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IUCN Guidelines for amphibian reintroductions and other conservation translocations

First edition

Edited by Luke J. Linhoff, Pritpal Soorae, Gemma Harding, Maureen A. Donnelly, Jennifer M. Germano, David A. Hunter, Michael McFadden, Joseph R. Mendelson III, Allan P. Pessier, Michael J. Sredl and Mallory E. Eckstut

IUCN/SSC Conservation Translocation Specialist Group

SSC Species Survival Commission

Conservation Translocation SPECIALIST GROUP

calgaryzoo

ASG IUCN SSC Amphibian Specialist Group

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Cover photo: (clockwise from top-left) Hellbender (*Cryptobranchus alleganiensis*) © Brian Gratwicke, Leopard Frog (*Lithobates pipiens*) © Lea Randall, Natterjack Toad (*Epidalea calamita*) © Jim Foster, Great Crested Newt (*Triturus cristatus*) © Brett Lewis

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Tribute to Phillip John Bishop (1957-2021)

There are times in our lives when we meet people that change how we see things, and the way that we conduct ourselves. These changes can be personal or professional. In the case of Phil, it is both.

It can be argued that Phil's involvement in global amphibian conservation began in 2000, when he was appointed Chair of the New Zealand Regional Working Group for the IUCN SSC Declining Amphibian Populations Task Force (DAPTF), established by the IUCN Species Survival Commission (SSC) in late 1990 to investigate instances of global amphibian declines and disappearances.

Later, Phil was appointed to three key roles: 1) Chief Scientist for the Amphibian Survival Alliance (ASA) in 2011, coordinating a global response to the amphibian biodiversity crisis; 2) Co-chair of the IUCN SSC Amphibian Specialist Group (ASG) in 2012, whose aim is to provide the scientific foundation to inform effective amphibian conservation action; and 3) Steering Committee and Executive Committee member of the Amphibian Ark in 2011, which seeks the global survival of amphibians, focusing on those that cannot be safeguarded in nature. Phil's contributions to global amphibian conservation with these roles were numerous, and he always ensured that amphibians were well represented in the global conservation arena. These positions were all voluntary in nature, demonstrating Phil's commitment to global amphibian conservation.

Reintroductions and translocations were near and dear to Phil. He presented on his translocation research at scientific meetings, and co-authored a book chapter and papers on the subject, including a highly-cited review on herp reintroductions. Phil was involved in translocations of three threatened species of native New Zealand frog, *Leiopelma* spp., in various ways (collecting and releasing individuals, carrying out post-release monitoring, and providing technical advice on translocation methods). Furthermore, Phil supervised several postgraduate student projects focusing on translocation of *Leiopelma*, providing guidance on the topic, including technical aspects of translocations (suitability of release sites, assessment of success), and advocating for the involvement of local community members in translocation and monitoring. In 2017 he was also actively involved in the reintroduction of an extirpated frog (*Pelophylax lessonae*) to Norfolk (UK).

Phil firmly believed in supporting new generations to advance amphibian conservation and was an award-winning and dedicated teacher. His brilliance, commitment, and no-nonsense approach, together with his irreverent sense of humour, made him stand out for all the right reasons.

We miss him terribly. But we also know that it is our turn to pick up the baton and continue the legacy that Phil worked so hard to build.

Thank you, Phil.

Executive summary

The number of amphibian reintroductions and other conservation translocations has increased in recent decades. Clearer guidance to plan, implement, and obtain resources for amphibian reintroductions is needed to improve conservation outcomes. The vast diversity within Class Amphibia, which contains 8000+ species, makes generalisations difficult, but many common themes exist concerning amphibian reintroductions. This document is designed to provide guidance, best practices, and links to helpful resources that will be useful for a wide variety of practitioners involved in amphibian reintroductions.

Reintroductions are highly interdisciplinary. Information useful for undertaking amphibian reintroductions is scattered, and the available information may not be known to many conservation practitioners. Therefore, we have included links to numerous resources and planning tools that were collated by multiple experts. We understand that each amphibian species will likely require unique strategies for successful translocation. Furthermore, poorly known species may require a large amount of novel research, creativity, and trial and error. The technologies required to successfully reintroduce some species may not even exist yet. Amphibian reintroductions are challenging and may not always work, but amphibian reintroductions may be the best or only option for conserving some species. This document outlines the most important considerations for each stage of an amphibian reintroduction and provides a brief overview of each topic with references to numerous specific resources for further information.

Key messages related to amphibian conservation translocations and reintroductions

- Amphibian reintroductions are challenging, and they require substantial planning, resources, and long-term commitment.
- Clear and concise objectives, goals, and an exit strategy should be defined before commencing a project.
- Amphibian reintroductions are not particularly well understood and should be considered experimental. Many amphibian reintroductions have failed; however, successful examples do exist. Released with these guidelines, we have compiled a separate collection of amphibian reintroduction case studies to assist conservation planners.
- What works for one amphibian species may not work for another. When working with a poorly studied amphibian species, conservation practitioners may need to develop and test entirely original tactics and protocols. Although dozens of amphibian species have undergone reintroductions, many taxonomic families and genera of amphibians do not include any species that were previously utilised in a reintroduction.
- Designing amphibian reintroductions as experiments will help develop the overall understanding of what works and what does not. However, only the dissemination of protocols, experiments, and case studies that both succeeded and failed will enhance our understanding of amphibian translocations.
- Difficulties associated with keeping amphibians in captivity should not be

Key topics at each stage of implementing a successful amphibian reintroduction

1. Pre-translocation, planning and risk assessment

- Feasibility & risk assessment areas:

- Habitat
- Demographics
- Genetics
- Captivity
- Environmental and ecological impacts
- Disease issues
- Animal welfare
- Local people and communities
- Selecting a new release site
- Integrating research into translocation design

2. Implementation

- Capture plan

- Transporting animals

- Post-capture husbandry and health screening

- Release methodology

3. Post-release actions

- Post-release monitoring

- Continuing management

- Applying adaptive management

- Dissemination of information

underestimated. Developing amphibian husbandry protocols to effectively keep and manage captive amphibian populations may take considerable time, research, and creativity.

- The diversity of amphibian life history strategies makes selecting the optimal life stage (e.g. eggs, larvae, sub-adults, or adults) for amphibian translocations important for success. Because the best life stage(s) for release will vary between species, modelling and experimental trials will help practitioners develop optimal protocols.
- The spread of disease through translocated amphibians is a very real risk and concern; however, with proper disease risk management and appropriate caution, most disease risks can be ameliorated.
- After release, post-release monitoring may be difficult because of the cryptic nature and small size of most species, but monitoring should be carried out at the best level possible.
- Because of the highly experimental nature of amphibian reintroductions, adaptive management is critical for success. Learning from mistakes, operational flexibility and being creative to solve on-the-ground problems will greatly contribute to increasing chances of success.

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Acronyms & abbreviations

AArk - Amphibian Ark

ASA - Amphibian Survival Alliance

ASG - Amphibian Specialist Group (ASG)

ACAP - Amphibian Conservation Action Plan

Bd - *Batrachochytrium dendrobatidis*

Bsal - *Batrachochytrium salamandrivorans*

CTSG - Conservation Translocation Specialist Group

DAPTF - Declining Amphibian Populations Task Force

GAA - Global Amphibian Assessment

IUCN - International Union for Conservation of Nature

OIE - The World Organisation for Animal Health (formerly the Office International des Epizooties (OIE))

PCR - Polymerase chain reaction

SDM - Structured Decision Making

S.M.A.R.T. - Specific, Measurable, Attainable, Relevant, Timely

SSC - Species Survival Commission

Introduction and scope of guidelines

Reintroductions and other conservation translocations are being performed for an increasing number of endangered species. The International Union for Conservation of Nature (IUCN) Species Survival Commission (SSC) has published generalised best-practice guidelines for conservation reintroductions and other translocations that are applicable to a wide variety of flora and fauna (IUCN/SSC, 2013). However, translocation methods are often difficult, interdisciplinary, and highly variable across taxa. Successful conservation translocations may require largely different and specialised methodologies that depend on both the type of organism being translocated and the programme's goals.

Amphibians are currently experiencing an unprecedented conservation crisis, with thousands of species threatened with extinction and many more in decline (Stuart et al., 2004). Because of the rapidity of these declines, the number of amphibian *ex situ* conservation programmes and amphibian conservation translocations have risen dramatically in the last two decades (Harding et al., 2016). Amphibians (class Amphibia) are an extraordinarily diverse and ancient vertebrate lineage of over 8,000 species (Frost, 2019) that belong to three orders: Anura (frogs and toads), Caudata (newts and salamanders), and Gymnophiona (caecilians). There is vast diversity of natural history, ecology, behaviour, life history, morphology, and physiology within Amphibia, and this makes generalisations for conservation management of amphibians difficult.

This document, the IUCN SSC Conservation Translocation Specialist Group's (CTSG) *IUCN Guidelines for amphibian reintroductions and other conservation translocations* (2021), was developed to provide guiding principles for conservation practitioners who work with amphibians. It is important that our current understanding of amphibian translocations is synthesised to direct future conservation efforts. We hope this document addresses many of the taxon-specific complexities of amphibian translocations. Additionally, we provide references to many resources throughout this document that should be useful for amphibian conservation managers engaged in or thinking of starting a conservation-focused amphibian translocation.

The process to create this document was initiated by a group of experienced translocation practitioners who identified the need for best-practice guidelines for amphibian translocations. An initial draft was started by several members of this group and others in 2012 but was not carried to completion. The project was restarted in 2015 by a new core group of authors. The core group developed an initial draft text. Then, there was a period of review of the draft guidelines by the IUCN SSC Amphibian Specialist Group, Wildlife Health Specialist Group, Invasive Species Specialist Group, Climate Change Specialist Group, and the CTSG in 2018. Additionally, a public consultation period was opened for one month between March and April 2019, during which the public could review and submit comments to the IUCN SSC CTSG.

As the number of amphibian translocation projects increases, research will be presented at professional meetings and publications will appear in peer-reviewed scientific journals. In fast-moving fields, including reintroduction biology, updates to best -practice guidelines tend to be frequent, and a web-located document can be more easily amended or edited than a “print” book project. As such, these Guidelines are produced primarily as an electronic document to enable updates to be accomplished quickly and efficiently as new information on translocations of amphibians accumulates. Additionally, everyone interested in the topic can access the information contained herein. The need for amphibian translocations is likely to increase in the future, and hopefully the results from current projects will be used to inform future work to ensure survival of threatened and endangered amphibian species. We hope that the publication of these guidelines stimulates future research on amphibian reintroductions and provides a clear guide to improve both current and future conservation-focused amphibian translocations.

Northern leopard frog © Lea Randall

Definitions and classifications

Translocation terminology has varied though time and by taxonomic disciplines of biology. In this guide, we follow terminology as defined by the *IUCN Guidelines for Reintroductions and Other Conservation Translocations* (IUCN/SSC, 2013).

Translocation – is the movement of an organism by human agency that is then released in a different area. Translocation is the most general and highest-order term referring to human-mediated movement of a species, subspecies, or lower taxon (including any part, gamete, or propagule that might survive and subsequently reproduce). Conservation translocations are defined as the intentional movement and release of a living organism where the primary objective is for conservation purposes. These translocations usually attempt to improve the conservation status of the focal species locally or globally, and/or to restore natural ecosystem functions and processes. The following describe more specific types of translocation.

a. **Population restoration –** is a conservation translocation within the species historic, indigenous range and comprises two activities:

   i. **Reinforcement –** is the intentional movement and release of an organism(s) into an existing population of conspecifics, and is synonymous with the terms augmentation, supplementation, and restocking. Reinforcement may be done for several reasons, including to enhance population viability, increase genetic diversity, or increase the representation of specific demographic groups or stages.

   ii. **Reintroduction –** is the intentional movement and release of an organism(s) inside the species' indigenous range from which the species has disappeared.

b. **Conservation introduction –** is the movement of an organism, by human agency, outside its species natural range (past or present) to achieve a conservation goal. Non-conservation introductions may be performed for a variety of reasons, but are not the focus of this document (e.g. pest control, aesthetics, or religious purposes). Conservation introductions comprise two activities:

   i. **Assisted colonisation –** is the intentional movement and release of an organism outside its indigenous range to avoid extirpation of populations or extinction of the focal species. Assisted colonisation is primarily carried out where protection from current or likely future threats in the current range is deemed less feasible than at alternative sites outside the current range.

ii. **Ecological replacement –** is the intentional movement and release of an organism outside its indigenous range to perform a specific ecological function.

c. **Mitigation translocation –** is the intentional removal of organisms from habitat that will be lost through anthropogenic land-use change or threat, and release at an alternative site.

Conservation translocations for amphibians usually employ one or more of the following:

- Direct translocation of eggs and/or individuals (tadpoles, juveniles, or adults) from one site to another with minimum time spent in captivity.
- Head-starting, including rearing wild-collected early life stages (eggs, larvae, and juveniles) to later life stages (sub-adults and adults) in captivity before releasing them. Head-starting is typically done to circumvent periods of high mortality in early life stages of wild amphibians.
- Release of captive-bred animals of any life stage to the wild.

Translocations of the critically endangered Southern Corroboree frog (*Pseudophryne corroboree*) have included introducing them to fenced enclosures within their wild distribution © Michael McFadden

Reasons for amphibian translocation

Most translocations involving amphibians are undertaken for the goal of improving the conservation status of the translocated species. This involves a goal to enhance the population size, range and/or future prospects of the species. Some translocations are driven by non-conservation motives, such as pest management or religious reasons, but these occur far less often than conservation-driven projects. There have also been unintentional translocations of amphibians which are outside the scope of these guidelines (e.g. coqui frogs, *Eleutherodactylus coqui*, introduced to Hawaii); see section Environmental and Ecological Impacts within this document for further discussion.

Conservation translocations

The first documented conservation translocations and reintroductions involving amphibians were in the 1960s. Translocations of Natterjack toad (*Epidalea calamita*) spawn between ponds in the UK started in 1966. The head-starting programme for the Houston toad (*Anaxyrus houstonensis*), led by the Houston Zoo, followed in 1978. In the 1980s, concerns about amphibian declines were being voiced globally, and prompted increased focus on conservation management. Several critical conservation breeding and reintroduction programmes started around this time, such as reintroduction of the Mallorcan Midwife Toad (*Alytes muletensis*) to Mallorca, Spain in 1985. Beginning in the 1990s, the number of amphibian conservation reintroductions started to rapidly increase.

The emergence of infectious diseases such as chytridiomycosis and other large-scale threats that result in rapid amphibian population declines and extinctions in the wild has led to a resurgence of the ark concept, whereby at-risk species are rescued and placed in captive assurance populations until the threats can be mitigated (Bowkett, 2009; Soulé et al., 1986). Following the Global Amphibian Assessment (GAA) in 2004 and the Amphibian Conservation Action Plan (ACAP) in 2007, *ex situ* conservation measures, such as captive breeding and reintroduction, were considered necessary to curb declines.

The organisation Amphibian Ark (AArk) was formed in 2007 and given the task of implementing the *ex situ* components of the ACAP. To date, AArk has evaluated the conservation needs of around 2,700 (approximately 34%) of the world's amphibian species through workshops in 41 countries. Since publication of the ACAP, captive breeding programmes for conservation have increased by almost 30% (Harding et al., 2016). The AArk estimates that there are 500 species in need of intervention by *ex situ* conservation programmes. Currently, approximately 213 amphibian species have been involved in *ex situ* conservation programmes, most of which had or have reintroduction as their main objective (Harding et al., 2016).

Mitigation conservation translocations

Mitigation translocations are initiated when human land-use change conflicts with the continued persistence of a species' population at a particular site (Germano et al., 2015). These translocations are usually implemented in response to legislation or governmental regulation, with the intent of mitigating, minimising, or offsetting a development project's effects on animals or plants that inhabit the site (IUCN/SSC, 2013).

Mitigation translocations are becoming increasingly common, especially for herpetofauna (Germano et al., 2015; Sullivan et al., 2004). Although there has been a great deal of progress in the field of reintroduction biology, results from scientific research are often not applied to mitigation projects. Mitigation translocations may be economically motivated, with anthropocentric pressures and constraints, or mandated by state/federal wildlife protection laws (Germano et al., 2015). Outcomes may be less successful than releases designed to serve the biological needs of the species. In contrast to many conservation translocations for endangered species, mitigation translocations often involve large numbers of individuals (thousands to tens of thousands).

Species-specific mitigation guidelines were developed for a few mitigation translocations (e.g. Great Crested Newt Mitigation Guidelines [English Nature, 2001]). Additionally, the Guidelines for Mitigation Translocations of Amphibians: Applications for Canada's Prairie Provinces (Randall et al., 2018) provides an outline of mitigation best-practices tailored for several Canadian Provinces. In many cases, however, there are no species-specific guidelines, and no requirements to conduct and report post-translocation monitoring of these translocations.

Translocation of amphibians as a regulatory tool may be ill-suited for mitigating environmental damage caused by development projects. Evidence demonstrates that many mitigation-driven translocations have failed (Sullivan et al., 2015) because of lack of application of scientific principles and best practices. If mitigation-driven translocations are to continue, it is imperative that the scale and effects of these releases be transparent, fully documented, reported, and evaluated.

Suitability of amphibians for translocations

Because amphibians are generally described as having a relatively small body size and/or high fecundity, and being low maintenance in captivity (compared with mammals and birds), they are often considered suitable candidates for reintroduction programmes. However, this assumption masks the diversity of life histories and husbandry requirements within the class (Tapley et al., 2015a). Amphibian diversity encompasses great variation, and generalisations are difficult for best practices for translocations. Amphibians may have a larger body size and/or lower fecundity, and may require a higher degree of husbandry expertise compared with many mammal or bird species.

Additionally, amphibian conservation translocations are often unsuccessful or take many years to achieve their goals.

Amphibian translocations are still largely experimental, with no guarantee for success. What may work for one species may be inappropriate for others. Some taxonomic groups or species may require much more time, research, and expense to successfully translocate, and there is no clear guide to which amphibians are more or less suitable for translocation. The case study section included with this document provides many examples and highlights some of the difficulties faced in recent amphibian translocation programmes. Before deciding if translocation will play an important role in your conservation programme, alternative conservation strategies should be seriously considered.

Future directions in amphibian translocations

Conservation translocations have become a tool in global amphibian conservation efforts. The rescue of threatened populations and species carried out as a result of population declines due to disease and other unmanageable threats has led to dozens of species being held in captive assurance programmes, with numerous captive husbandry challenges and few plans for immediate reintroduction. Nevertheless, the process of identifying which amphibian species in captive colony programmes can be successfully released will soon need to be determined, and there should be increased focus on those reintroductions that are most feasible and likely to succeed. Further research and strong partnerships will be needed to establish which species in captivity fit reintroduction criteria and how best to achieve them; this is one of the greatest challenges for amphibian reintroductions in the future. Furthermore, with inevitable habitat fragmentation and destruction, mitigation translocations may become increasingly common for amphibian conservation. Mitigation translocation effectiveness, best practices, and value are important areas of future study that will have direct application to many current conservation problems.

Deciding when a translocation is the best option

Amphibian translocations are typically labour-intensive, expensive, and may take many years to achieve success. Alternatively, some translocations may even be impossible with current technology, methodologies, and understanding of amphibian translocations. Serious consideration is required when deciding if a translocation is the best option for conservation of a population or species. Expert advice must be sought and a critical assessment of the translocation's potential for success should be completed. Practitioners should consult Section 3 and Annex 3 of the *IUCN Guidelines for Reintroductions and Other Conservation Translocations* (2013) for a thorough discussion.

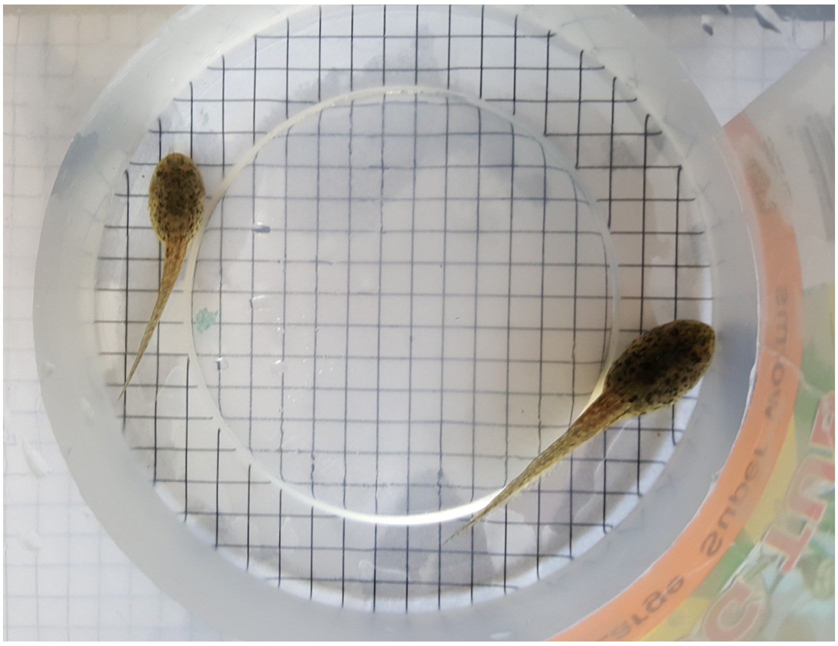
Amphibian translocations have had mixed success, indicating that they are both difficult and have no guarantee of success. Management of a species in their natural habitat is often a more cost-effective and lower-risk conservation option than translocations (National Species Reintroduction Forum, 2014). In a review of 20 years of published herpetofaunal translocations, Germano & Bishop (2009) found a 41% success rate of reported translocations. This figure is consistent with success rates of translocations of other animal taxa (e.g. Fischer & Lindenmayer, 2000; Griffith et al., 1989; Miskelly & Powlesland, 2013; Wolf et al., 1998). However, it was shown that publication bias exists in reintroduction biology; therefore, these figures may overrepresent success rates (Miller et al., 2014). Although translocation success rates have increased over time via incorporation new knowledge and tools (Germano & Bishop, 2009), careful consideration must be given to determine whether a conservation translocation is the best and most effective use of conservation resources.

There are well-developed non-taxon-specific translocation guidelines in place to inform conservation translocation programmes (IUCN/SSC, 2013). Additionally, resources for undertaking comprehensive disease risk assessments can help guide decision makers (e.g. Jakob-Hoff et al., 2014). There are also successful model programmes that can be used as precedents when planning a new programme (e.g. case studies provided in this document). Given these resources, attention should focus on what constitutes an appropriate situation for initiating an amphibian translocation i.e. where the determined threats outweigh the potential risks associated with any translocation.

The first thing that needs to be discussed among stakeholders is whether action is required, because inaction also represents a deliberate conservation decision (Minteer & Collins, 2005; Schwarz et al., 2012). Well-constructed risk assessments should move projects forward with proper precautions, whereas poor (or absent) risk assessments typically result in disagreement, which generally results in delayed or non-implemented programmes. Such conservation paralysis misapplies the precautionary principle, as it implies (rather than decides) that inaction or delayed action is the best course of action for a given situation.

Situations appropriate for amphibian reintroduction or translocation programmes may be emergencies (e.g. in the form of unexpected contamination, disease outbreaks, or habitat perturbation), or predictable on different time scales, as informed by modelling of future climate shifts or spreading invasive pathogens or other organisms. In other situations, the timeframe for implementation of a translocation programme is within the control of the programme managers (e.g. captive stock is ready and available, and the recipient site is prepared, so managers simply wait until circumstances such as weather and habitat restoration seem ideal). Consequently, action plans that may require future translocation efforts should include specific information, risk assessments, and preliminary plans for reintroductions, especially for wild-to-wild translocations in the event that an unanticipated situation arises.

Emergency amphibian conservation actions have occurred multiple times. These instances can serve as valuable case studies; two examples include the rapid response to amphibian declines in Panama due to spread of chytridiomycosis (Gagliardo et al., 2008), and threats to the Kihansi Spray Toad (*Nectophrynoides asperginis*) by the construction of a hydro-electric dam (Channing, 2006; Lee et al., 2006).



Measuring tadpoles prior to release © Lea Randall

Pre-translocation planning and risk assessment

Tadpole cages © Luke Linhoff

"Assessment of any translocation proposal should include identification of potential benefits and potential negative impacts, covering ecological, social and economic aspects. This will be simpler for a reinforcement or reintroduction within indigenous range compared to any translocation outside indigenous range"

(IUCN, 2013)

Setting objectives and defining success

- Setting clear objectives provides a means to measure the performance of released organisms against programme goals, develop a basis for adjusting objectives through adaptive management, and activate an exit strategy.
- The acronym S.M.A.R.T. is useful for defining translocation goals as they should conform to the following criteria: Specific, Measurable, Attainable, Relevant, and Timely.
- However, setting objectives is a difficult task for most reintroduction programmes (Chauvenet et al., 2016). Although, it is easy to make a generalised objective such as, "to create a new self-sufficient population", the objectives should ideally include clear and precise defining parameters surrounding the objective. How long exactly will the population be monitored until it is considered self-sufficient? How big of a population is required? Objectives may vary widely depending on the initial goals and desires of managers, and not all objectives may be possible.
- To assist in defining objectives and success, the use of Structured Decision Making (SDM) is recommended. Besides requiring the methodical creation of objectives, SDM promotes easier and more transparent decision-making processes throughout a translocation process and is particularly useful when many stakeholders are involved. Although, a full description of SDM is beyond the scope of these guidelines, they have been used successfully in reintroductions, and implementation guides exist (e.g. Chauvenet et al., 2016, Converse & Armstrong, 2016).

