is nocturnal and prefers to hide during the day. It is attractively marked and both sexes possess sharp bony spines along the back of the skull creating the appearance of a *crown*; the spines are hypothesized to be used for combat in territorial disputes among males. Eggs are deposited above water in large bromeliads, bamboo internodes, tree hollows, or other suitable sites, and the female returns every few days to feed unfertilized eggs to the larvae. Threats include habitat loss and *Batrachochytrium dendrobatidis* throughout their range in Mexico and Central America. They are best exhibited utilizing a reverse light cycle so that they can be seen by the public and can be housed with other large anurans such as red-eyed tree frogs.

Other Notes: Currently, there are two populations available and should be maintained separately (i.e. *do not interbreed*): (1) a larger more robust Central American/Panamanian form that originated via Atlanta Botanical Gardens; and (2) a smaller form in the private sector believed to be from Mexico

Interpretive Messages:
Tropical amphibian biodiversity
Unique morphology
Parental care
Amphibian chytrid fungus
Global amphibian declines

Care and Facilities: A moderately-sized enclosure (18"L x 18"W x 24"H) is appropriate for a pair of animals, and males should only be housed together if the enclosure is large enough and provided with multiple breeding and hiding sites. Maintain enclosure temperatures between 68-82F. Suitable live plants and furnishings such as large branches and cork tubes provide animals with climbing, hiding, and breeding spaces. Daily misting and employing a moisture-retentive substrate can help boost humidity, but good ventilation is very important as the species does not tolerate stagnant, wet conditions. Water containers should be placed throughout the enclosure, and if breeding is desired place upright cork tubes/branches in the containers for egg deposition. Eggs should remain undisturbed; tadpoles will hatch and fall into the water containers to be fed by the dam as they do not do well on common fish foods like other amphibians. Larvae do require water quality similar to dendrobatid tadpoles, and metamorphosis can take two months or more. Feeding of frogs should consist of appropriately-sized crickets or other conventional feeder insects dusted with a quality multivitamin/calcium supplement. UVB lighting is not required to raise and reproduce this species, and the frogs typically prefer lower light levels. (*Submitted by Nick Hanna, Nashville Zoo*)

Aquatic Caecilian

Typhlonectes natans

Species Summary: This interesting amphibian represents the often-overlooked amphibian order, Gymnophiona. It is one of several wholly-aquatic species of caecilians and is the most common caecilian in the pet industry, often (incorrectly) called a *rubber eel* or *conger eel*. Legless and dark-grey in appearance, this species can reach up to 22" in length; the largest-sized animals almost always tend to be females. Native to northwestern South America, this highly-resilient species can tolerate silty and muddy areas with poor water quality and habitat degradation. Formerly imported by the thousands as a *fish* for the pet trade, populations are sufficiently stable to be listed as Least Concern by the IUCN. They possess two lungs, although one is usually smaller in size; specimens will gulp air at the surface but can also breathe through their skin if needed.


Fort Worth Zoo

Interpretive Messages:
Amphibian biodiversity
Species resiliency
Unique reproductive strategies and parental care
Amazon ecosystem health

Care and Facilities: Under managed care, this species thrives under varying temperature and water quality parameters. Temperatures should be 75-82F, although 68-92F have been tolerated for short periods of time. Water should be slightly acidic (pH 6.5), although a pH of 8.5 has been tolerated. One or two specimens can be kept in typical 30-gallon aquariums, and more room should be provided for larger groups. Breeding can occur either with pairs or in groups; a group of 6-8 seems to be ideal for effective breeding. Males will insert their phallodeum (an intromittent organ) into the female's cloaca, and pairs can be conjoined for many hours. During the 6 to 7-month gestation, the developing fetuses utilize specialized fetal teeth to scrape the interior walls of the dam's uterine lining. Newly-born aquatic caecilians can be seen on and around the female scraping off skin secretions. Typically lost prior to birth, some individuals are born with large, filamentous gills which fall off right after birth. Adult aquatic caecilians have been successfully housed and exhibited with many species of fish and

Surinam toads (*Pipa pipa*). Depending on a specimen's size, a diet will consist of earthworms, small fish, pinky mice, and other invertebrates. Longevity remains unknown. (*Submitted by Andy Snider, Brookfield Zoo*)

Emperor Newts

Tylototriton spp.

Species Summary: Although emperor newts produce toxins and caution must be taken, these salamanders make for an active and colorful display that guests will enjoy. Originally from montane wetlands and shrublands of Indo-China they prefer cooler environments. They appear barrel-bodied with knobby skin and bold conspicuous coloration. Notorious for their defensive and anti-predation displays, they also possess warning colorations to indicate that they have highly-toxic skin secretions which can denature proteins. Regardless of the month, animals housed in small groups will regularly breed once water depth and food are increased following interesting reproductive courtship behaviors and displays for guests to see. Clear eggs develop providing guests and staff an opportunity to monitor larval development. Several emperor newt species are typically available from other AZA institutions.

Interpretive Messages:
Amphibian biodiversity
Salamander chytrid fungus, *Batrachochytrium salamandrivorans* (Bsal)
Skin glands that produce toxins (proteolytic enzyme)/warning colorations
Unique behaviors and courtship displays
Eft life stage


Tylototriton kweichowensis Photo: Max Steinberg

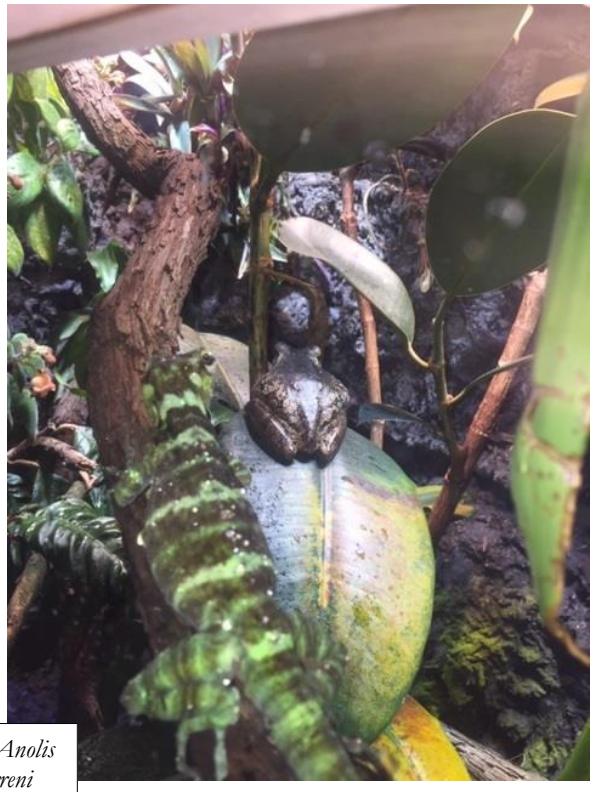
Care and Facilities: Emperor newts are easily maintained in small enclosures (e.g., a pair of adults in a 10-gallon aquarium). The enclosure should be a semi-aquatic terrarium with varying terrestrial gradients. If breeding is desired, enclosures should allow for flooding up to a depth of 8 inches and still provide enough land and horizontal refugia for the newts to bask or hide. Year-round ambient air temperatures are cool (63-73F) although basking spots of 75-90F should be provided. A canister filter provides ideal water quality and clarity; water temperature should not exceed 73F and can be maintained can be easily maintained with an inline chiller. To breed emperor newts, adults are hibernated seasonally (55F) with a shallow water dish for three months, followed by a short warming period as water depths slowly increase to 8 inches. Eggs are laid on damp, soft surfaces (e.g., moss or under plant roots) and hatch at 7-21 days. Larval newts can be offered brine shrimp nauplii, diced black worms, diced red worms and diced red wigglers. Terrestrial efts will consume the same diet as the semi-aquatic adults, including small crickets, diced earth worm, diced black worms, and diced red wigglers.
NOTE: Due toxic skin secretions, keepers should always wear gloves when handling any *Tylototriton* sp. and avoid placing animals in small containers/spaces where toxins can be concentrated and cause them harm. (*Submitted by Kelsey Barron, Fort Worth Zoo*)

Mixed-species exhibits

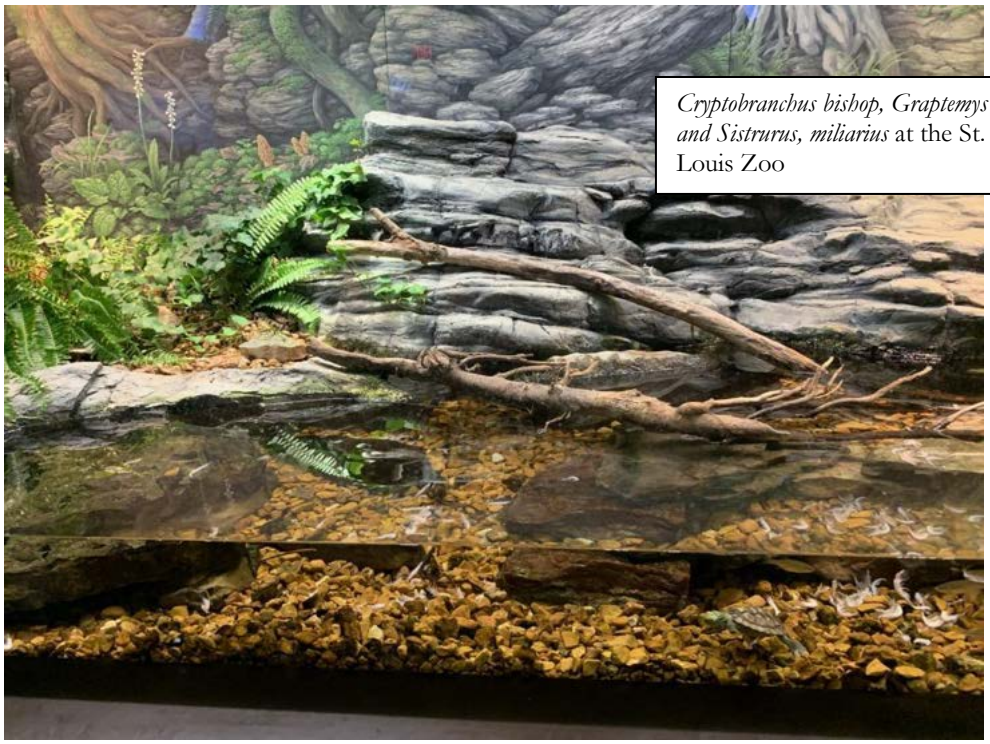
Species Summary: Mixed-species exhibits remain a popular way to generate a niche, ecosystem approach to displays which provides another interpretation to species' natural history. Many amphibian taxa can be displayed with many other reptiles and amphibians but work best when enclosures can provide containment & adequate humidity.

Interpretive Messages:
Ecosystems
Habitats/microhabitats
Zoogeographic regions
Natural History

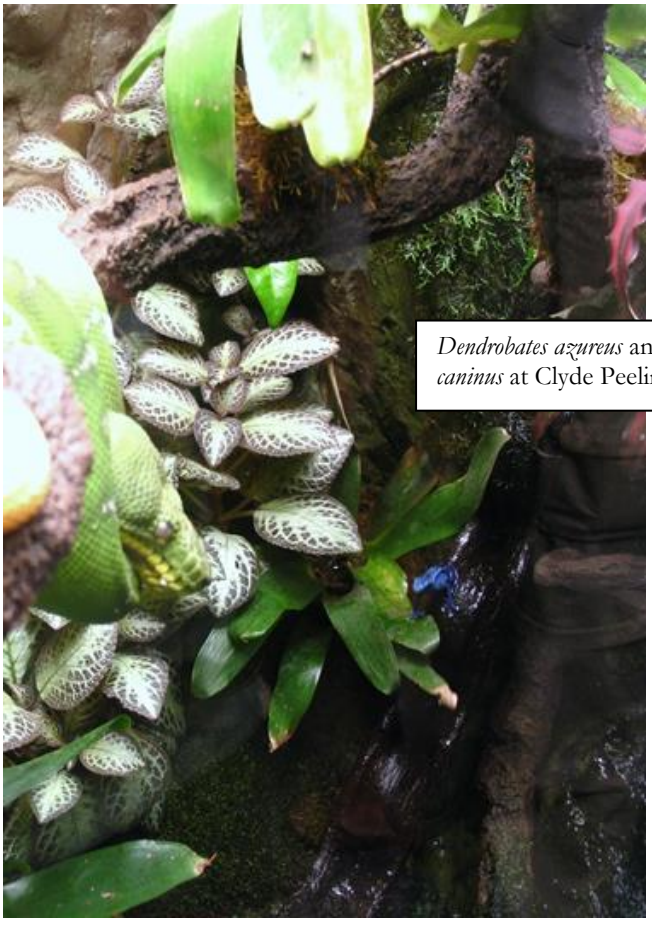
Atelopus zeteki, Dendrobates auratus, and *Pipa parva* at the Elmwood Park Zoo



Osteopilus dominicensis, Anolis ricordi, and *Celestus warreni*



Cryptobranchus bishop, Graptemys sp., and Sistrurus, miliarius at the St. Louis Zoo



Dendrobates azureus and *Corralus caninus* at Clyde Peeling's Reptiland

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. 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. The majority of species suggested can be obtained from zoos and reputable private breeders following AZA's Policy on Responsible Population Management: **https://www.aza.org/board-approved-policies-and-osition-statements**..

Staff member interpreting live newts, larvae, for eggs for zoo guests during an amphibian-focused *Salamander Saturday* educational event at the Fort Worth Zoo.

Table 9: Suggested Taxa for Outreach

Common Name	Scientific Name	Husbandry Level	Experience Level
Central American caecilian	*Dermophis mexicanus*	Moderate	Moderate
Two toed amphiuma	*Amphiuma means*	Moderate	Moderate
California newt	*Taricha torosa*	Moderate	Moderate
Eastern newt	*Notophthalmus viridescens*	Moderate	Moderate
European fire salamander	*Salamandra*	Hardy	Novice
Tiger salamander	*Ambystoma tigrinum*	Hardy	Novice
African bullfrog	*Pyxicephalus adspersus*	Hardy	Novice
Bullfrog	*Lithobates (Rana) catesbeiana*	Hardy	Novice
Cuban tree frog	*Osteopilus septentrionalis*	Moderate	Moderate
Horned frogs	*Ceratophrys spp.*	Hardy	Novice
Mexican burrowing tree frog	*Smilisca dentata*	Moderate	Moderate
North American tree frog	*Hyla spp.*	Moderate	Moderate
Poison dart frog	*Dendrobates spp.*	Moderate	Moderate
Red-eyed tree frog	*Agalychnis callidryas*	Moderate	Moderate
Tomato frog	*Dyscophus spp.*	Moderate	Moderate
White's tree frog	*Litoria caerulea*	Moderate	Novice
Wood frog	*Lithobates (Rana) sylvaticus*	Moderate	Moderate
Fire-bellied toad	*Bombina orientalis*	Hardy	Novice
Marine toad	*Rhinella (Bufo) marinus*	Hardy	Novice
North American toad species	*Anaxyrus (Bufo) spp., Ollotis (Bufo) spp., & Incilius spp.*	Hardy	Moderate
Red-legged walking frog	*Kassina maculata*	Moderate	Moderate
Spadefoot toad species	*Scaphiopus spp. & S. multiplicata*	Hardy	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 Recommendations

Responsible Population Management: Humane Euthanasia

See AZA's Policy on Responsible Population Management: **https://www.aza.org/board-approved-policies-and-osition-statements**. Recommended approved forms of euthanasia for amphibians are summarized in **Appendix III**.

Amphibians and Outreach

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 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 effects 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 (**Table 9**).

Amphibians in Classroom Settings

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.

Amphibian Welfare

The welfare of animals under human care is of utmost importance to AZA institutions and the Amphibian TAG. References for quality care can be found in the Amphibian Husbandry Resource Manual (Poole and Grow, 2012). Welfare needs and indicators in amphibians vary on a species by species basis, making welfare assessment of these animals a challenge. Since amphibians are so closely linked to their environment, it is important to use external indicators in assessments, such as taking into consideration how closely the enclosure and environmental parameters match the animals' natural habitat. When large numbers of amphibians are housed together or in mixed species' enclosures, it is beneficial to assess them by species grouped within a common enclosure. Within an overall group assessment, individual animals that are not meeting the group welfare standards can be isolated and assessed separately. If moderate to large number of individuals within a group do not meet the group standards over time, this can alert the caretakers to an underlying issue within the group's environment that has a prolonged, inconspicuous effect. A general example of an amphibian assessment is included in **Appendix IV**.

