Principles of Program Development and Management for Amphibian Conservation Captive Breeding Programs

By Luis Carrillo, Kevin Johnson and Joseph R. Mendelson III

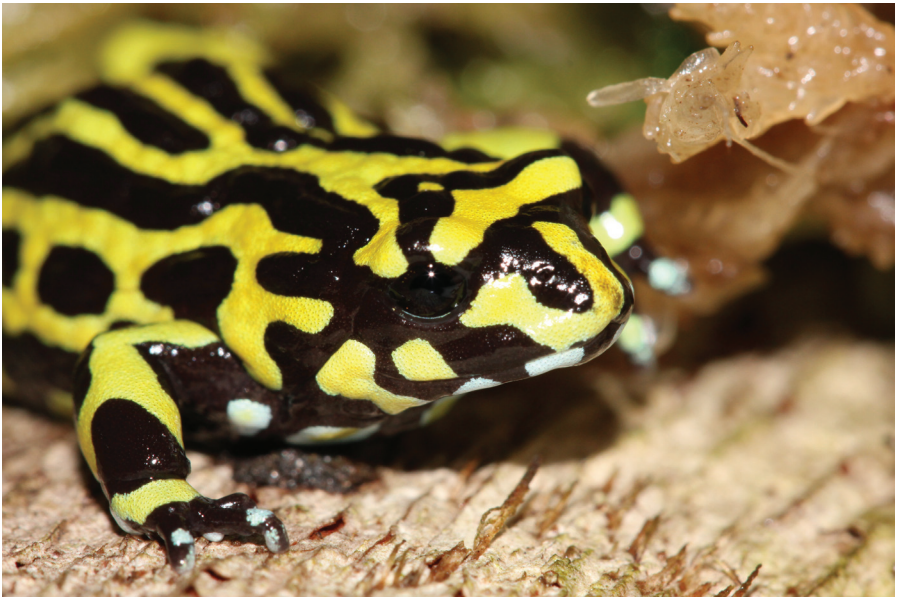
The multiple threats to global amphibians require multifaceted conservation programs to ensure continued survival of what remains of amphibian diversity. The IUCN Amphibian Conservation Action Plan (ACAP; Gascon et al., 2007) identified such programs, based on information available at that time. Among the conservation responses recommended was an organized approach to build capacity and inform a global network of independent captive breeding programs for the most endangered species. In response, Amphibian Ark (AArk; www.amphibianark.org) was launched by three principal organizations: the World Association of Zoos and Aquariums (WAZA), the IUCN-SSC Conservation Breeding Specialist Group (CBSG), and the Amphibian Specialist Group (ASG). To meet its mission, AArk has been helping zoos, aquariums and other ex situ (captive) facilities to address the captive components of the ACAP to save as many species as possible.

One of the biggest challenges in dealing with amphibian conservation is the huge number of species threatened worldwide. Based on our experience, expertise and observations of various programs worldwide, and on interactions with stakeholders participating in our workshops and courses, Amphibian Ark has developed a set of general principles to be considered in the development phase of an amphibian conservation breeding program.

Species selection for ex situ conservation programs

Amphibian conservation biologists face the dilemma of which criteria should be used to prioritize the species to conserve, and thus the resources necessary to commit to the conservation of those species. Conservation resources always are limited, species face different classes of threats at different levels, species may be considered to be of differential value based on subjective criteria such as human cultural importance, or arguably objective criteria such as phylogenetic distinctiveness or ecological roles. While no extinctions are tolerable, some situations may be prioritized as being of more immediate concern than others or the necessary threat mitigation may be more, or less, tractable in some situations. Thus, Amphibian Ark and its partners have designed a tool (Amphibian Conservation Needs Assessment, www.amphibianark.org/pdf/AArk_Conservation_Needs_Assessment_tool.pdf) that is as objective as possible to guide the difficult, and sometimes contentious, process of prioritizing species for conservation efforts, and assessing which forms of

conservation response are appropriate. The assessment tool identifies a broad suite of conservation actions, including habitat restoration or augmentation, other threat mitigation such as pollution control, community awareness and involvement, and/or captive breeding for eventual reintroduction.



Northern Corroboree Frog

Captive conservation programs for the Northern and Southern Corroboree Frogs (*Pseudophryne pengilleyei* and *P. corroboree*), are jointly managed by a range of ex situ organizations and government wildlife departments in Australia, as part of well-structured recovery programs, which include a wide range of stakeholders. Photo: Meaghan O'Connor, Tidbinbilla Nature Reserve.