Feasibility and design

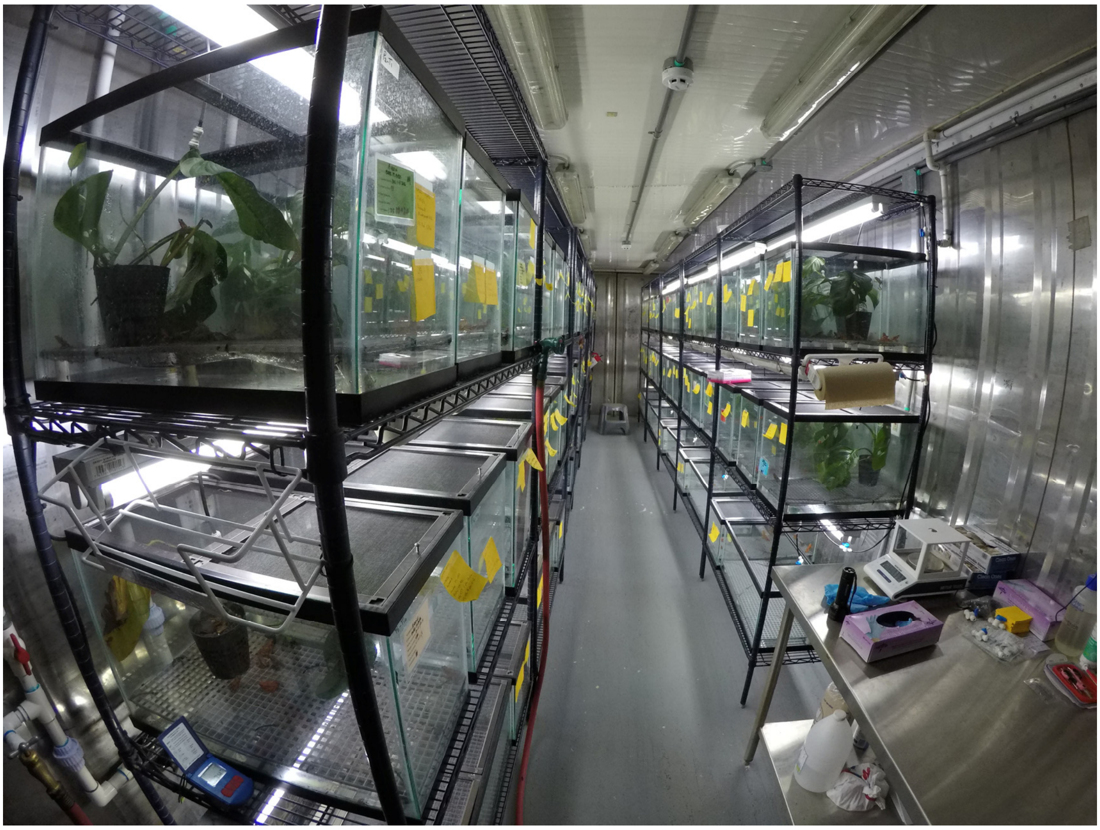
Resource availability

- *Ex situ* conservation breeding programmes, reintroductions, and translocations require long-term commitments and monitoring to have maximal conservation value (Gagliardo et al., 2008; Carrillo et al., 2015; Foster et al., 2018). Amphibian translocations require sufficient funding; personnel; physical resources; multi-year commitments from stakeholders; and careful planning, coordination, and management. Emergency situations may lead to launching a programme that only has funds sufficient for short-term operation, may generate disagreements among stakeholders, and result in the implementation of a programme before it is properly planned out.
- Many current amphibian reintroduction programmes have become decades-long commitments (e.g. programmes for the Houston toad, *Anaxyrus houstonensis*; Wyoming toad, *Anaxyrus baxteri*; and Puerto Rican Crested toad, *Peltophyne lemur*), and long-term financial planning and commitment should be expected. When developing project budgets, it is important to estimate resources for monitoring the pre- and post-release project phases (Nichols & Armstrong, 2012).

- Launching a translocation programme without a clear, validated plan linked to sufficient and realistic financial, human, and logistical resources over the requisite timeframe will jeopardise the success and may nullify the value of the project.

Resource categories for consideration

- *Physical resources:* Are the appropriate agreements in place for long-term access to the site? If the site is remote, how will personnel get there? Will 4×4 vehicles, helicopters, or boats be needed? If animals must be kept in captivity, are husbandry materials (e.g. animal enclosures, building space, clean water, quarantine facilities) locally available? Will specialised survey or monitoring equipment be needed (e.g. radio telemetry gear or audio call loggers)? Captive amphibian populations can consume large numbers of prey items. How will prey items be acquired? Will they be bought or cultivated on-site?
- *Personnel:* Are trained staff available? What expertise is required? Personnel skilled



Converting shipping containers into "frog pods" is a method successfully utilized by multiple translocation programs to house amphibians. The relatively low cost of shipping containers and modular design allows for easy implementation of biosecurity procedures. Because of the small size of most amphibians, large numbers of animals can be kept in a single, compact frog pod © Brian Gratwicke

at amphibian surveying, collecting, husbandry, veterinary, insect cultivation, and/or monitoring may be difficult to find. Is it possible to train additional personal? Will it be feasible for personnel to live or work fulltime at the translocation site if it is remote or in difficult conditions?

- *Financial:* Is sufficient financial capital available for the project timeline? How will the initial funds be raised? What happens if the project takes longer or is costlier than planned? Is there a plan to attract additional donors or fundraising sources? How will money be managed, and who will do it?

Developing knowledge about the focal species and environment

- Understanding the basic biology, natural history, current and former range, threats, and ecology of the amphibian species is vital for their conservation. Information can be gathered from publications, reports, species action plans, and consultations with relevant species experts and those carrying out field research and in situ conservation of the species. However, many amphibian species are poorly studied and associated information may not be available. Targeted research on wild populations or captive animals, which can be a costly and lengthy, yet necessary, process, may be required to fill gaps in knowledge prior to undertaking a conservation translocation.
- Factors influencing survival and fecundity are likely to be the most relevant to a translocation. Other natural history elements will depend on the species. For example, the life stage of translocated amphibians may be key to success of the release. For amphibian species with strong homing instincts, release of eggs or juveniles without strong home site associations may improve the ability to anchor a translocated population to a new site (Bloxam & Tonge, 1995; Germano & Bishop, 2009; Semlitsch, 2002; Tocher & Brown, 2004).
- If captive breeding or extended captivity is part of the programme, recording and measuring habitat data, including environmental parameters and biological parameters (such as diet, spatial ecology, causes of mortality, and habit use of the animal) is particularly important. A comprehensive list of parameters to measure in the field and examples of how they can impact success of captivity and translocation of amphibians was summarised by Michaels et al. (2014). Evidence shows that keeping or breeding captive amphibian species in conditions that differ from natural conditions can have a significant impact upon its fitness and breeding success in captivity (Michaels et al., 2014).
- For threatened and poorly understood amphibian species, obtaining such data can be extremely difficult. Knowing how many individuals, and what life stages and time of year to release is often poorly understood. In cases where such questions are unanswered, experimental, pilot releases can provide essential data and are a good way to test threat neutralisation and confirm causes of decline (Caughley, 1994). For

example, reintroduction of the Iberian frog (*Rana iberica*) and Common Midwife toad (*Alytes obstetricians*) in Spain revealed that larval stages were heavily predated during the winter releases. Consequently, the programme was able to ameliorate the problem by releasing larvae in a different season with lower predation rates (Martín-Beyer et al., 2011).

Topics for developing a knowledge base that may be useful for assessment

- *Biological and natural history knowledge:* This may cover aspects such as physiology, individual growth and development, reproduction, mating systems, social structure, behaviours, physical adaptations, existence of parental care, and population dynamics in the indigenous range.
- *Ecological knowledge:* Categories include biotic and abiotic habitat requirements, intraspecific variation, adaptations to local ecological conditions, seasonality and phenology, dispersal, and interspecific relationships, including feeding, predation, disease, commensalism, symbiosis, and mutualisms.

Identifying initial causes of declines prior to amphibian reintroductions

- Prior to translocation, it is essential to identify all threats to the species or population, both direct and indirect, which might jeopardise the success of the translocation. Amphibians often face unknown or unmanageable threats (Shoo et al., 2011; Stuart et al., 2004). When reasons for decline are unknown, research into reasons for decline should be undertaken to identify and assess possible translocation alternatives.
- In situ conservation and monitoring are vital to understanding how threats are developing. Even where amphibian populations are considered to be extinct in the wild, in situ research and surveys to monitor other amphibian species, habitats, and environmental change should continue.
- Performing pilot releases with robust post-release monitoring can provide useful data on various threats, such as pathogen presence in the environment or predator impact. Models that predict the outcome of a translocation under various scenarios (e.g. with or without threat mitigation measures) can provide useful insight, and highlight risks and how these risks may influence translocated amphibians. Models developed for reintroductions of other taxa may also be useful for amphibians (e.g. Bertolero et al., 2007).

Addressing threats and/or known causes of decline

- An amphibian translocation is not advisable if threatening factors are sustained or uncontrolled in the release area (IUCN/SSC, 2013; Kleiman et al., 1994). However, even if known threats still exist and no other suitable release sites exist, small experimental or pilot releases may be useful for testing threat mitigation strategies, developing practical experience, or gaining a better understanding of a poorly known amphibian's ecology, habitat use, and behaviour that may be otherwise impossible to collect in captivity and is useful for future releases.

- Threats that cause only local extinctions, such as predators or collection for the pet trade, are often acute and controllable, but threats that operate over a large part of the species' range (such as pathogens, widespread land-use change, pollutants, and climate change) are difficult to manage.
- Multiple threats may also interact synergistically, having a cumulatively larger impact on a population relative to each of the threats individually.
- Severity of impact or sensitivity to a threat may vary with demography or life stage. Because species traits can vary regionally (for example, a species may have ecotypes adapted to different habitat types), justifications for translocations must specify the spatial extent of a threat.
- Threat assessments need to consider the adaptive capacity of the target amphibian species; such capacity will tend to be highest in populations with high genetic diversity, long-range dispersal ability, effective colonisation ability, short life spans, high reproductive rates, phenotypic plasticity, and/or rapid evolutionary rates.

Considerations for source and release sites

- It is critical that an evaluation of potential direct and indirect impacts of an amphibian translocation are assessed for the recipient habitat, even if the release site is within the known range of the species in question and primary agents of decline have been mitigated. If a population does not already exist at the proposed release site, there may be clear ecological cause(s). For example, the presence of competitive congeners, predators, and parasite assemblies may be present, and the release may either fail or cause negative impacts on the local biotic community at the site.
- Amphibians are ectothermic and highly sensitive to environmental fluctuations because of their moist, permeable skin. Amphibians translocated between two sites with similar environmental conditions (e.g. water chemistry or soil type) would likely experience less stress than moving animals to a vastly different environment. When performing any type of amphibian translocation, the source and release site should match each other as closely as possible.
- The source site may not be optimal habitat if the source population was actively experiencing declines at the time of collection. Additionally, if a species has experienced major declines throughout much of its range, the location of the last known surviving population(s) of the amphibian species might be atypical, peripheral habitat compared with that of the species' previous core range.

- Recording environmental and biological variables of the source site may serve as a guide when later selecting a release site. If the focal species is no longer present at a site within its historical range (e.g. the species is killed by disease), the ecology and physical parameters at the site may shift in the absence of the focal species' ecological role in the environment. For example, trophic shifts, prey population changes, or expansion of niche competitors may occur after species extirpation, thus render the release site's environment sub-optimal and complicating reintroduction. Thus, it is important to record biological and environmental characteristics prior to both collection and release of animals. Physical and biological parameters outlined by Michaels et al. (2014) provide a list of data to collect at the source site that is useful for both husbandry and release site selection.

Selecting a release site

The four main categories of criteria to consider when selecting a release site are (adapted from Fiedler & Laven, 1996):

- *Physical criteria:* Physical characteristic assessment of a release site should include any parameters that will be critical to the survival of released animals, such as temperature, water pH and dissolved oxygen, alkalinity, humidity, micro-habitat availability, breeding habitat, soil type, and how much habitat is available. The site should by environmentally suitable for year-round survival of the species. If physical criteria of the release site (e.g. water supply or water quality) rely on outside areas, an assessment of surrounding habitat should be done. Are there long-term agreements to protect the stability of the release site's habitat? Will nearby or upstream habitat be developed in the future that may threaten physical criteria of the release site? The probability of stochastic events that may negatively impact the translocation, such as flooding of the release site, should also be assessed. The general size and quality of the habitat will indicate how many translocated individuals can survive at the release site.
- *Logistical criteria:* A release site's logistical criteria include its accessibility to humans and site protection status. If a site is remote, it may increase difficulty for regularly monitoring translocated animals or undertaking research projects. Whether the site requires remediation or restoration prior to a translocation must be considered. Public access and the risk of disturbance or collection of reintroduced animals by members of the public are other factors to be considered as part of any translocation project. When selecting a site, it is important to know what the security of the release site is. Is it a protected nature reserve? If so, does the nature reserve status have implications for the amphibian reintroduction, for example conflicting management objectives or funding arrangements?" Is the land private or publicly owned? Will it be developed in the future? Is it at risk of habitat degradation from threats such as illegal logging? Is there a risk of released animals being collected for the pet trade or food? If it is not protected, can it be protected? Does the site require long-term

management of habitat or security? Will political instability or other local issues impact animals or the safety of people working with them?

- *Biological criteria:* Presence of predators, competitors, and parasites; availability of food resources for all life stages; and risk of hybridisation should be assessed prior to release. Conservationists should ascertain whether the focal species exists already at the site before translocating; if it is, then population augmentation could be considered, or it may be better to choose an unoccupied site.
- *Historical criteria:* Whether a site is known or potential habitat of the species should be assessed. It should not be assumed that habitat formerly occupied by a species is still suitable. The same causes of decline that caused the need for reintroduction may still exist at the release site and thus the site may be inappropriate for use.

Planning for long-term stability and climate change

- When designing translocations that consider the possible effects of climate change, an important starting point of in situ or *ex situ* conservation is an understanding of the basic biology and ecology of the focal species (Michaels et al., 2014; Semlitsch, 2002). Many amphibians are highly sensitive to environmental variation, and changes in climate may influence amphibians more than other taxa (Blaustein et al., 1994; Stebbins & Cohen, 1995).
- Local extinctions of amphibian populations due to climate change have already occurred and will likely increase (Wiens, 2016). Climate change may interact synergistically with other factors such as disease and habitat loss or conversion (Shoo et al., 2011). Additionally, climate change will not affect all amphibian species the same (Blaustein et al., 2001; Lowe et al., 2015; Nowakowski et al., 2016). It is also important to consider the impact that climate change may have on altering key factors, such as predator and prey abundance, seasonality and quality of aquatic and terrestrial habitats, and disease dynamics.
- The effects of climate change on regional populations of amphibians differ (Araújo et al., 2006; Bickford et al., 2010; Duan et al., 2016; Mokhatla et al., 2015). Therefore, it is important that the climate at the translocation release site is suitable for the foreseeable future. To assess the degree to which climate change may affect the region in which the destination site is located, climate change metrics or bio-climate envelope models can be used to assess the likelihood of the climate changing beyond the species' tolerance limits (Garcia et al., 2016). Wright et al. (2015) recommend using multiple climate projection models to address the uncertainty in any one particular model. If climate change alters the conditions beyond the focal species' tolerance, active management of the translocation site may be necessary or the selected site may no longer be deemed suitable. Alternatively, sometimes currently unsuitable land will become climatically "suitable" for a species in climate projections.

- If future climate regimes are beyond the focal species' tolerance in a substantial portion of its range, assisted colonisation may be necessary for species survival (Hoegh-Gouldberg et al., 2008; Gallagher et al., 2015). However, assisted colonisation is not a proven conservation method, and may even prove impossible with current technologies. No assisted amphibian colonisations in response to climate change have been performed at the time of writing. Furthermore, any future amphibian assisted colonisation attempts would likely be controversial among stakeholders and conservation practitioners because of their experimental nature.
- Potential management actions to ameliorate the effects of climate change and negate the necessity for translocations of amphibians affected by climate change should be pursued first. Some non-translocation-focused management actions to ameliorate the effects of climate change on amphibian include (adapted from Shoo et al., 2011): installation of microclimate and microhabitat refugia, breeding site enhancement and restoration, and hydroperiod or water level manipulation at breeding sites. However, solutions requiring a permanent budget and human resource needs should be a last resort.
- Helping species naturally adapt or move is preferable to perpetual intensive management. Maintaining habitat corridors that allow for the natural migration of amphibians to suitable areas or climatic zones under the pressure of climate change should not be overlooked. Some amphibians can travel or migrate long distances if suitable habitat is available. Maintenance of connectivity between watersheds and streams/important wetlands/breeding areas can increase resilience of amphibian populations to the impacts of a shifting climate.

Demographic and population considerations

Modelling reintroduced amphibian populations

- Modelling reintroduced populations typically uses various mathematical and statistical methods to evaluate different parameters (e.g. population growth rate or survival) and predict outcomes or probabilities that certain scenarios will occur in an amphibian translocation. Although a full discussion of population modelling is beyond the scope of this document, using modelling tools while planning or undertaking an amphibian translocation may facilitate assessment of population viability; optimisation of population growth, dispersal, genetic viability, and habitat suitability and selection; and prediction of the effects of climate change on populations in the future. Modelling can also help improve risk analysis prior to reintroductions. Models can be improved by incorporation of post-release findings to improve any future translocations.
- An array of modelling techniques has been developed for reintroduced populations (see Resource Box: Modelling). It is important to realise that methods developed and applied to translocations of different taxa (e.g. mammals or fish) can often be directly applied to amphibian translocations.
- If modelling expertise is lacking in your programme, consult someone who is experienced in conservation modelling and can provide useful and scientifically rigorous recommendations.

Demographic source and release site considerations

- Many amphibian populations and species have high levels of taxonomic and/or genetic uncertainty, which can make demographic planning challenging. Thus, it is important to study the lineage of the population being translocated. Where was it originally collected? Was there historical connection between populations? Is the translocated population the same genetically as animals already at the source site? What impacts might occur if populations are mixed? Is there a benefit to translocating one life stage over another (e.g. larva versus adults)? Will removing animals from the source site impact the long-term viability of the wild source population? The answer to these questions will vary widely depending on the specifics of each translocation. Luckily, a wide array of resources exists to help practitioners make informed decisions (see resource box Demographic Considerations).
- The selection of source site(s) and the number of animals to collect from the wild may vary widely. The impacts on the source population should be considered within the conservation goals of the target species. For example, translocations requiring large numbers of individuals could impact the viability of a healthy source site population and lead to its extirpation. Alternatively, if the goal is to establish a captive colony by using the last known individuals of a rapidly declining species, the entire wild population might be collected if deemed necessary to save the species from extinction. This scenario has happened several times, such as with the Wyoming

RESOURCE BOX: Modelling

IUCN SSC Climate Change Specialist Group (CCSG) has a Modelling Support team that aims to provide technical guidance to SSC specialist groups who want to develop niche models, demographic models, or integrated niche-demographic models for conservation purposes. For further information, see https://iucn-ccsg.org.

Further reviews of modelling reintroduced populations can be found in the following sources, and are applicable to amphibian translocation planning and modelling:

Armstrong, D.P. & Reynolds, M.H. (2012). Modelling Reintroduced Populations: The State of the Art and Future Directions. In: J.G. Ewen, D.P. Armstrong, K.A. Parker and P.J. Seddon (eds.) *Reintroduction Biology: Integrating Science and Management*, pp.165–222. Oxford, UK: John Wiley & Sons.

Beissinger, S.R. & Westphal, M.I. (1998). On the Use of Demographic Models of Population Viability in Endangered Species Management. *The Journal of Wildlife Management* 62:821–841.

Converse, S.J., Moore, C.T. & Armstrong, D.P. 2013. Demographics of Reintroduced Populations: Estimation, Modeling, and Decision Analysis. The *Journal of Wildlife Management* 77:1081–1093.

Fordham, D. A., M. J. Watts, S. Delean, B. W. Brook, L. M. B. Heard, & C. M. Bull. (2012). Managed relocation as an adaptation strategy for mitigating climate change threats to the persistence of an endangered lizard. *Global Change Biology* 18:2743–2755.

Kraaijeveld-Smit, A.F.J.L., Griffiths, R.A., Moore, R.D., Trevor, J., Beebee, C., Journal, S., Apr, N., Kraaijeveld-Smit, F.J.L. & Beebee, T.J.C. (2014). Captive Breeding and the Fitness of Reintroduced Species: A Test of the Responses to Predators in a Threatened Amphibian. *Journal of Applied Ecology* 43:360–365.

Osborne, P.E. & Seddon, P.J. (2012). Selecting Suitable Habitats for Reintroductions: Variation, Change and the Role of Species Distribution Modelling. In: J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon (eds.) *Reintroduction Biology: Integrating Science and Management*, pp.73–103. Oxford, UK: John Wiley & Sons.

toad (*Anaxyrus baxteri*) (Hammerson, 2004). Demographic information from source sites will assist in evaluating the potential impacts of harvesting, and provide a basis for designing and planning a successful translocation.

- An understanding of population genetics will help determine how many individuals should be collected and from which sites. Establishing an inventory of potential pathogens that occur at source sites will provide a basis for evaluating potential disease risks associated with reintroducing animals to a new area.
- In contrast to the source site, demographic and population-level considerations for the release site are primarily associated with the capacity for the release site to support adequate survivorship across all life stages of an amphibian. Key threats that are still operating or are not entirely known may be partially alleviated by consistently high survivorship across important life stages.
- For many amphibian species, it is important to consider their metapopulation dynamics and ensure there is sufficient habitat extent and quality to support the spatial and temporal dynamics of the species across a landscape (Heard et al., 2015; Marsh & Trenham, 2001). Given the capacity for amphibians to be locally adapted to specific environmental conditions, it is desirable that release sites are sufficiently similar to the source sites to maximise the fitness of the released animals.

RESOURCE BOX: Demographic considerations

Demographic considerations are an important component of a successful translocation. Further information reviewing demographic considerations, which can be applied to amphibian translocations, includes the following sources:

Canessa, S., Hunter, D., McFadden, M., Marantelli, G. & McCarthy, M. A. (2014). Optimal Release Strategies for Cost-Effective Reintroductions. *Journal of Applied Ecology* 51:1107–1115.

Converse, S. J., Moore, C.T. & Armstrong, D.P. (2013). Demographics of Reintroduced Populations: Estimation, Modeling, and Decision Analysis. *The Journal of Wildlife Management* 77:1081–1093.

Converse, S. & Armstrong, D. (2016). Demographic Modeling for Reintroduction Decision-making. In: *Reintroductions of Fish and Wildlife Populations*, pp.123. Oakland: University of California Press.

Frankham, R., Ballou, J. D., Ralls, K., Eldridge, M., Dudash, M. R., Fenster, C. B., Lacy, R.C. & Sunnucks, P. (2019). *A Practical Guide for Genetic Management of Fragmented Animal and Plant Populations*. Oxford University Press. New York, NY.

Kissel, A.M., Palen, W.J., Govindarajulu, P. & Bishop, C.A. (2014). Quantifying Ecological Life Support: The Biological Efficacy of Alternative Supplementation Strategies for Imperiled Amphibian Populations. *Conservation Letters* 7:441–450.

Genetic considerations for amphibian reintroductions

Genetics issues associated with reintroductions is a topic with a wide array of available resources (see Resource Box: Genetic considerations). Luckily, the genetic issues that impact reintroductions of most flora and fauna have a lot in common and are directly relatable to amphibian reintroduction programmes. Therefore, this document will not provide a comprehensive summary of genetic considerations, because they are typically not specific to amphibians. It is essential before undertaking any amphibian translocation to research and assess the genetic considerations of how the translocation may impact animals at source and release sites. If genetic expertise is lacking in a programme, assembling a small group of experienced conservation geneticists familiar with translocations to provide advice and assist with planning is highly recommended.

Basic genetic principles in amphibian translocations

- For amphibians maintained in captivity, practitioners should use methodology that retains and maximises genetic diversity. Using studbooks and/or computer programmes (e.g. Allele Retain; Weiser et al., 2012) to optimise genetic diversity may be essential for multi-generation maintenance of captive populations. Luckily, resources for managing the genetics of captive populations have been developed to help with this process.
- For some amphibians, there may still be taxonomic uncertainty. Many species of amphibians remain to be formally described and given a scientific name. There may also be cryptic species diversity within known populations. For example, what we assume might be one species is actually two, or there may be several genetically distinct subspecies within one species which should be managed separately. Checking with qualified amphibian taxonomists about the status of a focal species may help determine if foundational taxonomic research needs to be done to make informed decisions. Generally, managers want to maintain genetically distinct populations and avoid mixing lineages.
- A sufficient number of founders with genetic diversity (e.g. not all siblings) is a key factor in long-term, multi-generational survival of a translocated population and creates the best chance of long-term success.
- Laboratory tools to assess genetic relatedness of different populations or individuals exist but may require significant expertise, time, and monetary commitment to perform. However, some tools, such as the Amphibian Ark Founder Calculation Tool (www.amphibianark.org/founder_calculation_tool.htm), can be useful for calculating the necessary number of founders for a given species.
- An assessment of what effect the collection of animals will have on the source

population is needed to prevent overharvest.

- Mixing different genetic populations is a complex and potentially controversial objective that may benefit or harm wild populations. Consultation and careful assessment with experienced experts in conservation genetics should be done prior to mixing any populations.
- Consideration of the natural history and life history of an amphibian may help guide methodology and collection techniques to maximise genetic diversity. For example, if 500 tadpoles of the same developmental stage are collected from a single site, the tadpoles could consist of an entire sibling group. Alternatively, the same tadpoles could consist of many sibling groups produced by a group breeding event, thus representing great genetic diversity.
- It may be necessary to consider some form of assisted evolution to mitigate a threat such as disease to successfully restore a species to its prior range where the threat persists.

The Natterjack toad (*Epidalea calamita*) has been the focus of translocation related conservation projects in the United Kingdom for several decades. It is an example of the long-term commitment that may be required to successfully conserve some amphibian species © Jim Foster/ARC

RESOURCE BOX: Genetic considerations

Genetic considerations are a critical component of a successful translocation and represent a wide field of study beyond the scope of these amphibian-specific guidelines. Further information on genetic considerations, which can be applied to amphibian translocations, may be found in the following sources:

Beauclerc, K.B., Johnson, B. & White, B.N. (2010). Genetic Rescue of an Inbred Captive Population of the Critically Endangered Puerto Rican Crested Toad (*Peltophryne lemur*) by Mixing Lineages. *Conservation Genetics* 11:21–32.

Buckley, J., & Foster, J. (2005). Reintroduction Strategy for the Pool Frog *Rana lessonae* in England. *English Nature Research Report* 642.

Jamieson, I.G. & Lacy, R.C. (2012). Managing Genetic Issues in Reintroduction Biology. In: J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon (eds.) *Reintroduction Biology: Integrating Science and Management*, pp. 448–482. Oxford, UK: John Wiley & Sons.

Frankham, R. (2008). Genetic Adaptation to Captivity in Species Conservation Programs. *Molecular Ecology* 17:325–333.

Frankham, R., Ballou, J.D., Ralls, K., Eldridge, M., Dubash, M.R., Fenster, C.B., Lacy, R.C. & Sunnucks, P. (2017). *Genetic Management of Fragmented Animal and Plant Populations*. Oxford, UK: Oxford University Press.

Moritz, C. (1999). Conservation Units and Translocations: Strategies for Conserving Evolutionary Processes. *Hereditas* 130:217–228.

Schad, K., (ed.) (2008). *Amphibian Population Management Guidelines*. Amphibian Ark Amphibian Population Management Workshop; 2007 December 10-11; San Diego, CA, USA. Amphibian Ark.

Weeks, A., Moro, D., Thavornkanlapachai, R., Taylor, H.R., White, N.E., Weiser, E.L. & Heize, D. (2015). Conserving and Enhancing Genetic Diversity in Translocation Programs. In: D. Armstrong, M. Hayward, D. Moro & P. Seddon. (eds.) *Advances in Reintroduction Biology of Australian and New Zealand Fauna*, pp.127–140. Clayton South VIC, Australia: CISIRO Publishing.

Williams, S.E. & Hoffman, E.A. (2009). Minimizing Genetic Adaptation in Captive Breeding Programs: A Review. *Biological Conservation* 142:2388–2400.

Wilson, G.A., Fulton, T.L., Kendell, K., Scrimgeour, G., Paszkowski, C.A. & Coltman, D.W. (2008). Genetic Diversity and Structure in Canadian Northern Leopard Frog (*Rana pipiens*) Populations: Implications for Reintroduction Programs. *Canadian Journal of Zoology* 86:863–874.

Environmental and ecological impacts

- A translocated animal does not represent a single species, but is rather a biological package containing a selection of viruses, bacteria, protozoa, helminths, and arthropods (Nettles, 1988).
- Translocation beyond the native range of a species may also risk the introduction of parasites and pathogens both infecting the amphibians being moved and via equipment and substrates. Assuming appropriate quarantine procedures are maintained during transport and housing, the need to consider environmental and ecological impacts are primarily associated with reintroductions outside a species' historical range.
- The spread of pathogens, particularly undescribed pathogens for which screening tests do not exist, pose a very significant risk for assisted colonisation programmes (see section on Disease and Parasite Consideration). Typically, species should not be moved beyond their naturally occupied bioregions or areas connected by continuous habitat that are appropriate for the focal species.
- In addition to issues associated with disease, assisted colonisation programmes must consider hybridisation with other species, competition, and predation, or other forms of disruption to the host environment. Reintroductions should not occur in areas where there is any possibility that hybridisation may disrupt the genetic integrity of a taxon. Translocated amphibians may also impact other threatened species at the release site, such as those that they may compete with or prey upon.