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Links to Amphibian Resources

Amphibian Conservation and Husbandry
https://www.aza.org/amphibian-conservation
http://saveamphibians.org/wp-content/uploads/2015/09/Amphibian_Resource_Manual.pdf
http://saveamphibians.org/wp-content/uploads/2015/09/AmphibianHusbandryResourceGuide2012.pdf

Conservation Funding Sources
https://www.aza.org/conservation-funding-sources/

FrogWatch USA
https://www.aza.org/frogwatch

Amphibian Medicine Tutorials
http://www.youtube.com/channel/UCaOhxmTP7asO5zyZQwYzh-A/videos

Amphibian Ark
http://www.amphibianark.org

Amphibiaweb
http://amphibiaweb.org

IUCN
https://www.iucnredlist.org/
https://www.amphibians.org/asg/

CITES
http://www.cites.org/eng

Partners in Reptile and Amphibian Conservation
http://www.parcplace.org

ARKive
http://www.arkive.org/amphibian-conservation

Amphibian Species of the World
http://research.amnh.org/vz/herpetology/amphibia/

EDGE
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Appendix I: AArk Amphibian Species Prioritization Ranking Results and Process

SUMMARY REPORT RESULTS for each species taxon referenced under this RCP are accessible through links from ASG/AArk Prioritization by species and country (if evaluated), downloadable at the following link - http://www.amphibianark.org/resources/assessment-results/ (reports last accessed 24, December 2019):

Country/ region	**Ark (A)**	**Rescue (inc. Supplementation) (A)**	***In situ* Conservation (B)**	***In situ* Research (C)**	***Ex situ* (Husbandry) Research (D)**	**Conservation Education (E)**	**Bio-banking (F)**	**None**
Canada			1	3	9	21		22
United States	1	7	87	96	49	118	7	120
Mexico		23	49	80	3	4	23	
Puerto Rico		7	14	8	5	22	7	
Panama[6]		1	5	4	2	2	1	
Honduras[7]		19	76	66	16	29	18	
Guatemala[8]		34	77	83	11	12	34	29

The AArk PRIORITIZATION METHODOLOGY utilized globally is delineated on the following pages (Johnson, et al, 2020) including an example.

[6] Prioritization information for the country of Panama is only included in reference to *Atelopus zeteki* and *A. varius* since this is an existing AZA SSP program; the conservation programs for all other Panamanian species is to be addressed within the country of Panama in accordance to the AArk global plan.

[7] Prioritization information for the countries of Honduras and Guatemala included in reference to *Bolitoglossa dofleini* since this is a proposed PIP species for within AZA; the conservation programs for all other range-country species are to be addressed within their own country in accordance to the AArk global plan.

SUPPLEMENTARY MATERIAL 1 *Assessment Questions and Answer Scores.*

Section One – Review of external data

1. **Extinction risk:** What is the current IUCN Red List category for the taxon?

The Red List category can be modified accordingly (for the purposes of this assessment only) if new/additional information is available, or if country-level Red List assessments exist. If the assessors consider that the Red List category of threat would change if the species was re- assessed using more current data than that which was used previously, or if a more recent national Red List assessment exists, a revised estimate of the new category can be chosen, and this will be used to calculate priorities and conservation actions.

If a national Red List assessment exists, the national category of threat is used rather than the global category.

Extinct	20
Extinct in the wild	20
Critically Endangered	16
Endangered	12
Vulnerable	8
Near Threatened	4
Data Deficient	8
Least Concern	0
Not Evaluated	0

If there is a proposal to modify the Red List category, a note must be added explaining the rationale for the proposed change.

2. **Possibly extinct:** Is there a strong possibility that this species might be extinct in the wild?
If there is a strong possibility that the species might have already gone extinct in the wild, this should be indicated, as it is also likely that the species will be included as a high priority for conservation actions, however, the likelihood of some of these actions (e.g. collection for *ex situ* rescue or research) is highly unlikely.

Yes
No

Note: If the answer is Yes, a note should be added to justify this reasoning.

3. **Phylogenetic significance**: The taxon's Evolutionary Distinctiveness (ED) score, as generated by the ZSL EDGE program. (These data are added by AArk staff, and are not editable by Assessors).

Using a scientific framework to identify the world's most Evolutionarily Distinct and Globally Endangered (EDGE) species, the EDGE of Existence program highlights and protects some of the weirdest and most wonderful species on the planet. EDGE species have few close relatives on the tree of life and are often extremely unusual in the way they look, live and behave, as well as in their genetic make-up. They represent a unique and irreplaceable part of the world's natural heritage, yet an alarmingly large proportion are currently sliding silently towards extinction unnoticed. A higher ED score indicates a more unique species.

Additional information about the EDGE scoring process can be found at www.edgeofexistence.org/about/edge_science.php.

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

4. **Protected habitat:** Is a population of at least 50% of the individuals of the taxon included within a reliably protected area or areas?

Protected habitat is defined as a clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values. Protected habitat might be within a national system of protected areas or privately-owned land which is actively managed to protect natural biodiversity.

Initial data were extracted from the IUCN Red List of Threatened Species (2008), www.iucnredlist.org and the Alliance for Zero Extinction (2010), www.zeroextinction.org.

Yes
No
Unknown

Note: If the answer is Yes, a note should be added, providing details of the protected habitat(s).

Section Two – Status in the wild

5. **Habitat for reintroduction:** Does enough suitable habitat exist, either within or outside of currently protected areas that is suitable for reintroduction or translocation?

This question provides information on particular areas of existing habitat that are suitable for reintroduction of captive-bred animals. When prioritizing species for possible *ex situ* conservation and reintroduction programs, priority should be given to those species that are known to have suitable release habitat available.

Yes	10
No	0
Unknown	0

Note: If the answer is Yes, a note should be added to provide details of the suitable reintroduction areas.

6. **Previous reintroductions:** Have reintroduction or translocation attempts been made in the past for this species?

This question does not affect the conservation action(s) assigned to the species, and nor does it affect the scoring. It is included purely to help guide, and to indicate the potential for demonstrable success with future reintroduction or translocation attempts.

Yes, successfully
Reintroduction or translocation attempts have been made for this species in the past, and post relocation monitoring has shown that the reintroduction or translocation was successful, with animals persisting in the wild.

Yes, but unsuccessfully
Reintroduction or translocation attempts have been made for this species in the past, and post relocation monitoring has shown that the reintroduction or translocation attempts were not successful - the relocated animals did not survive in the wild.

Yes, but outcome is unknown
Reintroduction or translocation attempts have been made for this species in the past, insufficient monitoring has been undertaken to indicate whether the relocated animals survived in the wild.

No
No known attempts have been made to reintroduce or translocate this species in the past.

Note: If the answer is Yes, a note should be added to provide details.

Section Three – Threats and recovery

7. **Threat mitigation:** Are the threats facing the taxon, including any new and emerging threats not considered in the IUCN Red List, potentially reversible?

It is often helpful to turn each of the answers into questions, and ask each question in turn until the correct answer is obtained, e.g.

- Does the species require conservation action at this time? If not, select answer (a).
- Is the species effectively protected? If it is, select answer (b).
- Are the threats this species is facing known? If not, select answer (c).
- Are the current threats being actively managed? If they are, select answer (d).
- Are the threats this species is facing potentially reversible before the species becomes extinct? If they can, select answer (e).
- Can the threats be reversed in time to prevent the species becoming extinct? If not, select answer (f).

(a) Species does not require conservation action at this time 0
This species is not currently facing any major threats in the wild, and no conservation action is currently required to safeguard this species in the wild.

(b) Species is effectively protected 0
All, or the majority of the population of the species in the wild is sufficiently protected to prevent further decline in numbers (e.g. the bulk of the population occurs in protected areas).

(c) Threats unknown 8
Either no knowledge about the threats to this species exists, or there is so little information known about the distribution of the species in the wild, that the threats cannot be determined.

(d) Threats are being managed - conservation dependant 10
Without the current management of the threat, the species would disappear in the wild. Examples of this sort of management include actions such as filling temporary ponds each year for breeding, diverting a dam to create a torrent, or harvesting predatory species.

(e) Threats are potentially reversible in a timeframe that
will prevent further decline/extinction 2
The threats to the species can, or will likely be removed or reversed, in a timeframe that will prevent further decline of the species in the wild.

(f) Threats cannot/will not be reversed in time to prevent
likely species extinction 20
The species will very likely go extinct in the wild before anything can or will be done to save it, but in principle the threats to the species could be reversed and the animals in *ex situ* colonies could be used to re-stock the wild if/when the threats are reversed.

8 **Over-collection from the wild:** Is the taxon suffering from unsustainable collection within its natural range, either for food, for the pet trade or for any other reason, which threatens the species' continued persistence in the wild?

Yes	10
No	0
Unknown	0

Note: If the species is suffering from over-collection, the reason (pet trade, food, etc.) should be included in a note.

9. **Population recovery:** Is the known population of this species in the wild large enough to recover naturally, without *ex situ* intervention if threats are mitigated?

Yes
No
Unknown

Section Four – Significance

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

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

Note: If the species is identified as being biologically distinct, a note should be included to explain this.

11. **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	10
No	0

If the species is identified as being of cultural or socio-economic importance, a note should be included to explain this.

12. **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), *within the Class* Amphibia.

Research dependent upon species	5
Research dependent upon <6 species (including this taxon)	3
No research dependent on this species	0

Note: If the species is identified as being of scientific importance, a note should be included to explain this.

Section Five* – Ex situ *activity

13. ***Ex situ* research:** Does conserving this species (or closely related species) *in situ* depend upon research that can be most easily carried out *ex situ*?

Yes
No

14. **Husbandry analog:** Do the biological and ecological attributes of this species make it suitable for developing husbandry regimes for more threatened related species? i.e. could this species be used in captivity to help to develop husbandry and breeding protocols which could be used for a similar, but more endangered species at a later stage?

Yes
No

Notes: Resources for *ex situ* programs are scarce, and analog species should only be specified for target species that are threatened, and have not previously been successfully kept in captivity. A note should be included which lists the target species for this analog.

15. **Captive breeding:** Has this species been successfully maintained and bred in captivity?

Yes, bred to F2

In this instance, successful captive breeding to F2 refers to animals which were bred and raised to adulthood in captivity, and they have then subsequently reproduced, with these second generation offspring also reaching adulthood. This second generation breeding and rearing to adulthood should be a repeatable event.

Yes, bred to F1

In this instance, captive bred to F1 refers to animals which were both bred and raised to adulthood in captivity. This first generation breeding and rearing to adulthood should be a repeatable event.

Maintained but no successful breeding

Animals have been successfully maintained in captivity for a long enough period of time to show that their husbandry and dietary needs are being met effectively, although the species is yet to regularly reproduce offspring that have reached adulthood.

Not held in captivity to date
Attempts to maintain this species in captivity have not yet been made.

Note: If the species has previously been maintained or bred in captivity, a note should be included providing details of institutions, zoo associations and contact person(s), if known.

Section Six – Education

16. **Educational potential:** Is the species especially diurnal/active/colourful and therefore suited to be an educational ambassador for amphibian conservation?

Yes
No

Section Seven* – Ex situ *Program Authorization/Availability of animals

17. **Mandate:** Is there an existing conservation mandate recommending the *ex situ* conservation of this taxon?

Yes
No

The decision about which species should be protected in *ex situ* conservation programmes should not be made by the *ex situ* community alone because such programs must be part of broader plans for species conservation. The *ex situ* 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 species can come from a number of recognised sources, such as:

- An IUCN SSC taxonomic specialist group (e.g. the Amphibian Specialist Group (ASG)).
- The IUCN - the IUCN Guidelines on the Use of *Ex situ* Management for Species Conservation 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/document-repository).
- An IUCN SSC Conservation Breeding Specialist Group (CBSG) Conservation Assessment and Management Plan (CAMP) process. (www.cbsg.org/document-repository).
- A published Species Action Plan.
- A local, regional or national government request.

Notes: If the answer is No, there is insufficient authorisation for an *ex situ* initiative at this time. Seek mandate from the appropriate IUCN taxonomic specialist group or other authority. If the answer is Yes, identify the source of the recommendation.

18. **Range State approval:** Would a proposed *ex situ* initiative for this species be supported (and approved) by the range State (either within the range State or out-of-country *ex situ*)?

    Yes
    No

Notes: If the answer is No, there is insufficient authorisation for an *ex situ* initiative at this time. Seek approval from range country (with help from the appropriate IUCN SSC taxonomic specialist group as required) before proceeding.

19. **Founder specimens:** Are sufficient animals of the taxon available or potentially available (from wild or captive sources) to initiate the specified *ex situ* program? It is recommended that a minimum of twenty active breeding pairs of animals be used as founder animals, ideally including several different locations or populations.

    Yes
    No
    Unknown

Notes: If the answer is No, there are insufficient potential founder specimens to initiate the ex situ program. Evaluate options for alternative conservation strategy including gamete biobanking.

20. **Taxonomic status:** Has a complete taxonomic analysis of the species in the wild been carried out, to fully understand the functional unit you wish to conserve (i.e. have species limits been determined)?

Typically this unit is a species; however, because species are continuously changing units evolving through time, there are often distinct but not yet unique subunits (evolutionary significant unit or ESU) in the process of divergence within the species and which might warrant independent consideration.

    Yes
    No
    Unknown

Notes: Typically this unit is a species; however, because species are continuously changing units evolving through time, there are often distinct but not yet unique subunits (evolutionary significant unit or ESU) in the process of divergence within the species and which might warrant independent consideration.

If the answer is No, there is insufficient knowledge of the species, and a taxonomic study, including phylogenetic analyses of DNA, should be undertaken before considering an *ex situ* program for the species.

Undertake appropriate research in conjunction with local field biologists (with help from the appropriate IUCN taxonomic specialist group as required) in order to confirm that the specific program encompasses only ONE evolutionary distinct unit (ESU) before proceeding.

SUPPLEMENTARY MATERIAL 2 *Conservation Actions.*

One or more conservation actions can be recommended for each species, and these are calculated for each species, based on the data provided during the assessment workshop (Appendix 1). The triggers described for each conservation action are compared to the responses to the assessment questions to determine which actions are relevant for each species.

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

Triggers for Ark species are:

- IUCN Red List category = Extinct in the Wild (EW)

Rescue
A species that is in imminent danger of extinction (locally or globally) and requires *ex situ* management, as part of an integrated program, to ensure its survival.

Triggers for Rescue species are:

- IUCN Red List category is not Extinct in the Wild (EW) **and**
- Threat Mitigation = Threats cannot/will not be reversed in time to prevent likely species extinction.

Note: Threats that constitute imminent danger of extinction include:

- Threats for which we currently have no remedy:
  - *Bd*, including any species known or suspected to be susceptible
  - Climate change, including any species documented to be drastically contracting its range, e.g., mountaintop salamanders in Central America (per Wake et al.) and mountaintop frogs in Madagascar (per Raxworthy et al.)
- Threats for which we have a remedy but not the resources or will to intervene
  - Imminent destruction of more than 50% of habitat, e.g., dam construction, mining/pollution
  - Species collected to brink of extinction
- All other threats are considered to be “reversible in time frame”.

***In Situ* Conservation**
A species for which mitigation of threats in the wild may still bring about its’ successful conservation.

Triggers for *In Situ* Conservation species are:

- Threat Mitigation = Threats are reversible in time frame that will prevent further decline/extinction **or**
- Threat Mitigation = Threats cannot/will not be reversed in time to prevent likely species extinction (species is in Rescue role) **and** Protected Habitat = No (species will need a secure place to go back to).

***In Situ* Research**
A species that for one or more reasons requires further *in situ* research to be carried out as part of the conservation action for the species. One or more critical pieces of information is not known at this time.

Triggers for *In Situ* Research species are:

- IUCN Red List category = Data Deficient (DD) **or**
- Threat Mitigation = Unknown **or**
- Habitat for Reintroduction is Unknown **or**
- Protected Habitat = Unknown **or**
- Population Recovery = Unknown **or**
- Over-collection status = Unknown **or**
- Taxonomic Status = No **or**
- Founder Specimens = Unknown **or**
- Conservation role = Rescue.

***Ex Situ* Research**
A species currently undergoing, or proposed for 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).