Ex situ conservation of a threatened amphibian species should be considered a necessity when in situ conservation cannot by itself ensure the survival of a species and its ecosystem. Institutions considering the development of an amphibian ex situ conservation program should begin by reviewing and considering the results of previous Conservation Needs Assessments in the country/region. The results of all conservation need assessments conducted so far may be viewed on the AArk Assessment Results page on the AArk web site (www.amphibianark.org/assessment-results/). Assessing species for conservation actions both in situ and ex situ will guide institutions in deciding, with the resources it has (space, staff, funding, etc.) which species should be prioritized for the development of new ex situ programs, which species urgently need field research or protection, etc. The assessment process may also help with gaining governmental authorization and support from relevant organizations such as the IUCN. In conjunction with AArk's Amphibian

Conservation Needs Assessment process, the *Guidelines on the Use of Ex Situ Management for Species Conservation* (IUCN/SSC, 2014) should be used to help confirm that an ex situ program for each species is warranted, and that appropriate planning, monitoring and evaluation are considered and documented.

Amphibian Ark strongly recommends that at least twenty pairs of animals (or groups of individuals) be available as founder animals for establishment of a new ex situ colony. Ideally these would be unrelated and will successfully reproduce, but of course that cannot be guaranteed. Realize that many more than this number may have to be captured to ensure that twenty pairs actually survive and successfully reproduce. You should ensure that there are sufficient numbers of founder animals available, and that they can be legally collected. Amphibian Ark has developed a tool to help calculate the number of founders that should be collected, based on the reproductive biology of the species being considered. The tool is available on the AArk web site (www.amphibianark.org/founder_calculation_tool.htm) and uses data from our Amphibian Population Management Guidelines: (www.amphibianark.org/pdf/AArk-Amphibian-Population-Management-Guidelines.pdf).

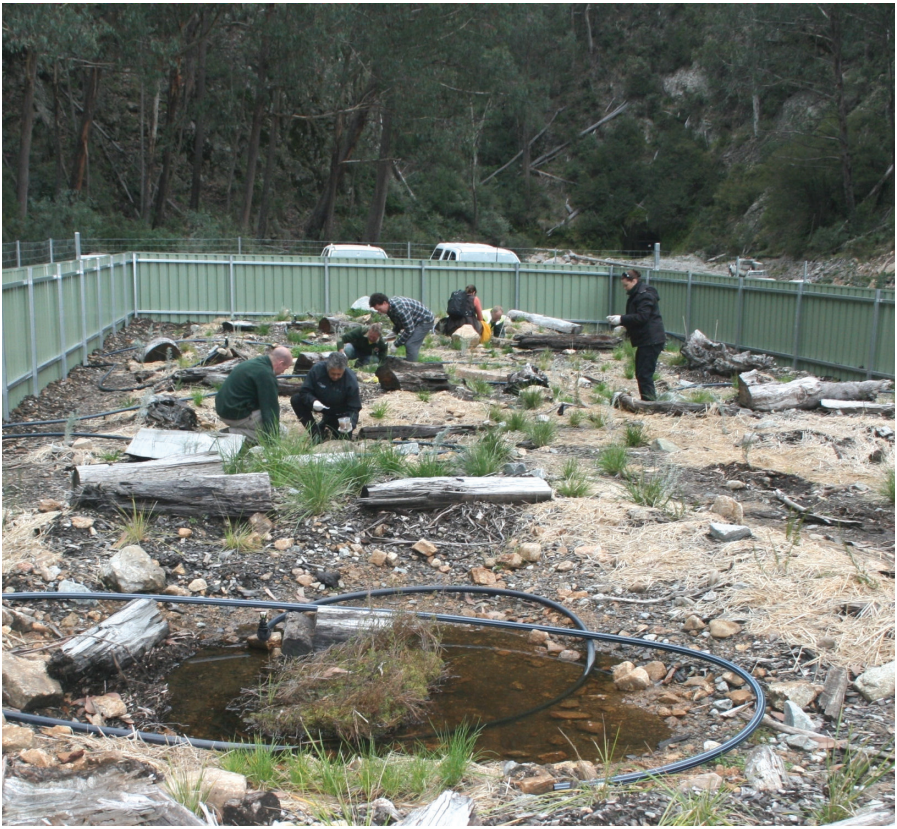
It is also vital to ensure that there is adequate information to understand what the functional unit is that you wish to conserve (i.e. is the "species" you wish to conserve really a valid species?). Species are continuously evolving through time and there are often distinct but not yet unique subunits (evolutionary significant unit or ESU) in the process of divergence within the species and which might warrant independent consideration. If there is insufficient knowledge of the species, a taxonomic study, including phylogenetic analyses of DNA, should be undertaken before considering an *ex situ* program for the species. This should be carried out by, or in conjunction with local field biologists to confirm that the proposed program encompasses only ONE evolutionary distinct unit (ESU) before proceeding**.**