A global invasive alien species the Asian Common Toad
© Pritpal Soorae

- Translocated amphibians have the potential to become invasive. For example, coqui frogs (*Eleutherodactylus coqui*), Asian common toads (*Duttaphrynus melanostictus*) and cane toads (*Rhinella marina*) have damaged native ecosystems after introduction (e.g. Choi & Beard, 2012; Soorae et al., 2020; Shine, 2010). Amphibians translocated outside their native range must be carefully studied to assess their potential to become problematic invasive species.

Captivity-specific considerations

Importance of husbandry in amphibian translocations

All translocations inherently require some level of husbandry. Even if the animal spends a few minutes being transported, the animal is still captive and the responsibility of the capturer. However, many amphibian translocation programmes require substantial levels of husbandry expertise, and animals may be kept in captivity for generations because of the inability to mitigate causes of decline. Good husbandry practices are necessary to ensure that captive animals are cared for in a way that is both ethically responsible and prevents a wide range of husbandry-related problems for translocated animals.

Husbandry is the foundation of *ex situ* conservation and many translocations. Amphibians may be kept in captivity for many reasons, including producing animals used for reintroductions, providing an assurance against extinction in the wild, performing research beneficial to undertaking a translocation that would be difficult on wild individuals, and use as educational animals to raise awareness for the conservation of the species *in situ*. Animals can also be held until the causes of decline in the wild can be ameliorated, with the goal for collected animals to be reintroduced. However, this is most useful when there are known threats with a short-term impact on the habitat. Maintaining captive populations may not be the best use of conservation funding when long-term threats with few apparent solutions prevents all reintroductions.

The rapidity of declines in many amphibian populations has made the role of amphibian husbandry particularly important because of the large number of amphibian species that require urgent *ex situ* attention. Additionally, many of the species brought into captive breeding programmes likely have not been well-studied, making the development of husbandry protocols largely based on trial-and-error (e.g. Gagliardo *et al.*, 2008; Coloma & Almeida-Reinoso, 2012).

The difficulties of keeping amphibians in captivity should not be underestimated. Many amphibian reintroduction programmes have struggled with captive husbandry, resulting in both short-term husbandry problems after initial capture (e.g. Gagliardo et al., 2008; Coloma & Almeida-Reinoso, 2012; Soorae, 2010, 2011, 2016) and long-term husbandry problems that result in a decline of captive animal health (e.g. Lee et al., 2006; Pessier et al., 2014; Shaw et al., 2012; Soorae, 2011, 2016).

Developing amphibian husbandry protocols

- Because of the life history diversity within Amphibia, generalised husbandry recommendations for amphibians is a challenge. Only a small fraction of the known 8,000+ species of amphibians have ever been kept in captivity. Furthermore, the natural history of many amphibian species is unknown, which makes initial husbandry recommendations for housing, nutrition, and captive environmental parameters difficult (Ferrie et al., 2014; Michaels et al., 2014; Tapley et al., 2015a; Pessier & Mendelson, 2017). It is critical to involve husbandry practitioners with sufficient experience to develop husbandry protocols for a species. Depth and breadth of knowledge regarding amphibian husbandry will allow the individual to detect and react to unexpected problems and challenges with the animals, which may be frequent when working with poorly studied species.
- There is an array of available information on amphibian husbandry. Most published husbandry information is generalised and can be applied to a wide range of amphibian species; however, generalised information may be of limited utility for specialised species. Therefore, a substantial amount of research may need to occur before some species can be reliably kept long-term and/or produce offspring in captivity.
- When planning to keep an amphibian species in captivity, locate any literature that describes husbandry methods for the species. Speaking with anyone who has husbandry experience with the species may be highly beneficial. If no taxon-specific husbandry information is available, information on species that are closely related or that have similar ecological traits may be useful. Husbandry information for other species should be used cautiously, because amphibian species, even within the same genus, may have very different husbandry needs (Staniszewski, 1995). Furthermore, optimal husbandry parameters may vary between populations of the same species (Räsänen et al., 2003).
- Developing initial species-specific husbandry protocols may involve trial and error, a steep learning curve, and an associated high level of mortality within the amphibian captive population (e.g. Lee et al., 2006; Gagliardo et al., 2010; Michaels et al., 2014; Tapley et al., 2015a). If a species is hard to acquire or has few individuals, it may be useful to gain experience working with closely related surrogate species that are more common. The use of surrogate taxa may prevent husbandry-related mortality of the species of conservation concern.
- Prior to the collection of wild animals, habitat and environmental parameters from the location of collection should be recorded. Basic parameters, such as water chemistry, temperature fluctuations, and substrate, should be recorded. Michaels et al. (2014) outlines various environmental parameters that would be useful for developing husbandry protocols.
- Recent studies demonstrated that captive-reared amphibians may suffer from

nutritional deficiencies that are not always clinically visible, including nutritional metabolic bone disease and vitamin A deficiency (Ferrie et al., 2014; Michaels et al., 2014; Tapley et al., 2015b). Such nutritional issues should be carefully considered during rearing to ensure that animals with compromised fitness are not being released. Addressing these issues includes providing sufficient vitamin supplementation and ultraviolet lighting.

Planning for population management of amphibians in captivity

- If captive breeding is used to maintain the captive population or produce animals for reintroduction, the genetics of the captive population should be closely managed. There is abundant literature regarding genetic adaptation in captivity and its implications for reintroductions (reviewed by Gilligan & Frankham, 2003; Frankham, 2008; Groombridge et al., 2012; Keller et al., 2012; Weeks et al., 2015).
- The genetics of amphibian populations in captivity can be managed similarly to those of most vertebrate taxa (see section genetic considerations for amphibian reintroductions for a list of resources). Additionally, the Amphibian Population Management Guidelines (Schad, 2008) were developed by AArk to provide specific guidelines for amphibians in captivity.

Amphibian holding cages © Brian Gratwicke

Behavioural issues of amphibians in captivity

- Captive populations may develop maladaptive behaviours which may adversely impact reintroduction efforts (Griffin et al., 2000; Mcphee & Silverman, 2004; McDougall et al., 2006; Teixeira et al., 2007; Lacey et al., 2013; Mendelson & Altig, 2016).
- The assumption that amphibians are good candidates for reintroductions because their behaviour is largely hardwired and would thus prevent captivity-related behavioural problems has little scientific backing (Crane & Mathis, 2011; Ibáñez et al., 2014; Tapley et al., 2015a). For example, captive-bred amphibians may be predator-naive and benefit from predator avoidance training prior to reintroduction (Teixeira et al., 2007; Price-Rees et al., 2013). Teixeira & Young (2014) demonstrated that captive-bred American Bullfrog (*Lithobates catesbeianus*) tadpoles could be taught to avoid an avian predator, thus showing that behaviours in captive amphibians can be experimentally modified. Predator avoidance training of Hellbender (*Cryptobranchus alleganiensis*) salamander larvae was also shown to be effective (Crane & Mathis, 2011). Other studies have indicated varied responses, with some evidence of learned predator avoidance (Semlitsch & Reyer, 1992; Murray et al., 2004).
- Although training responses and benefits are likely to be species-specific, future studies are needed to demonstrate if behavioural conditioning could have significant impacts on post-release mortality. Understanding how maladaptive behaviours may impact translocation outcomes and developing new ways to mitigate these problems appears to be a fertile area for study.

Translocations for the Booroolong frog (*Litoria booroolongensis*) have taken place in Australia after the species severely declined due to threats including chytrid fungus and drought © Michael McFadden

Animal welfare of amphibians in translocations

- Animal welfare is a goal for any translocation project, and conservation practitioners must comply with regulations concerning research and welfare of amphibians.
- Stress associated with translocation should not be underestimated and may occur at multiple stages of a wildlife translocation (reviewed by Teixeira et al., 2007; Dickens et al., 2010). Consequently, amphibian translocations should consider animal welfare issues and supportive management actions during all stages of the translocation process. Harrington et al. (2013) developed a decision tree to help identify relevant animal welfare considerations throughout the translocation process.
- Many amphibian translocations include captive breeding as a component and may involve animals kept in captivity for generations; thus, welfare considerations should play a prominent role in both planning and decision-making. Amphibians can experience acute pain and distress, and it is the responsibility of all parties working with amphibians to treat them as well as possible.
- Poor animal welfare in an amphibian translocation may cause increased stress and may be directly linked to problems that may impact the outcome of a conservation translocation (e.g. decreased reproductive capacity and reduced pathogen resistance; Moore & Jessup, 2003; Teixeira et al., 2007). Also negative perceptions and loss of support from stakeholders or the public may result from inadequate attention to animal welfare.
- Ideally, once a captive amphibian is returned to the wild, the animal should experience what a wild conspecific would in a healthy, self-sustaining population. Released amphibians should be free to express normal behaviour in suitable habitat. It may be important for released populations to experience typical natural selection processes to ensure subsequent generations are adapted appropriately.
- Practitioners should use their best judgement so released animals have the greatest chance for success. For example, knowingly releasing animals into exceedingly poor habitat or areas with extremely low chances of survival (lower than they would naturally experience) would be irresponsible. Likewise, releasing individuals that have fared poorly in captivity can hinder the success of a release programme (Mendelson & Altig, 2016).
- High mortality rates of released individuals may be difficult to avoid because of the poorly studied nature of some amphibian species. For example, the optimal habitat or time of year to release individuals may not be known, and may require experimental releases of small groups of animals to examine survival and success.
- If low survival occurs, utilising adaptive management policies to continuously try to improve release survival should improve translocation welfare.

RESOURCE BOX: Amphibian welfare and use in research

There are a variety of print and web resources available for persons interested in amphibian welfare in reintroductions and care, and the use of amphibians in research settings, which may be applicable for aspects of reintroduction programmes.

Reviews of animal welfare in reintroductions:

Harrington, L.A., Moehrenschlager, A., Gelling, M., Atkinson, R.P., Hughes, J. & Macdonald, D.W. (2013). Conflicting and Complementary Ethics of Animal Welfare Considerations in Reintroductions. *Conservation Biology* 27:486–500.

Swaisgood, R.R. (2010). The Conservation-Welfare Nexus in Reintroduction Programmes: A Role for Sensory Ecology. *Animal Welfare* 19:125–137.

Online resources for amphibian care and use in research & translocation settings:

1) The OIE working group on Animal Welfare was founded in 2002.Their website has links to the OIE Terrestrial Animal Health Code (www.oie.int/en/international-standard-setting/terrestrial-code/access-online/) and the Aquatic Health Code (https://www.oie.int/en/standard-setting/aquatic-manual/access-online/).
2) The American Society of Ichthyologists and Herpetologists has a set of guidelines for the use of live amphibians and reptiles in research (https://asih.org/animal-care-guidelines). The guidelines are used by several university Institutional Animal Care and Use Committees in the United States to evaluate research protocols proposed by faculty members.

Disease and amphibian reintroductions

The extent and rapidity of global amphibian population declines have required development of captive survival assurance populations for myriad species. The scale of this effort is unique and includes many poorly known species (often kept in captivity for the first time), which poses challenges to veterinarians and other animal health professionals advising reintroduction programmes. Infectious diseases are a risk to the success of any translocation or reintroduction programme conducted as part of a species conservation effort. Of particular concern is the potential to introduce harmful pathogens from the source population to conspecific or sympatric species at a release site. Similarly, newly released animals may be exposed to unfamiliar parasites or pathogens in wild populations to which they may have little resistance. Finally, because many amphibian species have not previously been maintained in captive settings, health problems related to husbandry and nutrition can impact a reintroduction programme's success.

- The issues related to infectious disease are highlighted for amphibians because of the important role played by the chytrid fungi (*Batrachochytrium dendrobatidis*) (Bd) and *B. salamandrivorans* (Bsal) in the global amphibian extinction crisis. Notably, Bd was documented to have been dispersed by anthropogenically mediated animal movement, including reintroduction programmes (Walker et al., 2008).
- The practice of housing amphibian species from a wide range of geographic locations in a single facility (so-called cosmopolitan collections) combined with a relative lack of information on amphibian pathogens compared with fish, bird, and mammal pathogens has led to real concerns about the possibility of spreading new population-limiting agents through reintroduction programmes.
- Numerous diseases exist that may impact amphibians and are pertinent to translocations, such as chytridiomycoses (Bd and Bsal), ranaviruses, parasites, bacterial and fungal infections, and deficiencies of nutrition or husbandry.

A complete treatment of disease mitigation for captive breeding and reintroduction programs should consult *A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs* produced by the IUCN SSC Conservation Breeding Specialist Group and can be found at the following link: http://www.cpsg.org/sites/cbsg.org/files/documents/AMPHIBIAN_DISEASE_MANUAL_2017.pdf

General disease considerations of amphibian reintroductions

It is impossible to completely eliminate the risk of infectious disease in any translocation or reintroduction programme. Each programme will need to evaluate the risk level acceptable to its stakeholders. Determining risk level is best achieved through a formal process of disease risk assessment. The World Organization for Animal Health (OIE) and the IUCN Conservation Breeding Specialist Group and Wildlife Health Specialist Group have established excellent guidelines for performing formal disease risk assessments for reintroduction programmes (Jakob-Hoff et al., 2014).

Fortunately, the infectious disease risks of amphibian reintroductions are substantially reduced by implementation of a few key practices (Murray et al., 2011; Pessier & Mendelson, 2017). Where direct wild-to-wild translocations are being considered, care should be taken to minimise the transfer of substrates that may contain pathogens of other taxon groups as well as amphibians. Whenever possible, amphibian breeding and reintroduction programmes should operate within the native range of the species. Programmes that keep amphibians outside of their native range pose a higher risk of introducing novel pathogens to wild amphibian populations. If operation of the programme inside the native range is impossible because of lack of resources, expertise, or time, animals destined for translocation or reintroduction should be kept in long-term isolation from amphibians that originate outside their native range. Under some conditions, allopatric populations of the same species may be considered to have different native ranges.

The process of long-term isolation is especially important for institutions (e.g. zoos) that have cosmopolitan collections. Long-term isolation is conceptually simple and does not need to be as expensive or logistically complex as it sometimes is viewed. Detailed examples of creative, practical, and affordable facilities developed to facilitate long-term isolation are available (Poole & Grow, 2012; Pessier & Mendelson, 2017). Amphibians that are part of a release or translocation programme should be held in isolation from other populations of captive or wild amphibians during the entire period that they are in captivity; this minimises the risk that they could acquire extremely harmful pathogens that were not previously present in the population.

The biosecurity conditions needed for long-term isolation are:

- Permanently housing animals that will be reintroduced in dedicated rooms or buildings away from other species that originate outside their native range.
- Implementation of basic biosecurity measures (e.g. hand-washing, and dedicated footwear and clothing) that functionally isolate dedicated rooms and buildings, thus preventing indirect exposure of isolated amphibians to cosmopolitan collections.

Pre-release disease screening

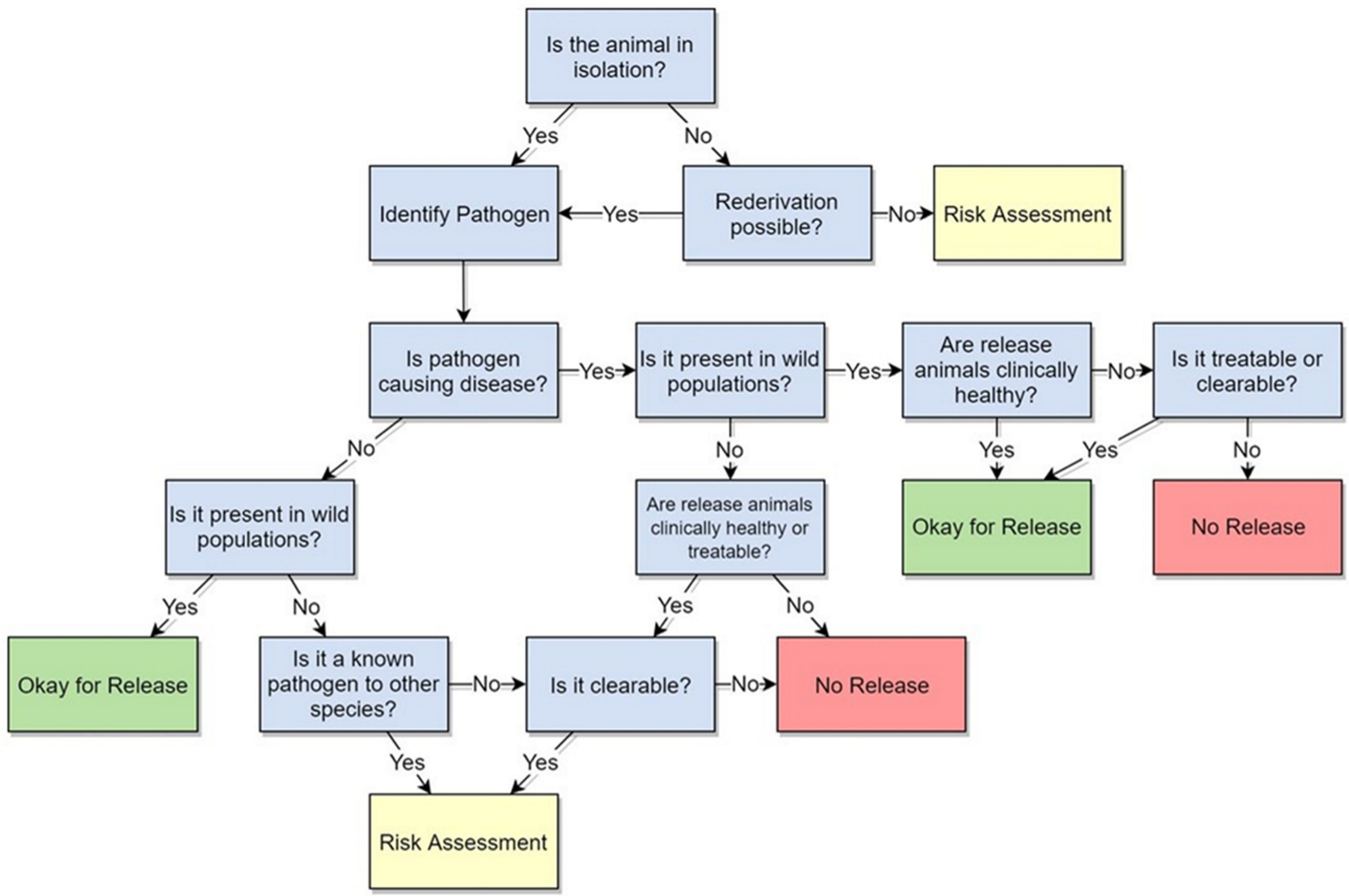
If pathogen transmission is prevented between animals or via contaminated substances (e.g. water, substrate, shoes, and husbandry tools), then animals maintained in long-term isolation will require minimal pre-release disease screening. Disease screening can be expensive and of limited sensitivity to unknown pathogens, so this is a financial value-added component to consider as the concept of isolated amphibian colonies begins to gain agency among stakeholders. In contrast, animals that were exposed to cosmopolitan collections would require extensive pre-release infectious disease screening (Pessier & Mendelson, 2017; Khatibu et al., 2013). In fact, many programme leaders would argue that such animals should never be reintroduced to the wild, in part because of the possibility of yet unknown pathogens that they may bear. We generally endorse this view. However, large mammal translocations from cosmopolitan collections for conservation purposes are very common (e.g. Seddon et al., 2005); some researchers think that, “there is rarely cause to consider translocation as unfeasible due to disease or parasites” (IUCN/SSC, 2013). The conceptual disparity in perspectives likely results from the relatively advanced state of knowledge of pathogen and disease prevention in mammals compared with amphibians and the greater impact that disease spread has had on amphibians.

- Basic common-sense precautions include not releasing animals that appear to be sick, nor seemingly healthy animals from a source population that has experienced recent mortality events.
- Animals should be assessed for non-infectious diseases (e.g. metabolic bone-disease) to make sure animals have the best chance of surviving upon release.
- Because of its known potential to cause devastating disease outbreaks, polymerase chain reaction (PCR)-based screening of a population for Bd and Bsal (reviewed by Pessier & Mendelson, 2017) should be a common component of pre-release protocols. Formal disease risk assessments within each translocation programme are helpful for determining the extent and type of pre-release disease screening needed.

Risk assessment of amphibian diseases in reintroductions

- Basic information is required to inform a proper risk assessment such as the full history and background (e.g. original source) of the animals under consideration should be known. Ideally, baseline information on diseases occurring in both captive and wild populations should be gathered and compared; including disease data for all extant amphibian species at the reintroduction site and not just for the focal species, which may not even occur there at the time of assessment.
- Several examples of disease risk analysis for herpetofauna exist and are useful guides (e.g. Sainsbury et al., 2017; Suarez et al., 2017).
- Collection of these baseline data could include typical veterinary disease screenings, such as skin swabs (e.g. Bd PCR), faecal parasite examinations, and blood smears. Necropsy examination with histopathology of animals that are found sick or dead, or apparently healthy (euthanised) is one of the most valuable approaches to detecting health problems not covered by other techniques. In some cases, euthanasia of healthy wild or captive individuals may not be possible because ethical or regulatory concerns.

Flow chart 1. Decision tree for dealing with pathogens in amphibian translocations.



- The results gathered from these reviews of history and disease screenings will form the basis for the disease-related portions of the overall conservation risk assessment for the reintroduction or translocation protocol. If an identical pathogen is present in both the target and source (e.g. captive) population, the presence of this pathogen may not impact the decision to proceed with the reintroduction or translocation. Alternatively, in some situations, knowledge of pathogen strain differences between locations may be valuable. For example, different Bd strains may have significantly different virulence (Jenkinson et al., 2016).
- Pre-release screenings of amphibians at the release site can help identify endemic pathogens and parasites, and including low levels of those same pathogens and parasites in captive animals to maintain natural degrees of resistance. In all cases, each factor involved in the risk assessment will be specific to the details of the species and sites involved; it is not possible to create blanket recommendations that will suit all situations. Flow chart 1 depicts a logical chart of our recommended sequence of questions and factors to be considered in a risk assessment.

Chytrid fungus swabbing © Lea Randall

Social feasibility

The social feasibility of an amphibian reintroduction or translocation project represents how local people and communities will interact with the project. Conservation practitioners should not underestimate the potential for controversy between local stakeholders and conservationists when planning an amphibian translocation (e.g. the Houston Toad; Brown & Mesrobian, 2005). However, amphibian translocations may greatly benefit from integrating community involvement, and there may be excellent opportunities for educating the public about broader amphibian conservation topics.

Amphibians are part of folklore and traditions in many cultures. A review of all social uses and customs involving amphibians is outside the scope of our reintroduction guidelines, but social feasibility is part of any conservation translocation project. Crump (2015) provides a thorough summary about the lore and mythology of amphibians and reptiles. There are a variety of websites that detail myths, stories associated with amphibians, and cultural practices used by local groups. These stories can provide insight into potential attitudes and reactions of humans to amphibian reintroduction projects. Individuals who fear amphibians may dislike any project that aims to increase the size of amphibian populations. Some communities may view amphibians as a natural resource for consumption, which could increase their involvement in population conservation because they want to preserve the food source, or, alternatively, increase their resistance because they do not care about conservation. Some cultures associate amphibians with renewal, luck, or creation, and individuals in those cultures may care passionately about amphibians in their natural habitats. Translocation programmes may be able to harness local attitudes to increase conservation support. For example, the Panamanian Golden frog (*Atelopus zeteki*) was culturally important to the people of Panama, but currently only survives in a multi-national captive-breeding programme. After the conservation programme for this frog lobbied the government, legislation was passed in Panama in 2010 which designated August 14 as “National Golden Frog Day”, thus greatly increasing awareness and support for the frog’s reintroduction programme (Amphibian Rescue and Conservation Project, 2013).

Any reintroduction project conducted with species living near indigenous people needs to work closely with local stakeholders to ensure project success (Sheil & Lawrence, 2004). In many cases, local people can be involved in the project, ensuring that there is local support for translocation efforts. Moreover, attitudes and opinions can change with education. One key to success of any translocation project is assessment of local knowledge and attitudes prior to project initiation, and incorporation of local stakeholders in the project if possible. In some countries, especially where amphibians are consumed as food, used for biomedical research, or used medicinally, reintroduction projects may conflict with local traditions and practices, resulting in human–wildlife conflict, which must be foreseen and managed for the project to be a success. Isolating local people from projects runs counter to the lessons learned from successful conservation projects, and humans are part of the equations for success, especially as we continue to convert natural areas for human uses. Achieving long-term conservation goals is as much about economics, politics, and building relationships as it is about the biology of the target taxon or ecosystem. Social feasibility will have to be assessed and managed on a case-by-case basis for conservation translocations. Reintroduction teams should include social scientists to help facilitate project success when reintroductions put amphibians and humans in close contact.

Incorporating experimental research into amphibian translocations

Amphibian translocations should be designed as experiments to test explicit hypotheses, which will improve the likelihood of achieving translocations goals (reviewed by Kemp et al., 2015). For example, monitoring two groups of translocated individuals released under different conditions (e.g. site location, time of release, or life history stage at release) allows for direct comparisons between different protocols to optimise methodology. A clear process that both reviews and approves research proposals integrated within an amphibian translocation programme should exist.

Designing experiments for translocations of some amphibians may have advantages compared with translocations of many other vertebrates. The monetary costs per translocated animal for many amphibian species are likely much lower than those for translocations of other vertebrates, such as hoof-stock, primates, or large carnivores. Most amphibians are relatively small (although there are notable exceptions, such as cryptobranchid salamanders) and not dangerous to humans, which greatly reduces many expense and logistical concerns faced by other programmes. Additionally, housing captive amphibian populations for research may require much less space and food, and fewer staff than comparably sized mammal or bird populations, which makes amphibian research a productive avenue of study for zoos or aquariums. Moreover, large experimental reintroductions of other highly fecund taxa in well-studied systems, such as some fish (e.g. Milot et al., 2013), may provide unique experimental ideas that could be implemented in amphibian reintroductions.

Translocation implementation

Frog release © Arizona Game & Fish

"Any translocation bears risks that it will not achieve its objectives and/or will cause unintended damage. Consequently, the full array of possible hazards both during a translocation and after release of organisms should be assessed in advance"

(IUCN/SSC, 2013)

Capture-specific considerations

The impact of removing individuals from the source population should be assessed. Ideally, it should not negatively impact source population viability (except in the rare case where removal of the source population is a goal). When individuals are collected, age, life stage, sex, and phenotypic variation are all important factors that should be considered. Amphibians display a vast array of social interactions and behavioural considerations which are relevant to translocations. Factors such as tadpole schooling, parental care, territoriality, and monogamy may necessitate careful planning of the source group structure, number of individuals, and life stages that have the best chances of being successfully translocated.

- Amphibians are often cryptic, and collection efforts may bias certain demographic inferences. Calling males may be easier to locate and capture than females.
- Certain phenotypes or behavioural temperaments may be more cryptic to humans and thus collected less frequently. Potentially, the animals that are the worst at avoiding collection might be preferentially collected over more cryptic conspecifics. Moreover, animals with temporal activity patterns that most coincide with collection timing may also bias collection.
- Care should be taken to capture a wide range of phenotypic and behavioural variety within the source population.