Triggers for *Ex Situ* Research species are:

- The species has been identified as a husbandry analogue for a more threatened species **or**
- IUCN Red List category = Critically Endangered (CR) **or** Endangered (EN) **or** Vulnerable (VU) **or** Near Threatened (NT) **or** Data Deficient, **and** conserving this species depends on *ex situ* research **and** Threat Mitigation = Threats unknown or Threats are reversible in time frame **or**
- IUCN Red List category = Extinct in the Wild (EW) **or** Critically Endangered (CR) **or** Endangered (EN) **or** Vulnerable (VU) **or** Near Threatened (NT) **or** Data Deficient, **and** the species has not been successfully maintained and bred in captivity **and** the species is biologically or evolutionarily distinct.

Mass production in captivity
A species threatened through wild collection (e.g. as a food resource), which could be or is currently 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.*

Triggers for Mass Production in Captivity species are:

- IUCN Red List category = Critically Endangered (CR) **or** Endangered (EN) **or** Vulnerable (VU) **and**
- Species is suffering from over-collection from the wild.

Conservation Education
A species that is specifically selected for management – primarily in zoos and aquariums - to inspire and increase knowledge in visitors, in order to promote positive behavioural 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).

Triggers for Conservation Education species are:

- The species has a high Evolutionary Distinctiveness score **or**
- The species is biologically, culturally, or scientifically significant **or**
- The species is suited to be an educational ambassador for amphibian conservation.

Supplementation
A species for which *ex situ* management benefits the wild population through breeding for release as part of the recommended conservation action.

Triggers for Supplementation species are:

- Threat Mitigation = Threats are being managed **or** Threats are reversible in time frame that will prevent further decline/extinction **or** Species is effectively protected **and**
- The (sub)population of the species in the wild is too small to recovery naturally **and**
- There is suitable habitat available for reintroduction.

Biobanking
A species for which the long-term storage of sperm or cells to perpetuate their genetic variation is urgently recommended, due the serious threat of extinction of the species.

Triggers for Biobanking species are:

- Recommended conservation role is Ark or Rescue

None
Species that do not require any conservation action at this point in time. This list may also contain species that were not evaluated during the workshop due to lack of data being available.

Triggers for these species are:

- Species does not match the criteria for any of the previous roles **or**
- Insufficient data available during the workshop to properly evaluate the species.

SUPPLEMENTARY MATERIAL 3 *An example assessment.*

The following assessment for *Mantella aurantiaca* in Madagascar was made by Devin Edmonds from Association Mitsinjo. It shows how each species assessment is prioritized and how conservation actions are recommended.

Subject	Question Text	Response	Score	Comments
Extinction risk	Current IUCN Red List category. [Data obtained from the IUCN Red List.]	Critically Endangered (CR)	16	
Possibly extinct	Is there a strong possibility that this species might be extinct in the wild?	No	0	
Phylogenetic significance	The taxon's Evolutionary Distinctiveness (ED) score, as generated by the ZSL EDGE program. (These data are added by AArk staff, and are not editable by Assessors).	ED value < 20	0	
Protected habitat	Is a population of at least 50% of the individuals of the taxon included within a reliably protected area or areas?	No	0	Found within Ramsar Site of Torotorofotsy, though this site is not reliably protected. New Protected Area of Mangabe supports over 50% of *Mantella aurantiaca* population and is in development, but currently is not protected or managed effectively.

Habitat for reintroduction	Does enough suitable habitat exist, either within or outside of currently protected areas that is suitable for reintroduction or translocation?	Yes	10	Torotorofotsy, Mangabe, Ambatovy Conservation Zone, etc. however habitat needs to be modified by creation of breeding ponds to support population and this technique tested and monitored long-term.
Previous Reintroductions	Have reintroduction or translocation attempts been made in the past this species?	Yes, but outcome is unknown	0	Translocations from Ambatovy mine footprint, where breeding sites have been cleared, to created ponds ("receptor sites") in Conservation Zone surrounding the mine have been carried out by NGO Madagasikara Voakajy since 2011. Their outcome is unknown. A major limitation is finding suitable breeding sites that are not already occupied by the species. Creating new breeding ponds by modifying habitat for translocations currently undertaken but outcome not yet known.
Threat mitigation	Are the threats facing the taxon, including any new and emerging threats not considered in the IUCN Red List, potentially reversible?	Threats cannot/will not be reversed in time	20	Habitat loss is the main threat, and although actions are being carried out to address this threat in some locations they do not guarantee the species survival. See Randrianavelona, R., Rakotonoely, H., Ratsimbazafy, J., Jenkins, R. K. B. 2010. Conservation assessment of the critically endangered frog *Mantella aurantiaca* in Madagascar. African Journal of Herpetology 59(1): 65 — 78 and Randrianavelona R., Randrianantoandro J. C., Rabibisoa N., Randrianasolo H., Rabesihanaka S., Randriamahaleo S., Jenkins R. K. B. 2010. Stratégie de Conservati on de l'Espèce *Mantella aurantiaca* (grenouille dorée) 2011-2015 for threats facing *M. aurantiaca* and actions being done to address them.
Over-collection from the wild	Is the taxon suffering from unsustainable collection within its natural range, either for food, for the pet trade or for any other reason, which threatens the species' continued persistence in the wild?	Yes	10	Not known if collection is sustainable in the long-term, likely has been or is still unsustainable at highly targeted sites. CITES II. Collected in high numbers into the early 2000's, with at least 30,000 individuals collected in a single year in the late 1990's (see Rabemananjara, F., et al. 2008.Malagasy poison frogs in the pet trade: a survey of levels of exploitation of species in the genus Mantella. Amphibian & Reptile Conservation 5(1): 3-16); however, moratorium on exports in mid 2000's and annual CITES quota reduced in recent years to as low as 280 individuals/year. Collection supposed to occur only at a few breeding sites that are supposed be or have been monitored recently. Research into effects of trade ongoing. Mortality after collection means CITES export quotas are lower than the actual number of frogs collected.
Population recovery	Is the known population of this species in the wild large enough to recover naturally, without *ex situ* intervention if threats are mitigated?	Yes	0	If threats can be mitigated effectively then the wild population should be large enough at most sites to recover naturally.
Biological distinctiveness	Does the taxon exhibit, for example, a distinctive reproductive mode, behaviour, aspect of morphology or physiology, within the Class to which the species belongs (Amphibia, Mammalia etc.)?	No aspect of biology known to be exception al	0	Not biologically distinct.

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	10	Socio-economic importance related to trade. Flagship species at Mangabe and Torotorfotsy Wetland.
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), within the Class to which the species belongs (Amphibia, Mammalia etc.)?	No research dependen t on this species	0	Not known to be scientifically important.
Ex situ research	Does conserving this species (or closely related species) *in situ* depend upon research that can be most easily carried out *ex situ*?	Yes	0	Population ecology and dynamics for potential sustainable trade; habitat requirements for breeding pond creation regarding translocations at Ambatovy sites
Husbandry analog	Do the biological and ecological attributes of this species make it suitable for developing husbandry regimes for more threatened related species? i.e. could this species be used in captivity to help to develop husbandry and breeding protocols which could be used for a similar, but more endangered species at a later stage?	No	0	Husbandry of this species and closely related species already well-understood.
Captive breeding	Has this species been successfully maintained and bred in captivity?	Yes, bred to F2	0	Genetically viable captive assurance colony maintained in range (Andasibe) and bred to F2 generation. Outside of Madagascar bred widely within cosmopolitan zoo collections and by private breeders.

Educational potential	Is the species especially diurnal/active/ colourful and therefore suited to be an educational ambassador for conservation of this group of species?	Yes	0	
Mandate	Is there an existing conservation mandate recommending the *ex situ* conservation of this taxon?	Yes	0	
Range State approval	Would a proposed *ex situ* initiative for this species be supported (and approved) by the range State (either within the range State or out-of- country *ex situ*)?	Yes	0	The Sahonagasy Action Plan is ratified by the Malagasy government and states support for *ex situ* initiatives for all amphibian species in Madagascar. Already maintained in country.
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	0	
Taxonomic status	Has a complete taxonomic analysis of the species in the wild been carried out, to fully understand the functional unit you wish to conserve (i.e. have species limits been determined)?	Yes	0	
		Total (priority)	**66**	

Appendix II: Creating Isolation Spaces for Amphibian Programs (2020 Update)

Creating Isolation Spaces for Amphibian Programs (2020 Update)

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INTRODUCTION

Establishments have been keeping amphibians in captivity for more than a century for research and exhibit purposes. Over this time period, understanding of amphibians and their husbandry requirements has increased substantially, as has the focus on amphibian conservation. In 1984, the first Association of Zoo and Aquarium's (AZA) Species Survival Program® (SSP) was formed for the Puerto Rican crested toad (*Peltophryne lemur*) and in 1993 collaborative breeding efforts began for the Wyoming toad (*Anaxyrus baxteri*). Both of these long-running reintroduction programs have served as models for intensive *ex situ* management of amphibians and contributed to the premise of modern assurance colonies within AZA facilities.

The scope of amphibian assurance colony programs quickly expanded from regional to international following the International Union for the Conservation of Nature's (IUCN) *Amphibian Conservation Summit* (ACS) in 2005. This gathering was held in response to global amphibian declines that were documented through the 1980's and 1990's and the overall lack of action being taken to stop this rapid loss. The *Amphibian Conservation Action Plan* (ACAP) (Gascon et al., 2007) produced during that summit provided direction for the global community to address the amphibian extinction crisis. Over 500 threatened species were identified as candidates for immediate *ex situ* conservation action, and the zoological community was asked to begin creating biologically secure isolation spaces for assurance populations of these species. In 2006, the IUCN Conservation Breeding Specialist Group (CBSG) and the World Association of Zoos and Aquariums (WAZA) held the *Ex situ Conservation Planning Workshop* in El Valle, Panama. From this workshop, guidelines were developed for biosecure containment of amphibian assurance colonies (Zippel et al., 2006). These guidelines, along with the first edition of the *Amphibian Husbandry Resource Manual* (Poole and Grow, 2008) and the *Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs* (Pessier and Mendelson, 2010) have laid the foundation for responsible management of amphibian assurance colonies and are informing the way species are managed for reintroduction.

Throughout this initial push to take action, many organizations became confused about how to create appropriate amphibian isolation spaces and discouraged by the lofty goals that were identified in these various documents. A misunderstanding and an overwhelming sense of burden has developed among some within the zoological community due to the large number of species in need

and the resources perceived necessary to meet the suggested level of research and biosecurity for recovery efforts. While some have found it difficult to locate existing space for amphibians within their facilities, many more have found it challenging to identify the resources and support needed to build new structures. Despite this, many AZA-accredited institutions have found the means to respond to the call of action.

This chapter includes numerous examples of isolation areas that have been created for amphibians (descriptions and photos offered for inspiration). The individuals that have contributed to this chapter have encountered challenges that may be unique to their own situations but have also found solutions through creative ingenuity that may be applicable to others. It is hoped that these examples, ranging from modified existing space to the creation of new facilities, can be used as tools for creating more places for amphibians world-wide.

BIOSECURITY (BIOSAFETY) – WHAT DOES IT MEAN?

While the term "biosecurity" may conjure up images of people in hazmat suits breathing through respirators in sterile white rooms, it actually refers to "biosafety," which is defined as *safety from exposure to infection agents*. Although intimidating to some, everyone should remain diligent about following protocols to reduce the spread of infectious agents.

It is now widely understood that costly, sterile environments for amphibians are not necessary, however questions still arise regarding the appropriate level of biosafety. The answer is that it depends upon the situation. It is recommended that assurance or reintroduction populations remain in permanent isolation (i.e., species separated into a room within a building with species from other regions or housed in an entirely separate building). When working with local or regional species, biosafety measures may be less extensive than with species from outside the region. Important considerations for designing amphibian isolation spaces include acceptable levels of biosafety/quarantine needed, and disposal of waste material and wastewater (Pessier and Mendelson, 2010; Poole and Grow, 2012).

TYPES OF FACILITIES

When starting a new program that requires dedicated amphibian space, review what areas and resources may already be available at the facility; these may range from new construction to modification of existing spaces. For some species, it may be preferable to create outdoor housing options, either in place of or in addition to, indoor housing to meet their needs. Most often these spaces can be found on the grounds of our existing facilities, but these same principles may be applied internationally, as needed. The following sections offer current examples of different amphibian isolation facilities, with the goal of serving as models and inspiration for new programs that may be developed.

Outdoor Space

Perhaps the simplest and least costly type of facility is one that is created for a local species that can be housed outdoors. Working with local species that are exposed to the same local environments and pathogens are the most ideal, as biosafety measures are minimal and species can be housed outdoors within secure enclosures or areas. The Riverbanks Zoo (Example 1) is working with local dwarf sirens (*Psuedobranchus sp.)* which are native to their area and are housed in large, plastic stock tanks outdoors, and the Saint Louis Zoo (Example 2) in Missouri has created a large, fast-running stream on zoo property for a population of Ozark hellbenders (*Cryptobranchus bishopi*). The hellbenders are used as a study population to learn more about their natural history and potentially to augment local populations. A new effort replicates microhabitats for amphibian breeding and metamorphosis, such as the North Carolina Zoo's gopher frog (*Lithobates capito*) head-starting

mesocosms (Example 3). Each of these semi-natural enclosures require little maintenance and provide moderate protection from predators. They also expose the animals to the same elements and light cycles they would experience in their natural habitat, which helps maintain animal health and stimulate reproduction with little manipulation by caregivers. This type of situation is a LOW biosecurity risk (as described in Pessier and Mendelson, 2010), although dedicated equipment should still be used and proper hygiene techniques followed (Poole and Grow, 2012).

Detached Spaces

When referring to detached spaces in this document, the author is implying construction of a new or modified facility that is detached from existing buildings and may include shipping containers and sheds. These may be created in range country or on zoological grounds and are an inexpensive alternative to construction/modification of an entire building. Careful planning is warranted, as hidden costs can often drive budgets higher than anticipated.

A small, prefabricated storage shed that can be located near a building with accessible power and water is easily assembled by novice staff and is less expensive than acquiring a refurbished and outfitted shipping container/freight trailer (i.e., pod). The Fort Worth Zoo added a shed used for flexible amphibian isolation/quarantine needs (Example 4), and the Toronto Zoo acquired a small prefabricated building and turned it into their *Amphibian Rescue Center* to expand the amount of isolation space available for species in need (Example 5).

Shipping containers can hold many amphibians, can be outfitted in one location and transported fully-assembled to another, and may be ready for use more quickly than a building that is newly constructed or modified. However, freight costs and the installation of power, water, and possibly sewer/septic system to a container can cause costly delays and logistical nightmares. To avoid headaches in the long-run, it is imperative to conduct thorough research (e.g., identify utility sources), determine the legal classifications (i.e., temporary or permanent) of the structures, and identify permitting requirements. Planning meetings with local officials prior to installation are important and may help resolve these issues. Memoranda of Understanding (MOU) or other contractual agreements should be considered when placing mobile units in remote regions to clearly define areas of responsibility for partners prior, during, and post- installation. Atlanta Botanical Garden purchased a fully-outfitted shipping container, the *FrogPOD,* which was placed on grounds for assurance colonies of frogs from Panama (Example 6).

Modified Spaces

One of the easiest ways to create space for amphibian assurance populations with limited funds is to modify existing rooms or buildings because amphibians generally require less space compared to other vertebrates and most can be housed at ambient air temperatures. Vacant 8 x 8 ft office spaces or facilities built for other species could be transformed into amphibian holding areas. Although floor drains are nice, they are not required since many options are available for pumping or moving wastewater.

A number of facilities have repurposed spaces to increase their amphibian conservation capacity. Omaha's Henry Doorly Zoo transformed empty, drain-less hallways into twelve *Isolated Amphibian Rooms,* using greenhouse material for walls and plastic storage vats for water (Example 7). Northwest Trek Wildlife Park enclosed a free-standing garage to create a rearing room for local Oregon spotted frog (*Rana pretiosa*) tadpoles and an outdoor area for staging juvenile and adult frogs prior to release (Example 8). Jacksonville Zoo and Gardens modified a building that once held koalas into a *Save the Frogs* exhibit, featuring numerous interpretive graphics and behind-the-scenes viewing of isolation rooms (Example 9). Even historical buildings can be resourcefully altered at moderate cost while still

maintaining their integrity; Toledo Zoo renovated the interior of a Depression-era museum into *Amazing Amphibians,* which included a large exhibit area for visitors and quarantined isolation space for four species assemblages (Example 10). Abandoned buildings *in situ* may be modified relatively quickly and at low cost if the overall structure is sound, providing an alternative to an outfitted shipping container; a vacated forest station in Madagascar provided the framework and foundation for a community-run amphibian rearing facility for local species (Example 11). The Houston Zoo modified several available spaces to create their large-scale Houston toad (*Anaxyrus houstonensis*) facilities shown in Example 12.