Long-term planning for amphibian conservation programs

When ex situ management of an amphibian species is considered necessary and appropriate, the priority should be to establish the initiative within the range country/area of ecological origin. However, if the perceived urgency of the situation requires it and appropriate infrastructure is not available regionally, then ex situ programs may sometimes be set up outside of the range country/area - ideally while appropriate infrastructure is being established in the home country/range. All ex situ initiatives should be temporary in nature and viewed as just one of the tools that can help in the overall conservation of a species. It therefore follows that strong links between fully integrated ex situ and in situ programs are fundamental to the long-term success of species conservation. This is normally best highlighted through the establishment of a formal Taxon Management Plan that explicitly states the short, medium and long term goals of each component of the conservation initiative. In cases where an ex situ conservation initiative has been established prior to, or in the absence of, a concurrent in situ initiative (e.g. where a political situation currently prohibits in situ conservation measures, or where a disease problem currently invalidates measures to

protect wild populations), emphasis should be placed on establishing the appropriate in situ links as soon as it becomes possible to do so, in order to achieve the end goal of having the species safely back in nature.

At the organization level, a genuine commitment to captive amphibian conservation programs is essential, from the most senior management to the animal husbandry staff who will manage the program on a daily basis. This is especially critical in organizations such as zoos and aquariums that typically house a wide range of species, where there is competition for funds and resources, and where the primary focus is frequently on larger and more charismatic species. For any medium-long term program to effectively reach its goals there must be commitment to resourcing the program, and to ensuring its effective management.



Southern Corroboree Frog release

As part of the exit and release strategy for Southern Corroboree Frogs, captive-bred and captive-reared animals are released into chytrid-free exclosures within the species' natural distribution. Regular releases to augment the wild population have been taking place for the past 6 years. Photo: Michael McFadden, Taronga Zoo.

A key challenge to the success of ex situ conservation programs is ensuring the long-term viability of the program, until such times as the threats facing the species in the wild have been eliminated, and the wild population is once again self-sustaining. In many cases, this can take years, and so ex situ rescue programs might be required to be maintained from five to ten years or more. Long-term viability involves all aspects of the program, such as secure sources of continued funding, consistent staffing and infrastructure, successful health and husbandry protocols, genetic health of the population, and advance planning for housing or reintroduction to the wild of the predicted offspring.

Resources

Adequate resources, in predictable and steady supply, are crucial to the success of an ex situ program. Resources include skilled staff, live food, funding, veterinary services, etc. and must be available for the entire duration of the program. Sufficient resources to support the program for its anticipated lifetime must be available for the program to be successful. Establishing facilities and collecting rescue populations is only the first, albeit perhaps the greatest, expense. However, it is insufficient to support only those first-year expenses without operational support for the long term, which may amount to years or even decades. In addition to financial planning, ex situ programs should begin with an established plan for working with partners to mitigate threats in the wild and, where necessary, getting animals back into the wild, as well as how to distribute and properly manage the progeny of captive animals in the interim. AArk has developed a tool that should be utilized before the implementation of any new ex situ program, to ensure that adequate resources are in place (www.amphibianark.org/program_implementation_tool.htm).

Adequate information of natural history of the species or a related one

It cannot be emphasized enough how important it is to ensure that enough and adequate information of the natural history of the species is in place before bringing animals into captivity. This information and knowledge will not only help in the assembly of adequate enclosures but also in the attempts to breed the species and to eventually reintroduce it.

Genetic diversity

Simple successful breeding of an endangered species in captivity is not sufficient to declare a program a success. In some cases, the founder animals of a species breed one time, soon after being brought into captivity, but never again breed successfully. Attrition of offspring due to natural causes, faulty husbandry, or uncontrollable accident prior to F1 animals reaching sexual maturity may reduce or eliminate the real success of a breeding event. A surprising number of amphibian species may produce seemingly healthy F1 progeny but ultimately fail to produce viable successive generations. There will inevitably be some loss of founder diversity in

populations that prove to be difficult to produce F2 offspring, and this needs to be considered when determining founder numbers. Additionally, in some species, a single pair of founder animals may produce many thousands of offspring, and the capacity of the facility is instantly overwhelmed by a single reproductive event (i.e., a single cohort representing a single genetic line) that is relatively unimportant in the absence of establishment of multiple genetic lineages.