Post-capture and pre-release health screening

The degree of health screening prior to amphibian translocation or reintroduction can range from minimal to very extensive, depending on the outcome of the disease risk assessment. Using the information gathered above, health screening protocols are customised for each translocation and reintroduction programme, and for each group of animals destined for release. The *Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs* produced by the IUCN SSC Conservation Breeding Specialist Group and can be found at the following link: http://www.cpsg.org/sites/cbsg.org/files/documents/AMPHIBIAN_DISEASE_MANUAL_2017.pdf

Disease risk assessment to determine suitability of a group of animals for translocation and reintroduction is facilitated by:

- Knowledge of the history of the animals to be released. Those maintained in the native range or in long-term isolation without exposure to species from outside their native range need the least pre-release disease screening. Animals that have been directly or indirectly exposed to amphibians from outside their native range need more intensive disease screening.

- Collection of background information on the health of the captive population that will be used for reintroduction, and conspecific and sympatric species of amphibians already present at the reintroduction site is essential. Methods that can be used to develop a database of health information include:
    - ⇒ Necropsy (including histopathology) of animals that die or that are culled, which is essential for health evaluation of both individuals and populations. Histopathology is valuable for detecting new or unsuspected infectious diseases; it allows for evaluation of the nutrition programme, and is an opportunity to collect samples and characterize parasites and parasite loads.
    - ⇒ Testing of populations for specific amphibian pathogens, such as Bd or ranaviruses (see below), that are known to be significant causes of mortality.
- Creation of captive populations free of treatable pathogens that cause significant morbidity or mortality in amphibian populations, such as Bd. Breeding populations that are free of specific pathogens may be necessary to maintain sustainability by limiting disease-related mortality of founder animals, and reducing the need for some disease testing prior to release.
- If infectious diseases are identified in either captive animals destined for release or animals already present at the release site, attempts should be made to define the potential impact of the pathogen.
    - ⇒ In general, if the same pathogen is present in both the captive and wild populations, then infected animals can be released. However, it should be noted that it can be difficult to easily determine if similar pathogens are truly identical. For example, different strains of frog virus 3-like ranaviruses can have identical major capsid protein gene sequences (Schock et al., 2008). Additionally, important strain differences between Bd isolates are also being recognized (Retallick & Miera, 2007; Farrer et al., 2011, Dang et al., 2017). The level of resolution needed to make appropriate decisions will vary with each programme.
- Individual animals that are sick or members of a captive population that is experiencing a mortality event should not be released into the wild until after identification and resolution of the illness. This is true for both opportunistic pathogens and pathogens already known to occur in the wild population. If the cause of morbidity or mortality cannot be identified, then the animals should not be released.
- Animals considered for release into the wild should be considered high risk if they have been directly or indirectly exposed to amphibians from outside their native range (e.g. a cosmopolitan collection) or exposed to animals with infectious diseases not already present in the captive population. If high-risk animals are considered for release because the species is extinct in the wild and alternatives do not exist, extended disease screenings and quarantine, risk assessments, and mitigation plans

are needed. Components of this plan can include establishing breeding populations in long-term isolation and potentially re-derivation of breeding populations from eggs.

- Animals placed directly into long-term isolation conditions that are then returned to the same location may not require any disease screening if appropriate biosecurity measures were in place.

Transport-specific considerations

When transporting amphibians to the release location, minimise stress levels that may lead to higher mortality immediately after release. Although no studies on transport stress have been undertaken on amphibians, studies on fish demonstrated elevated levels of stress during and immediately after transport, which lead to lower recapture rates (Iversen et al., 1998).

Considerations to be taken during transport:

- Transport duration should be minimised by packing the specimens immediately prior to transport, and choosing an appropriate and rapid mode of transport.
- If transporting amphibians by air, guidelines on shipping can be viewed in the International Air Transport Association Live Animal Regulations (www.iata.org/whatwedo/cargo/live-animals/Pages/index.aspx). Throughout transportation, ensure that extreme cold or hot temperatures are avoided, and appropriate temperatures will be species-specific (Poole & Grow, 2012). Such conditions may be achieved by choosing appropriate weather conditions to transport the animals, the use of air conditioning within the mode of transport, or the use of cold/warm packs within insulated transport crates. Transport crates should never be left in direct sunlight or in a closed, unventilated vehicle parked in direct sunlight that may quickly overheat.
- Adding an environmental data logger that will record temperature to the transportation package is useful retrospectively if any issues arise. Transporters can then be held accountable if they guarantee certain parameters. Temperature data can also help inform managers how to better pack animals for thermal stability in future shipments.
- Containers to maintain the frogs should be chosen so that they are large enough to comfortably contain the amphibians but not large enough to permit jumping, which may cause injury. Plastic, disposable food containers with tight-fitting lids are well suited for this purpose. Substrate should be placed in the container to permit access to moisture and to absorb any excretory waste; rinsed and moistened sphagnum moss, coco-fiber, or unbleached, moist paper towels may be suitable for this purpose. Containers should be stocked with conspecifics of a similar size, and the stocking density must be considered to minimise stress and waste production.
- Ventilation may be provided by drilling, punching, or soldering small holes into the lid or side of the enclosures. If providing ventilation via the lids, care should be taken to

ensure that water cannot drip onto the container. Holes must be sanded flat or drilled from the inside of the container to ensure there are no sharp edges that may abrade the amphibian skin. The smaller containers can be stacked within a larger rigid transport crate using shredded or crumpled paper between containers to buffer against excessive movement. The larger container should also be ventilated and appropriately labelled.

- Tadpoles or aquatic amphibians should be transported in a similar manner to live fish. In some situations, especially with aquatic salamanders, transportation in tanks with oxygenation systems may be appropriate (Pramuk et al., 2011; Essner et al., 2012); however, in most cases with tadpoles, round- or square-bottomed aquarium bags should be used to prevent tadpoles from being trapped in corners. The bag should be filled between 40-50% with water from the amphibian's original source water, or with a combination of the original water source and filtered water. Oxygen from a medical cylinder should then be added to oxygenate the water. Bags should be tied shut with a rubber band and placed within a second bag, and tied shut with a rubber band. The bags should then be placed in an insulated styrofoam box for careful temperature control.

Captive-bred *Atelopus varius* frogs ready for release in Panama after selecting a safe and suitable site along a steep mountain stream © Brian Gratwicke

Pre-release monitoring

Monitoring includes pre- and post-release phases. Depending on the phase of the programme, the specific monitoring needs differ (Nichols & Armstrong, 2012). Pre-release monitoring encompasses monitoring of the source population or release site before the translocation occurs.

Some general considerations for pre-release monitoring include (adapted from IUCN/SSC, 2013):

- Monitoring animals, habitat, and ecological variables prior to undertaking a translocation can be a valuable opportunity to help train staff, develop solutions to unexpected problems, and develop on-the-ground experience to improve the skill of searchers in locating cryptic animals.
- Baseline ecological data can add great value to the programme design when collected prior to release.
- Collecting data related to relevant habitat variables that are positively or negatively associated with site suitability should result in selection of a better release site (Nichols & Armstrong, 2012). It is important to consider the habitat suitability for supporting the species in both the short- and long-term (Ewen & Armstrong, 2007).
- Presence of predators and competitors along with features of the aquatic and terrestrial habitats required or preferred by the focal species should be assessed.
- General features of the habitat that are important are often species-specific for amphibians, but may include microsites suitable for calling, oviposition and development, foraging, thermoregulation, and refuge.
- Depending on the state of knowledge, additional species-specific natural history data should be considered and assessed prior to release (Michaels, 2014).
- Changes in relevant habitat variables over time may also be useful during post-release monitoring or in adaptive management (Nichols & Armstrong, 2012).

Release-specific considerations

A translocation's release methodology typically falls into two categories; soft or hard release. Soft and hard release options are not dichotomous, but represent opposite ends of a release strategy continuum (Mendelson & Altig, 2016). A soft-release strategy means the animal receives some sort of support after being brought to the release site. The options may vary widely depending on the life stage of the translocated animal. The animals are provided with assistance, such as delayed release, habitat enrichment, or predator management at the release site, to improve chances of establishment (Parker et al., 2012). In contrast, a hard-release strategy does not acclimate or support the animal at the release site - the animal is simply released.

- Many environmental and rearing variables may impact an amphibian's health and post-translocation survival. Because amphibians are ectothermic, it is prudent to acclimate animals to local environmental conditions, such as water conditions (e.g. pH and temperature), prior to release (of both soft and hard releases) to minimise post-release stress. For aquatic life stages, this may also involve gradually mixing the transport water with water from the release site to gradually acclimate the organisms to water quality parameters at the release site. Additionally, a risk assessment of what might be transported in that water (e.g. pathogens or other organisms unintentionally translocated) is prudent so as not to have undue impact at the release site.
- It is important to note that release methodologies for amphibians have not been thoroughly studied and, as such, is an area fertile for research. The complex life history of most amphibians and the variety of reproductive modes make release methodologies for amphibians particularly varied compared with other vertebrate taxa.

Inspecting pool frog spawn before release © Jim Foster

- The optimal amphibian life history stage (e.g. eggs, larvae, juveniles, or adults) for translocation depends on the translocation goals and the animal's natural history and behaviour. Particular attention to the translocated species' natural history, behaviour, and reproductive mode should be considered when deciding a release method.
- When animals are held in a soft-release enclosure, the environmental/physical criteria of the enclosure should be closely monitored. Do the animals have enough food, water, sunlight, and refugia? Will the animals get too hot during the day or cold at night? Can predators access soft release enclosures?

Types of soft-release methods for amphibians include:

- *Delayed release:* Holding the translocated animal in a pen or enclosure at the release site for a period of time prior to full release into the environment may allow the animal to acclimate to the release site (e.g. Polasik et al., 2015). Delayed release tactics have not been thoroughly studied in amphibians; however, in some non-amphibian taxa, delayed release was shown to increase site fidelity, establishment, and survival (reviewed in Parker et al., 2012). Delayed releases are likely an area fertile for study in amphibians.
- *Environmental enrichment:* The practice of modifying the release environment to increase likelihood of translocated animal establishment. For example, providing an artificial substrate or modifying the environment to provide supplemental breeding sites, shelter, water sources, or food may constitute environmental enrichment for

Translocated, captive-bred *Atelopus limosus* carries a 0.3 g radio-transmitter attached to the frog via a small silicon belt © Brian Gratwicke

amphibians.

- *Disease management:* Translocating amphibians into an environment with an existing pathogen (e.g. infectious chytrid fungus or ranavirus) may require disease management or support for released animals; this may include eliminating disease vectors and reservoirs or removing the pathogen from the environment (e.g., Bosch et al., 2015; Stockwell et al., 2014). Individuals should be monitored for disease in the field post-release (e.g. Brannelly et al., 2016).
- *Predator management:* Techniques such as using predator-proof fencing or enclosures may be necessary to reduce predation (e.g. Polasik *et al.*, 2015). Additionally, trapping or removal of local predators (e.g. invasive mice/rats) from the release site may be warranted.

Multi-species amphibian reintroductions

Given the dramatic decline of whole amphibian communities, reintroductions or conservation translocations involving several species at the same location could become necessary. However, multi-species reintroductions with amphibians have not been well studied, and there are few examples in the literature. Gagliardo et al. (2008) only found partial success in removing several species from the wild to establish captive assurance colonies in Panama prior to community collapse from Bd. Successfully translocating multiple species may greatly compound the complexity and difficulty compared with translocating a single species. Although multi-species translocations involving wildlife are rare, reintroductions of multiple plant species to restore a community during habitat restoration have occurred at some sites and is a subject of study (e.g. Plein et al., 2016).

Multi-species translocations of amphibians should not be taken lightly given the lack of evidence supporting them, but they may be suggested for several reasons:

- Reintroducing several amphibian species of a community may be advisable if a site experienced a catastrophic decline of numerous species from a novel threat (e.g. disease, introduced predators, or natural disaster), but the habitat is still good quality.
- Conservation mitigation plans may try to salvage an amphibian community by translocating as many individuals as possible of multiple species prior to a habitat being destroyed.
- Restoration of previously degraded habitats may include translocating multiple species of amphibians to bolster or reintroduce amphibian communities. For example, isolated fragments of secondary growth forest that has returned to good quality habitat may be targeted.

Post-release monitoring and reporting

Corroboree frog © Michael McFadden

"Translocation management is a cyclical process of implementation, monitoring, feedback and adjustment of both biological and non-biological aspects until goals are met or the translocation is deemed unsuccessful"

(IUCN/SSC, 2013)

Post-release monitoring and continuing management

Objectives determining success or failure should have been set prior to any other work being carried out, and a monitoring plan will test whether these objectives have been met. Additionally, given the monitoring methods available, objectives should only be set if the success can be tested. Monitoring in conjunction with clear objectives will also aid in determining if a project should be adjusted or terminated.

- Monitoring amphibians after translocation should be integrated into any translocation plan. The key to determining success or failure of many translocations is demographic monitoring of released populations. Are the animals surviving after translocation? Is breeding occurring? What is the age structure of the population? Is the translocated population viable without further intervention for the foreseeable future? Are actions such as predator management or habitat enrichment needed to support the translocated animals to ensure their survival? Monitoring can help answer all these questions, and is integral for developing a successful translocation programme.
- Monitoring is designed to inform recurrent decisions and update translocation procedures when uncertainty exists (Rout et al., 2009; McCarthy et al., 2012). Furthermore, there is value in modelling each stage of the adaptive management cycle, including conservation actions, monitoring strategy, and triggers that lead to management intervention (Bearlin et al., 2002).
- The complex life cycle of most amphibians, their diverse modes of reproduction and behaviour, and varied ecological requirements make monitoring populations difficult. The difficulty may be compounded by low detectability, thereby making generalisations for monitoring amphibian populations extremely difficult. However, standardized amphibian monitoring techniques have been developed over decades for wild populations, and they should be used when designing protocols (reviewed by Heyer et al., 1994; Dodd et al., 2012).
- Because amphibians are often highly cryptic, controlling for search effort and detectability is essential for obtaining accurate and comparable data. The skill of individuals searching for amphibians may vary considerably depending on the experience of personnel and how cryptic the animals are.
- Monitoring is frequently done for several years after translocation, and it may continue for decades to monitor long-term population stability.
- Monitoring programmes should not cause significant disturbance to either the target species or its habitat. All general principles for minimising stress in monitoring programmes should be maintained (reduced handling time, avoiding temperature or moisture stress, and appropriate hygiene). If individual identification is required and involves harm (e.g. PIT tagging and toe-clipping), then marking should be undertaken in captivity and wounds given sufficient time to heal prior to release. Any post-release monitoring programme involving moving substrates (e.g. rocks and logs) should

avoid long-term habitat degradation.

- Specific post-release monitoring examples can be found in the Amphibian Reintroduction Case Studies included as a supplement to this document.

Using standardized methods

- A critical component of amphibian monitoring is using standardised methods, which allows robust comparisons between periods of time to observe trends. For example, standardised surveying methods, such as those outlined in Heyer et al. (1994), may include visual encounter surveys, drift fences, audio surveys, trap surveys, and/or other methods effective for monitoring the target species.
- Well-defined standardised methods, data sheets, and training protocols can help maintain high levels of scientifically rigorous monitoring over many years regardless of changing personnel. Assessing the effectiveness of different translocation methods is easier if documentation of methods and outcomes are standardised (Sutherland et al., 2010).

Categories of monitoring for a translocation (reproduced and adapted from IUCN, 2013):

- *Behavioural monitoring:* Comparative data from natural populations or the same individuals before translocation are necessary to assess how animals may be adapting post-release (McDougall et al., 2006). For example, spatial movement patterns, feeding behaviour, or habitat use may all be pertinent indicators of establishment and the well-being of released animals. Direct comparisons of behaviours between different release locations or between wild translocated and captive-bred amphibians may be useful for developing successful management policies.
- *Genetic monitoring:* Where genetic issues are identified as critical to the success of a translocation, monitoring can be used to assess genetic diversity in establishing populations, or the effects of reinforcement or other management efforts (Groombridge et al., 2012; Jamieson et al., 2012; Keller et al., 2012).
- *Health and mortality monitoring:* Assessing the extent that an establishing population is experiencing or spreading disease, or adverse welfare conditions or mortality provides a basis for identifying underlying causes (Ewen et al., 2012). However, monitoring the mortality of released amphibians may be very difficult without intensive mark-recapture or radio telemetry methods.
- *Social, cultural, and economic monitoring:* Assessing how public support for the project has changed may be useful. Political or economic changes may alter funding for the project. Participation in amphibian monitoring may be a practical means of engaging the interest and support of local communities, and can be used to assess

attitudes towards the translocation, and any direct and indirect costs and benefits that arise.

Some considerations for post-release monitoring include (adapted from IUCN/SSC, 2013):

- The emphasis of post-release monitoring is typically directed towards the performance of demographic state variables (abundance and proportion of area occupied) and vital rates (survival, recruitment, immigration, and emigration) of the focal species (Nichols & Armstrong, 2012). Monitoring only for survival and breeding events at the release site is not ideal, but should be performed and reported at a minimum if no other option is available.
- The intensity and duration of post-release monitoring should be appropriate to each programme and species. This will be related to the reproductive strategy of the species, and the ability to detect the different life stages (e.g. aquatic larvae versus

RESOURCE BOX: Post-release monitoring

Post-release monitoring is an important component of a successful translocation. Further information reviewing monitoring considerations which can be applied in amphibian translocations can be found in the following sources:

Ewen, J.G. & Armstrong, D.P. (2007). Strategic Monitoring of Reintroductions in Ecological Restoration Programmes. *Ecoscience* 14:401–409.

Gitzen, R., Keller, B., Miller, M., Goetz, S., Steen, D.A., Jachowski, D.S., Godwin, J.C. & Millspaugh, J. (2016). Effective and Purposeful Monitoring of Species Reintroductions. In: D.S. Jachowski, J.J. Millspaugh, P.L. Angermeier & R. Slotow (eds.), *Reintroduction of Fish and Wildlife Populations*, pp.283–318. Oakland: University of California Press.

Heyer, W.R., Donnelly, M.A., Foster, M. & McDiarmid, R. (eds.). (1994). *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Washington, D.C.: Smithsonian Institution Press.

Nichols, J.D. & Armstrong, D.P. (2012). Monitoring for Reintroductions. In: Ewen, J.G., Armstrong, D.P., Parker, K.A. and Seddon, P.J. (eds.) *Reintroduction Biology: Integrating Science and Management*, pp.223–255. Oxford, UK: John Wiley & Sons.

Sutherland, W.J., Armstrong, D., Butchart, S.H., Earnhardt, J.M., Ewen, J., Jamieson, I., Lee, R., Newbery, P., Nichols, J.D. & Parker, K.A. (2010). Standards for Documenting and Monitoring Bird Reintroduction Projects. *Conservation Letters* 3:229–235.

terrestrial adults) and longevity of the species.

- Estimation of vital rates and state variables at a particular translocation site can be informative and may serve as a useful metric for evaluating the success of translocations (Muths & Dreitz, 2008), but this method can be labour- and cost-intensive when implemented across a large landscape. In these instances, occupancy modelling may offer a more efficient metric, especially if geographic spread of the focal species is of interest and stage-based models are developed (Nichols & Armstrong, 2012).

Dissemination of information

Publishing the results, both successes and failures, of amphibian translocations is an important step to increase the understanding of amphibian translocations. The dissemination of results and new methodology will increase our ability to achieve reintroduction goals. Frequently reporting results should be a critical component in any amphibian translocation, and programmes should not underestimate the value of their results improving other current or future amphibian translocations. The diversity in Amphibia reinforces the importance of reporting results of less-studied species. Both peer-reviewed publications and popular publications (magazines, newspapers, or other grey literature) provide useful formats for disseminating results.

Sharing plans, project status, and results with local communities and stakeholders may also benefit long-term project goals. Outreach through schools and environmental education programmes may provide new amphibian conservation-based connections within the community. Zoos are foci for amphibian-related conservation projects, and many are the source of assurance populations for reintroductions. Exhibits and programmes at these zoological institutions are an excellent way to increase public awareness of amphibian conservation and *ex situ* programmes. Furthermore, articles for hobbyist magazines and the popular press are another way to share information about amphibian reintroduction projects.

The Internet facilitates new communication formats, and sharing information online can be a useful way to connect with a broad audience worldwide. Formats such as blog posts and social media are increasingly common forms of outreach. Posting pictures, videos, and updates may reach thousands of people per day, and can be an invaluable tool for fundraising and developing support within the local and wider community.

RESOURCE BOX: Places to disseminate amphibian translocation results

Some resources to publish the results of amphibian translocations include:

- The IUCN CTSG publishes the “Re-introduction Perspectives” book series, which publishes case studies: (https://iucn-ctsg.org/resources/ctsg-books/)
- Alytes (www.amphibians.org/alytes/)
- Amphibian Ark Newsletter (www.amphibianark.org/aark-newsletter/)
- Amphibian Ark Husbandry Documents ((www.amphibianark.org/husbandry-documents/)
- Amphibia-Reptilia (booksandjournals.brillonline.com/content/journals/15685381)
- Amphibian and Reptile Conservation (amphibian-reptile-conservation.org/)
- Biodiversity and Conservation (www.springer.com/life+sciences/ecology/journal/10531)
- Biological Conservation (www.journals.elsevier.com/biological-conservation/)
- Conservation Biology (onlinelibrary.wiley.com/journal/10.1111/%28ISSN%291523-1739)
- Conservation Evidence (www.conservationevidence.com)
- Diversity and Distributions (onlinelibrary.wiley.com/journal/10.1111/%28ISSN%291472-4642)
- Froglog (www.amphibians.org/froglog)
- Herpetologica (https://bioone.org/journals/herpetologica)
- Herpetological Review (ssarherps.org/publications/journals/herpetological-review/)
- Ichthyology & Herpetology (https://meridian.allenpress.com/copeia)
- Journal of Applied Ecology (www.journalofappliedecology.org)
- Journal of Herpetology (ssarherps.org/publications/journals/journal-of-herpetology)
- Journal of Zoo & Aquarium Research (www.jzar.org)
- Oryx (https://www.cambridge.org/core/journals/oryx)
- Phyllomedusa (www.phyllomedusa.esalq.usp.br/)

Bibliography

Amphibian Rescue and Conservation Project. (2013). Long live the frog: the 2013 frog festival. [blog post] <http://amphibianrescue.org/tag/national-golden-frog-day/> (accessed 7 February 2019).

Araújo, M. B., Thuiller, W., & Pearson, R. G. (2006). Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography* 33: 1712–1728.

Bearlin, A. R., Schreiber, E. S., Nicol, S. J., Starfield, A. M., & Todd, C. R. (2002). Identifying the weakest link: simulating adaptive management of the reintroduction of a threatened fish. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1709–1716.

Bertolero, A., Oro, D., & Besnard, A. (2007). Assessing the efficacy of reintroduction programmes by modelling adult survival: the example of Hermann's tortoise. *Animal Conservation* 10: 360–368.

Bickford, D., Howard, S. D., Ng, D. J., & Sheridan, J. A. (2010). Impacts of climate change on the amphibians and reptiles of Southeast Asia. *Biodiversity and Conservation* 19: 1043–1062.

Blaustein, A. R., Belden, L. K., Olson, D. H., Green, D. M., Root, T. L., & Kiesecker, J. M. (2001). Amphibian breeding and climate change. *Conservation Biology* 15: 1804–1809.

Blaustein, A. R., Wake, D. B., & Sousa, W. P. (1994). Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* 8: 60–71.

Bloxham, Q. M. C., & Tonge, S. J. (1995). Amphibians - suitable candidates for breeding-release programs. *Biodiversity and Conservation* 4: 636–644.

Bosch, J., Sanchez-Tomé, E., Fernández-Loras, A., Oliver, J. A., Fisher, M. C., & Garner, T. W. (2015). Successful elimination of a lethal wildlife infectious disease in nature. *Biology Letters* 11: 20150874.

Bowkett, A. E. (2009). Recent captive-breeding proposals and the return of the ark concept to global species conservation. *Conservation Biology* 23: 773–776.

Brannelly, L. A., Hunter, D. A., Skerratt, L. F., Scheele, B. C., Lenger, D., McFadden, M. S., Harlow, P. S. & Berger, L. (2016). Chytrid infection and post-release fitness in the reintroduction of an endangered alpine tree frog. *Animal Conservation*, *19*(2), pp.153-162.

Brown L. E., Mesrobian, A. (2005). Houston toads and Texas politics. In: M.J. Lannoo (ed.) *Amphibian Declines: The Conservation Status of United States Species*, pp.150–167. Berkeley: University of California Press.

Carrillo, L., Johnson, K., & Mendelson, J. R., III. (2015). Principles of program development and management for amphibian conservation captive breeding programs. *International Zoo News* 62: 96–107.

Caughley, G. (1994). Directions in conservation biology. *The Journal of Animal Ecology* 63: 215–244.

Channing, A., Finlow-Bates, K. S., Haarklau, S. E., & Hawkes, P. G. (2006). The biology and recent history of the critically endangered Kihansi spray toad *Nectophrynoides asperginis* in Tanzania. *Journal of East African Natural History* 95: 117–138.

Chauvenet A. L. M., Canessa S., Ewen J. G. (2016). Setting objectives and defining the success of reintroductions. In: D. S. Jachowski, J. J. Millspaugh, P. L. Angermeier, and R. Slotow, (eds.). *Reintroduction of Fish and Wildlife Populations.* University of California Press, Berkeley, CA, USA. pp. 105–121.

Choi, R. T., & Beard, K. H. (2012). Coqui frog invasions change invertebrate communities in Hawaii. *Biological Invasions* 14: 939–948.

Coloma, L. A., & Almeida-Reinoso, D. (2012). *Ex situ* management of five extant species of *Atelopus* in Ecuador—progress report. *Amphibian Ark Newslett*er 20: 9–12.

Converse, S. J., & Armstrong D. P. (2016). Demographic modeling for reintroduction decision-making. In: D. S. Jachowski, J. J. Millspaugh, P. L. Angermeier, and R. Slotow, (eds.). *Reintroduction of Fish and Wildlife Populations.* University of California Press, Berkeley, CA, USA. pp. 123–146

Crane, A. L., & Mathis, A. (2011). Predator-recognition training: a conservation strategy to increase post release survival of hellbenders in head-starting programs. *Zoo Biology* 30: 611–622.

Crump, M. L. (2015). *Eye of Newt and Toe of Frog, Adder's Fork and Lizard's Leg: The Lore and Mythology of Amphibians and Reptiles*. Chicago: University of Chicago Press.

Dang, T. D., Searle, C. L., & Blaustein, A. R. (2017). Virulence variation among strains of the emerging infectious fungus *Batrachochytrium dendrobatidis* (*Bd*) in multiple amphibian host species. *Diseases of Aquatic Organisms*, 124(3), pp.233-239.

Dickens, M. J., Delehanty, D. J., & Romero, L. M. (2010). Stress: an inevitable component of animal translocation. *Biological Conservation* 143: 1329–1341.

Dodd, C. K., Jr., J. Loman, D. Cogalniceanu, & Puky, M. (2012). Monitoring amphibian populations. In: H.H. Heatwole & J. W. Wilkenson (eds.) *Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans and Management*, pp.3577–3635. Amphibian Biology, Volume 10. New South Wales, Australia: Surrey Beatty & Sons.