New Spaces

New construction dedicated exclusively to amphibians is rare. New amphibian spaces can be added to construction plans for an education building, animal hospital, primate facility, etc.; the options are limitless both in range and out of range country. Although exhibit space may help engage visitors, it is not crucial that amphibian assurance colonies are placed within public view.

The Detroit Zoo opened the first large-scale facility built entirely for amphibians in 2000, and the *National Amphibian Conservation Center* remains a popular exhibit for visitors today. The Atlanta Botanical Garden collaborated with the National Zoo in Chile to create breeding space for Darwin's frog (*Rhinoderma darwinii*) within a new building that also houses terrestrial invertebrates and flamingos (Example 13). The Fort Worth Zoo added four permanent isolation rooms for amphibians in an off-exhibit area within their newly constructed herpetarium, *Museum of Living Art* (Example 14), as well as a building for and the Phoenix Zoo created a conservation center for rearing and breeding rare Arizona species, which includes a large room for native amphibians (Example 15).

Additional Resources

Garnering support and obtaining resources for small creatures such as amphibians can be difficult, but it is not impossible. Amphibian programs need leaders to champion their species and conservation efforts within their own facility in order to garner resources comparable to those dedicated to other taxa. It is essential to share information and engage directors, boards, city officials, and/or state agencies regarding the need for action. Public outreach can expand a program's exposure, which may lead to unexpected external resources. Presentations and one-on-one conversations about amphibian declines have led to the development and completion of many of the amphibian facilities and programs presented in this manual.

Additional information on initiating amphibian conservation projects and identifying opportunities for grant support are available (Grow and Poole, 2008).

CONCLUSION

A mass extinction event is occurring and space and resources for amphibians needs to be committed immediately. Although space and resources are at a premium, these obstacles can be overcome if allocated to prioritize amphibian conservation; the longer we procrastinate, or wait for others to take action, the more species will disappear. All that is needed to bolster conservation efforts for amphibians is foresight and creative planning with key personnel. Through examples and discussions, this chapter has provided a foundation for people to create new functional space for amphibians. Grab a hammer and start building today!

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Example 1.
RIVERBANKS ZOO AND GARDEN NATIVE AMPHIBIAN HOLDING AREA (2012)
Information and photos submitted by Scott Pfaff, Riverbanks Zoo and Garden

Introduction
Little is known about the status of dwarf sirens (*Pseudobranchus sp.*) in the wild and few are held in zoos. Although they have a wide range, habitat requirements may be narrow. In South Carolina, habitat preferences of *Pseudobranchus s. striatus* are limited to vernal ponds and pocosins occurring in pine flat woods and long-leaf pine forest. These habitats are currently undergoing significant alteration due to forestry practices and coastal development. *P. s. striatus* is listed by the South Carolina Department of Natural Resources as a state threatened species. By maintaining colonies in outdoor enclosures (Figures 1 and 2), the Zoo has learned more about their reproductive strategies and husbandry requirements and is prepared to offer assistance should there be a need to bolster wild populations or to help other species of dwarf sirens in the future.

Type of Construction and/or Modification
Modification of an existing outdoor service area for Riverbanks' *Aquarium Reptile Complex*

Estimated Total Square Footage
Approximately 1,500 sq. ft.

Initial Set-up Costs for Facility
About $2,000

Major Challenges
Exclusion of native predators including mink, raccoons, and natracine snakes.

Useful Additions and Features
The facility has close proximity to a water system that provides water directly from the Saluda River. The Saluda is classified as a *State Scenic River* and is relatively-free from contaminants. Access to the river water system allows the facility to use open water systems in the outdoors amphibian

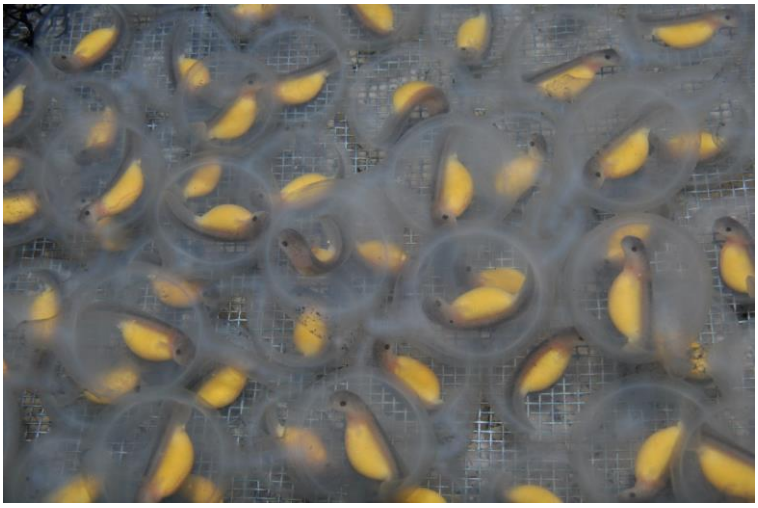
enclosures. Native amphibians are exposed to natural changes in photoperiod and temperature, and feed on the many invertebrates that colonize the tanks.

Areas for Improvement (i.e., planned differently or improved)
There is a need to secure all of the outdoor amphibian enclosures within a screen barrier to exclude small predators, yet allow entry of insects, natural light, rain, etc.

Figure 1. Stock tank enclosure with wire mesh lid removed.

Figure 2. Rubbermaid tub with wire mesh

Example 2.
THE RON GOELLNER CENTER FOR HELLBENDER CONSERVATION AT THE SAINT LOUIS ZOO'S WILDCARE INSTITUTE (2020)
Information and photos submitted by Mark Wanner, Saint Louis Zoo



Introduction
The Saint Louis Zoo's WildCare Institute, *Ron Goellner Center for Hellbender Conservation*, has created isolated amphibian space for the Ozark and Eastern Hellbenders of Missouri.

In 2011, the Saint Louis Zoo and the Missouri Department of Conservation announced the world's first breeding of the Ozark Hellbender (*Cryptobranchus a. bishopi*). The next year the Zoo was successful at repeating this achievement with the breeding of all three artificial streams (indoor and outdoor streams). The reproduction of these three Missouri river systems was the catalyst for over 10,000 eggs produced and over 7000 young Hellbenders released to present.

A recently updated Hellbender Husbandry manual is completed and available to anyone interested.

Type of Construction and/or Modification
New construction beginning in September 2011.

Estimated Total Square Footage
2600 sq. ft. of isolated amphibian space and two 40 ft. outdoor streams. The current indoor space encompasses four isolated rooms (Figures 1-5). Each stream is about 40 ft. in length with a 400 sq. ft. building constructed to house the life support equipment.

Initial Set-up Costs for Facility
Approximate costs for the construction of streams, life support building, and life support equipment were over $200,000.

Useful Additions and Features
Outdoor hellbenders are exposed to natural seasonal changes in photoperiod and temperature, as compared to those housed in the indoor facilities (Figure 6). Wastewater is treated using chlorine infusion. Stainless steel hydraulic lids were added after the initial construction was completed, are lifted manually, and contain hellbenders while excluding predators (Figure 7). The streams are deep enough for staff to snorkel to monitor the hellbenders, and are outfitted with chillers, boilers, UV

sterilizers, carbon towers, bag filters, and outdoor bio-towers maintained within the Life Support System (LSS) room (Figure 8).

Keepers have dedicated tools and have developed a "stoplight" work-order system for husbandry procedures: **Green** = *B.d.* negative; **Yellow** = *B.d.* status unknown; **Red** = *B.d.* positive; an intermediate color (**Orange**) may be used for eggs or a new Hellbender to the facility with a status that is most likely *B.d.* positive, but unknown. The order of operation is always Green, Yellow, Orange, then Red last.

Figure 1. Room 1 consists of multiple rack systems with varying sized aquaria, the egg tray systems, and a 32 ft. stream for propagation.

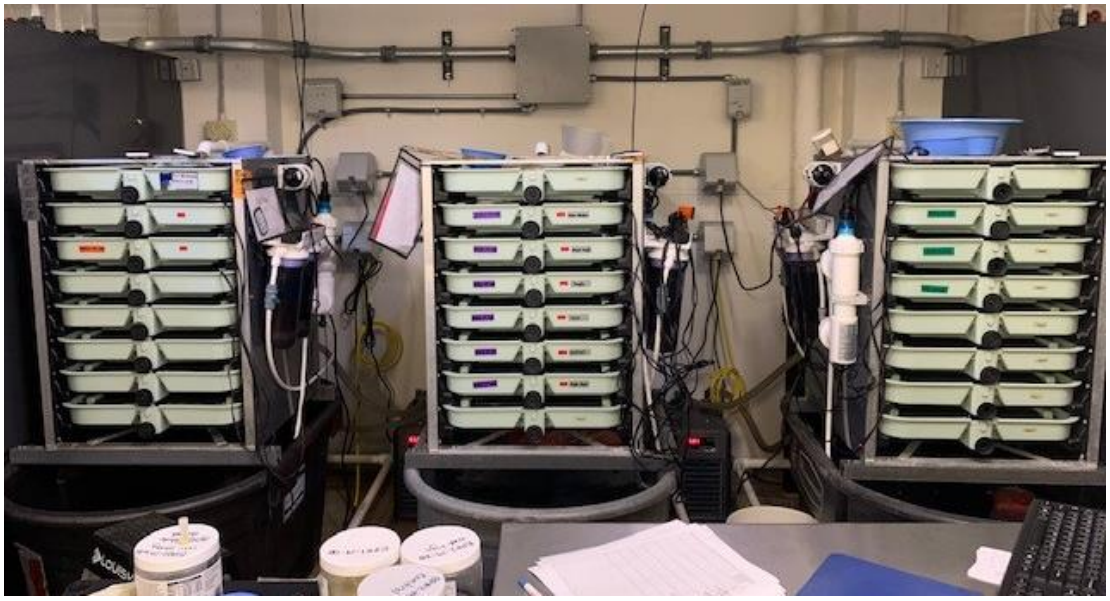


Figure 2. The flow-through hellbender egg tray system.

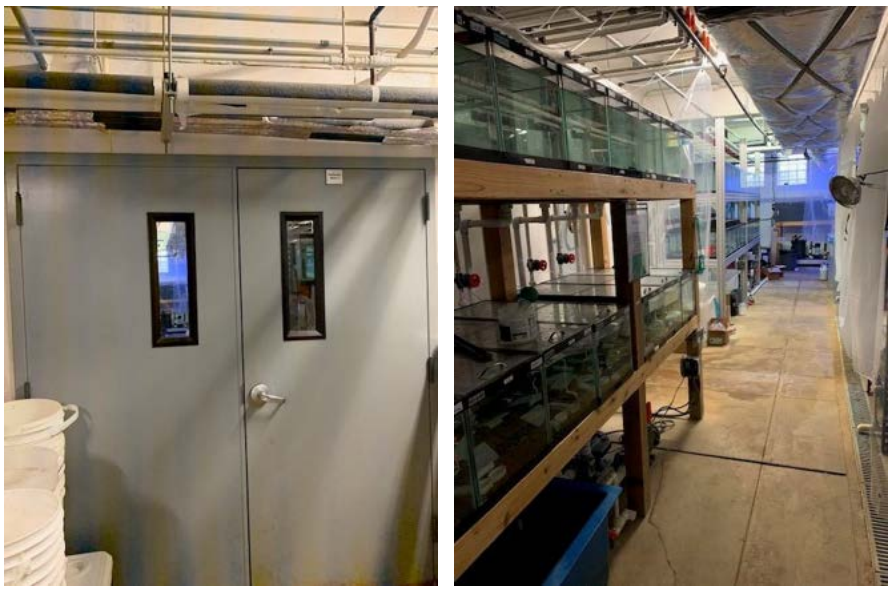


Figure 3. Room 2 consists of more rack systems with aquaria. This room generally houses hatchling and younger Hellbenders.

Figure 4. Room 3 houses larger juvenile and sub-adult Hellbenders and consists of the largest rack system with aquaria.

Figure 5. Room 4 is the new Hellbender quarantine room.

Figure 6. Two artificial, outdoor streams

Figure 7. Hydraulic lids on stream.

Figure 1 Hydraulic lid

Figure 8. Interior view of stream life support system (LSS) building.

Example 3:
GOPHER FROG HEAD-STARTING MESOCOSMS - NORTH CAROLINA ZOO (2020)
Information and photos submitted by Dustin Smith, North Carolina Zoo

Introduction

Starting in 2016, the North Carolina Zoo (NCZ) began a partnership with the North Carolina Wildlife Resources Commission (NC WRC) to oversee head starting the NC Endangered gopher frog (*Lithobates capito*) outdoors at the zoo. The example provided here has slowly evolved from mesocosms used at other institutions to what works best for the NCZ in this situation.

Type of construction/modification

New construction of an array of modified 300-gallon stock-tanks. The number used can vary depending on available space, staffing, and program needs.

Initial set up cost for facility

Between $350 - $400 per mesocosm; for planning, many of the items used come in bulk quantities that work well when constructing 10 to 12 mesocosms (a startup cost of $3500 - $4000 for ten mesocosms). Most costs are one-time startup cost (tub, plumbing, crayfish traps). Some items will last for multiple years before needing to be replaced (e.g., covers, bungees, nets), while others will need to be replenished annually (e.g., algae wafers); cost for subsequent years management is greatly reduced.

List of Materials needed per mesocosm

- 300-gallon stock tank (Rubbermaid™)
- 8' square of heavy duty (black) pet screen (Phifer Pet Screen – can order 8' x 100' roll)
- ~8' of ¼" bungee cord (for securing the screen)
- (2) 15" lengths of 1" x 2" PVC board (won't rot like wood, and will hold the screen in place)
- (2) small carabiners (secured by cable ties to the lip of the mesocosm …the bungee cords run through these to hold the cord in place)
- (1) double threaded 1" bulkhead fitting (used for the overflow at desired water level) hole size 1-3/4"

- (1) 1" threaded / slip PVC fitting and (1) 4" length of 1" PVC (screwed into the bulkhead on the inside of the mesocosm (at desired water level) – cover opening with a piece of pet screen cable tied in place.
- (2 or 3) coated crayfish traps (hung at water surface of mesocosms as approaching time of metamorphosis.
- Maidencane (dry) – used at a rate of 13g / tadpole (harvested from near the breeding pond in fall and dried in paper bags over the winter)
- Algae wafers – We use Hikari™ algae wafers (available through Amazon)
- ~8" soft mesh net x2 – Used to remove old food and metamorphs

Gopher frogs generally breed in the late winter or early spring, but sometimes they may breed in the early fall as well (heavy rainfall events). It is best to be prepared for both scenarios to not miss any breeding events and subsequent head starting opportunities. The NCZ husbandry method follows:

- Once the previous season's metamorphs have been removed from the mesocosms tubs and screens should be drained, scrubbed, bleached, rinsed and sundried for the next season. *If this is a new tub, this is the time to drill a hole for the overflow bulkhead and add the covered 1" x 4" PVC extension to the inside of the bulkhead.*
- Pet screen is cut to 8' squares, placed over the tub and then the bungee put in place to prevent any unwanted amphibians or invertebrates from entering. Then the PVC trim boards are screwed into the tub lip (on both sides) to prevent the mesh from slipping out of place. The carabiners can then be added to the lip below the PVC boards to keep the bungee in place while servicing the mesocosm.
- Make sure that the mesocosms are in the desired location, as it is now time to allow them to start collecting rainwater. *If available utilize well water. City treated water should be avoided however as this can be problematic due to the presence of chlorine, chloramines and fluoride.*
- The next action does not occur until the frog eggs have been collected. When eggs are collected, an additional 1-gallon of natal pond water (per mesocosm) is transported back to the zoo and acclimated to the mesocosms. The eggs are maintained in smaller tubs until hatching has begun, which is also when the maidencane (Figure 1) should be added to mesocosms at a rate of 13g / tadpole. Tadpole stocking densities may vary, but our target is 50 tadpoles / mesocosm.
- 10 days post-hatching the tadpoles are released into the mesocosms. This is done by moving the tadpoles from their hatching containers into a 5-gallon bucket. Then a slow drip line starts the slow acclimation process to the water (composition and temperature) in the mesocosm. This process generally takes ~ 30 minutes. *Fish bags have been tried in the past, however there is some undesired mortality due to tadpoles swimming into the corners of the bag.*
- For about the first month, there is little algae wafer consumption as there is enough algal growth on the maidencane and mesocosm walls to sustain the tadpoles. During this period, it is only necessary to offer 1 wafer per feeding. Feeding protocol is to offer wafers on Mon/Wed/Fri and remove uneaten wafers on Tue/Thu/Sat. It is recommended to document amount offered and removed (i.e. not consumed). Once wafer consumption increases, amount should be increased by 2 wafers every time the amount removed is 10% or less.
- Crayfish traps should be suspended around the edge of the mesocosm (at the water line) so that metamorphs swimming around the edge can enter, but high enough that tadpoles do not enter (Figure 2). *These should be installed when hind limbs are first noted on the tadpoles.*
- Once installed, crayfish traps should be checked minimally once daily. Tadpoles that may have found their way into the traps should be released and metamorphs with less than ½" of tail should be

transferred from the mesocosm into a holding container that will be used to transport to the release site. At this time, the metamorph should be weighed and measured (SVL).