Because amphibians often are maintained in groups, and individual identification sometimes can be difficult, amphibians can be challenging to manage in captivity in terms of maintaining well-documented pedigrees or genetic bloodlines. Nevertheless, the basic principles of genetic health that pertain to all ex situ conservation programs apply to amphibians. AArk recommends this online tool (http://popfrog.org/) to review the basics of successful genetic management of a long-term multi-generational population and to inform the start-up strategy regarding important factors such as number of unrelated founder animals.

Northern Corroboree Frog breeding facilities

Priority considerations when establishing a new program are that under normal circumstances, it should be within the range country/area of ecological origin, and adequate resources, including skilled staff, live food, funding, veterinary services etc., must be available for the entire duration of the program. Photo: Meaghan O'Connor, Tidbinbilla Nature Reserve.

Exit strategy and reintroductions

Viable and genetically robust cohorts of offspring produced by well-managed breeding programs must be maintained in the long term in appropriate facilities. Once successful breeding begins, the progeny need to be managed in order to liberate space for subsequent generations. These animals, and ideally there should be a great number of them, will require adequate space, food, and staffing that, generally, should be allocated across multiple partner facilities. The exit-strategy or final goal ideally would be to raise them until they may be reintroduced into the wild as part of a coordinated in- and ex- situ conservation program involving all relevant government agencies and regional stakeholders. All captive programs which will result in reintroductions must include appropriate goals, objectives and actions, risk assessments, release strategies and ongoing post-release monitoring (IUCN/SSC, 2013).

There certainly are situations where ex situ programs may be started before it is clear that the threats in the wild have been - or can be - mitigated. This realistic situation is a very difficult challenge to confront and may lead to conflicts in priorities and values among stakeholders. Unfortunately, the situation of diseases, such as amphibian chytridiomycosis that is now endemic in the historical ranges of many threatened species, can represent just such a conservation challenge. Such is the case with the Species Survival Plan® (AZA-SSP) for the Panamanian Golden Frog (*Atelopus zeteki*), led by the Association of Zoos and Aquariums in which a large number of genetically healthy lineages of frogs derived from an appropriate diversity of founder animals are maintained in a number of North American zoos. However, the intractable threat of chytridiomycosis across the native range means that the exit strategy is unclear. Alternatively, another AZA-SSP program for the Puerto Rican Crested Toad (*Peltophryne lemur*) is similarly robust but, after adequate resources applied to habitat restoration and protection in the native range, the exit strategy of reintroductions is accomplishing the goal of establishing viable wild populations. These two examples highlight the importance of developing a robust exit-strategy during the planning phase of any new ex situ program. At a high level, this is factored into AArk's Conservation Needs Assessment process so that the reality of mitigating the primary threat(s) to any particular population or species is considered and appropriate priorities given to those species.

Alternative rationale for ex situ breeding programs

Planning an ex situ program for amphibians that is relevant to conservation may include goals other than ultimate reintroduction of captive-produced offspring of a critically endangered species. AArk has identified a number of such programs that are important and may well fit the needs, mission, and possible limitations of specific institutions. These alternative conservation roles, outlined in the Conservation Needs Assessment tool (web link above) include "Ex Situ Research" in which animals in the program are explicitly being produced for purposes of research, which may be in the

realms of laboratory research (e.g., controlled studies of the pathology or veterinary treatment of chytridiomycosis) or to develop and refine husbandry techniques. Such exploratory husbandry research may involve a small number of a threatened species, or a surrogate species that is closely related with a similar biology. In either case, the goal is to inform subsequent ex situ programs with the focal threatened species. An ex situ program for a non-endangered species may be warranted if the stakeholders decide that a surrogate program is the best plan, for example in order to train new staff or troubleshoot new facilities or protocols using a non-endangered species but closely related or ecologically similar species.

Discussion

The principles outlined here derive from our experience in working with amphibian conservation breeding programs around the world in a wide variety of types of facilities, institutions, and programmatic scopes. Important concepts such as biosecurity, genetic management, and long-term secure funding are simple to consider. However, maintaining their integrity over the many years that a new program will likely be needed is a real challenge given the realities of personnel changes, or changes in the nature of threats to amphibians. Because most ex situ conservation programs are developed in response to emergency situations, stakeholders are sometimes required to balance the contradictory realities of developing a secure long-range plan in a short amount of time. This can result in the ironic situation where funds are available to build a new building, for example, but program managers cannot be assured that funding will be in place for two keepers in that facility ten years from now.