Duan, R. Y., Kong, X. Q., Huang, M. Y., Varela, S., & Ji, X. (2016). The potential effects of climate change on amphibian distribution, range fragmentation and turnover in China. *PeerJ* 4:e2185.

English Nature. (2001). Great Crested Newt Mitigation Guidelines. Peterborough, UK: English Nature.

Essner Jr, R. L., Jorgensen, M. E., Corbin, C. E., Robins, J. H., Suffian, D. J., & Toft, N. A. (2012). A technique for field maintenance and transport of cold-water amphibians. *Herpetological Review* 43: 247–249.

Ewen, J. G., & Armstrong, D. P. (2007). Strategic monitoring of reintroductions in ecological restoration programmes. *Ecoscience* 14: 401–409.

Ewen, J. G., Acevedo-Whitehouse, K., Alley, M. R., Carraro, C., Sainsbury, A. W., Swinnerton, K., & Woodroffe, R. (2012). Empirical consideration of parasites and reintroduction. In: Ewen, J. G., Armstrong, D. P., Parker, K. A., & Seddon, P. J. (eds.) *Reintroduction Biology: Integrating Science and Management,* pp.290–335. Oxford, UK: Jon Wiley & Sons.

Farrer, R. A., Weinert, L. A., Bielby, J., Garner, T. W., Balloux, F., Clare, F., Bosche, J., Cunningham A. A., Weldon, C., du Preez, L. H., Anderson, L., Pond, S. L. K., Shahar-Golan, R., Henk, D. A., & Fischer, M. C. (2011). Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proceedings of the National Academy of Sciences* 108: 18732–18736.

Ferrie, G. M., Alford, V. C., Atkinson, J., Baitchman, E., Barber, D., Blaner, W. S., Crawshaw, G., Daneault, A., Dierenfeld, E., Finke, M., & Fleming, G. (2014). Nutrition and health in amphibian husbandry. *Zoo Biology* 33: 485–501.

Fiedler, P. L., & Laven, R. D. (1996). Selecting reintroduction sites. In: D. A. Falk, C. I. Millar, & M. Olwell (eds.) *Restoring Diversity: Strategies for Reintroduction of Endangered* Plants, pp.157–169*.* Washington, DC: Island Press.

Fischer, J., & Lindenmayer, D. B. (2000). An assessment of the published results of animal relocations. *Biological Conservation* 96: 1–11.

Foster, J, Buckley, J, Martin, Y, Baker, J & Griffiths, RA (2018). Re-introduction of the pool frog to the United Kingdom. In: Soorae, P (ed.) *Global Reintroduction Perspectives: 2018. Case studies from around the globe*. IUCN/SSC Re-introduction Specialist Group.

Frankham, R. (2008). Genetic adaptation to captivity in species conservation programs. *Molecular Ecology* 17: 325–333.

Frost, D. R. (2019). Amphibian Species of the World: An Online Reference. Version 6.0. [Database] New York, New York: American Museum of Natural History. <research.amnh.org/herpetology/amphibia/index.html> (accessed *3 July 2019*).

Gagliardo, R., Crump, P., Griffith, E., Mendelson, J., Ross, H., & Zippel, K. (2008). The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *International Zoo Yearbook* 42: 125–135.

Gagliardo, R., Griffith, E., Hill, R., Ross, H., Mendelson, J. R., Timpe, E., & Wilson, B. (2010). Observations on the captive reproduction of the Horned Marsupial Frog *Gastrotheca cornuta* (Boulenger 1898). *Herpetological Review* 41: 52–58.

Gallagher, R. V., Makinson, R. O., Hogbin, P. M., & Hancock, N. (2015). Assisted colonization as a climate change adaptation tool. *Austral Ecology* 40: 12–20.

Garcia, R. A., Cabeza, M., Altwegg, R., & Araújo, M. B. (2016). Do projections from bioclimatic envelope models and climate change metrics match? *Global Ecology and Biogeography* 25: 65–74.

Germano, J. M., & Bishop, P. J. (2009). Suitability of amphibians and reptiles for translocation. *Conservation Biology* 23: 7–15.

Germano, J. M., Field, K. J., Griffiths, R. A., Clulow, S., Foster, J., Harding, G., & Swaisgood, R. R. (2015). Mitigation-driven translocations: are we moving wildlife in the right direction? *Frontiers in Ecology and the Environment* 13: 100–105.

Gilligan, D. M., & Frankham, R. (2003). Dynamics of genetic adaptation to captivity. *Conservation Genetics* 4: 189–197.

Griffin, A. S., Blumstein, D. T., & Evans, C. S. (2000). Training captive-bred or translocated animals to avoid predators. *Conservation Biology* 14: 1317–1326.

Griffith, B., Scott, J. M., Carpenter, J. W., & Reed, C. (1989). Translocation as a species conservation tool: status and strategy. *Science* 245: 477–480.

Groombridge, J. J., Raisin, C., Bristol, R., & Richardson, D. S. (2012). Genetic consequences of reintroductions and insights from population history. In: Ewen, J. G., Armstrong, D. P., Parker, K. A., & Seddon, P. J. (eds.) *Reintroduction Biology: Integrating Science and Management*, pp.395–440. Oxford, UK: Jon Wiley & Sons.

Hammerson, G. 2004. *Anaxyrus baxteri*. The IUCN Red List of Threatened Species (2004): e.T54583A11155140. <http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T54583A11155140.en> (accessed 4 January 2018).

Harding, G., Griffiths, R. A., & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology* 30: 340–349.

Harrington, L. A., Moehrenschlager, A., Gelling, M., Atkinson, R. P., Hughes, J., & Macdonald, D. W. (2013). Conflicting and complementary ethics of animal welfare considerations in reintroductions. *Conservation Biology* 27: 486–500.

Heard, G. W., Thomas, C. D., Hodgson, J. A., Scroggie, M. P., Ramsey, D. S. L., & Clemann, N. (2015). Refugia and connectivity sustain amphibian metapopulations afflicted by disease. *Ecology Letters* 18: 853–863.

Heyer, W. R., Donnelly, M. A., McDiarmid, R. W., Hayek, L. C., Foster, M. S. (1994). Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington, DC.

Hoegh-Guldberg, O., Hughes, L., McIntyre, S., Lindenmayer, D. B., Parmesan, C., Possingham, H. P., & Thomas, C. D. (2008). Assisted colonization and rapid climate change. *Science* 321: 345–346.

Ibáñez, A., Caspers, B. A., López, P., Martín, J., & Krause, E. T. (2014). Is the reaction to chemical cues of predators affected by age or experience in fire salamanders (*Salamandra salamandra*)? *Amphibia-Reptilia* 35: 189–196.

IUCN/SSC. (2013). *Guidelines for Reintroductions and Other Conservation Translocations*. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission. https://doi.org/10.2305/IUCN.CH.2018.08.en

Iversen, M., Finstad, B., & Milssen, K. J. (1998). Recovery from loading and transport stress in Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture* 168: 387–394.

Jakob-Hoff, R. M., MacDiarmid, S. C., Lees, C., Miller, P. S., Travis, D., & Kock, R. (2014). *Manual of procedures for wildlife disease risk analysis*. World Organization for Animal Health, Paris, 160 pp. Published in association with the International Union for Conservation of Nature and the Species Survival Commission.

Jamieson, I. G., & Lacy, R. C. (2012). Managing issues in reintroduction biology. In: Ewen, J. G., Armstrong, D. P., Parker, K. A., & Seddon, P. J. (eds.) *Reintroduction Biology: Integrating Science and Management*, pp.441–475. Oxford, UK: Jon Wiley & Sons.

Jenkinson, T. S., Betancourt Roman, C. M., Lambertini, C., Valencia-Aguilar, A., Rodriguez, D., Nunes-de-Almeida, C. H. L., Ruggeri, J., Belasen, A. M., Silva Leite, D., Zamudio, K. R., & Longcore, J. E. (2016). Amphibian-killing chytrid in Brazil comprises both locally endemic and globally-expanding populations. *Molecular Ecology* 25: 2978–2996.

Keller, L. F., Biebach, I., Ewing, S. R., & Hoeck, P. E. (2012). The genetics of reintroductions: inbreeding and genetic drift. In: Ewen, J. G., Armstrong, D. P., Parker, K. A., & Seddon, P. J. (eds.) *Reintroduction Biology: Integrating Science and Management*, pp.360–394. Oxford, UK: Jon Wiley & Sons.

Kemp, L., Norbury, G., Groenewegen, R., & Comer, S. (2015). The roles of trials and experiments in fauna reintroduction programs. In: D. Armstrong, M. Hayward, D. Moro, & P. Seddon (eds.) *Advances in Reintroduction Biology of Australian and New Zealand Fauna*, pp.73–90. Melbourne, Australia: CSIRO Publishing.

Khatibu, F. H. A., Church, D. R., Moore, R. D., Lewis, J. P., McAloose, D., Magurisha, H. B., Hanley, C., Pessier, A., Misinzo, G., Malago, J. J., Howell, K., Kalokola, P., Ngalason, W., Pramuk, J. Newmark, B. Weldon, C., Odum, A., Gibbs, J., Mturi, F., Mbuta, C., Masunzu, C., Sabuni, F., Ferdinard, F., Pilla, J., Jeswald, U., Manusa, M., Shayo, C., Mwikila, D., Sylivester, B., Maembe, A., Kibassa, J. Kihaule, J., Kaoneka, D., O'Connor, M., Soorae, P., & Msuya, C. *(*2013). *Kihansi spray toad re-introduction guidelines*. <http://www.amphibians.org/wp-content/uploads/2013/07/kihansi-spray-toad-re-introduction-guidelines.pdf>

Kleiman, D. G., Stanley Price, M. R. & Beck, B. B. (1994). Criteria for reintroductions. In P. Olney, G. Mace, & A. Feistner (eds.) *Creative Conservation: Interactive Management of Wild and Captive Animals*, pp.287–303*.* London, UK: Chapman & Hall.

Lacy, R. C., Glen A., & Walsh, A. (2013). Evolution of *Peromyscus leucopus* mice in response to a captive environment." *PloS One* 8: e72452.d

Lee, S., Zippel, K., Ramos, L., & Searle, J. (2006). Captive-breeding programme for the Kihansi spray toad *Nectophrynoides asperginis* at the Wildlife Conservation Society, Bronx, New York. *International Zoo Yearbook* 40: 241–253.

Lowe, K., Castley, J. G., & Hero, J. M. (2015). Resilience to climate change: complex relationships among wetland hydroperiod, larval amphibians and aquatic predators in temporary wetlands. *Marine and Freshwater Research* 66: 886–899.

Marsh, D. M., & Trenham, P. C. (2001). Metapopulation dynamics and amphibian conservation. *Conservation Biology* 12: 40–49.

Martín-Beyer, B., Fernández-Beaskoetxea, S García, G., & Bosch, J. (2011). Re-introduction program for the common midwife toad and Iberian frog in the Natural Park of Peñalara in Madrid, Spain: can we defeat chytridiomycosis and trout introductions? In: P. S. Soorae (ed.) *Global Re-introduction Perspectives, 2011: More Case Studies From Around the Globe*, pp.81–84. Gland, Switzerland: IUCN/SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi. https://portals.iucn.org/library/node/10058

McCarthy, M. A., Armstrong, D. P., & Runge, M. C. (2012). Adaptive management of reintroduction. In: Ewen, J. G., Armstrong, D. P., Parker, K. A., & Seddon, P. J. (eds.) *Reintroduction Biology: Integrating Science and Management*, pp.256–289. Oxford, UK: Jon Wiley & Sons.

McDougall, P. T., Réale, D., Sol, D., & Reader, S. M. (2006). Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. *Animal Conservation* 9: 39–48.

McPhee, M. E., & Silverman, E. D. (2004). Increased behavioral variation and the calculation of release numbers for reintroduction programs. *Conservation Biology* 18: 705–715.

Mendelson, J. R., III, & Altig, R. (2016). Tadpoles, froglets, and conservation: A discussion of basic principles of rearing and release procedures. *Amphibian and Reptile Conservation* 10: 20–27.

Michaels, C. J., Gini, B. F., & Preziosi, R. F. (2014). The importance of natural history and species-specific approaches in amphibian *ex-situ* conservation. *The Herpetological Journal* 24: 135–145.

Miller, K. A., Bell, T. P., & Germano, J. M. (2014). Understanding publication bias in reintroduction biology by assessing translocations of New Zealand herpetofauna. *Conservation Biology* 28: 1045–1056.

Milot, E., Perrier, C., Papillon, L., Dodson, J. J. and Bernatchez, L. (2013). Reduced fitness of Atlantic salmon released in the wild after one generation of captive breeding. *Evolutionary Applications*, *6*(3), pp.472-485.

Minteer, B. A., & Collins, J. P. (2005). Ecological ethics: building a new tool kit for ecologists and biodiversity managers. *Conservation Biology* 19: 1803–1812.

Miskelly, C. M., & Powlesland, R. G. (2013). Conservation translocations of New Zealand birds: 1863–2012. *Notornis* 60: 3–28.

Mokhatla, M. M., Rödder, D., & Measey, G. J. (2015). Assessing the effects of climate change on distributions of Cape Floristic Region amphibians. *South African Journal of Science* 111: 1–7.

Moore, I. T., & Jessop, T. S. (2003). Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Hormones and Behavior* 43: 39–47.

Murray D. L., Roth, J. D. & Wirsing, A. J. (2004). Predation risk avoidance by terrestrial amphibians: The role of prey experience and vulnerability to native and exotic predators. *Ethology* 110: 635–647.

Murray, K., Skerratt, L., Marantelli, G., Berger, L., Hunter, D., Mahony, M., & Hines, H. (2011). Hygiene protocols for the control of diseases in Australian frogs: report for the Australian Government Department of Sustainability, Environment, Water, Population and Communities. <https://www.environment.gov.au/biodiversity/invasive-species/publications/hygiene-protocols-control-diseases-australian-frogs> (accessed *3 July 2019*).

Muths, E., & Dreitz, V. J. (2008). Monitoring programs to assess reintroduction efforts: a critical component in recovery. *Animal Biodiversity and Conservation* 31: 47–56.

National Species Reintroduction Forum. (2014). The Scottish Code for Conservation Translocations. *Scottish Natural Heritage*. <http://www.snh.gov.uk/docs/A1327922.pdf> (accessed *3 July 2019*).

Nettles, V. F. (1988). Wildlife relocation: disease implications and regulations. In: *Proceedings of the 37th Annual Conference of the Wildlife Disease Association*, Athens, Georgia, p. 52.

Nichols, J. D., & Armstrong, D. P. (2012). Monitoring for reintroductions. In: Ewen, J.G., Armstrong, D.P., Parker, K.A., & Seddon, P.J. (eds.) *Reintroduction Biology: Integrating Science and Management*, pp.223–255. Oxford, UK: Jon Wiley & Sons.

Nowakowski, A. J., Watling, J. I., Whitfield, S. M., Todd, B. D., Kurz, D. J., & Donnelly, M. A. (2016). Tropical amphibians in shifting thermal landscapes under land use and climate change. *Conservation Biology* 31: 96–105.

Parker, K. A., Dickens, M. J., Clarke, R. H., Lovegrove, T. G., Ewen, J., & Armstrong, D. (2012). The theory and practice of catching, holding, moving and releasing animals. In: Ewen, J. G., Armstrong, D. P., Parker, K. A., & Seddon, P. J. (eds.) *Reintroduction Biology: Integrating Science and Management*, pp.105–137. Oxford, UK: Jon Wiley & Sons.

Pessier, A. P., & Mendelson, J. R. III (eds.) (updated 2017). (2017). *A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs.* Apple Valley, Minnesota: IUCN/SSC Conservation Breeding Specialist Group.

Pessier, A. P., Baitchman, E. J., Crump, P., Wilson, B., Griffith, E., & Ross, H. (2014). Causes of mortality in anuran amphibians from an *ex-situ* survival assurance colony in Panama. *Zoo Biology* 33: 516–526.

Plein, M., Bode, M., Moir, M. L., & Vesk, P. A. (2016). Translocation strategies for multiple species depend on interspecific interaction type. *Ecological Applications*, 26: 1186-1197.

Polasik, J. S., Murphy, M. A., Abbott, T., & Vincent, K. (2015). Factors limiting early life stage survival and growth during endangered Wyoming toad reintroductions. *The Journal of Wildlife Management* 80: 540–552.

Poole, V. A., & Grow, S. (eds.). (2012). *Amphibian Husbandry Resource Guide, Edition 2.0*. Silver Spring, Maryland: Association of Zoos and Aquariums.

Pramuk, J., V. Titus, & Wagner, J. (2011). An effective method for transporting hellbenders (*Cryptobranchus alleganiensis*). *Herpetological Review* 42: 532–534.

Price-Rees, S. J., Webb, J. K., & Shine, R. (2013). Reducing the impact of a toxic invader by inducing taste aversion in an imperilled native reptile predator. *Animal Conservation* 16: 386–394.

Randall, L., N. Lloyd, and A. Moehrenschlager. (2018). Guidelines for Mitigation Translocations of Amphibians: Applications for Canada's Prairie Provinces. Version 1.0. Centre for Conservation Research, Calgary Zoological Society. Calgary, Alberta, Canada.

Räsänen, K., Laurila, A., & Merilä, J. (2003). Geographic variation in acid stress tolerance of the moor frog, *Rana arvalis*. II. Adaptive maternal effects. *Evolution* 57: 363–371.

Retallick, R. W., & Miera, V. (2007). Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and non-permanent, sub-lethal effects of infection. *Diseases of Aquatic Organisms* 75: 201–207.

Rout, T. M., Hauser, C. E., & Possingham, H. P. (2009). Optimal adaptive management for the translocation of a threatened species. *Ecological Applications* 19: 515–526.

Sainsbury, A. W., Yu-Mei, R., Ågren, E., Vaughan-Higgins, R. J., Mcgill, I. S., Molenaar, F., Peniche, G. & Foster, J. (2017). Disease Risk Analysis and Post-Release Health Surveillance for a Reintroduction Programme: the Pool Frog *Pelophylax lessonae*. Transboundary and Emerging Diseases, 64(5), pp.1530-1548.

Schad, K (ed.). (2008). *Amphibian Population Management Guidelines*. Amphibian Ark Amphibian Population Management Workshop, 10–11 December 2007; San Diego, CA, USA. Amphibian Ark.

Schock, D. M., Bollinger, T. K., Gregory Chinchar, V., Jancovich, J. K., & Collins, J. P. (2008). Experimental evidence that amphibian ranaviruses are multi-host pathogens. *Copeia* 2008: 133–143.

Schwartz, M. W., Hellmann, J. J., Lachlan, J. M. M., Sax, D. F., Borevitz, J. O., Brennan, J., Camacho, A. E., Ceballos, G., Clark, J. R., Doremus, H., Early, R., Etterson, J. R., Fielder, D., Gill, J. L., Gonzalez, P., Green, N., Hannah, L., Jamieson, D. W., Javeline, D., Minteer, B. A., Odenbaugh, J., Polasky, S., Richardson, D. M., Root, T. L., Safford, H. D., Sala, O., Schneider, S. H., Thompson, A. R., Williams, J. W., Vellend, M., Vitt, P., & Zellmer, S. (2012). Managed relocation: integrating the scientific, regulatory, and ethical challenges. *BioScience* 62: 732–743.

Seddon, P. J., Soorae, P. S., & Launay, F. (2005). Taxonomic bias in reintroduction projects. *Animal Conservation* 8: 51–58.

Semlitsch, R. D. (2002). Critical elements for biologically-based recovery plans for aquatic breeding amphibians. *Conservation Biology* 16: 619–629.

Semlitsch, R. D., & Reyer, H. (1992). Modification of anti-predator behaviour in tadpoles by environmental conditioning. *Journal of Animal Ecology* 61: 353–360.

Shaw, S. D., Skerratt, L. F., Kleinpaste, R., Daglish, L., & Bishop, P. J. (2012). Designing a diet for captive native frogs from the analysis of stomach contents from free-ranging *Leiopelma*. *New Zealand Journal of Zoology* 39: 47–56.

Sheil, D., & Lawrence, A. (2004). Tropical biologists, local people and conservation: new opportunities for collaboration. *Trends in Ecology & Evolution* 19: 634–638.

Shine, R. (2010). The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *The Quarterly Review of Biology* 85: 253–291.

Shoo, L. P., Olson, D. H., McMenamin, S. K., Murray, K. A., Van Sluys, M., Donnelly, M. A., Stratford, D., Terhivuo, J., Merino-Viteri, A., Herbert, S. M., Bishop, P. J., *et al.* (2011). Engineering a future for amphibians under climate change. *Journal of Applied Ecology* 48: 487–492.

Soorae, P.S., Frankham, G.J., Mohamed, A.A. (2020). The first record of the Asian common toad *Duttaphrynus melanostictus* Schneider, 1799 in Abu Dhabi, United Arab Emirates. BioInvasions Records 9(2): 434–443, https://doi.org/10.3391/bir.2020.9.2.29.

Soorae, P. S. (ed.) (2021). *Global conservation translocation perspectives: 2021. Case studies from around the globe*. Gland, Switzerland: IUCN SSC Conservation Translocation Specialist Group, Environment Agency - Abu Dhabi and Calgary Zoo, Canada. xiv + 353pp.

Soorae, P. S. (ed.) (2018). *Global Reintroduction Perspectives: 2018.Case studies from around the globe*. IUCN/SSC Reintroduction Specialist Group, Gland, Switzerland and Environment Agency, Abu Dhabi, UAE. xiv+ 286pp.

Soorae, P. S. (ed.) (2016). *Global Re-introduction Perspectives: 2016. Case-Studies from Around the Globe*. Gland, Switzerland: IUCN/SSC Reintroduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi. https://portals.iucn.org/library/node/45889

Soorae, P. S. (ed.) (2013). *Global Re-introduction Perspectives: 2013. Further case studies from around the globe*. Gland, Switzerland: IUCN/SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi. xiv + 282 pp.

Soorae, P. S. (ed.) (2011). *Global Re-introduction Perspectives: 2011. More Case Studies from Around the Globe.* Gland, Switzerland: IUCN/SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi. https://portals.iucn.org/library/node/10058

Soorae, P. S. (ed.) (2010). *Global Re-introduction Perspectives: Additional Case-Studies from Around the Globe*. Gland, Switzerland: IUCN/SSC Reintroduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi. https://portals.iucn.org/library/node/9774

Soorae, P. S. (ed.) (2008). *Global Re-introduction Perspectives:* Re-introduction case-studies from around the globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE. viii + 284 pp.

Soulé, M., Gilpin, M., Conway, W., & Foose, T. (1986). The millenium ark: how long a voyage, how many staterooms, how many passengers? *Zoo Biology* 5: 101–113.

Staniszewski, M. (1995). *Amphibians in Captivity*. Neptune City, N.J., T.F.H. Publications.

Stebbins, R. C. & Cohen, N. W. (1995). *A Natural History of Amphibians*. Princeton, New Jersey: Princeton University Press.

Stockwell, M. P., Storrie, L. J., Pollard, C.J., Clulow, J. & Mahony, M. J. (2015). Effects of pond salinization on survival rate of amphibian hosts infected with the chytrid fungus. *Conservation Biology*, 29(2), pp.391-399.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783–1786.

Suarez, M. B., Ewen, J. G., Groombridge, J. J., Beckmann, K., Shotton, J., Masters, N., Hopkins, T. & Sainsbury, A.W. (2017). Using qualitative disease risk analysis for herpetofauna conservation translocations transgressing ecological and geographical barriers. *EcoHealth*, 14(1), pp.47-60.

Sullivan, B. K., Kwiatkowski, M. A., & Schuett, G. W. (2004). Translocation of urban Gila Monsters: a problematic conservation tool. *Biological Conservation* 117: 235–242.

Sullivan, B. K., Nowak, E. M. & Kwiatkowski, M. A., (2015). Problems with mitigation translocation of herpetofauna. *Conservation Biology*, *29*(1), pp.12-18.

Sutherland, W. J., Armstrong, D., Butchart, S. H. M., Earnhardt, J. M., Ewen, J., Jamieson, I., Jones, C. G., Lee, R., Newbery, P., Nichols, J. D., Parker, K. A., Sarrazin, F., Seddon, P. J., Shah, N., & Tatayah, V. (2010). Standards for documenting and monitoring bird reintroduction projects. *Conservation Letters* 3: 229–235.

Tapley, B., Bradfield, K. S., Michaels, C., & Bungard, M. (2015a). Amphibians and conservation breeding programmes: do all threatened amphibians belong on the ark? *Biodiversity and Conservation* 24: 2625–2646.

Tapley, B., Rendle, M., Baines, F. M., Goetz, M., Bradfield, K. S., Rood, D., Lopez, J., Garcia, G. & Routh, A. (2015b). Meeting ultraviolet B radiation requirements of amphibians in captivity: A case study with mountain chicken frogs (*Leptodactylus fallax*) and general recommendations for pre-release health screening. *Zoo Biology* 34: 46–52.

Teixeira, B., & Young, R. J. (2014). Can captive-bred American bullfrogs learn to avoid a model avian predator? *Acta Ethologica* 17: 15–22.

Teixeira, C. P., De Azevedo, C. S., Mendl, M., Cipreste, C. F., & Young, R. J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. *Animal Behaviour* 73: 1-13.

Tocher, M., & Brown, D. (2004). *Leiopelma hamiltoni* (NCN.) homing. *Herpetological Review* 35: 259–261.

Walker, S. F, Bosch, J., James, T.Y., Litvintseva, A.P., Valls, J.A., Pina, S., Garcia, G., Rosa, A., Cunningham, A.A., Hole, S., Griffiths, R., & Fisher, M. (2008). Invasive pathogens threaten species recovery programs. *Current Biology* 18: 853–854.

Weeks, A. R., Moro, D., Thavornkanlapachai, R., Taylor, H. R., White, N. E., Weiser, E. L., & Heinze, D. (2015). Conserving and enhancing genetic diversity in translocation programs. In: D. P. Armstrong, M. Hayward, & D. Moro (eds.) *Advances in Reintroduction Biology of Australian and New Zealand Fauna,* pp.127–140. Melbourne, Australia: CSIRO Publishing.

Weiser, E. L., Grueber, C. E., & Jamieson, I. G. (2012). AlleleRetain: a program to assess management options for conserving allelic diversity in small, isolated populations. *Molecular Ecology Resources* 12: 1161–1167.

Wiens J. J. (2016). Climate-Related Local extinctions are already widespread among plant and animal species. *PLoS Biology* 14: e2001104.

Wolf, C. M., Garland, T., & Griffith, B. (1998). Predictors of avian and mammalian translocation success: reanalysis with phylogenetically independent contrasts. *Biological Conservation* 86: 243–255.

Wright, A. N., Schwartz, M. W., Hijmans, R. J., & Shaffer, H. B. (2015). Advances in climate models from CMIP3 to CMIP5 do not change predictions of future habitat suitability for California reptiles and amphibians, *Climatic Change* 1–13.