- As metamorphs are removed from the mesocosm and consumption decreases, the amount of food offered should also decrease.

Major Challenges

For many of us, our site selection is based less on what would be ideal parameters for the amphibians, but more likely based on what usable space is actually available, so the challenge is how to best make the space suitable. Considerations should include the following

- Is the site secure from unwanted visitors (animal or human)?
- Is the site isolated from other amphibian species?
- What does the sun exposure during the season(s) that the mesocosms will be in use look like? Do the species you will be working with prefer full sun or partial sun? Will a black tub create too much unwanted heat?
- What environmental features in the vicinity might become problematic at some point in time? (e.g., heavy tree pollen area that might foul the pond, areas with excessive noise, vibration, exposure to pollutants, etc.)
- Indoor space available for storage of equipment and holding amphibians at various life stages (is climate control needed?)?

Useful additions and features

Even though these are native species, biosecurity measures should be provided to minimize disease risk to the program animals. These may include insuring that every mesocosm has a tight-fitting cover that prohibits predatory invertebrates and/or native amphibians from entering the mesocosm; following a best practices approach to the care provided; using dedicated equipment, wearing gloves when servicing animals and equipment.

Areas for improvement

Nature's byproducts (e.g., pollen or foam) can build-up on water surface during prolonged dry periods, but a fine mesh brine shrimp nets can be used to skim this from the surface daily. Filamentous algae growth will occur if maidencane is added to the mesocosm too soon before the tadpoles., but it can be removed by netting out larger clumps. It is possible to address poor water clarity by transferring some water from a mesocosm with a daphnia bloom as they will consume any suspended algae.

Figure 1. A Gopher frog mesocosm full of maidencane

Figure 2. The crayfish trap added at the surface level which gathers metamorphs but not tadpoles.

Example 4.
FORT WORTH ZOO TOAD SHED (2020)
Information and photos submitted by Diane Barber & Vicky Poole, Fort Worth Zoo

Introduction
This outdoor tool shed is a relatively inexpensive way to isolate a group of amphibians and can be placed next to a building for easy access to electricity and water (Figure 1). Similar units can be purchased at any major hardware store or on-line and have the flexibility to be shipped or transported as an unassembled kit if needed in range-country.

Type of Construction and/or Modification
The original shed kit was purchased in 2004 from a local hardware store and was constructed on site by staff. Originally built to house Pecos pupfish, a newer pre-fabricated shed with windows and a porch purchased in 2017 has replaced the original structure and is now used as a flexible isolation space. Windows allow natural lighting which is a benefit to species. The shed's interior surfaces are easily disinfected using a large steam cleaner prior to moving in the toads. Two custom shelves are installed along the walls to accommodate glass aquariums and lighting. The shed is equipped with a sink and water filtration/storage area (Figure 2) and is cooled/heated by small portable units.

Estimated Total Square Footage
This new structure is a single room that is about 120 sq. ft.

Initial Set-up Costs for Facility
Estimates for constructing a similar set-up today would be approximately $2,220, not including labor for construction, or electrical and plumbing installation. Expenses are estimated as follows:

Metal Racks	$400
Roof shingles	$150
Flooring material	$200
Lighting/timers	$400
Tanks/lids	$280
Sink and fixtures	$60
Water storage tubs	$60
Water filtration	$120
Window air conditioner	$250
Small Heater	$300

Major Challenges

The shed lacks a central floor drain, so tank drains are routed to the sink and the linoleum covered floor is mopped. If the air conditioner or heater malfunctions, room temperatures fluctuate quickly, and so staff must be diligent about monitoring the building.

Useful Additions and Features

Commercially-available, easy-to-assemble units are available with windows/skylights, which would also provide natural light cycles. The addition of a screened vestibule or covered entryway is beneficial for staff preparing to enter the room, especially in inclement weather.

Areas for Improvement (i.e., planned differently or improved)

As space for a growing population may be a limiting factor, the size of the unit selected should reflect program needs. A hoseable floor surface with a central drain would be an improvement for keeper staff. Normal wear and tear allow for a

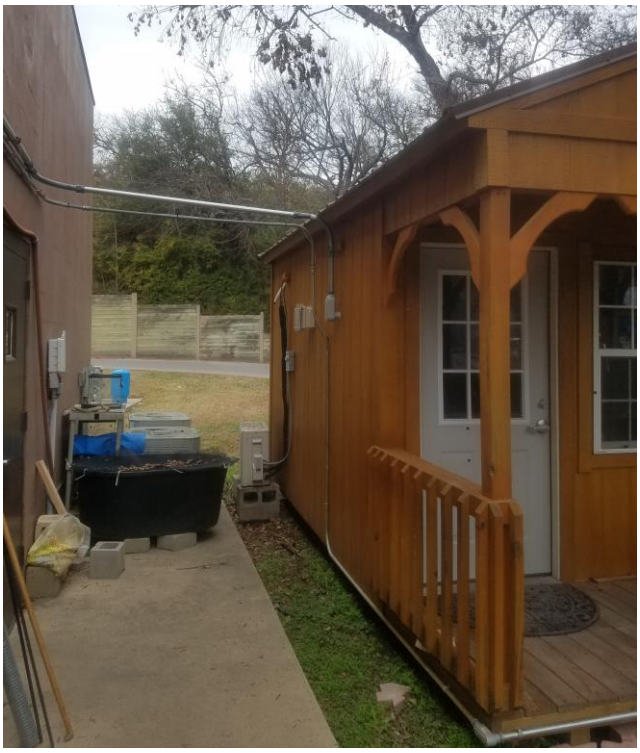


Figure 1. Overhead lines from nearby building provide electricity for the shed.

Figure 2. *Left*: The Original shed's water filtration and storage areas. *Right*: New shed with a sink and R/O storage vat visible to the left of the entry door. Shelves provide ample isolation space for amphibians as needed.

Example 5.
TORONTO ZOO AMPHIBIAN RESCUE CENTER (ARC) (2020)
Information and photos submitted by Bob Johnson & Andrew Lentini, Toronto Zoo

Introduction
This building was purchased to expand isolated space for amphibians at our facility.

Type of Construction and/or Modification
This is a prefabricated building that was purchased new (Figure 1).

Estimated Total Square Footage
The *Amphibian Rescue Center* has a total of 280 sq. ft., containing two isolation rooms (Figure 2) that are 90 sq. ft. each and a vestibule (Figure 3).

Initial Set-up Costs for Facility
The building costs were approximately $84,000 (USD), shelving and tanks were about $1,500, and roughly $3,000 was spent to run the water lines and power to the facility.

Useful Additions and Features
If needed, the HVAC system allows for manipulation of temperatures in order to hibernate amphibians. Windows that open to exterior for air exchange and to facilitate cooling in winter (in a northern climate).

Areas for Improvement (i.e., planned differently or improved)
Several issues arise when temperatures are lowered to hibernate adult amphibians. First, the room becomes too cold for the juvenile amphibians, which has been overcome by staging the hibernation in the following manner: the room temperature is lowered by 10-15F, which is still comfortable for the juveniles; once stabilized, the adults are moved to separate aquatic chiller systems where they are further cooled, while the room is returned to a normal temperature range for the growing juveniles.

High humidity within the rooms at low temperatures is also problematic as moisture condenses on the walls. A de-humidifier (with *auto-dry* function) was added to reduce condensation on cool surfaces during cooling/brumation periods. A Reverse-Osmosis filter and reservoir was added to provide the ability to better control water chemistry parameters in the source water.

As space has become a limiting factor, the rooms should have been made about three feet larger so that three more tanks or a water reservoir could have been added.

Figure 1. Moving the Amphibian Rescue Center onto the concrete pad

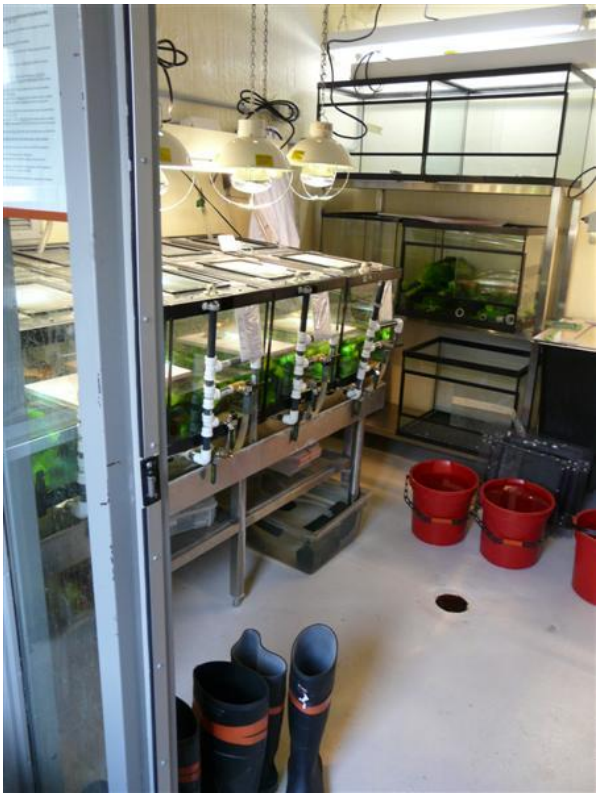


Figure 2. Interior amphibian room

Example 6.
ATLANTA BOTANICAL GARDEN'S FROGPOD (2012)
Information and photos submitted by Robert L. Hill, Atlanta Botanical Garden

Introduction
The *FrogPOD* was purchased in order to house an assemblage of amphibians that were collected in Panama as an assurance population. This off-exhibit facility currently houses approximately 200 juvenile to adult animals but could potentially house many more if needed.

Type of Construction and/or Modification
In 2008, the shipping container was purchased new as a complete, fully equipped/outfitted unit. Doors, windows, floor drain, and electrical outlets were installed by the company offering the unit, while plumbing, enclosures, and additional "after market" accessories were installed on-site.

Estimated Total Square Footage
Approximately 3,200 sq. ft.

Number of Isolation Rooms
The pod includes two rooms: an 800 sq. ft. entry room used for general storage and changing footwear, and a single 2,400 sq. ft. amphibian room (Figure 1).

Initial Set-up Costs for Facility
$53,000

Major Challenges
Inadequate heating and cooling systems were initially installed, and the grated flooring/drain system has been problematic.

Useful Additions and Features

The heating and cooling problems have been remedied by upgrading to a more powerful split-unit heat pump and installing back-up window air-conditioning units and small space heaters, to be utilized as necessary. The box shape of the unit makes design and layout of enclosures quite simple, as the pod lacks the odd columns or strangely placed corners often found in many herp buildings.

Areas for Improvement (i.e., planned differently or improved)

Modification of the grated floor would be nice, as drainage has been a constant problem due to the lack of solid-surface floor material. The initial purchase of a split-unit heat pump would have solved many of the heating and cooling issues from the start. In areas that may reach warm seasonal temperatures for extended periods of time (i.e., the southeastern USA), inexpensive window a/c units may prove to be inadequate.

Figure 1. Amphibian holding room

Example 7.
OMAHA'S HENRY DOORLY ZOO'S ISOLATED AMPHIBIAN ROOMS (2020)
Information and photos submitted by Jessi Krebs and Derek Benson, Omaha's Henry Doorly Zoo

Introduction

In February of 2006 a CBSG/WAZA Amphibian Ex Situ Conservation Planning Workshop was held in El Valle, Panama. One of the many purposes of the meeting was to re-evaluate and make recommendations for husbandry standards for amphibians that are part of reintroduction programs or animals that may one day be returned to the wild. Many of these recommendations were made to "upgrade" the current housing and quarantine standards practiced by many institutions.

What follows is Omaha's Henry Doorly Zoo's (OHDZ) methods to provide simplified modular amphibian care spaces that are easily replicated, with financial considerations documented. These technologies and practices presented here have been implemented by the Johannesburg Zoo of South Africa for their Amphibian Conservation Center and the Honduras Amphibians Rescue and Conservation Center, demonstrating the transferability of the techniques not only within AZA institutions but is more remote areas as well.

Type of Construction and/or Modification

Isolated Amphibian Rooms (IARs) are versatile rooms constructed out of commercially available greenhouse materials with all construction completed by zookeepers. They have been set-up within the hallways of an unused, existing building on the Henry Doorly Zoo grounds (Figure 1).

Estimated Total Square Footage

Total indoor square footage is over 4,200 sq. ft. Outdoor square footage is over 1500sq. ft for local amphibian head start mesocosms.

Number of Isolation Rooms

Currently there are 13 rooms that vary in size from 44 sq. ft. to 256 sq. ft. filling all indoor space.

Initial Set-up Costs for Facility

Prices to completely set up the last few 8x8 ft. rooms were approximately $8,000. For estimating purposes, the rough expenses are as follows:

Room materials	$1,200	
Shelving	$300	
Heater/AC	$850	
Frog tanks	$150	each x 18 = $2,700
Lighting	$220	each x 9 = $1,980
Plumbing	$500	
Electrical/duct work	$250	
TOTAL for one room	$7,780	

Major Challenges

Staffing remains a challenge for managing so many isolation spaces.

Useful Additions and Features

IARs at the zoo range from 8x4x8 ft. (2.4x1.5x2.4 m) in size to 10x16x8 ft. (3x4.9x2.4 m). The walls are made of 1 ½" x 1 ½" (3.8x3.8 cm) hollow aluminum tubing with two-ply polycarbonate sheeting (Figure 2). Individual walls are connected with 1" (2cm) aluminum angle pieces. All joints and cracks are sealed with 100% silicone to prevent water from leaking out or between isolation rooms. Commercially purchased storm doors are used to access each room. All joints and cracks are sealed with 100% silicone to prevent water from leaking into common areas or into other isolation rooms. Seals are pressure-tested before installation of equipment and animals and visual inspections are ongoing to maintain biosecure levels. The storm door is placed at the lowest point and the one-inch threshold allows each room to hold at least 175 gallons (796 L) before overflowing into a common hallway with a drain. List of items used for the construction of an 8x8 ft. room:

Cap[8]	18 @ 8 ft. (2.4 m)
Splice[10]	3 @ 8 ft. (2.4 m)
Lexan®[10]	6 @ 6x8 ft. sheets (1.8x2.4 m)
Aluminum Tubing[9]	18 @ 8 ft. [1.5x1.5 in. (3.8x3.8 cm); 1/8 in. (0.3 cm) thick]
Storm door[12]	standard sizes
Hardware, screws, washers[12]	

Portable heating/air condition units (price estimate $750) are used to control the ambient temperature in each room. Units can be purchased with different BTU ratings for different size rooms: 8x8x8 ft. (2.4x2.4x2.4 m) rooms use 10,000 BTU units; the 10x16x8 ft. (3x4.9x2.4 m) use 12,000 BTU units[10]. Recently, combined heating/cooling window units (price est. $400-$850 depending on BTUs) have been added above the storm door frame to provide more thorough temperature control and declutter available floor space. For even colder temperature requirements, a CoolBot[11] (price est. $350) may be installed on the window unit to achieve temperatures as low as

[8] **www.stuppy.com**
[9] **www.statesteel.com/omaha.htm**
[10] **www.samsclub.com** or from materials acquired at local hardware stores
[11] **https://www.storeitcold.com/product/coolbot-walk-in-cooler-controller**

38F with proper distribution and insulation; for example, the Omaha Zoo has been able to maintain Mountain Yellow Legged Frogs (*Rana muscosa*) at ambient temperatures of 52F in the polycarbonate IARs with no extra insulation (Figure 3).

Once the shelving[12] is installed, tubs and lids[12] used for amphibian enclosures are fabricated from food-grade polycarbonate material to prevent the leaching of toxins sometimes found in plastic materials (Figure 4). Though glass fish-tanks may be a less expensive, the polycarbonate tubs are far more durable and versatile, making them suitable for housing terrestrial or aquatic species. Drilling each tub does not require a specialized drill bit nor do they crack or break as easily as glass. The volume of the tanks used ranges from 5-16 gallons.