We cannot emphasize strongly enough the crucial importance of planning for the logistical and genetic management of the animals in the program and the need for a realistic exit strategy. Our experience has repeatedly shown us the mistakes and resources wasted when programs are overly ambitious in scope - trying to save too many species, for example - or when they failed to plan for the simple eventuality of raising or placing thousands of metamorphs. Similarly, significant changes to the long-range plan must be very carefully considered and can involve making difficult or controversial decisions, such as declining to take in a large group of critically endangered frogs that were unexpectedly confiscated at the airport or culling offspring if the captive population outgrows available holding space, and reintroduction to the wild is still not yet possible.

Amphibian Ark's mission is to ensure the survival and diversity of amphibian species, focusing on those that cannot currently be safe-guarded in their natural environments. We help to coordinate conservation programs implemented by partners (zoos, aquariums, museums, universities, NGOs, and private conservationists) around the world, with our primary emphasis on programs within the range countries of the species, and with a constant attention to our obligation to couple captive conservation measures with necessary efforts to protect or restore species in their

natural habitats. The AArk web site (www.amphibianark.org) contains a wealth of information to assist with all aspects of ex situ amphibian conservation programs, and AArk staff (info@amphibianark.org) are always available to provide specific advice, training and support to individual amphibian programs.

With appropriate planning, resources and commitment, all ex situ organizations are able to implement and support conservation programs for threatened amphibians, thereby taking a positive step towards saving some of our most threatened species. If more organizations are willing to follow the principles outlined in this article, to work with species that have been assessed as needing urgent ex situ programs, and to be well-prepared to commit to a potentially long-term program, we can reduce the chance of losing more species, and ensure the survival of those species that most need our help.

References

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APPENDIX I

Husbandry and Biosecurity Standards

Husbandry and biosecurity standards can be divided into three categories based on the intended Role of the animals in captivity.

Basic Specimens maintained *ex situ* for **Conservation Educational*** purposes with no requirement for research and no prospect of release to the wild
• Separate footwear per room and/or footbaths at entry/exit
• Treatment/decontamination of **any and all** waste water from enclosures and rooms housing amphibians prior to discharge/disposal
• Incineration of all amphibian enclosure waste – soil, leaves, plants, food items, faeces, bodies (after post-mortem examination), if the species is held in an area outside of the range area of local origin.
• Scheduled water changes – automated or manual
• Water free of pathogens and other chemical contaminants
• Escape-proof housing of a size appropriate for species
• Pest-proof housing (rodents, cockroaches, ants etc) to prevent pathogen transfer and/or predation/escape of amphibians
• Appropriate cage furnishings wherever necessary
• Exposure to natural light (or good artificial equivalent) if exposure is normal in natural history of the species
• Appropriate temperature for natural history of the species (mean temp)
• Appropriate food, dependent on species – with supplementation (vitamin/mineral)

Intermediate Specimens maintained *ex situ* for **Ex Situ Research*** purposes with no prospect of release to the wild
All Basic standards, but also:
• Individual instruments (tongs, nets, bowls, tanks, pumps, filters etc) per enclosure and/or species
• Change gloves (non-powdered) for each enclosure
• Design of enclosure should minimize keeper/animal contact
• Maximize use of automation in water quality maintenance/watering
• Maintain a consistent/directional flow of husbandry routine – from low risk and high importance species/individuals to high risk and lower importance species/individuals
• Climatic conditions (lighting, photoperiod, temperature, rainfall, humidity, etc) should follow the natural cycle for the species and be automated wherever possible
• Highest level of record-keeping

Advanced Specimens maintained *ex situ* for conservation breeding purposes **(Ark/Rescue/ Supplementation)*** with the ultimate expectation of release to the wild
All Basic and Intermediate standards, but also:
• One species or local assemblage of species per room/unit
• Separate uniforms per room (stays in room)
• Food coming from known and trusted source; 3-month period of familiarization with natural food types recommended prior to any release, if the species is a dietary specialist that might find it difficult to locate a particular food. (Ensure that natural foods are collected from the release site, as a number of pathogens, including chytrid, can live or survive on insects).
• During pre-release familiarization, monitor condition of specimens to determine fitness for release – thorough health screening including regular and frequent PCR screening for chytrid fungus over several months

NB – *ex situ* includes any and all animals removed from their wild habitat whether within or outside of their native range and country.

APPENDIX II

Conservation Roles

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

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

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

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

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

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