Appendix I

Global reintroduction perspectives amphibian case-studies: 2008, 2010, 2011, 2013, 2016, 2018 and 2021

GLOBAL RE-INTRODUCTION PERSPECTIVES

Re-introduction case-studies from around the globe

Edited by

Pritpal S. Soorae

RSG

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Cover photo: Clockwise starting from top-left:
- Formosan salmon stream, Taiwan
- Students in Madagascar with tree seedlings
- Virgin Islands boa

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Translocation of Romer's Tree Frog in Hong Kong SAR, China

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Introduction

Romer's tree frog used to be called ***Philautus romeri,*** but a recent taxonomic review places it tentatively in the genus ***Chirixalus*** due to its free-swimming larval stage. This species is listed as Endangered by the IUCN and is protected in Hong Kong under the "Wild Animals Protection Ordinance". It is endemic to Hong Kong and is naturally known from four off-shore islands. The species became threatened when Chek Lap Kok, one of the four islands originally inhabited by this species, was chosen as the site for the new airport in 1989. In late 1991, the Royal Hong Kong Jockey Charities Ltd. supported the University of Hong Kong to conserve Romer's tree frog. Rescue operations were carried out from November 1991 to December 1992 and captive-breeding programs were established at the University of Hong Kong (UHK) and at Melbourne Zoo (MZ). Habitat requirements, ecology and genetic relationships among the different populations were also studied. Suitable release sites were identified in the New Territories and Hong Kong Island where natural populations were absent and translocations were carried out from 1993 to 1996.

Goals

- Goal 1: To establish viable populations of the Chek Lap Kok population of Romer's tree frogs in the release sites.
- Goal 2: To increase the number of individuals through captive breeding.
- Goal 3: To gain knowledge on the ecology, breeding biology, genetics and captive care of this species through field study and captive observations.

Success indicators

- Indicator 1: Viable populations established in the release sites and their range expanded.
- Indicator 2: The captive-breeding program is successful, producing the required number of individuals for the releases.
- Indicator 3: Enough knowledge

Romer's tree frog (*Philautus romeri*)

gained on this species to ensure a high degree of success in both the captive breeding and translocation programs.

Project Summary

Feasibility Stage: Funding was secured by the UHK. A literature search was carried out to determine important success factors and concerns in cases of amphibian and reptile re-introductions. Field work was carried out on Chek Lap Kok to assess the species' distribution and a small number of frogs were captured and maintained in captivity before the project started.

Implementation Stage: Rescue operations were carried out from 1991 to 1992 when construction had already started. Field studies were conducted into habitat requirements and ecology. Partners in captive-breeding programs were sought through the IUCN/SSC Captive Breeding Specialist Group. Melbourne Zoo and Frankfurt Zoo agreed to join the program and breeding was successful in the UHK and MZ. Frogs bred at MZ were transferred to UHK for subsequent release. Genetic studies were undertaken to look at the genetic relationships among the different insular populations and it was found that there was some genetic differentiation among them. Hence, release of the Chek Lap Kok frogs to the other three islands was ruled out. Potential release sites were identified in the mainland New Territories and Hong Kong Island. Discussions were carried out with the relevant government departments and Kadoorie Farm & Botanic Garden (KFBG) to select sites where frogs would be protected in the future and to carry out habitat management work to provide suitable breeding habitats. In 1993, trial release of tadpoles was carried out in three sites and they were monitored weekly. Marked adults were only released when tadpoles survived and grew. The released individuals were again monitored regularly. Translocation was expanded to five additional sites in 1994 after tadpoles succeeded in metamorphosing and calling males were located in the three trial sites.

Post-release Monitoring Stage: The released populations were monitored at least once every year during the breeding season to locate individuals (in particular calling males and tadpoles) and to map their distribution. Follow-up work was needed for some sites to maintain the breeding habitats. Even after the project finished, monitoring was carried out initially by the project implementer (Michael Lau at the UHK) and later taken up by the Agriculture, Fisheries & Conservation Department and KFBG.

Breeding tubs for released individuals

Major difficulties faced

- Very little was known about this species when the project started.

- The rescue work had a very limited time frame as construction had already started before the project began.
- The captive-breeding program consumed a lot of time and manpower as this species matures in less than a year and produces several clutches per year.
- Not many well-documented successful amphibian re-introduction examples to draw from.

Major lessons learned

- Adequate understanding of the species' ecology, biology and genetics is essential.
- A project of this nature takes at least five years (even on a species with very short generation time). This might be more than a funding agency is willing to cover and more than the normal time span of a post-graduate project.
- Captive-breeding can be very time-consuming and resource demanding and partnerships should be established with other organizations, especially zoos as they have the expertise and facilities.
- If the project requires captive-breeding, this should involve more than one institution to reduce the impact of potential accidents.
- Captive-breeding and re-introduction programs are good at attracting media and public attention. This should then be used to raise community awareness and promote conservation of the species and its habitats.
- Open exchange of information and experiences very important for project success.
- Continual monitoring is required to prevent habitat degradation and to maintain suitable conditions for the target species.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reasons for success/failure:

1. Major funding to enable the necessary studies to be undertaken.
2. A committed individual with the necessary skills and expertise to work consistently on the project from the outset.
3. Having consistent institutional support.
4. An external partner organization to provide captive management/breeding support, which was important in the initial stages to spread the risk of captive management failure.

Re-introduction of Puerto Rican crested toads to historic range in Puerto Rico

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Introduction

The Puerto Rican crested toad (*Peltophryne lemur*) is listed as threatened by the United States Fish and Wildlife Service and critically endangered by the IUCN. Two genetically distinct northern and southern populations once existed in Puerto Rico. The northern populations are extirpated in the wild. The only known wild population is found in Guanica National Forest and over 25 years the population has fluctuated between 500 and 2,000 adults. A stable breeding population of northern and southern toads is held in captivity. Addressing threats is important given the small population, single breeding pond and potential for a catastrophic event to cause extinction. The American Zoo Association Species Survival Plan (SSP) for the crested toad was approved in 1984. A USFWS Service Recovery plan was written in 1991. The SSP has merged management goals with those of the recovery plan. Recovery partners: 21 zoos and aquariums (US, Canada, UK and Puerto Rico), USFWS, Puerto Rico Department of Natural and Ecological Resources, University of Puerto Rico, Puerto Rican National Park Company at Juan Rivero Zoo, Iniciativa Herpetologica, Inc. and Citizens of the Karst. Recovery priorities for this species are coordinated through the FWS Puerto Rican Crested Toad Recovery Plan and Population and Habitat Viability Analysis Working Group.

Puerto Rican Crested Toad
(*Peltophryne lemur*)

Goals

- Goal 1: Creation of new ponds to support six self-sustaining meta-populations (three in the north and three in the south).
- Goal 2: Expansion of ecological research.
- Goal 3: Protection and restoration of existing habitat.
- Goal 4: Island-wide education and outreach.
- Goal 5: Re-introduction of tadpoles from captive genetically and demographically managed population.
- Goal 6: In-country training and

capacity building.

Success Indicators

- Indicator 1: To meet demographic and genetic goals of captive management, expansion of captive population to over 400, supplemented by tadpoles collected from wild.
- Indicator 2: Post-release survival to maturity in wild of captive bred tadpoles.
- Indicator 3: Breeding of adult toads released as tadpoles within 10 years; ongoing until six meta-populations breeding for 10 years.
- Indicator 4: No net loss of breeding habitat.
- Indicator 5: Increased profile and awareness of threats to toads.
- Indicator 6: Increase in number of constructed breeding sites (to support meta-population persistence) on protected lands.
- Indicator 7: In-country training and establishment of captive breeding and release in Puerto Rico.

Project Summary

Feasibility: Focus would remain on protection, hydrological research, and addressing threats to the single remaining natural breeding wetland in Guanica forest. Only tadpoles (to maintain a potential founder group of 20) from separate tadpole schools or pond sections would be collected to establish captive populations. Several research projects were initiated on the captive populations (genetic, growth, health screening, and nutritional). Lack of awareness of the existence of the toad and the threats to its survival were identified and stakeholder groups identified. Forging working partnerships with shared goals was initiated through working meetings with USFWS, DNER and AZA SSP with invited stakeholders. Working groups expanded to include all stakeholders and formalized in a PHVA Masterplan. A GIS based survey of potential release sites was subjected to further on site analysis to select best sites to establish satellite populations.

Implementation: Recovery efforts are directed through a Memorandum of Understanding between the USFWS, Department of Natural and Ecological Resources (DNER), Puerto Rican National Park Company and the AZA. Permit requirements are met through annual issue of blanket permit listing participating institutions to facilitate and expedite (within six days of hatching) movement of tadpoles back to Puerto Rico. All tadpoles are released at the earliest age possible to ponds outside the existing migratory range of the single extant

Tamarindo breeding site

Puerto Rican crested toad mascot

population and within ground truthed habitat profiles in the historic range of the toad. All tadpoles are subject to health screening prior to release; random testing for disease; and no tadpoles are released from groups with parents with illness or death and tadpole groups with unexplained deaths prior to release.

Post-release monitoring: Marking techniques for tadpoles and technology to efficiently track toads through a labyrinth of subterranean limestone caverns has yet to be developed. Subsequently, post metamorphic survival and movements have been the subject of graduate projects. All natural and constructed breeding ponds are monitored for breeding activity under guidelines establishing windows for searches. Monitoring of historic and release sites has begun using automated frog call loggers. Health assessment studies of sympatric species and crested toads is ongoing. This also includes chytrid fungus screening.

Major difficulties faced

- Difficulty of monitoring either adults or juvenile toads in natural habitat.
- Lack of protected release sites in the north.
- Loss of protected wild habitat.
- No formal biological research program to understand natural history and severity of identified threats paralleling efforts to maintain assurance populations.
- Funding for inter-disciplinary research.

Major lessons learned

- Large number of early age metamorphs required to mimic natural life stage mortality tables (i.e. ramp up partners to meet numbers before releases attempted).
- Importance of establishing in-country partnerships and agreement on shared goals at earliest stages.
- Need to establish assurance populations early even while protection of natural habitat and addressing threats is being undertaken.
- Need for and value of social marketing skills and trained professionals to deliver these skills.
- Need for leadership to win small short-term victories in the face of overwhelming odds and to show success while formal long-term programming is under development.
- It may take up to 10 years before establishment of a re-introduced population;

highly variable dependant upon number of offspring released.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reasons for success/failure:

1. Juvenile recruitment has been confirmed at one southern location (the other two release sites are less than two years old); ongoing construction of ponds for increasing protected breeding habitat is underway.
2. Breeding of adult toads themselves released as tadpoles into ponds constructed for release has been confirmed over two breeding seasons.
3. Increased awareness of threats and partnerships for conservation action.
4. We are seeing recruitment at the main release site and the Puerto Ricans are finally taking ownership of this project. This program has also been used as a model for many other release programs). Long-term population persistence has not been documented, so partially successful in that regard.

Re-introduction of the Mallorcan midwife toad, Mallorca, Spain

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Introduction

The Mallorcan midwife toad (***Alytes muletensis***, Sanchíz & Alcover, 1977) or ***ferreret*** was first described in the 1970s as ***Baleaphryne muletensis*** from upper Pleistocene fossils, and was considered extinct. The discovery of live tadpoles in 1980 led to further research which confirmed the species as extant and endemic to Mallorca (Mayol & Alcover, 1981). Subfossils suggest that the species was once widespread across the island, but today it is confined to a few gorges within the Serra de Tramuntana mountains in the north-west part of the island. There are currently about 34 populations within the mountains and adjacent areas (16 original wild populations plus 18 re-introductions). These are largely isolated from each other by physiographic barriers, but there is little evidence of any inbreeding depression. Re-introduction of captive bred toads started in 1989 and it is estimated that about 25% of the wild toads stem from captive bred stock. The successful re-introduction program contributed to the downgrading of the species from 'Critically Endangered' to 'Vulnerable' in the Global Amphibian Assessment of 2004. There is little evidence that wild populations are continuing to decline, but the recent discovery of chytridiomycosis in four populations gives cause for concern.

Mallorcan midwife toad (*Alytes muletensis*)

Goals

- Goal 1: Identification of potential re-introduction sites within the species' historic range.
- Goal 2: Habitat management and creation at potential re-introduction sites.
- Goal 3: Sustainable populations of toads established in all areas where there is suitable habitat, hydrology and absence of introduced predators.
- Goal 4: Annual monitoring of all toad populations (both natural and

re-introduced).

Success Indicators

- Indicator 1: Self-sustaining populations established at re-introduction sites.
- Indicator 2: Overall geographical distribution of the species extended.

Toad tadpoles in a natural pool

Project Summary

A captive breeding program was initiated at Jersey Zoo in 1985 following the collection of 8 animals from the wild. This was supplemented by a further 12 individuals in 1987 and the species was bred for the first time in 1988. Further breeding colonies were subsequently established at other collection-based institutions and Universities in Europe, with the Balearic Island government retaining formal ownership of all animals. Following an assessment of potential re-introduction sites by the Mallorcan conservation authority (Conselleria d'Agricultura i Pesca), 76 tadpoles were returned to Mallorca and released at 2 sites in 1989. Since that time releases of both toadlets and tadpoles occurred on an annual basis up to 1997 (Buley & García, 1997), and then less regularly until 2001.

Meetings of all project partners have occurred at approximately two-yearly intervals to evaluate progress and decide upon future goals. In 1996 an extensive health screening program of captive toads was established (probably the first for any amphibian in a captive-breeding program). Toads underwent parasitological and bacterial screening for three months prior to release, and fecal samples were collected from both captive and wild toads for analysis by the veterinary department at Jersey Zoo. As all toads in captivity were descended from the original 20 founders collected in 1985 - 1987, and three new bloodlines were established in captivity in 1997 with the collection of 25 tadpoles from each of three wild populations (Buley & Gonzalez-Villavicencio, 2000; Roca ***et al.***, 1998, 2000).

With concerns growing towards the end of the 1990s about the global impact of emerging infectious diseases on amphibians, a recommendation was made that no further re-introductions should be carried out until i) the disease implications of further re-introductions became clearer; and ii) genetic analysis of both wild and captive populations was carried out. Microsatellite DNA analysis was completed in 2006, and revealed that although populations in different gorges were largely isolated, wild populations retained relatively high levels of genetic diversity. Equally, there was no evidence that reintroduced or captive toads had suffered any loss of fitness or genetic variability for up to eight generations of captive breeding (Kraaijeveld-Smit ***et al.***, 2005; 2006). Screening for chytridiomycosis

Artificial cistern which is used by ***Alytes*** (now constructed as a conservation management measure)

(***Batrachochytrium dendrobatidis***) was added to the health screening protocol in 2005, and chytrid-positive animals have subsequently been identified in four populations. The impact of chytrid remains unclear, but successful breeding still appears to be occurring in the populations concerned.

A complete census of all ***Alytes muletensis*** breeding sites is carried out annually. As the adult toads spend most of their lives underground and are very difficult to survey, the censuses consist of counts of tadpoles observed in each pool. Although it is difficult to relate such simple counts to actual population sizes, the presence of abundant tadpoles spread across several size classes provides a useful index of breeding success. Breeding populations of toads have become established at all 18 sites where re-introductions were carried up to 2001, and wild populations appear to be stable, and in some cases, increasing. Since its early days, the conservation program for the Mallorcan midwife toad has embraced a multidisciplinary approach to species recovery. In this respect, the wider components of the project have included conservation education initiatives, publicity, applied ecological research, predator control, conservation genetics, health screening and habitat management and creation. In addition to using natural torrent pools as breeding sites, the toad also breeds successfully in artificial cisterns constructed for the watering of livestock. Construction of such cisterns in suitable areas has proved to be a successful supplementary conservation action.

Major difficulties faced

- Alien predators and competitors – notably the viperine snake (***Natrix maura***) and Spanish marsh frog (***Rana perezi***) – remain a widespread and very significant threat and are very difficult to control.
- A burgeoning human population coupled with climate change means that water is in short supply on Mallorca. Consequently, torrents flow less frequently than they once did and breeding pools may be more prone to desiccation.
- Because of the two points mentioned above it is impossible to completely neutralize the threats to the toads on the island, and re-introductions may therefore need to be accompanied by management measures to minimize the impact of alien predators and desiccation.

Major lessons learned

- A small partnership of co-operative stakeholders that meet regularly enabled decisions to be made quickly and appropriate actions implemented.
- A health screening program was in place before reliable methods for the detection of chytridiomycosis were known. Chytridiomycosis (and possibly other emerging infectious diseases not yet known to science) may therefore have gone undetected for several years.
- Management decisions have been informed by scientific research (more scientific papers have been published on ***Alytes muletensis*** than on any other amphibian species in a captive breeding/re-introduction program).
- The program has been running for nearly 30 years, and during this time has tried to embrace new ideas and protocols in re-introduction practice as they have been developed. Consequently the whole program has 'evolved' rather than been 'planned'.

Success of project

Highly Successful	Successful	Partially Successful	Failure
√			

Reasons for success/failure:

- The Mallorcan midwife toad was the only amphibian species in the Global Amphibian Assessment to be downgraded from 'Critically Endangered' to 'Vulnerable' in 2004.
- All of the 18 re-introductions appear to have been successful. This has resulted in a doubling of the original geographical range of the species.

References

Buley, K.R. & García, G. (1997) The recovery programme for the Mallorcan midwife toad ***Aytes muletensis***: an update. Dodo 33: 80 - 90.

Buley, K.R. & Gonzalez-Villavicencio, C. (2000) The Durrell Wildlife Conservation Trust and the Mallorcan midwife toad, ***Alytes muletensis*** – into the 21st century. Herpetological Bulletin 72: 17 - 20.

Kraaijeveld-Smit, F.J.L., Beebee, T.J.C., Griffiths R.A., Moore, R.D. & Schley, L. (2005) Low gene flow but high genetic diversity in the threatened Mallorcan midwife toad ***Alytes muletensis***. Molecular Ecology 14: 3307 - 3315.

Kraaijeveld-Smit, F.J.L., Griffiths, R.A., Moore, R.D. & Beebee, T.J.C. (2006) Captive breeding and the fitness of reintroduced species: a test of the responses to predators in a threatened amphibian. Journal of Applied Ecology 43: 360 - 365.

Mayol, J. & Alcover, J.A. (1981) Survival of Baleaphryne Sanchíz and Adrover, 1979 (Amphibia: Anura: Discoglossidae) on Mallorca. Amphibia-Reptilia 3/4, 343 - 345.

Roca, V., García, G., Carbonell, E., Sánchez-Acedo, C. & Del Cacho, E. (1998) Parasites and conservation of ***Alytes muletensis*** (Sanchiz & Adrover, 1997) (Anura: Discoglossidae). Revista Española de Herpetología 12: 91 - 95.

Roca, V., Galdón, M. A., Martín, J. E., García, G. & Lopez, J. (2004) Primeros datos acerca de la población natural del sapillo balear ***Alytes multensis*** (Sanchiz et Adrover, 1977) (Anura: Discoglossidae). Bolletín Asociación Herpetológica Española 15: 44 - 49.

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Re-introduction of the natterjack toad in the UK

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Introduction

The natterjack toad (*Epidalia [Bufo] calamita*) has a broad range in north central and western Europe but it is rarer - and in many places declining - towards the northern parts of its range. This is the case in the UK, where it reaches its north-western limits. Although it is listed as ‘Least Concern’ by IUCN, it is afforded both habitat and species protection in the UK. This level of protection is due to a substantial national decline of the species by 70-80% since the beginning of the 20th century (Beebee, 1977). Population decline in Britain has historically been attributed to habitat change through afforestation, urbanisation, agricultural practice, seral succession on neglected heathlands, acidification of breeding ponds and invasion by competitively superior species (Beebee, 1977; Beebee *et al.*, 1990; Denton & Beebee, 1994). Conservation management began in the 1970s focusing on aquatic and terrestrial habitat conditions, translocations to re-establish extirpated populations and control of competitors and predators. Natterjacks are confined to three main habitat types in the UK - lowland heathlands, coastal sand dunes and upper saltmarshes. Populations are broadly scattered across southern, eastern and north-western England, extending into south-west Scotland. There have also been recent re-introductions into north Wales.

Adult natterjack toad

Goals

- Goal 1: To re-establish the historical range of the natterjack toad in the UK.
- Goal 2: To increase the number of natterjack toads in the UK by establishing new populations.

Success Indicators

- Indicator 1: Increase the number of breeding females in the UK from 2,500 to 3,500 by 2010.

- Indicator 2: Increase the range of the species in the UK from 27 to 28 occupied 10 km grid squares by 2010.
- Indicator 3: Increase the range of the species in the UK from 17 to 21 occupied vice-counties by 2010.

Project Summary

Conservation management of the natterjack in Britain began on a significant scale in the 1970s and consisted of survey and monitoring, habitat management and translocations to re-establish populations on heathlands. As a result of the survey effort, the number of natterjack sites known increased, but no new populations have been discovered since 1993. The distribution of the species is now considered to be completely known within the country (Buckley & Beebee, 2004). By 1990, five new populations had been established using translocations (Denton *et al.*, 1997). In 1992, English Nature (now Natural England) implemented a three-year Species Recovery Program, which increased management effort at native sites and initiated a further eight populations through translocation (Denton *et al.*, 1997). Conservation efforts for the natterjack continue today led by the Amphibian and Reptile Conservation Trust, through implementation of targets set out in its Species Action Plan. This aims to maintain or improve existing populations through habitat management and restoring natterjacks to areas from where they have been lost. To this end, 10 additional translocations have taken place since 2000 in areas with authenticated historical records of natterjack toads. These efforts have seen the number of known natterjack sites in the UK increasing from about 40 in 1970 to 69 today. As a result natterjack sites in the UK consist of native sites where toads continue to persist and those that have been re-established via translocation, either pre- the Species Recovery Program or as part of it.

Re-introductions have mainly occurred through the translocation of spawn and tadpoles from existing populations, although head-starting of tadpoles and captive breeding have also played a role in some cases. Although re-introductions started in 1975, standardised monitoring protocols were not established until several years later. However, since 1985 all natterjack populations (i.e. natural and re-introductions) have been monitored on a near-annual basis and the data compiled within a national site register. The first definite successful natterjack toad translocation in Britain was one initiated in 1980 at a heathland site at Sandy (breeding to at least the second generation of animals). In 1982, Holme was the first successful translocation to a dune habitat, establishing a large population of >200 adults. The 1985 translocation at Minsmere was the first example of the successful use of artificial ponds but compared to other translocations, the population here grew more slowly and the total population size remains small (Beebee & Rowe, 2001). Translocations of spawn strings and tadpoles to Hengistbury, occurred in 1989, 1990 and 1991 and resulted in the establishment of a rapidly expanding population of >50 adults. In total, translocations have been carried out at 29 sites since 1975. Of these, 27 are at stages where the level of success can be judged. Nineteen of the 27 (70%) have been successful at least in the short- to medium-term, with adults returning to breed successfully and self-sustaining populations established at some sites. Re-establishing natterjacks on


Desiccating natterjack pond on heathland

heathland (57% success) has proved much more difficult than on dune or saltmarshes, where the overall success of translocations is much higher (85%).

All management interventions have been supported by an ongoing program of applied research, which has embraced population dynamics, identification and neutralisation of threats, genetics, reproductive biology and population modelling. Chytridiomycosis has emerged in natterjack populations in one region and is the subject of current research to determine its impacts. Conservation efforts for the natterjack to date have been encouraging, and translocations have resulted in an increase in both the number of populations and the range of the species within the UK. However, some populations are still declining despite management efforts to counter this. Ongoing research will continue to refine management methods and re-introduction techniques.

Major difficulties faced

- Understanding the scale to which habitats, particularly heathlands, have historically deteriorated in the UK and hence the level of restoration and management required.
- Limited re-introduction sites, because sites not under conservation management continue to deteriorate.
- Opportunistic - rather than planned - progress due to limits imposed by staffing and funding.

Major lessons learned

- Most sites in the UK have reduced potential for natural rejuvenation and translocation sites need to be under conservation management to maintain the key habitat features for natterjacks.
- A dedicated site manager (or a keen volunteer) is essential for the success of translocation projects, especially in the early stages.
- Population genetics research may be needed to inform the choice of donor stock.
- Captive breeding is a reliable source of animals for translocation only when biosecurity measures are in place to reduce disease risk.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- Long-term monitoring of several re-introduction projects has revealed self-sustaining populations.
- A small number of re-introduction projects, mostly on heathland, have failed for reasons that are unclear (preventing the project being classified as 'Highly Successful').

References

Beebee, T. J. C. 1977. Environmental change as a cause of natterjack toad *Bufo calamita* declines in Britain. Biological Conservation 11: 87-102.

Beebee, T. J. C., Flower, R. J., Stevenson, A. C., Patrick, S. T., Appleby, P. G., Fletcher, C., Marsh, C., Natkanski, J., Rippey, B. & Battarbee, R. W. 1990. Decline of the natterjack toad *Bufo calamita* in Britain: Palaeoecological, documentary and experimental evidence for breeding site acidification. Biological Conservation 53: 1-20.

Beebee, T. J. C. & Rowe, G. 2007. Defining population boundaries: use of three Bayesian approaches with microsatellite data from British natterjack toads (*Bufo calamita*). Molecular Ecology 16: 785-796.

Beebee, T. J. C. & Rowe, G. 2001. Application of genetic bottleneck testing to the investigation of amphibian declines: a case study with natterjack toads. Conservation Biology 15: 266-270.

Buckley, J. & Beebee, T. J. C. 2004. Monitoring the conservation status of an endangered amphibian: the natterjack toad *Bufo calamita* in Britain. Animal Conservation 7: 221-228.

Denton, J. S. & Beebee, T. J. C. 1994. The basis of niche separation during terrestrial life between two species of toad *Bufo bufo* and *Bufo calamita*: competition or specialisation? Oecologia 97: 390-398.

Denton, J. S., Hitchings, S. P., Beebee, T. J. C. & Gent, A. 1997. A recovery program for the natterjack toad *Bufo calamita* in Britain. Conservation Biology: 11: 1329-1338.

Reclaiming the lost world: Kihansi spray toad re-introduction in Tanzania

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Introduction

The Kihansi spray toad (*Nectophrynoides asperginis*) was first discovered in 1996 and listed in CITES App. I, and as critically endangered species endemic to the Kihansi river gorge in Tanzania. Its original population declined following diversion of water for hydropower production resulting in reduced flow of less than 2 m^3/s from the initial 16 m^3/s thereby causing the toad habitat to desiccate. Despite installing artificial sprinklers to generate sprays for the habitat, in late 2003 the population crashed to less than ten toads from more than 20,000 since its discovery (Lee *et al.*, 2006; Poynton *et al.*, 1999). Now, the species is extinct in the wild. The population and habitat viability assessment done in 2007 cited presence of chytridiomycosis, toxic pesticide chemicals released from dam flushing and pollution from agricultural activities as the probable causes of decline. Species conservation approach has included captive breeding in the USA since 2000 and due to commence shortly in Tanzania, ecological monitoring of the gorge habitat and Kihansi ecosystem restoration. The government of Tanzania has started plans to re-introduce the toad to Kihansi gorge using a captive population of approximately 4,000 toads presently available in the USA.

Goals

- Goal 1: A reasonable number of captive populations established at Bronx and Toledo zoos in the USA.
- Goal 2: Establish fully recovered toad habitat at all three spray wetland meadows in Kihansi gorge through management of the installed artificial spray system and the wetland vegetation.
- Goal 3: Cultivate healthy and substantial local captive population at Dar-es-Salaam and Kihansi to be used for re-introduction into the wild.
- Goal 4: Developing effective biological control for chytridiomycosis that will be used to eradicate the disease in Kihansi gorge and other infested areas.
- Goal 5: Viable and self-sustaining Kihansi toad population reinstated at the gorge and other prescribed suitable areas that are free from diseases and predators.
- Goal 6: Long-term monitoring of the re-introduced population carried out.

Success indicators

- Indicator 1: Control and treatment for chytrid fungus successfully developed.
- Indicator 2: Healthy captive population established and natural habitat restored in Tanzania.
- Indicator 3: Sustainable Kihansi spray toad population established at Kihansi gorge.