Lighting on every rack system is made available in two forms: LED shop lights[13] above each shelf to provide for ambient photoperiod and plant growth, and small halogen track-lighting heat lamps on each enclosure to offer basking sites for species requiring higher temperatures. Lids can be modified to allow UVB penetration by cutting a square in the top and securing appropriately sized mesh to the hole. Use the largest size possible for each species to allow for the best UVB penetration, which should be monitored regularly with a solarmeter (Figure 4).

All water is treated going into and out of the IAR facility. For source water, the department-wide Reverse-Osmosis (RO) water filtration unit is used to strip pathogens, chemicals, and other contaminants from city water, and is reconstituted to make it safe for amphibians. RO water is mixed with appropriate salts and minerals (*reconstituted*) in a 300-gallon tank for 30 minutes then distributed to holding tanks for each isolation room (Figure 5).

The wastewater for each individual enclosure runs out through a standpipe (with mesh attached to prevent animals escaping) attached to a bulkhead fitting, then into a common piping system located under every shelf. Drain system lines are 2 in. (3 cm) diameter to allow for large volumes of water to pass through them without backing up into adjacent enclosures (Figure 6). Each room's wastewater pipes lead to into a one or more corresponding 55-gallon waste water barrels dedicated for disinfection, although it can be safely directed to a municipal sewage system (Figure 7). At the OHDZ all waste water from within the isolation rooms is sterilized with a 65% powder bleach (respirators required for safety; agitated with an air stone) overnight before entering the area's central treatment station, which is then additionally treated with household bleach (4-6%) for twelve hours before being released into the city sewer system.

At the time of design of the IARs, wastewater treatment was highly recommended for all amphibian isolation facilities, however current practices only require wastewater treatment for those facilities which do not directly flow into a municipal sewage system. The following information on a centralized wastewater treatment plan is offered for facilities desiring such an example. A sink combination (price est. $450) is used to collect all wastewater from each isolation room and is created by stacking two inexpensive utility sinks together (Figure 8 *Right*). The bottom tub (without legs) is set directly on the floor un-drilled. The second sink (with legs) is set within the tub below and plumbed to drain into the lower tub without splashing. A sump pump[14] with an automatic on/off switch is set within the lower tub to pump wastewater to the central treatment station (Figure

[12] **www.webstaurantstore.com**
[13] Available at lighting or hardware stores, **www.usalight.com**, or **www.1000bulbs.com**
[14] **www.flotecpump.com**

8 *Left*). If desired, the upper tub can be plumbed for use as a working sink or else dedicated hose-lines can be run into each room and provide filtered source-water.

Rather than using individual air pumps for maintaining tadpoles, air is provided to the entire area from an large air blower (3-5psi) which is routed to each room via PVC pipe (Figure 9 *Left* & *Center*). Each room has a manifold of plastic air valves following an larger inline valve so rooms can be isolated if needed (Figure 9 *Right*). The main blower is on a backup generator, so in the event of power failure oxygen will still be supplied to tanks.

<u>Areas for Improvement (i.e., planned differently or improved)</u>
Figures 10 – 12 offer some unique modifications which OHDZ has created for select species which require specialized care, *including Mountain yellow-legged frogs* (*Rana muscosa*), Wyoming toads (*Anaxyrus baxteri*), and Puerto Rican crested toads (*Peltophryne lemur*).

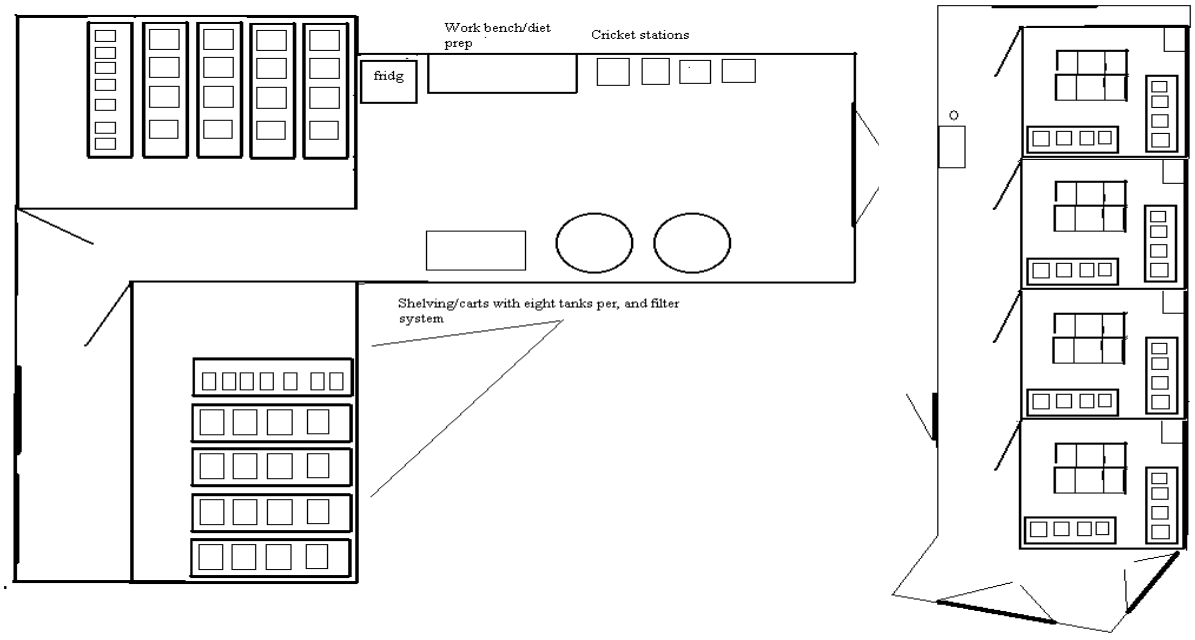


Figure 1. *Left:* Floor plan of one Isolation Amphibian Room (IAR) with dedicated food preparation area. *Right*: Floor plan of additional IARs.

Figure 2. Close-up of the 1 in. (2 cm) aluminum angle pieces holding the 1.5x1.5 in. (3.8x3.8 cm) aluminum tubing and storm door.

Figure 3. *Left*: The portable heating/air condition unit and dedicated footwear placed in each room. *Right*: Combined heating/cooling window units added above the door frame to provide colder temperatures. A CoolBot controller (seen to the left of the unit) has been added to the unit to achieve temperatures as low as 38F.

Figure 4: *Left*: Shelves of polycarbonate amphibian enclosures with shop lights installed typical of IARs. *Right:* Enclosure lids showing modifications for UV light penetration. Larger mesh can be used for larger amphibians (which feed on larger food items), but finer mesh should be used for smaller species.

Figure 5: City source water is pre-filtered using a Reverse-Osmosis (RO) system (*along the wall*) and then reconstituted with appropriate salts and minerals (*visible on the shelf*) in the 300-gallon tank (*to the right*) for 30 minutes before distribution to holding tanks in each isolation room.

Figure 6. *Left:* Shelving with removable stand-pipe drains visible within the front of each polycarbonate enclosure. *Right*: The installation of the over-sized drain system under each shelf in the IAR; bulkhead from each enclosure fits within the "T" fitting below for passive flow when the interior standpipe is removed.

Figure 7. Wastewater vats (*left*) typical for each IAR which are treated with concentrated bleach (r*ight)* overnight before being disposed into the municipal sewage system.

Figure 8: *Left*: Stacked room wastewater collection tubs with sump pump below to pump water to the central treatment station. *Right*: The two black barrels serve as the central treatment station for the entire area allowing for wastewater disinfection before release into the municipal wastewater system through floor drain. Note: All drain pipes are thoroughly-directed into the tubs/barrels/drains to minimize splashing of potentially diseased or chemically contaminated wastewater.

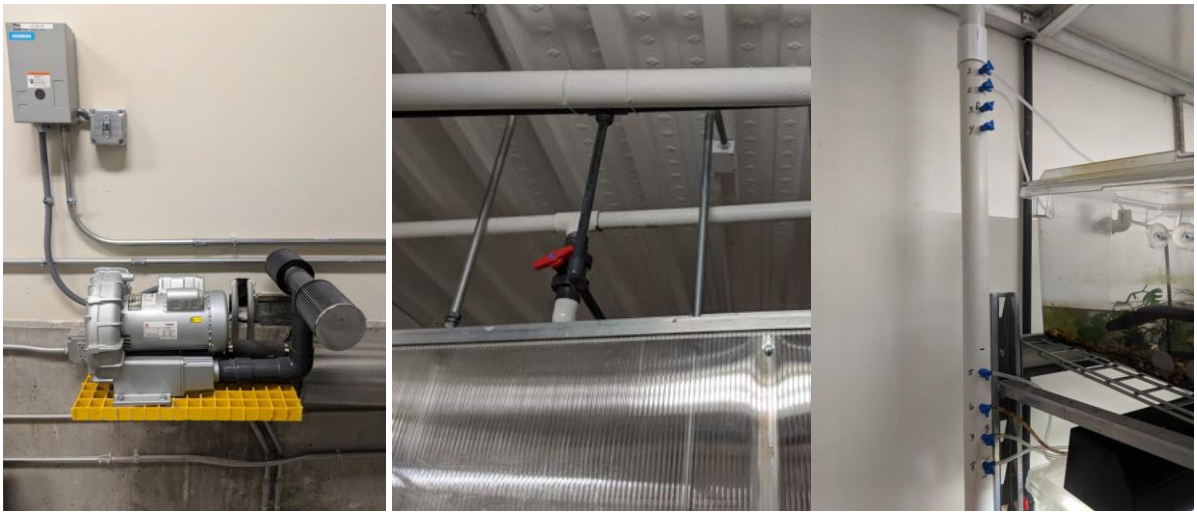


Figure 9: *Left*: Air blower for the entire IAR facility. *Center*: Overhead PVC pipe with isolating valves that direct the air to each IAR. *Right*: Main air manifold within an IAR with small commercially available plastic airline valves tapped into the PVC; airline tubing is attached and run into enclosures as needed.

Figure 10: Specialized set-up for Mountain yellow-legged frogs (*Rana muscosa)* that provides improved water quality and allows water depths to vary 5-10" depending on breeding needs. *Left*: Flow through tubs with floating turtle docks for basking and feeding. *Right*: The sump houses filtration components such as K1 biological media, granular ferric oxide for phosphate removal, and filter socks as mechanical filtration.

Figure 11: Wyoming toads (*Anaxyrus baxteri*) "condos" where two tubs are connected via a PVC tunnel (*visible at the top of the photo*) for easy access to offer toads the options of burrowing in soil substrate (*left*) or utilizing a deep-water pool (*right*). All aquatic tubs can also be linked to a single sump for common filtration. OHDZ has found that these condos provide varied environments for better animal welfare and allows staff to maintain more animals better with less maintenance time.

Figure 12: Single tub enclosures for up to five adult Puerto Rican crested toads (*Peltophryne lemur*). PVC hide tubes have been created to mimic natural limestone caverns where the animals hide during the daytime. This modification increases the usable surface area of the tub and encourages natural climbing and hiding behaviors.

Example 8.
NORTHWEST TREK WILDLIFE PARK'S OREGON SPOTTED FROG REARING FACILITY (2012)
Information and photos submitted by Allison Abrahamse, Northwest Trek Wildlife Park

Introduction
The Northwest Trek Wildlife Park participates in the recovery efforts for the Oregon spotted frog (*Rana pretiosa*) with several other zoos and agencies. Since this is a regional species, they can be housed in outdoor enclosures. Although not every facility maintains Oregon spotted frogs in the same type of setting, this is one example of how facilities can accommodate local species.

Type of Construction and/or Modification
NTWP remodeled a free-standing garage into a workspace complete with sink, refrigerator, stove, and space necessary for diet preparation and storage of materials. The insulated garage is temperature controlled and utilizes a specialized filter to eliminate excess iron from local well water. In addition, a chain link kennel was constructed outside of the garage to house adult frogs (Figure 1). The frogs are reared in 300 gallon holding tanks[15] within the kennel, with each tank able to hold approximately 100 animals. Separate 300-gallon reservoir tanks with de-chlorinated water are adjacent to each respective holding tank, along with a dedicated pump used for water changes (Figure 2). The size of kennel needed is relative to the number of tanks used for rearing. To prevent predation from above, the kennel is covered with nylon netting, and hot wire runs around the top edge to deter climbing predators (e.g., raccoons).

Estimated Total Square Footage
A total of 1,400 sq. ft. is used for the NTWP Oregon spotted frogs. The converted garage is approximately 600 sq. ft., the enclosed kennel is approximately 400 sq. ft., and the space with the reservoir tanks is about 400 sq. ft.

[15] Rubbermaid® stock tanks

Initial Set-Up Costs for Facility

The total cost to create this facility with four holding tanks and four reservoir tanks was approximately $7,745, which does not include costs for the "kitchen/storage" area remodel, labor, or installation. Cost approximations are detailed as follows:

Fencing	$1200
Netting	$150
Hot wire	$150
300-gallon stock tanks	$375 per tank
800W titanium aquarium heaters	$45 per tank
Temperature controller	$28
Timers	$25
Thermometers	$20
Air pump	$210
Air stones/tubing	$35 per tank
Water pump	$200 each
Hoses	$20
Lids (tight construction mesh w/bungee ring suspended over PVP pipe diameter support rod)	$100 each
Cleaning equipment (buckets, nets, turkey basters, etc.)	$30 per tank
Net-pens (pool noodles and aquarium nets; houses approx. 20 eggs each)	$12 each
Water quality testing kits	$50 each

Major Challenges

One challenge faced was a lack of amphibian expertise on staff, leading to a significant amount of time invested in reading related literature and talking to more experienced colleagues. Other beginners should not be surprised to do the same, and it is recommended to have one or more mentors from outside the institution if in-house experience is lacking. Even with more experience under one's belt, collaboration with other facilities is still one of the best resources.

The next challenge faced was an unexpected amendment to the rearing protocols that now incorporated the use of supplemental heat to accelerate the development of tadpoles and frogs. Adding heat precipitated the need to start improvising and acquiring items that had not been budgeted. Staff time dedicated to addressing this issue immediately ballooned, and the ultimate demands for electricity required significant investment in electrical infrastructure among other features.

Another challenge faced was related to water quality. The facility is located in a rural area and uses well water which requires extensive water quality testing; this would normally be conducted by a municipal water department in suburban or urban areas. Because the well water has high iron content and is not processed by a municipal water treatment plant, an iron filter was installed to produce an acceptable influent to the Oregon spotted frog facility.

Useful Additions and Features

The literature was mixed with respect to the need for full-spectrum lighting however, NWTP was conservative and invested in full-spectrum lighting for rearing eggs and young tadpoles indoors (Figure 3). Floating net-pens (Figure 4) in outside holding tanks negate the need for artificial full-spectrum lighting. A good quality water test kit with freshly stocked reagents is recommended.

In the author's opinion, institutional commitment for all departments and at all levels is the single most important *feature* needed to succeed.

Areas for Improvement (i.e., planned differently or improved)

Have seasoned amphibian experts on staff prior to beginning a project, ideally with experience working with the same or a closely related, species. Shy of that, employ committed seasoned generalists, who are willing to learn, hustle, adapt, communicate, and persevere.

Figure 1. Outdoor Kennel that houses adult frogs.

Figure 2. Each holding tank for live individuals (inside the fencing) has its own separate reservoir tank (outside the fencing) that holds de-chlorinated filtered water.

Figure 3. Tadpole rearing enclosures inside of remodeled garage.

Figure 4. Net-pens currently used for tadpole rearing outdoors.

Example 9.
JACKSONVILLE ZOO'S SAVE THE FROGS EXHIBIT (2020)
Information and photos submitted by Dino Ferri and Mark Beshel, Jacksonville Zoo and Gardens

Introduction
The Jacksonville Zoo decided to retrofit an old koala exhibit and dedicate the space to amphibian conservation.

Type of Construction and/or Modification
Retrofit that was completed in 2008 for *Year of the Frog*, which included biosecure rooms with dedicated water treatment systems (Figure 1).

Estimated Total Square Footage
About 1,500 sq. ft.

Number of Isolation Rooms
Currently there are two isolation rooms (120 sq. ft. each; Figure 2) and one exhibit room (80 sq. ft.; Figure 3).

Initial Set-up Costs for Facility
$30,000 to retrofit the entire building and set up the amphibian areas: $20,000 for building modifications and $10,000 for educational graphics, including a $2,500 mural. There is currently a plan to replace existing graphics with upgraded digital graphics.

Major Challenges
Quarantining new animals in accordance to best veterinary practices and standards can be challenging for this collection and space (i.e., minimizing the spread of potential pathogens within a retrofitted building that was not designed to this purpose).

Useful Additions and Features

The public is able to view the amphibians in quarantine while in a comfortably air-conditioned building. The isolation rooms have skylights, which not only help illuminate the space, but provide more natural photoperiods for the amphibians.

Areas for Improvement (i.e., planned differently or improved)

More space would be nice in order to work with additional species. Improved signage and interactive graphics would be preferred. Finally, increased staffing time from 4 hours to 8 hours daily would be ideal for this space and collection.

Figure 1. Sink and water treatment area for each room

Figure 2. Isolation room

Figure 3. Exhibit room

Example 10.
TOLEDO ZOO'S AMAZING AMPHIBIANS (2012)
Information and photos submitted by R. Andrew Odum, Toledo Zoo

Introduction
In May 2008, the Toledo Zoo opened its permanent dedicated *Amazing Amphibians* (AA) facility in the Depression-era Museum of Science building constructed in 1937. The area of AA was roughly divided into thirds, with one-third each dedicated to biosecure amphibian populations, keeper service, and public education. The first goal of this facility was to provide a conservation education experience for the Zoo visitor focused on both local amphibian species and the worldwide diversity of the Amphibia. The second goal was to provide four isolated and discrete biosecure amphibian facilities to serve as areas for *ex situ* conservation efforts. Three of these isolation areas were dedicated to amphibian reintroduction programs, although as of 2019 the AA has been eliminated and collection relocated due to building modifications.

Type of Construction and/or Modification
New space within an existing historical building.

Estimated Total Square Footage
Total square footage of AA is 2,000 sq. ft.

Number of Isolation Rooms
There are four biosecure isolation rooms that vary in size from 100-170 sq. ft.

Initial Set-up Costs for Facility

The total project budget was approximately $750,000. This amount included the structure; caging; display; interpretive graphics; environmental control systems; plumbing; new electrical service and electrical installations; keeper service areas; and biosecure facilities.

Major Challenges

Available funds did not cover the labor for equipping and fitting the reserve areas, life support systems, and biosecure facilities; these were completed by keeper staff. Fabrication of the elevated floors and curbs in AA required a more expensive alternative as the existing floors of the old Museum building were inadequate support the weight of concrete.

Useful Additions and Features

AA featured four separate isolation rooms connected to a common pre-isolation hallway (Figure 1). The hallway had a unique access point from the public area with no direct connection to other animal areas and was used for staff preparation prior to entering isolation areas, dedicated invertebrate culture area, and storage. It included a curtained area for privacy, where keepers wash and change into scrubs prior to entering any isolation rooms.

Elevated floors with curbs in all service areas allowed for drains and prevent water on the floor from flowing between service areas. A structure of wood framing, plywood, and composite-layered epoxy-flooring formed service area floors with drain troughs and continued up each wall for approximately one foot creating a waterproof barrier. Each isolation room entrance had an 8 in. sill to prevent water from entering or exiting the room at the doorway (Figure 2).

The walls were made of 6 in. metal framing, 5/8 in. plywood sheathing, and covered with a fiberglass reinforced panel (FRP) system with molding. This created a waterproof seal which facilitates cleaning. All walls were insulated with fiberglass, and a clear plastic vapor barrier was installed inside the plywood on both sides.

Each independent isolation room had its own air handling system, using commercially-available refrigeration equipment. There was no fresh air provided in the design of the isolation rooms, however, air was exchanged with the hallway when the keeper entered and exited for daily servicing. A constant unidirectional flow exhausts air that was exchanged between the hallway and isolation rooms, preventing cross-contamination between rooms as only one isolation room door was opened at a time. Direct-wired time-clocks and thermostats provided a day/night temperature change.

Hot and cold domestic water supplies, carbon-filtered water (for dechlorination)[16], and reverse osmotic (RO) water were available in AA. All wastewater from enclosures, keeper areas, and biosecure rooms was routed to a single common drain system in the floor trough, allowing disinfection if necessary; however, wastewater was routed to the municipal water treatment plant and no further treatment was considered warranted (Pessier and Mendelson, 2010).

A new electrical service was installed for the entire facility. All room circuits were protected with ground fault circuit interrupt breakers (GFCI) installed within the panels. The electrical outlets are

[16] 3M backwashing filter from Grainger® - Part # 3P971

installed in the raceway system at a height of about six feet and separate circuits were provided for timers and continuous power.

Shelves were created out of composite decking 2x4 in. lumber and stainless-steel fasteners. The shelf tops are made of half-inch high-density polypropylene purchased from a local plastic suppler. Cage lighting was provided by track light systems and 50W Eiko® EXT/SU 12V halogen bulbs with the lens removed (Browne et al., 2007).

All the glass tanks[17] were drilled and fitted with ¾ in. PVC bulkhead fittings. A false floor was installed, and the drains were piped to external standpipes to prevent cage flooding (Figure 3). A quick-drain method was provided by the PVC ball valves installed below the level of the tank bottom. These drains were piped into a common drain with an air-gap to prevent siphoning of water between cages. The common drain emptied into a floor trough drain piped to the building's wastewater system. Rain systems were later installed using the carbon filter water supply.

Staff maintained biosecure isolation for each of the rooms by donning dedicated footwear, head coverings, and clean scrubs; disinfecting hands before and after animal exposure; and utilizing latex gloves between each cage (Figure 4).

Areas for Improvement (i.e., planned differently or improved)

One issue that was not anticipated was the amount of dew produced each morning when the temperatures changed from nighttime lows to daytime highs; addressed by using off-the-shelf domestic dehumidifiers to help dry the air during temperature transitions.

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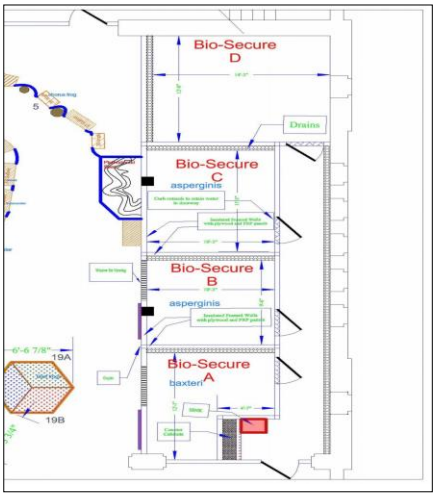

Figure 1. The isolation area hallway used to access the four individual biosecure rooms (labeled A-D) in AA is evident to the right.

[17] Zoo Med® terraria typical

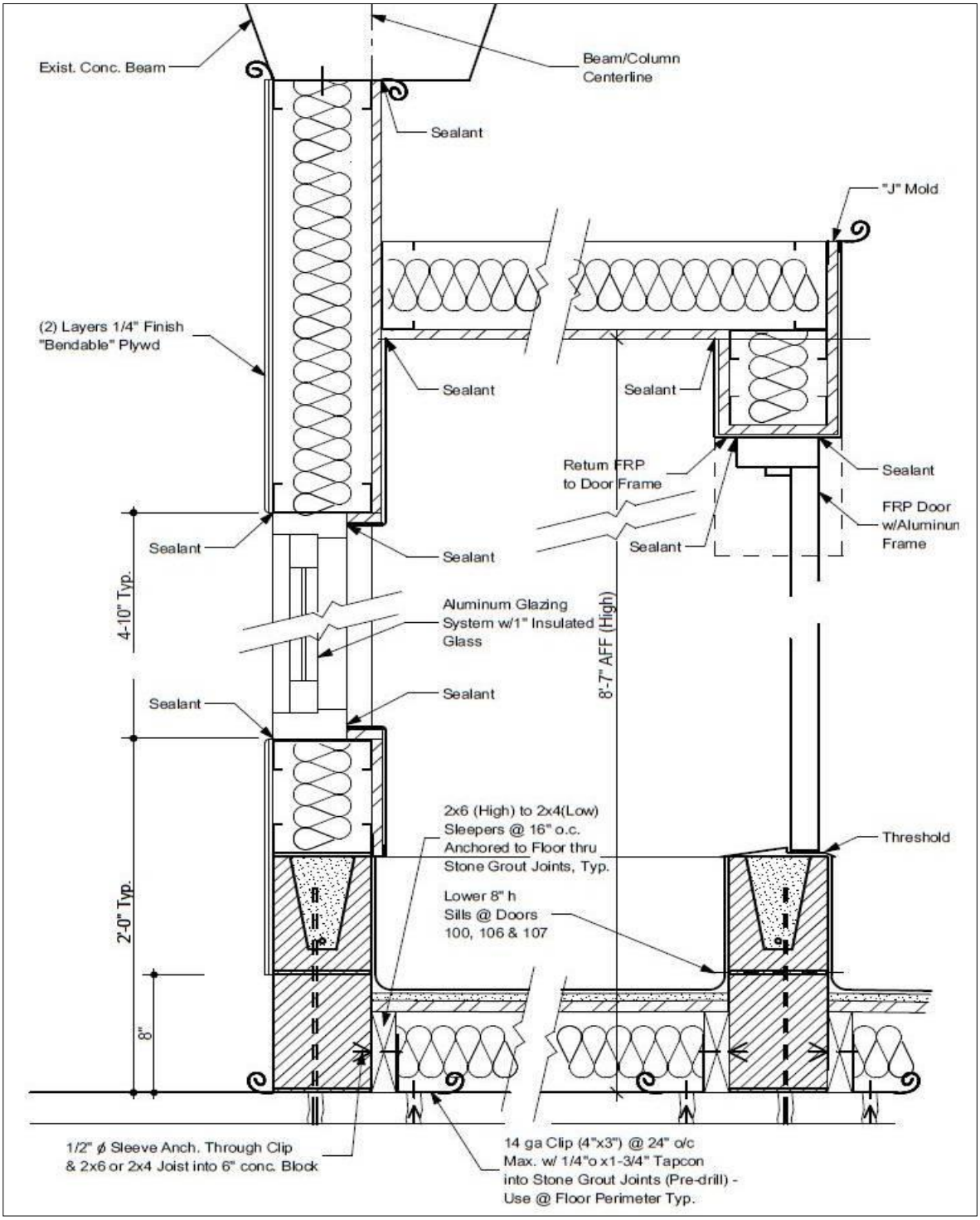

Figure 2. Detail for the floor, wall, and door structures. Note the elevated door threshold to prevent water from exiting and entering the room under the entrance door.

Figure 3. Each tank had its own standpipe overflow created out of "T" and flexible PVC, serviced by a quick-drain valve. All these drains flowed into a two-inch common drain pipe created by drilling a hole in the pipe and loosely fitting the ¾-inch drain into the pipe. This prevented siphoning between cages.

Figure 4. Elevated door threshold into an isolation room from common hallway. Note the dedicated footwear for hallway and each room.

Example 11.
MADAGASCAR AMPHIBIAN CONSERVATION PROJECT (2012)
Information submitted by Jennifer Pramuk, Woodland Park Zoo; Photos by Devin Edmonds

Introduction

Slightly more than one quarter of Madagascar's amphibian species are threatened with extinction. Habitat destruction and over-harvesting are the greatest factors contributing to this dramatic decline. The impending threat of amphibian chytrid fungus (*Batrachochytrium dendrobatidis; Bd*), which is responsible for many of the world's recent amphibian population declines and extinctions, is also of great concern. With the generous financial assistance of multiple organizations, the community-run Malagasy organization Association Mitsinjo has constructed a breeding facility in the Mitsinjo-managed Analamazaotra Forest, two km outside of Andasibe village in east-central Madagascar. This is the first in-country *ex situ* breeding and amphibian conservation project of its kind in Madagascar, and the facility will house managed populations of threatened amphibians to safeguard against current threats as well as the future introduction of *Bd*. The goal is for the facility to become a center for training and education in a bio-region of Madagascar which contains tremendous amphibian diversity and endemism. This project also has begun to develop additional value-added components, including collaborative efforts with the Amphibian Specialist Group (ASG) to perform local and country-wide *Bd* testing as a first line of detection for the disease in Madagascar. So far, four frog species have been selected for this project through discussions with colleagues that developed *A Conservation Strategy for the Amphibians of Madagascar* (ACSAM) and the ASG of Madagascar: *Blommersia blommersae*, *Boophis pyrrhus*, *Heterixalus betsileo,* and *Mantidactylus betsileanus*. These species are absent from zoological collections and little or nothing is known of their husbandry. They were selected based on their varied life histories and presumed correlated differences in care parameters to provide technicians with a diverse set of husbandry experience during training. Technicians from Andasibe have been trained in proper quarantine, biosecurity, and acclimation protocols, in addition to animal health procedures that ensure the health of each frog before it is transferred to permanent housing. The project's second phase will involve development of educational materials and graphics to

accompany an exhibit that will display some of the frogs to the public. Tourists will be charged a nominal fee to view the exhibit and these admission funds will augment the livelihoods of Malagasy technicians running the facility.

Type of Construction and/or Modification
This project was almost completely new construction, but was built upon the foundation of an old, abandoned forest station in the Analamazaotra Forest Reserve. The foundation included a partial concrete pad and walls. The facility is solidly constructed of bricks, mortar, wood, plaster, concrete, and a zinc sheet metal roof. Basic construction of the facility was completed in late winter of 2011.

Estimated Total Square Footage
The total square footage of the facility is 185 sq. m (Figure 1).

Number of Isolation Rooms
The facility includes three primary biosecure rooms for culturing live foods (Figure 2), maintaining managed populations of frogs (Figure 3), conducting husbandry research, and for quarantine (Figure 4).

Initial Set-up Costs for Facility
Initial set up costs were approximately $45,000 which included facility construction, on-going maintenance, tanks, shelving, electrical wiring, amphibian husbandry materials, and equipment for the production of live food. Early and significant funding was received from the AZA Conservation Endowment Fund and more recent support was awarded by Conservation International, Wildlife Conservation Society, Woodland Park Zoo, Cleveland Metroparks Zoo Africa Seed Grant, Durrell Ivoloina Training Course Small Grant, Tree Walkers International Amphibian Conservation Partnership, and the Amphibian Ark Seed Grant.

Major Challenges
Construction of municipal water and electrical lines to the facility has been the greatest challenge to the project. Politics have delayed the delivery and installation of a water line in the facility for almost seven months. Electricity is an even greater financial obstacle as the electric company wants to charge a substantial amount for installing power lines to the facility. Alternative energy, such as from solar panels, are being investigated to power the facility. Fortunately, a recent grant from Conservation International will allow the purchase and installation of large rain barrels that will be used for the primary water supply.

Additional challenges include sourcing materials in-country (e.g., plastic boxes, aluminum framing, glass, and silicone). Materials such as prefabricated aquariums, which are taken for granted in the United States, are nearly impossible to obtain in Madagascar.

Useful Additions and Features
A rainwater collection system is being developed so that the technicians do not have to hand-carry water to the facility. Solar panels are being sought to use as a reliable power source.

It was necessary to construct an exclusion fence for zebu and other large animals to prevent damage to the facility itself.

Areas for Improvement (i.e., planned differently or improved)

Improved communication with other international organizations prior to project initiation would have been tremendously beneficial. Relationships that were damaged due to poor communications early on are now being repaired; this could have been prevented if plans were more thoroughly vetted with other stakeholders from the start.

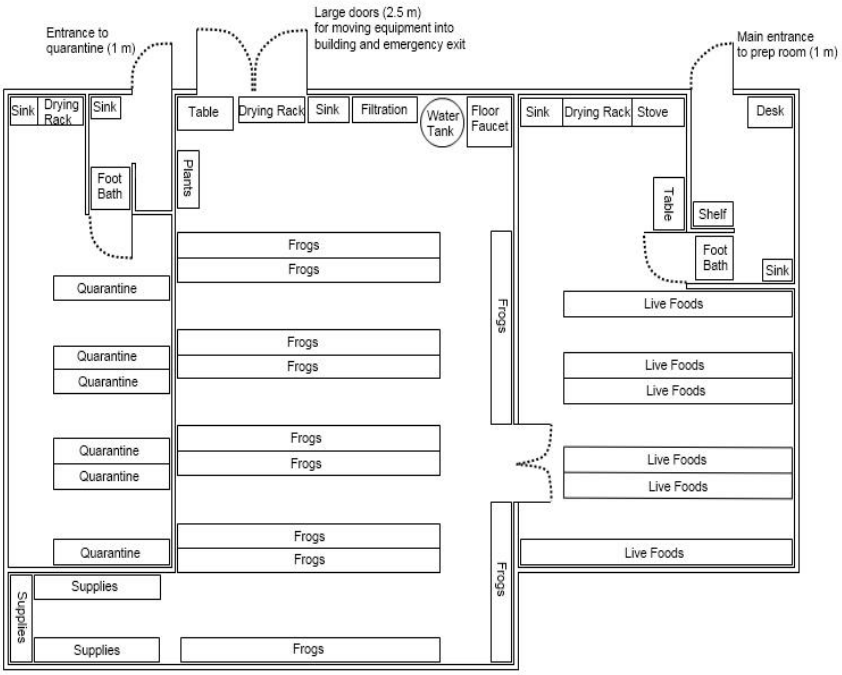

Figure 1. Floor plan

Figure 2. Insect Room

Figure 3. Anteroom to quarantine

Figure 4. Quarantine Room

Example 12.
HOUSTON TOAD FACILITY AT THE HOUSTON ZOO (2020)
Information and photos submitted by Melissa Spradley, Houston Zoo

Introduction
An underutilized building space on zoo grounds was modified to hold Houston toads (*Anaxyrus houstonensis*). Interior walls and a door were installed to isolate a section of the building and an HVAC unit was added to allow year-round temperature control. Adding this space allowed the Houston Zoo to increase their assurance colony by 250 toads on top of the existing main quarantine building that housed ~500 toads.