Project Summary

The diversion of Kihansi river for hydropower production left approximately 1.5-2 m^3/s water as bypass flow through the gorge. The water was insufficient to generate natural mists to maintain a healthy gorge ecosystem, consequently resulted in significant change of the gorge wetland hydrological regime. Gorge ecosystem alteration was evidenced by desiccation and rapid change in composition of the wetland vegetation and lower slope moist forests, from overgrowth of the toad's habitat herbaceous species to invasion of the wetlands by weeds, forest chameleons, lowland anurans and occasionally by safari ants (*Dorylus* sp.) (Lee *et al.*, 2006). This was followed by the toad population decline at Mhalala, Upper Spray Wetland, Lower Spray Wetland and Mid-gorge Wetland habitats. In response to these serious ecological and environmental changes in the gorge, artificial sprinklers were installed at all wetland meadows but Mhalala, to mimic the natural mists originally produced by the rapid falls. To ensure long-term perseverance of the species, about 500 toads were collected from various sites along the gorge and captive breeding was initiated at Bronx and Toledo Zoos in the USA. Captive breeding started in December 2000 by the United Republic of Tanzania (URT) and the Wildlife Conservation society (WCS) with support through the U.S. Fish and Wildlife Service, CITES and TRAFFIC. Initially, the breeding process presented unsurpassed challenges overwhelmed by sudden die-offs due to health and management issues and the population of the globally surviving Kihansi toad was soon reduced to only 37 individuals (Lee *et al.*, 2006). However, improved husbandry practices resulted in an increased population and recent reports from the Lower Kihansi Environmental Management Project (LKEMP), Tanzania which has been overseeing toad conservation show the population has reached 4,000 toads.

Other conservation measures toward sustaining the remaining wild population included, launching various field studies such as assessing diet spectrum of insects fed on by the spray toad, amphibian inventory studies, gorge microclimate

Mating Kihansi spray toads

Installed artificial sprinklers at one of the three spray wetlands toad habitat at Kihansi © Alfan A. Rija

and vegetation, working with policy makers to realize the kind of environmental flows required for the Kihansi gorge (now 2 m^3/s water) as prior to 2002, it was not legally recognized in Tanzania, toad screening for chytridiomycosis, construction of bridges and walkways within the toad habitat to reduce trampling damage and long-term ecological monitoring of the gorge by LKEMP. These measures provided invaluable data for the long-term conservation of the species, albeit were not able to sustain the Kihansi spray toad in the wild. In 2006, LKEMP launched a communication strategy to reach a wider Tanzania community to support the Kihansi toad recovery program. To gain more support for conservation by the local communities living around Kihansi catchment, LKEMP has been providing financial support for income generating projects in 21 surrounding community villages within the catchment. The projects could serve as alternative sources of cash income, thus help minimize serious negative environmental impacts emanating from human economic activities such as, valley and stream-side cultivation and use of pesticides such as endosulfan which is toxic to amphibians.

With the recovering habitat at Kihansi and the recent increase in captive population, the government of Tanzania is planning to re-introduce the toad back to the gorge. Essentially, the re-introduction program consists of four tentative stages; Pre-reintroduction phase. Main activities include, establishing possible causes of Kihansi toad crash, identifying strains and pathogenecity of chytrid fungus in Kihansi gorge, developing biological control measures for the fungus, investigating whether pesticide residues from the Kihansi dam caused population decline and determining the abundance of the toad's food habits at Kihansi. Other activities include designing pre and post release monitoring protocols as per the IUCN guidelines and selecting a task force to guide and monitor the re-introduction. Establishment of the local breeding colonies in Tanzania. Two captive breeding houses one at University of Dar-es-Salaam (already constructed) and Kihansi (not yet) will be furnished to further breed translocated Kihansi toads from the USA zoos. Capacity has been built for university technicians on husbandry practices for the toads, their feeding habits and habitat structure. Technicians have begun identifying and culturing feeder insects at the established breeding facility. Ongoing studies include, screening various amphibian species to determine chytrid fungus and other pathogens including rana viruses, survey of toad and frogs species at University of Dar-es-Salaam for histopathology studies against pathogens and testing for vegetation and diet requirements of the Kihansi spray toad.

Pre-release activities: Encompass construction of breeding house at Kihansi, translocating toads from Dar-es-Salaam to Kihansi facility for further breeding, developing monitoring indicators for soft release, site selection for soft release and final release to the wild. Long-term monitoring of the released population and the habitat. On a tentative schedule it is expected that soft releases will be done by December 2010.

Major difficulties faced

- Establishing solid re-introduction baseline data: Although there is substantial information on the habitat, food habits, and the biology of the spray toad than of any other amphibian species in Africa (D.W. Newmark, pers. comm. September 2009), important information pertinent to re-introduction is still lacking. Data are required on the suitability of potential release sites in relation to environmental variables, levels at which threats have been eliminated, nutrient dynamics in relation to habitat invasion by weeds, microclimate (temperature and relative humidity) effects on the emergence and severity of chytrid fungus, and on the best time and optimal temperature conditions to release the toads at the gorge. Such information if available would be useful for increasing chances of re-introduction success.
- Inadequate accounts of the causes of initial population decline and collapse: To date only chytrid fungus has been confirmed as the cause of population collapse. However, what caused the emergence of this disease has not been established. Ongoing studies include molecular characterization of the fungus species to determine its origin.
- Dam flushing: The impounded river dam gets flushed as part of routine maintenance work for the dam. While still investigated, this is a potential serious source of toxic substance that needs serious attention during the species re-introduction. Water and sediment samples that were collected during dam flushing in March 2009 indicated low levels of endosulfan present at the gorge. Further studies will be carried out to determine the lethal levels for amphibians, paying particular attention to the Kihansi spray toad.
- Anthropogenic issues: Despite the LKEMP investing in community development initiatives and environmental awareness, little has been appreciated by the locals. There have been serious environmental threats going on such as relentless wild fires, poaching, deforestation, stream-side and valley cultivation and use of toxic pesticides by the local communities, thereby increasing risks of damage to the gorge habitat. Although efforts have been increased to address the threats, they remain potentially critical to the survival of the re-introduced toad population.
- Healthy captive population in Tanzania: Final release of the toad to the gorge will probably depend on successfully bred colonies in Dar-es-Salaam and Kihansi. While managers are aware of the difficulty of establishing healthy colonies in Tanzania, there are also issues of longevity in captivity which may reduce species fitness to survive in the wild (McPhee, 2003). Research

(Kraaijeveld-Smith *et al.*, 2006) shows that long life in captivity up to eight generations may not reduce fitness traits. However, the recently bred colony of the Kihansi toad counts to eighth generation in captivity with perhaps more generations in Tanzania. Research is needed to test the ability of the spray toad on self defense against predators, on foraging ability and to changes in environmental conditions such as temperature and light to ascertain whether important traits are still retained by the toads in zoos.

- <u>Harmonizing with the socio and political atmosphere to support the toad recovery program</u>: Since commissioning of the Kihansi spray toad captive breeding in the USA, the toad conservation program has been ill-perceived with increasing comments from the press, some government officials, and the public being persistently negative largely due to its financial implications to a poor Tanzania nation (LKEMP, 2004). However, increased awareness raising by LKEMP to the public will probably help strengthen support for conservation of the spray toad.
- <u>Inadequate funding for re-introduction program</u>: Since its onset, toad conservation has been possible through financial support from the World Bank as part of the mitigation measures for negative environmental impacts emanating from the hydropower generation. Funding support will cease by December 2010 and all matters will be locally financed by the Tanzania government. In a poor country, the availability of internal funds is still a potential setback and a defining factor for successful implementation of the recovery program. There have been strategies to mainstream toad conservation activities into various government sectors in order to ease fund contributions from the sectors. However, the effectiveness of the mainstreaming strategy remains equivocal.

Major lessons learned

- Cultivating healthy captive colonies is a daunting undertaking that requires competent expertise as well as managerial and financial commitments. Experience acquired at Bronx and Toledo zoos will be useful for enhancing captive breeding in Tanzania.
- All threats caused initial population decline have not be completely and fully detected and addressed. Research is still required to effectively address and eliminate these threats.
- More socio-economic and political awareness at local and national level is still needed to gain support for successful recovery of the Kihansi spray toad.
- More data pertinent to re-introduction process are still needed to guide the recovery program. In the event of chytridiomycosis perseverance at the gorge, other options such as benign introduction will be explored as appropriately needed.
- A multidisciplinary team of both local and international experts is required for the Kihansi spray toad recovery program.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- The Kihansi spray toad population in captivity (Bronx and Toledo zoos, USA) has increased significantly reaching 4,000 toads recently.
- The natural habitats at Upper, Lower and Mid-gorge spray wetlands at Kihansi are recovering due to the artificial spray generated by the installed artificial sprinklers.
- Discovering of chytridiomycosis as the cause for population collapse has led to the ongoing research to develop its control treatment.
- Recovery program still at its infant stage with more research data still needed to guide the whole re-introduction process.

References

Kraaijeveld-Smith, F. J. L., Griffiths, R. A. & Moore, R. D. 2006. Captive breeding and the fitness of reintroduced species: a test of the responses to predators in a threatened amphibian. Journal of Applied Ecology, 43: 360-365.

Lee, S., Zippel, K., Ramos, L. & Searle, J. 2006. Captive-breeding program or the Kihansi spray toad (*Nectophrynoides asperginis*) at the Wildlife Conservation Society, Bronx, New York. Int. Zoo Yb, 40, 241-253.

LKEMP. 2004. Lower Kihansi Hydropower project: immediate rescue and emergency measures. Final Specialist report: amphibian studies: report produced for Tanzania Electric Supply Company Ltd (TANESCO), Dar-es-Salaam, Tanzania.

McPhee, M. E. 2003. Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. Biological Conservation, 115, 71-77.

Poynton, J. C., Howell, K. M., Clarke, B. T. & Lovett, J. C. 1999. A critically endangered new species of *Nectophrynoides* (Anura:Bufonidae) from the Kihansi Gorge, Udzungwa Mountains, Tanzania. African Journal of Herpetology, 47, 59-67.

Assessment of re-introduction methods for the Southern Corroboree Frog in the Snowy Mountains region of Australia.

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Introduction

The southern corroboree frog (*Pseudophryne corroboree*) only occurs in the Snowy Mountains Region of Kosciuszko National Park, and is one of Australia's most iconic frog species. This species occupies the sub-alpine zone between 1300 and 1750 m (Osborne, 1989), where it typically breeds in small ephemeral pools in sphagnum bog wetlands (Hunter *et al*., 2008). The southern corroboree frog has been in a continued state of decline over the past 20 years, and is likely to be extinct in the wild within the next 10 years if recovery efforts are unsuccessful. The primary cause of decline is chytridiomycosis, a disease caused by infection with the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Hunter *et al*., in press). Given the dire predicament faced by the southern corroboree frog (monitoring of all sites in 2010 suggests there are fewer than 40 males remaining in the wild, Hunter unpublished data) preventing the

Adult male southern corroboree frog © D. Hunter extinction of this species relies on successfully establishing a captive breeding and re-introduction program. The southern corroboree frog is listed as Endangered in Australia under the *Environment Protection and Biodiversity Act* 1999, and Critically Endangered by the IUCN.

Goals

- Goal 1: Develop a successful re-introduction program to ensure the persistence of the southern corroboree frog in the wild.
- Goal 2: Develop efficient re-introduction techniques to maximize the value of available resources.
- Goal 3: Use information on post-release survivorship to identify the number of offspring required from the captive breeding program for future re-introductions.

Success Indicators

- Indicator 1: Breeding populations of the southern corroboree frog increase in size.
- Indicator 2: Accurate estimates of post-release survivorship to breeding have been attained for comparing different re-introduction strategies and setting targets for the captive breeding program.

Project Summary

Feasibility: The recovery program for the southern corroboree frog has multiple partner organizations that are committed to the long term goal of achieving self-sustaining populations of this species in the wild. It is acknowledged by all partners that this program is likely to take several decades to achieve this goal. This program has considerable public and government support, and the recovery of this species is an important objective for the biodiversity management of Kosciuszko National Park. An experimental augmentation program has previously been undertaken, which involved harvesting eggs from the wild and rearing them through to a late tadpole stage before returning them back to their natal pools (Hunter *et al*., 1999). While this program successfully increased recruitment to metamorphosis (Hunter *et al*., 1999), it failed to noticeably reduce population decline (Hunter, 2008). The current program is aimed at assessing two alternative re-introduction techniques; releasing tadpoles into artificial pools, and releasing four-year-old frogs. The potential merits of releasing tadpoles into artificial pools (400 liter plastic tubs) is that it should reduce rates of chytrid fungus infection in tadpoles, there will be no tadpole mortality associated with early pool drying, and there are negligible rearing costs prior to release. The four year old frog release is being trialed because this strategy has the greatest potential to reduce infection and mortality prior to sexual maturity. However, this technique has considerable rearing costs, and relies on frogs that have been in captivity for an extended period being capable of surviving and breeding in the wild after release. The majority of the animals used in these trials were harvested from the wild as eggs.

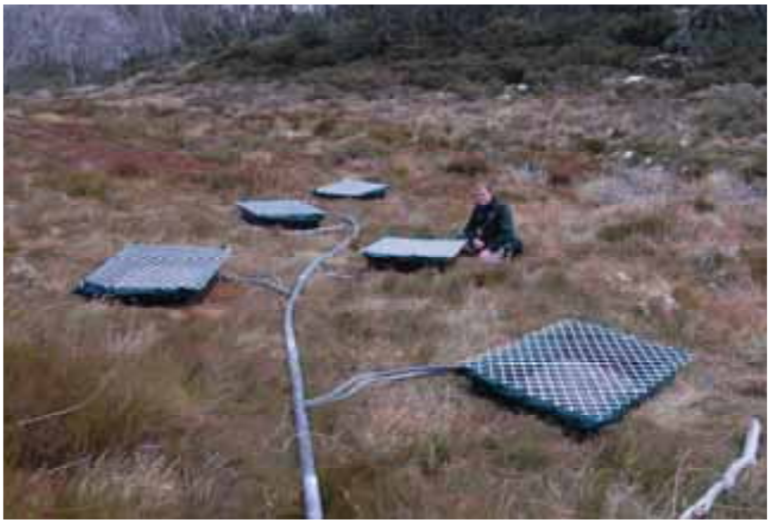
Implementation: *Release into artificial tubs* - Fifty eggs at hatching stage were placed in each of 20 artificial pools across four sites (five pools per site) in mid autumn (April or May) of 2008, 2009 and 2010. The artificial pools were 400 litre


Metamorph on net surface © D. Hunter

grey polypropylene tubs positioned within natural bog systems. Each tub had a constant flow from a nearby stream at a rate of approximately 20 litres per hour. A 2 cm layer of pond silt was placed on the bottom of each tub to provide a natural food source for the tadpoles. The top of the tubs were a minimum of 15 cm from the ground and positioned such that they could not be accessed by the common eastern froglet (*Crinia signifera*), which is a reservoir host for the chytrid fungus. Each pool was lined with shade cloth to provide an exit ramp for the metamorphosing frogs. Clumps of sphagnum moss were placed in two corners of each artificial pool to provide a moist refuge for the metamorphosing frogs.

Release of four year old frogs - In January 2006, 196 four-year-old frogs, and 15 five year old frogs, were released across two sites. Assuming an even sex-ratio for the released individuals, and since we only assessed male survivorship, the sample size of individuals for assessing the outcome of this study is half the number of individuals released. Prior to release, each individual frog was measured for snout-vent and tibia length, weighed, and their belly and throat photographed for individual identification using pattern recognition.

Post-release monitoring: *Release into artificial tubs* - The total number of tadpoles in each tub was assessed just prior to metamorphosis in late spring (November). Ten randomly selected tadpoles from each pool were also measured and staged. Upon reaching metamorphosis, a sample of the juvenile frogs were caught and swabbed for infection with the chytrid fungus. The mean survivorship from egg laying to metamorphosis across all pools was 35% in 2008, and 66% in 2009 (2010 has not been assessed at this stage). The results for 2008 are within the range of survivorship attained through augmenting recruitment in natural pools, while the results for 2009 are considerably greater (Hunter, 1999). The increase in survivorship during 2009 may have been due to better quality substrates provided in all pools, however this is unsubstantiated. The size of the tadpoles, and subsequent metamorphs, was typically greater than that observed in natural pools. Of the eleven artificial pools that attained survivorship through to metamorphosis in 2008, one pool was identified as infected with the chytrid fungus, which is lower than the 60% of natural pools identified as being infected in an earlier study (Hunter, 2008). Infection status of pools in 2009 and 2010 has not been analysed at this stage. While further assessment is required to determine the value of re-introducing eggs into artificial pools, the initial results are promising.



Artificial tubs © D. Hunter

Release of four year old frogs - Six surveys of calling males were undertaken at each release site during the last two weeks of January in 2007, 2008, 2009 and 2010 to identify the position of male nest sites for later inspection to determine if any of the released individuals had returned to breed. Surveys were also undertaken at all potential breeding habitats within a 2 km radius of the release sites to determine whether the released frogs had migrated to adjacent areas. Towards the end of the breeding season (first week in February), the males were removed from their nest sites to identify individuals, assess size, and swabbed for chytrid fungus infection. No re-introduced males were observed breeding in January 2007, however five breeding males were located at one of the sites in 2008. Males were observed at both breeding sites in 2009, and one site continued to have breeding adults in 2010. Chytrid fungus infection was detected in one individual in 2009. Based on the number of frogs returning to breed, estimated variation (95% conf. limits) for survivorship ranged from 1%-17%.

Major difficulties faced

- The length of time required to assess the value of the egg re-introductions (minimum seven years) has limited decision making by the recovery team in the interim.
- Severe drought immediately after the release of the four year old frogs may have greatly reduced survivorship and breeding activity, and thus produced atypical results.
- The relatively small number of four year old frogs released may have limited statistical inferences. A larger release is planned for December 2010, which will more specifically assess the role of chytridiomycosis in post-release survivorship.

Major lessons learned

- Given the relatively low post-release survivorship attained for the techniques assessed at this stage, future re-introductions will require substantial progeny from the captive breeding program.
- Post-release survivorship for the different release strategies can have substantial variation among years and sites, which should be considered in the

design of future re-introduction experiments to ensure robust results are attained.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- Re-introducing four year old frogs can be used to maintain populations in the wild, however, substantial resources will be required to produce sufficient numbers of individuals.
- The high survivorship to metamorphosis, and low chytrid fungus infection rates, for the eggs re-introduced into artificial pools suggests this technique may be an efficient re-introduction technique.

References

Hunter, D. 2008. Conservation management of two threatened frog species in south-eastern New South Wales, Australia. PhD thesis, University of Canberra.

Hunter, D., Osborne, W., Marantelli, G. and Green, K. 1999. Implementation of a population augmentation project for remnant populations of the Southern Corroboree Frog (*Pseudophryne corroboree*). Pp 158-167 in Declines and Disappearances of Australian Frogs ed. by A. Campbell. Environment Australia: Canberra.

Hunter, D., Osborne, W., Smith, M. and McDougall, K. 2009. Breeding habitat use and the future management of the critically endangered Southern Corroboree Frog. *Ecological Restoration and Management*, 10: 103-109.

Hunter D.A., Speare R., Marantelli G., Mendez D., Pietsch R., Osborne W. (in press) Presence of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, in threatened corroboree frog populations in the Australia Alps. Diseases of Aquatic Organisms.

Osborne, W.S. (1989) Distribution, relative abundance and conservation status of Corroboree Frogs, *Pseudophryne corroboree* Moore (Anura: Myobatrachidae). *Australian Wildlife Research*, 16: 537-547.

Captive management and experimental re-introduction of the Booroolong Frog on the South Western Slopes region, New South Wales, Australia.

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Introduction

The Booroolong frog (*Litoria booroolongensis*) is a medium-sized hylid frog, mostly restricted to the western flowing streams of the Great Dividing Range in New South Wales (NSW) and north-eastern Victoria, Australia. It was formerly considered to be widespread and abundant throughout its range until the mid-1980s when it suffered dramatic declines. It has almost disappeared from the northern part of its range, with many local extinctions occurring throughout the remainder of its distribution (Gillespie & Hines, 1999). The Booroolong frog is listed as Endangered nationally under the Environment Protection and Biodiversity Act 1999 and as Endangered under Schedule 1 of the NSW Threatened Species Conservation Act 1995. It is listed as Critically Endangered by the IUCN. There are numerous threatening processes that may have contributed to the decline of this species. These include disease (chytridiomycosis), habitat

An adult Booroolong frog

loss and alteration, introduced fish, invasive weeds and stream drying. During the summer of 2006-2007, drought threatened to cause the local extinction of Booroolong frogs in the Maragle Creek catchment as a result of stream drying (Hunter & Smith, 2006). This was particularly concerning as the Booroolong frog was a flagship for riparian restoration on private properties along Maragle Creek. To prevent the local extinction of this population, a small founder population was collected to initiate a captive breeding program.

Goals

- Goal 1: Ensure the persistence of the Booroolong frog in the Maragle Creek catchment on the South Western Slopes of NSW, Australia.
- Goal 2: Establish a captive insurance population and develop successful husbandry and breeding protocols for this species.
- Goal 3: Conduct a trial release of captive-bred animals and closely monitor survival to maturity and breeding from these individuals in the wild.
- Goal 4: Increase public awareness for the Booroolong frog, its declining population status and its habitat requirements in the local community.

Success Indicators

- Indicator 1: To establish successful captive breeding protocols
- Indicator 2: That released animals survive to maturity and breed in the wild.
- Indicator 3: To increase the awareness of the Booroolong frog in the local community.

Project Summary

Feasibility: Intensive surveys were undertaken for the presence of the Booroolong frog on the South West Slopes region of NSW during 2006 (Hunter & Smith, 2006). These surveys indicated that a number of populations were under threat of local extinction due to stream drying, including those in the Maragle Creek catchment. This was largely due to the modified, agricultural land-use and prolonged drought. Due to the short lifespan of this species and its reliance on streams for breeding (Anstis *et al.*, 1998), it is especially susceptible to reduced water flows. After two years of minimal rainfall, it was determined that the risk of losing this population was sufficiently high to warrant the collection of a small insurance population and initiate a captive breeding program. This would allow the release of captive bred individuals to supplement the depleted wild population should water flows increase, and provide an opportunity to assess the capacity to utilize re-introduction as a conservation tool for this species.

Implementation: In February 2007, a founder population of 32 juvenile frogs was collected by staff of the NSW Department of Environment, Climate Change and Water (DECCW) and Taronga Zoo from three separate sites along Maragle Creek. An additional nine frogs were collected to conduct an initial disease screening to establish parasite and pathogen levels in the wild population. The frogs were transported to Taronga Zoo and held in a biosecure room and maintained under strict quarantine conditions. In late 2007, captive breeding was achieved and the majority of founder animals produced fertile spawn. For the intended release, eight spawn were obtained from 16 founder animals, to


Frogs being released at Maragle Creek

maximize genetic diversity. These spawn were obtained in cohorts of five spawn and three spawn, spaced two months apart. The tadpoles and young frogs were reared in biosecure rooms, housing only this species, under strict quarantine. At the time of release, half of the frogs were four months old, whilst the other half were two months old. Six weeks prior to release, all 610 frogs were individually marked by clipping up to three toes. Additionally, the frogs underwent an intensive pre-release pathology screening of 30 tadpoles from each clutch. Frogs were released along a 1.5 km transect of Maragle Creek in February 2008, after it had been determined the captive stock did not contain any pathogens that were absent in the wild population. This conservation program also involved an educational campaign that provided an intensive educational experience for local primary and secondary school students at Taronga Zoo followed by an “Experts” day for students in the field. It concluded in a town-wide community expo day focusing on the conservation of the species in the town Tumbarumba, NSW, which is close to the release site.

Post-release monitoring: The release transect was surveyed four times during the two month period after release in 2008, and six times between October and February during both the 2008-2009 and 2009-2010 seasons, Surveys consisted of visual searches along the release transect at night to locate active frogs. Upon capture, each frog was identified, weighed, measured and swabbed for the presence of chytrid fungus. A total of 105 individual frogs were captured after release, with 29 frogs observed surviving through to sexual maturity and engaging in breeding activity (males calling or gravid female present in the breeding area). The size and condition of the released frogs at sexual maturity were equivalent to marked, wild frogs at the site. Only four released frogs were recorded in the 2009-2010 breeding season, suggesting that mortality to this point had been high, which is consistent with the rapid life-cycle of this species. Even so, two existing threats may have contributed to the high mortality of the released cohort, as much of the stream stopped flowing and dried out soon after release in autumn and then again the following summer, and high infection with the chytrid fungus was also recorded in the population.

Major difficulties faced

- The Booroolong frog had not previously been kept and bred in captivity. Additionally, the lifespan of the Booroolong frog in the wild is quite short, which did not allow much room for error in regards to establishing captive breeding.

- Some of the existing threats to the species were still operating at the release site, including high chytrid fungus infection rates and two stream drying events.

Major lessons learned

- The species has proven relatively easy to breed in captivity. In captivity the species grew to maturity and bred much faster than in the wild, and females had a much higher reproductive output, producing multiple spawn (of 400 to 1,250 eggs in each spawn) per season.
- During the first breeding season after release, the male captive-bred animals were observed engaging in breeding activity by exhibiting advertisement calling along the stream. During the second season post-release, both male and female captive-bred animals were observed engaging in breeding activity.
- This case study highlighted the importance of conducting initial pathology screening of wild individuals to establish which parasites and pathogens are present in the wild population. During the pre-release screening, a brain parasite was identified that would have aborted the intended release had it not been previously determined that it was a natural parasite in the existing wild population of this species.
- The local community has become well informed of this species due to the interactive educational campaign in the local township of Tumbarumba. As habitat loss and alteration is a significant threat to this species, educating the local rural community is an important conservation objective.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- The Booroolong frog has proven relatively easy to breed in captivity. Breeding was achieved from a large number of the collected founder animals.
- Captive-bred animals released into the wild survived to sexual maturity and engaged in breeding activity.
- Further stream drying and high levels of chytrid fungus infection may have contributed to the relatively low survivorship of released frogs.
- A successful educational campaign was undertaken in the local community of Tumbarumba.

References

Anstis, M., Alford, R. A. and Gillespie, G. R. 1998. Breeding biology of *Litoria booroolongensis* Moore and *L. lesueuri* Dumeril and Bibron (Anura: Hylidae). Trans Roy. Soc. S. A., 122: 33-43.

Gillespie, G. R. and Hines, H. B. 1999. Status of Temperate Riverine Frogs in South-eastern Australia. In: A. Campbell (Ed.) Declines and Disappearances of Australian Frogs. Environment Australia, Canberra, pp. 109-130.

Hunter, D. and Smith, M. 2006. Survey, Habitat Assessment and Management Recommendations for the Endangered Booroolong Frog (*Litoria booroolongensis*) in the Murray Catchment Management Area of New South Wales. Unpublished report to the NSW Department of Environment and Conservation.

Lessons learned from a series of translocations of the archaic Hamilton's frog and Maud Island frog in central New Zealand

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Introduction

A series of re-introduction case-studies from which conservation management lessons have been learned are provided for two threatened terrestrial frogs that survive on islands in the Marlborough Sounds, New Zealand. Until translocation, Hamilton's frog (*Leiopelma hamiltoni*; McCulloch, 1919) were restricted to a small 300 m^2 rock bank on Stephens Island (Is.) and the Maud Island frog (*Leiopelma pakeka*; Bell, Daugherty & Hay, 1998) to a remnant 16 ha forest patch on Maud Island (Is.) These evolutionarily distinct frogs are in one of the two earliest diverging genera of modern Anura. Formerly regarded as *L. hamiltoni*, *L. pakeka* was described as a cryptic phylogenetic species based on allozymes and morphometrics. However, more recent partial 12s RNA and Cyt b sequences, showed little variation between them (<1% for Cyt b), so the taxonomic status of *L. pakeka* requires further resolution.