Type of construction/modification
Interior modification to an existing building to isolate an area and allow climate control.

Estimated total square footage
516 square feet of additional space to the already existing 1100 sq. ft. of space.

Number of Isolation Rooms
The main toad area has two isolation rooms, a work/storage room, and an office space. This modified space adds one additional isolation room for a total of three isolation rooms.

Initial set up cost for facility
$5,838 for construction and lighting and an additional $12,000 for the R/O unit.

Major Challenges

This building was an old structure that required significant modifications to be suitable for holding amphibians. Specifically, adding an interior wall to isolate the space, insulating the roof and walls, and installing a new HVAC system for climate control. Floor drains existed in the structure, but floor slope was poor, so all rack systems had to be plumbed towards the floor drains. Electricians were required to add additional circuits and power outlets to meet the new power needs of the area.

Useful additions and features

An R/O water filtration unit and water holding tank was added to allow a ready source of clean filtered water for daily husbandry. The area is spacious which allows for easy moving about and maintenance of enclosures.

Areas for improvement

The modified structure is located some distance away from the larger, main toad quarantine building necessitating keepers to trek across zoo grounds to complete daily care and maintenance. Painted wood walls make it more challenging to clean and disinfect as compared to walls made of a non-porous material. Some old building features (e.g., a pipe leading from the ceiling into the floor) could not be removed and had to be worked-around when arranging the room.

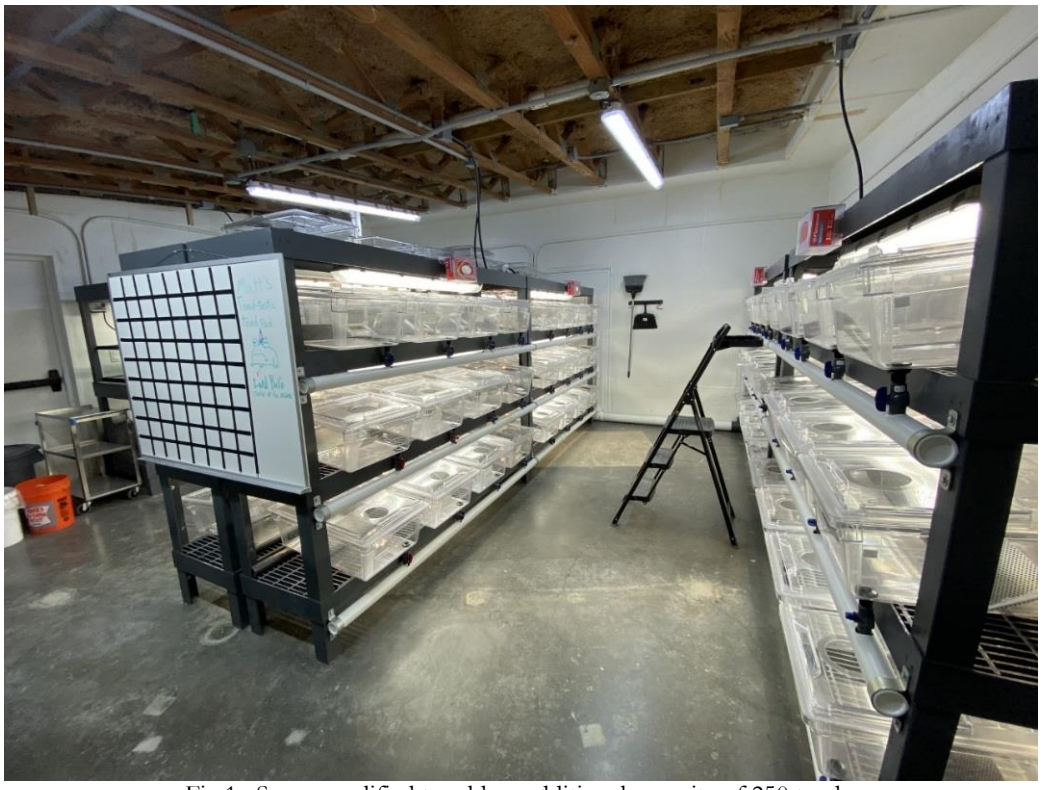

Fig 1: Space modified to add an additional capacity of 250 toads.

Fig 2: The same space from another angle, showing more holding as well as 10 breeding/tadpole rearing tanks. An inconvenient pipe, that could not be removed, is visible between the two shelves.

Fig 3: One of two isolation rooms in the main toad quarantine building.

Example 13.
DARWIN'S FROG BREEDING FACILITY AT THE NATIONAL ZOO OF CHILE (2012)
Information and photos submitted by Danté Fenolio, Atlanta Botanical Garden

Introduction
The Atlanta Botanical Garden helped the National Zoo of Chile (Santiago Zoo) complete a breeding facility for Darwin's frogs (*Rhinoderma darwinii*). This facility also includes two other areas dedicated to rearing Chilean flamingoes and feeder insects.

Type of Construction and/or Modification
A newly constructed building completed in 2009

Estimated Total Square Footage
The amphibian level is approximately 14 sq. m.

Number of Isolation Rooms
Two amphibian rooms, approximately 7 sq. m each. There is an additional insect culture room on the first level of the building that is also about 7 sq. m.

Initial Set-up Costs for Facility
The actual construction cost for all three levels of the building was approximately $360,000 (USD). The cost to equip (e.g., shelving, lighting, aquariums, etc.) the inside of the amphibian isolation rooms cost approximately $15,000 (Figure 1).

Major Challenges
One of the major challenges with setting up this facility was the development of preventative measures in the event of an earthquake. This resulted in the installation of a self-starting generator (Figure 2), a gravity-fed water storage system (Figure 3) and securing aquarium racks to walls. These

additions were proven effective after a large earthquake impacted the area shortly after building construction and left the zoo without electricity and water for a short period of time.

The amphibian isolation rooms are visible to the public through a large glass window (Figure 4). Initially, the window received a lot of sunlight in the mornings and overheated the rooms. Therefore, a large 4x1 m banner was installed above the upper portion of the windows, providing adequate shade from the sun and also serving as a graphic panel that describes the Darwin's frog project to visitors in both English and Spanish.

Useful Additions and Features

A large statue of a Darwin's frog was placed outside of the amphibian exhibit to attract children to the area and entice parents to read and interpret the graphics to them (Figure 5).

Areas for Improvement (i.e., planned differently or improved)

More space for juvenile frogs will be needed in the future. Two additional units were planned to accommodate at least six other critically endangered Chilean amphibians in 2012.

Figure 1. Lab service area

Figure 2. Generator

Figure 3. Water storage tank

Figure 4. Exhibit view

Figure 5. Frog sculpture

Example 14.
FORT WORTH ZOO AMPHIBIAN QUARANTINE ROOMS (2020)
Information and photos submitted by Diane Barber, Fort Worth Zoo

Introduction
The Fort Worth Zoo's herpetarium features four amphibian isolation rooms. Each room houses a different species that is part of a reintroduction program (Figure 1) or serves as an assurance population with the potential for reintroduction in the future. A limited number of staff have access to these rooms, and biosecurity procedures are followed when servicing each area (Figure 2). This type of single-room approach can be applied to retrofit spaces within existing buildings (e.g., hospitals, bird or mammal areas, office spaces, etc.) at minimal costs.

Type of Construction and/or Modification
The amphibian quarantine rooms were intentionally planned as part of the new construction of the *Museum of Living Art* (MOLA), which opened in 2010.

Estimated Total Square Footage
760 sq. ft.

Number of Isolation Rooms
Three rooms are approximately 10x12 ft., and the forth room is 10x20 ft. The amphibian room corridor is about 203 sq. ft., and the remaining 560 sq. ft. is dedicated quarantine space (Figure 3) divided into four rooms.

Initial Set-up Costs for Facility
The rooms were part of a multi-million-dollar construction project. Costs for equipment to initially outfit all four rooms [reverse-osmosis (RO) unit, water storage, sink, racks, tanks, lights, etc.] were approximately $8,225.

Major Challenges

Originally poured concrete curbs were requested to prevent water moving from the corridor under the doors, but due to Americans with Disabilities Act (ADA) regulations, it was not possible to add that feature. Instead, floor sweeps were added to the bottom of all doors and thresholds were installed on the interior floors to prevent water transfer. Daily cleaning and quarantine procedures overcome any minor water breeches that may occur.

Useful Additions and Features

Ultra-violet (UV) penetrating skylights were installed in each of the rooms. Each room has its own sink, hose, water filtration/storage area, and floor drain (Figure 4). A multitude of timed and untimed outlets are on each of the walls. Although the amphibian room's air conditioning system is shared with the adjacent kitchen area, the ducts that supply air to each of the rooms was equipped with a High-Efficiency Particulate Air (HEPA) filter.

Areas for Improvement (i.e., planned differently or improved)

It would have been ideal to have separate heating and cooling systems for each of the rooms, but it was too costly; instead, the four rooms are on a single thermostat. Bigger spaces, or additional rooms, are preferred where possible.

A decade of wear and tear have required each of these rooms to be disassembled, disinfected, repainted, and to have any rusting-shelves and cabinets replaced; this refresh has also allowed the staff to redesign better-functioning enclosures (Figure 5). Other recent improvements included switching these rooms to include more-efficient T5 fluorescent shelf lighting and UV sterilizers to each RO storage system to help overcome concerns about *Mycoplasma* sp.

Figure 1. Rooms are permanently marked to identify occupants and remind staff to follow protocols.

Figure 2. Dedicated footwear, gloves, and lab coat are worn by staff.

Figure 3. This large quarantine room with skylight housed larval hellbenders on the shelves and included a 12 ft. long enclosure to accommodate adults.

Figure 4. Service area within each quarantine room includes a cabinet, sink, hose, and water filtration/storage vat.

Example 15.
THE ARTHUR L. AND ELAINE V. JOHNSON FOUNDATION CONSERVATION CENTER AT THE PHOENIX ZOO (2012)
Information and photos submitted by Tara Sprankle, Phoenix Zoo

Introduction
This facility was built for head starting and managed breeding/rearing of Arizona native species.

Type of Construction and/or Modification
This conservation center is a new facility completed in the summer of 2007.

Estimated Total Square Footage
The building is 3,000 sq. ft. There are four separate labs used for Arizona species, one of which is a 1,250 sq. ft. room dedicated to amphibians (Figure 1).

Initial Set-up Costs for Facility
The total cost for construction of the building was approximately $750,000. The initial cost to outfit the amphibian room was about $5,150, detailed below:

Shelving	$1,400
Rubbermaid food storage tubs	$1,000
Filters	$ 750
Misc. supply	$2,000

Major Challenges
The facility has insufficient storage space.

Useful Additions and Features
The amphibian room is equipped with a deep sink (bathtub), which is ideal for disinfecting tanks and cleaning filters. The lab is visible to visitors so they can see the work being done with the

amphibians, including rearing tadpoles (Figure 2). Solar panels on roof help power the facility (Figure 3).

Areas for Improvement (i.e., planned differently or improved)
More storage space and additional electrical outlets would have been useful.

Figure 1. Interior view of the amphibian lab

Figure 2. Tadpole rearing tanks

Figure 3. Exterior of the facility exhibiting solar panels

Appendix III: Recommended Approved forms of Euthanasia for Amphibians

Amphibian embryos and eggs are resilient to agents commonly used in euthanasia of adult amphibians, and thus using those agents is considered irresponsible as death is not guaranteed. It is recommended that the below methods are used for the different life stages of amphibians.

Recommended methods for euthanasia of **amphibian eggs and embryos**:

- Freezing
    - This is mainly appropriate in tropical and neotropical species, as eggs and embryos of some temperate species can survive temperatures up to -20 C for over a week. If embryos and eggs can be sustained below -20 C for over 24 hours, this is acceptable for all species.
- Ethanol
    - Sedate in 5% ethanol for 15 minutes, then immerse in 95% ethanol for 1 hour.

Recommended methods for **amphibian larvae and adults** (dosing will vary by species, life stage, and size):

- MS-222/Tricane methanosulfonate (topical bath)
- Clove Oil (topical bath)
- Benzocaine (topical)
- Pentobarbital (injectable)

References

Underwood, W., Anthony, R., Gwaltney-Brant, S., Poison, A. S. P. C. A., & Meyer, R. (2020). S7.3.4 Acceptable Methods in ***AVMA guidelines for the euthanasia of animals: 2020 edition***. Schaumburg, IL: American Veterinary Medical Association.

Wright, K.N., and Whitaker, B.R. (2001). **Amphibian Medicine and Captive Husbandry** (pp.120-121). Malabar, FL: Krieger Publishing Company.

Appendix IV: General Example of an Amphibian Welfare Assessment

Please note that this assessment can be filled out for an individual housed singly, housed as part of a group, or for a group of individuals housed together. Results can differ between individuals housed as part of the same group. If a ***Somewhat*** or ***No*** column is selected, it is recommended to review that issue for that species/specimen and make appropriate adjustments.

	Species: Date: Individual or Group/Habitat:	Yes	Somewhat	No	N/A	Not clear	Notes
1.	Does it appear the physical environment meets the needs of the animals in terms of size?	☐	☐	☐	☐	☐	
2.	Is the density of animals appropriate for the housing space?	☐	☐	☐	☐	☐	
3.	Does it appear the physical environment meets the needs of the animals in terms of complexity (visual barriers, hiding places, climbing, burrowing, swimming, etc.)?	☐	☐	☐	☐	☐	
4.	Do animals make appropriate use of a variety of locations and features in the habitat?	☐	☐	☐	☐	☐	
5.	Does the environment provide conditions (temperature, humidity, temperature, lighting, water quality etc.) similar to natural environment appropriate for the species?	☐	☐	☐	☐	☐	
6.	Does the environment undergo natural changes, such as seasonal temperature or light cycle variation, to mimic the animal's natural climate?	☐	☐	☐	☐	☐	
7.	Does the habitat have adequate backup life support systems or a generator?	☐	☐	☐	☐	☐	
8.	Does it appear that social environment is appropriate in terms of number of animals, species, demographic composition (ages and sexes)?	☐	☐	☐	☐	☐	
9.	Does each animal interact with conspecifics in the manner expected?	☐	☐	☐	☐	☐	
10.	Do animals have the ability to avoid being disturbed by guests?	☐	☐	☐	☐	☐	
11.	Do animals have the ability to avoid being disturbed by animal care activities (e.g., cleaning, facility maintenance and repair activities, etc.)?	☐	☐	☐	☐	☐	
12.	Is the overall body condition of all of the animals in the habitat appropriate?	☐	☐	☐	☐	☐	
13.	Do all of the animals appear in overall good health?	☐	☐	☐	☐	☐	
14.	Do any of the animals have a history of disease or injury that requires changes in management?	☐	☐	☐	☐	☐	
15.	Are diets delivered in species-appropriate ways/schedules (content, texture, taste, frequency, and time of day)?	☐	☐	☐	☐	☐	
16.	Are the animals' diets varied for purposes of enrichment?	Frequently ☐	Sometimes ☐	Rarely ☐	☐	☐	
17.	Does each animal appear to be displaying a variety of species-appropriate behaviors?	☐	☐	☐	☐	☐	
18.	Are any species-specific behaviors prevented either through space or management?	☐	☐	☐	☐	☐	
19.	Have any welfare concerns been reported for this individual or environment? If yes, please note concern and outcome in Notes section.	☐	☐	☐	☐	☐	