*** - Zealandia (formerly known as Karori Sanctuary) is a predator-proof fully fenced urban wildlife sanctuary in Wellington on the North Is. of New Zealand**

Maud Is. and Stephens Is. have remained free from introduced rats, suggesting that such mammalian predators have led to their extinction elsewhere. Sub-fossils show a species identified as *L. hamiltoni* was formerly widespread across both the North Is. and South Is. of New Zealand.

Hamilton's frog (*above*) & Maud island frog (*below*)

Transfers of *L. pakeka* began in 1984-1985, when 100 frogs were moved to a restored site at Boat Bay on Maud Is. (Bell *et al.*, 2004). Subsequent transfers beyond Maud Is. were of 300 *L. pakeka* to Motuara Is. in 2001, 101 to Long Is. in 2005, and 60 into Zealandia* Sanctuary, Wellington, in 2006-2007 (Bishop, 2005; Tocher & Pledger, 2005;

Lukis & Bell, 2007). In 1992, 12 *L. hamiltoni* were transferred to adjacent newly created habitat on Stephens Is. (Brown, 1994), then 71 frogs were transferred over 2004-2006 to Nukuwaiata Is. (Tocher *et al.*, 2006). Additional *L. hamiltoni* and *L. pakeka* have been held in captivity, where they successfully bred and young were reared, but no re-introduction of captive bred frogs into the wild has taken place. No breeding sites have been found for either species in the wild. In the 2009 IUCN Red List, *L. hamiltoni* is ranked 'Critical' and *L. pakeka* as 'Vulnerable', while under the current New Zealand Threat Classification System these two taxa are listed as 'Nationally Critical' and 'Nationally Vulnerable' respectively. No chytridiomycosis has been found in these two island populations, or in any transferred populations, and neither source population has declined under conservation management over the past 30 years.

Goals

- Goal 1: Identification of potential re-introduction sites within the species' historic range.
- Goal 2: Successful breeding of released individuals, and persistence of each translocated population.
- Goal 3: Sustainable populations established in a range of suitable habitats, free of introduced mammalian predators, and where the risk of chytridiomycosis is minimal.
- Goal 4: Through adaptive management, re-establish populations on mainland sites where the risks of mammalian predators is managed.
- Goal 5: Annual monitoring of source populations and regular monitoring of translocated populations.

Success Indicators

- Indicator 1: Self-sustaining populations established at re-introduction sites.
- Indicator 2: Overall geographical distribution of the species extended.

Project Summary

The earliest transfer of these species was a re-introduction trial of *L. pakeka* that took place in regenerating forest at Boat Bay on Maud Is. in 1984-1985 at a site that had lost its presumed former frog population as a result of habitat changes induced by farming. In 1984 the first 43 frogs were transferred, then a further 57 in 1985, all being released at the same location. Population sampling has occurred at least annually, revealing high survival of founders, increased mean body condition, most settlement close to the release site (<26 m), steady recruitment (locally-bred individuals now exceed the number released), and a rising population level (Bell *et al.*, 2004). This intra-island re-introduction represents the most successful transfer to date. Once the Boat Bay re-introduction had demonstrated that these frogs could be successfully transferred and established in a new location, a transfer of 12 *L. hamiltoni* took place on Stephens Is. in 1992, to a specially excavated 'frog pit' filled with rocks in remnant forest 50 m from the original site (Brown, 1994). A predator-proof fence was built around the new habitat to exclude tuatara (*Sphenodon punctatus*), a known predator, and the area was seeded with invertebrate prey (Brown, 1994). In 2004, a fenced tuatara-excluded corridor was created to connect the two sites, and

Left Image: **Maud island the only location of *L. pakeka*, in 1984-1985, 100 frogs were translocated from remnant population “B” to forested gully “A”.**
Right Image: **Stephen’s island the only location of *L. hamiltoni,* which survived on a rock bank near the summit prior to translocation.**

while some frogs homed back to the original site, between 1996 and 2000 at least three frogs remained in the new ‘frog-pit’. In 1997, in the first island-island transfer of *L. pakeka*, 300 adult frogs were translocated from Maud Is. to Motuara Is., and this new population has been regularly monitored since. In August 2002, 155 individuals were recaptured as well as 42 new recruits (Tocher & Pledger, 2005).

Given that the only population of *L. hamiltoni* amounted to c.300 individuals living in 300 m^2 on Stephens Is., there was much to be gained by establishing a population on another island but risks were greater because of the low numbers (Tocher *et al.*, 2006). A long history of monitoring the source population provided data for predicting which of nine hypothetical translocation scenarios was likely to produce the best result for the species (Tocher *et al.*, 2006). A translocation of 40 female frogs (20 adults and 20 sub-adults) along with 40 male frogs (20 adults and 20 sub-adults) was chosen as it provided a balance between risk of extinction in the donor population and probability of success in the translocated population (Tocher *et al.*, 2006).

Consequently, in 2004 the first 40 *L. hamiltoni* were moved to a new site on Nukuwaiata Is. Data-loggers had been previously installed there to confirm that a suitable microclimate existed, and boardwalks were erected so that the frogs could be monitored without disturbing the habitat. By 2006, 25 had been encountered, and sub-adults were growing at a normal rate (pers. comm. H. Cooper). With these promising results the final cohort of 31 frogs were captured on Stephens Is. and shifted to Nukuwaiata during 2006. The first new recruit to the new population was discovered in 2008 with eleven further juveniles found in 2009.

A third island population of *L. pakeka* was initiated in 2005 when 101 frogs were translocated to a prepared site on Long Is. Their initial movements and adaptation to the new site were followed showing that there was a tendency to disperse downhill and that those shifted with near neighbors were just as likely to disperse as those released with unfamiliar frogs (Germano, unpubl. M.Sc., 2006). During the four years post-release, population numbers on Long Is. appear to be in decline, possibly due to poor habitat and kiwi (*Apteryx* sp.) predation.

In Zealandia, Wellington, 30 adult *L. pakeka* that had been held in captivity were placed in a 2 x 4 m predator proof mesh enclosure in February 2006. Their sizes (most >40 mm SVL) indicated they were predominately females, so 30 more frogs in the male size range (<40 mm SVL) were transferred from Maud Is. in October 2006, initially into another 2 x 4 m enclosure (Lukis & Bell, 2007). In April 2007, 58 surviving frogs were mixed into roughly equal numbers of males and females. Using an adaptive management approach, half were retained in an enclosure, the rest were released into the wild in adjacent forest, where there were at least two potential predators, the house mouse (*Mus musculus*) and little spotted kiwi (*Apteryx owenii*).

Survival in the enclosure remained high (27/29), but the number seen in the wild declined markedly, however, suggesting poor survival in the presence of even a limited range of predators. Despite this disappointment, by February 2008 the first breeding had occurred in the protected enclosure (two brooding males). Thirteen recently hatched larvae were moved to incubators to complete metamorphosis, eleven surviving until release into nursery pens at Zealandia in March 2008. In mid-March 2009, two males were found with a total of ten nearly metamorphosed young frogs, which again completed metamorphosis in incubators, before being placed in a nursery pen in Zealandia in late March.

Major difficulties faced

- Limited size of source population, requiring a modeling approach to determine optimal number to translocate to balance risks of over-cropping the source population against risks of insufficient pioneers in the transferred population - *L. hamiltoni* Nukuwaiata Is.
- Releasing low numbers (<30) could reduce likelihood of successful establishment - *L. pakeka* Zealandia.
- Probable predation from house mice (*L. pakeka*, Zealandia) and possibly little spotted kiwi (*L. pakeka*, Zealandia and Long Is.).
- In recreating suitable rocky frog habitats in sites of release, there may be a risk of inadvertently attracting mammalian predators e.g. house mice at *L. pakeka* release site in Zealandia.
- Finding suitable habitat on appropriate predator-free islands.

Major lessons learned

- The original Boat Bay transfer was a success and provides a model for future translocations - both *L. hamiltoni* and *L. pakeka*.
- Successful translocations require sufficient numbers and a mix of ages and sexes - both *L. hamiltoni* and *L. pakeka*.
- Founders likely to be at risk to potential mammalian/avian predators at mainland and island sites, so successful transfer likely to require exclusion and/or management of suspected predators. Remedial options at Zealandia

are to intensify house mouse control, or entirely eliminate mice, to exclude potential avian ground predators like kiwi by fencing, to provide more secure retreat sites around release area, to supplement release with a larger number of frogs (100+), and to consider a large fully enclosed predator-free release environment. Future island translocations should take into consideration potential conflicts with native predators and fencing should be used to help protect an establishing population at early stages - *L. pakeka* at Zealandia and Long Is.

- Construction of artificial rocky habitat piles or pits can enhance establishment in sites where such substrate is sparse or lacking, but may run risk of attracting predators where these occur – *L. hamiltoni* Stephens Is., *L. pakeka* Zealandia.
- These are K-selected species and long-term monitoring (>20 years) is required to confirm successful establishment – both *L. hamiltoni* and *L. pakeka*.
- Despite small home range sizes, these frogs can, and do, home following short-distance translocations. As homing instincts decrease with distance, future translocation should be at a sufficient distance to discourage homing.

Success of projects

Overall success summary, all transfers, both species (1984-2007):

Highly Successful	**Successful**	Partially Successful	**Failure**
1	**2**		**3**

***L. pakeka*, Boat Bay, Maud Is., Marlborough Sounds (1984-1985):**

Highly Successful	Successful	Partially Successful	Failure
√			

Reason(s) for success/failure:

- 75% of 100 founders recaptured at least 6 months post-release.
- Mean body condition index of founders increased after release.
- Mean body size growth in founding population greater than in source population.
- Increasing numbers of individuals being caught during annual sampling sessions.
- Founders now comprise a smaller proportion of captures, 34% of founders were still alive after 25-26 years.
- Immature frogs regularly observed and 136 individuals known to have been recruited into the population by 2010-more than the number of founders (100).

***L. hamiltoni*, Stephens Is., Marlborough Sounds (1992)**:

Highly Successful	Successful	Partially Successful	**Failure**
			√

Reason(s) for success/failure:

- Increased local habitat and range of existing population.
- No breeding at new site.
- The majority of translocated individuals homed to the point of capture, and very few sightings have been made of the translocated frogs that remained at the new site.

***L. pakeka*, Motuara Is., Marlborough Sounds (2001)**:

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- Maintained large numbers of individuals, though longer term monitoring required to confirm.
- Evidence of breeding at the site.

***L. pakeka*, Long Is., Marlborough Sounds (2005)**:

Highly Successful	Successful	Partially Successful	**Failure**
			√

Reason(s) for success/failure:

- Possibly unsuitable or suboptimal habitat, with too few rocks to provide retreat and/or breeding sites, though longer term monitoring required to confirm.
- Possible predation by little spotted kiwi. Recapture numbers have decreased substantially and one frog was caught with recent damage to one side of its face, which may be evidence of predation. Kiwi have been noted at the frog site during every monitoring session.

***L. hamiltoni*, Nukuwaiata Is., Marlborough Sounds (2004-2006)**:

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- Local breeding, short-term success, but still too early to confirm long-term success.

***L. pakeka*, Zealandia, Wellington (2006-2007):**

Highly Successful	Successful	Partially Successful	**Failure**
			√

Reason(s) for success/failure:

- Decline to extinction after release, despite high survival and successful breeding over two successive years by other frogs held in predator-proof enclosure.
- Probable predation from house mice and possibly little spotted kiwi.
- Low number of frogs released (29).

References

Bell, B. D., Pledger, S., & Dewhurst, P. L. 2004. The fate of a population of the endemic frog *Leiopelma pakeka* Anura: Leiopelmatidae translocated to restored habitat on Maud Island, New Zealand. New Zealand Journal of Zoology 31: 123-131.

Bishop, P. J. 2005. Re-introduction of endangered frogs to uninhabited, predator-free, islands in the Marlborough Sounds of New Zealand. Re-introduction NEWS, Newsletter of the Re-introduction Specialist Group of IUCN's Species Survival Commission (SSC), Abu Dhabi, UAE. No. 24: 44-45 (ISSN: 1560-3709).

Brown, D. 1994. Transfer of Hamilton's frog, *Leiopelma hamiltoni*, to a newly created habitat on Stephens Island, New Zealand. New Zealand Journal of Zoology 21: 425-430.

Lukis, K., & Bell, B. D. 2007. Restoring native frogs to a mainland island reserve: translocation and adaptive management of the Maud Island frog (*Leiopelma pakeka*) in the presence of house mice (*Mus musculus*) at Kaori Wildlife Sanctuary. New Zealand Journal of Zoology 34: 267.

Tocher, M. D., Fletcher, D., & Bishop, P. J. 2006. A modelling approach to determine a translocation scenario for the endangered New Zealand frog *Leiopelma hamiltoni*. Herpetological Journal 16:97-106.

Tocher, M.D., & Pledger. S. 2005. The inter-island translocation of the New Zealand frog *Leiopelma hamiltoni*. Applied Herpetology 2: 401-413.

A more detailed reference list can be obtained from the first author

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More case studies from around the globe

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IUCN/SSC Re-introduction Specialist Group (RSG)

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Houston toad population supplementation in Texas, USA

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Introduction

The Houston toad (*Bufo* [*Anaxyrus*] *houstonensis,* Saunders, 1953) is endemic to the forested, deep sandy soils of east-central Texas, USA. It was the first amphibian placed on the United States List of Threatened and Endangered Species in 1970, and is also listed as Endangered by the IUCN and the state of Texas. Since the 'Endangered' listing of the Houston toad, its populations have continued to decline across its range. This is largely synchronous with a reduction in habitat quantity, through conversion of forest to agriculture and urban development, and quality, due to fire suppression and fragmentation. Precipitous declines have been observed concomitantly with prolonged droughts (Brown & Mesrobian, 2005). There are two parcels of state owned property; the 2,400 ha Bastrop State Park and a separate 178 ha tract (Welsh Tract), both in Bastrop County, Texas. All other tracts are privately owned and only with the collaboration of landowners do these tracts provide habitat restoration and stewardship efforts for the species in the wild. The Welsh Tract, owned and administered by Bastrop County is the only tract managed primarily for toad recovery. Other conservation or stewardship tracts have other primary objectives and incorporate Houston toad stewardship alongside those goals.

Goals

- Goal 1: To increase juvenile survivorship above 1% on critical recovery sites, thereby decreasing the likelihood of extinction within the next decade.
- Goal 2: To facilitate natural recolonization of restored habitat by increasing population sizes.
- Goal 3: To establish a captive assurance colony of genetically representative Houston


Houston toad showing vocal sac

toads to supply individuals for re-introductions in the event of extinction in the wild.

Success Indicators

- Indicator 1: Increase in sub-population size (mean over 5 years) to 5,000 adult females for habitat fragments where head-starting has occurred.
- Indicator 2: Increase the number of robust sub-populations to at least two.
- Indicator 3: Achieve a sustainable captive assurance colony containing genetic diversity representative of the remnant wild populations.

Project Summary

Feasibility: A Population Viability Analysis (PVA) conducted by Hatfield *et al*. (2004) determined the Houston toad would likely go extinct within a decade if juvenile survivorship was below 1% and there was only one subpopulation. Field data suggested juvenile survivorship was 0.03% (Grueter, 2004), much lower than originally assumed, and that there might indeed be only one viable subpopulation. A subsequent model-based estimate concluded juvenile survivorship to be 0.75% - 1.5% (Swannack *et al.*, 2009), but again it appears that only one robust subpopulation exists. Thus, it was proposed by one of the authors to the United States Fish and Wildlife Service that without active stewardship the Houston toad would be extinct in the near future. We believe future recovery efforts should address pertinent biological weaknesses identified by the PVA, and focus on head-starting (to improve juvenile survivorship), habitat restoration (to increase the viability of additional subpopulations), and creation of a captive assurance colony. This would not be the first time Houston toads were collected for *ex situ* conservation purposes. In the 1980s nearly 500,000 eggs, tadpoles, toadlets and adult Houston toads were captive propagated and translocated to the Attwater Prairie Chicken National Wildlife Refuge in the hopes of creating a second population in a protected area. This previous Houston toad *ex situ* conservation program provided relevant experience and information for the current work. The 1980s effort has been largely viewed as a failure (Dodd & Seigel, 1991), yet recently generated genetic data from the dissertation work of McHenry (2010) revealed evidence that supports the potential long-term success of those early efforts. Significant pre-existing data from annual surveys, mark-recapture, and habitat restoration efforts were available for the Bastrop County sites, which enabled us to test the efficacy of supplementation at various life stages. With this backdrop, the most recent population supplementation project was initially focused on the robust Bastrop County sub-population, as well as the much less robust, but critically important, sub-population in Austin County, Texas.

Implementation: In the spring of 2007, the first Houston toad eggs were transported to the Houston Zoo for head-starting. For the head-starting efforts, egg strands or partial egg strands, are collected and transported to the zoo's "amphibian conservation quarantine" facility. The eggs are acclimated to captive water conditions and are introduced to the tadpole rearing aquarium rack system. As larvae approach Gosner stage 42 they are transferred to "emergence tanks", which are miniature ponds with a high temperature (32^{o} C - 35^{o} C) basking spot. Upon complete absorption of the tail the toadlets are then transferred to fully


Army of Houston toads ready for release

terrestrial enclosures. They are fed a series of gradually larger prey items (springtails, fruit flies, bean beetles, domestic crickets, wax worms, and mealworms) until achieving the scheduled release size. Actual releases are timed to coincide with rain events whenever possible. In 2010, larvae between Gosner stage 38 and 40 were released in an effort to determine if larvae releases would be as effective as toadlet releases (i.e., have the same, less, or more effects on juvenile survivorship). Pre-release protocols mandate a clear fecal parasite history (no parasites for at least 2 consecutive screenings), healthy and normal histopathology results from deceased or screened individuals from the group, and a negative amphibian chytrid qPCR test. The toadlets are released at or just after sunset into the forest surrounding the same pond from which the eggs were collected. For late stage larvae, releases are performed in the early afternoon. In 2007, 500 Houston toads were released, with an impressive 33.5% of juveniles surviving in captivity. In 2009 and 2010, 4,194 and 14,728 Houston toads were released, respectively, with captive survivorship increasing to 50 - 55%. Both 2008 and 2011 were exceptional drought years during which Houston toad reproduction in the wild was not detected, and may not have occurred.

Post-release monitoring: Differentiating between captive raised and wild individuals is challenging, as most techniques (e.g. toe clipping, elastomers, passive intergrated transponders) have innate failure rates that can reduce the detection of previously marked individuals if releases are made when the individuals are small. Specifically, it is extremely challenging to mark larvae for an evaluation of the success of releasing different life stages. Genetic markers can be used to differentiate individuals from different cohorts or sibling groups (Blouin, 2003), and if a cohort is adequately sampled and released at the same life stage, it is possible to genetically “tag” any individual and determine its origin when recaptured. Our previous population genetics work (McHenry, 2010) provides the highly polymorphic marker suite required, and research by Vandewege (2011) has confirmed the utility of those markers to detect kinship against unknown wild caught individuals.

Major difficulties faced

- Due to the rarity and secretive nature of the Houston toad, very little is known about commensal organisms and naturally occurring pathogens. This results in


Typical habitat with researchers

large delays to any releases when new organisms (e.g. *Mycobacteria sp.*) are detected in head-started toads.

- Juvenile amphibians consume a tremendous quantity of invertebrate prey, which is a testament to the ecological services of amphibians, but can become quite expensive in an *ex situ* conservation program.
- Determining the most effective (highest survivorship for the lowest cost) life stage to release is extremely important, but fraught with difficulties. As survivorship probability is positively linked to size, larger individuals should fare better after release, and the larger a female is, the closer she is to reproductive maturity. However, captive acclimation is likely to be more significant the longer an individual is reared in captivity. Likewise, cost is correlated to duration in captivity, requiring optimization of limited financial resources to either maximize numbers (larval population supplementation) or size (large juveniles). The data necessary to guide these decisions are not yet available.
- As Texas is primarily privately owned, Houston toad recovery will rely heavily on the ability of wildlife agencies to bring private landowners and other stakeholders to the table. Returning head-started endangered species into stakeholder communities, which have a mosaic of opinions about the toad and the government, can cause delays and even halt progress.

Major Lessons learned

State of the Science

- Amphibian declines and consequent stewardship programs are well established, but frameworks for optimizing amphibian population supplementation are not. Endangered species suffer from multiple impacts culminating in their declines. In many cases inherent rarity serves to increase the difficulty of accurate statistically supported assessment methods for a given management option. Seemingly too often, any population increases detected are assumed to be the results of a given management strategy, even if little or no data support those suppositions. We have found very little data to guide decisions about population supplementation strategy and success in amphibian populations. The lack of published evaluations of population supplementation using genetic markers or strong mark-recapture data was surprising to us.

- The math of survivorship reveals that any successful population supplementation effort in the Houston toad will require a much more industrial scale effort than was initially perceived. On average we have been able to head-start and release six egg strands or partial egg strands per year since 2007. On the one hand this is tremendously valuable, as those represent a significant proportion of the total reproduction in the wild, and an even larger proportion of the reproduction for the two largest sub-populations remaining for the species. Thus, reducing mortality from complete (i.e. drought desiccation losses) to "normal" is a significant contribution when reproduction is this rare in the wild. Unfortunately, that low level of overall reproductive success will not enable a population to rebound, much less recover. Wild egg strand head-starting also requires half of the overall program effort necessarily devoted to field monitoring, detection, and acquisition of wild egg strands. While the situation in the wild is improving and we have demonstrated that part of the positive change in abundance is a direct result of head-starts, it will not be enough and captive propagation must be carefully considered as a viable option.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:
Successes:

- As one of the goals is to significantly increase juvenile survivorship, this has been a remarkable success thus far. Both standard mark-recapture methods and genetic tracking have detected head-started individuals months and years after release, albeit at low total numbers but relatively high frequencies given the released life stage (~8% near adults and large juveniles, and ~0.1% among initial metamorphs of annual wild captures) as constrained by the expected natural survivorship frequencies in the wild.
- Another remarkable outcome has been the stakeholder response to head-starting and supplementation. The concept is easily grasped and the close involvement of those private stewards has provided a stronger engagement with the conservation efforts. Seeing juvenile toads hop away is not an abstract conservation program in the way that chorus monitoring or annual pitfall trapping can be. The response to the program has included media attention and the consequential additional public outreach.
- The captive assurance colony is in place and a genetic comparison of the wild populations and captive assurance colony has been completed. While results vary among subpopulations, 67% of the genetics detected in the wild is retained by the current captive colony.

Failures:

- Our field procedural techniques did not account for the resampling of recaptured individuals. We have completed more than a decade of mark-recapture and monitoring of the species at the field sites. Historically, animals that were recaptured and had been previously marked were not resampled for DNA, with the knowledge that they were sampled at initial capture. For our purposes during the first two years of the population supplementation, this had not been fully modified for the head-start tracking. Previously marked head-starts were recaptured but not resampled for DNA, decreasing the power of our DNA mark-recapture analyses and preventing final confirmation of those individuals as head-starts. This is particularly relevant for metamorphs with a cohort toe-clip released during the first two years of the study. It is less relevant, but still an issue, for larger releases that were microchipped but not resampled at recapture.

- Persistent drought conditions have resulted in very few wild egg strands, with attendant consequences to the study. Captive propagation should have been incorporated during the planning stages to compensate for this recurring problem.

References

Blouin, M. S. 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends in Ecology and Evolution 18: 503 - 511

Brown, L. E. & A. Mesrobian. 2005. Houston toads and Texas politics. Pages 150 - 167 in M. Lannoo, editor. Amphibian Declines. University of California Press, Berkeley.

Dodd, D., C. Kenneth & R. A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles: are they conservation strategies that work? Herpetologica 47: 336 - 350.

Greuter, K. 2004. Survivorship and growth in the endangered Houston toad. Masters Thesis. Texas State University, San Marcos, TX.

Hatfield, J. S., A. H. Price, D. D. Diamond & C. D. True. 2004. Houston toad (*Bufo houstonensis*) in Bastrop County, Texas: need for protecting multiple subpopulations. Pages 292 - 298 in H. R. Akcakaya, M. A. Burgman, O. Kindvall, C. C. Wood, P. Sjogren-Gulve, J. S. Hatfield, and C. A. McCarthy, editors. Species Conservation and Management. Oxford University Press, Oxford.

McHenry, D. J. 2010. Genetic variation and population structure in the endangered Houston toad in contrast to its common sympatric relative, the coastal plain toad. Ph.D. dissertation. University of Missouri, Columbia, MO, USA.

Swannack, T. M., W. E. Grant & M. R. J. Forstner. 2009. Projecting population trends of endangered amphibian species in the face of parametric uncertainty. Ecological Modeling 220: 148 - 159

Vandewege, M. W. 2011. Using Pedigree reconstruction to test head-starting efficiency for endangered amphibians: field tested in the Houston toad (*Bufo houstonensis*). Master's thesis. Texas State University. San Marcos, TX, USA.

Conservation and recovery of the mountain yellow-legged frog in Southern California, USA

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Introduction

The mountain yellow-legged frog (*Rana muscosa*) is endemic to California and occupies the Transverse Ranges of southern California and the southern extent of the Sierra Nevada Mountains. The mountain yellow-legged frog occurs almost entirely on protected lands yet has declined from more than 98% of its historic range (Vredenburg *et al.*, 2007). Currently in the Transverse Ranges, nine extant populations exist across three mountain ranges with less than 200 adult frogs remaining in the wild (USGS unpublished data). This species is listed as Endangered by the IUCN, Endangered by the U.S. Fish and Wildlife Service, Sensitive by the U.S. Forest Service, a Species of Special Concern by the California Department of Fish and Game and is being reviewed for listing as California State Endangered. An informal working group was assembled to address conservation activities for the mountain yellow-legged frog in 1999. This group consists of representatives from the U.S. Geological Survey, U.S. Fish and Wildlife Service, California Department of Fish and Game, U.S. Forest Service, San Diego Zoo, Los Angeles Zoo, and the Fresno Chaffee Zoo. This work involves monitoring known populations, surveying for new populations, habitat restoration, disease screening, captive breeding, and re-establishment to sites within the historic range.

Goals

- Goal 1: To establish self-sustaining populations of mountain yellow-legged frogs within the historic range of the species.
- Goal 2: Understand the genetic structure of remaining frog populations to guide captive breeding and reestablishment efforts.
- Goal 3: Understand the dynamics and challenges of restoring Bd positive wild populations.

Mountain yellow-legged frog © Adam Backlin

Success Indicators

- Indicator 1: Develop effective techniques and protocols for captive husbandry and breeding, translocation, and restoration for mountain yellow-legged frogs.
- Indicator 2: Develop successful captive breeding colonies for each of the three conservation units (mountain ranges).
- Indicator 3: Identify suitable reestablishment sites with compliance from all partners.
- Indicator 4: Increase the numbers of approved re-establishment sites.
- Indicator 5: Expand the available habitat to the mountain yellow-legged frogs at sites currently occupied through habitat restoration.

Project Summary

Feasibility: The mountain yellow-legged frog was historically abundant across the Transverse Ranges of southern California. Museum vouchers indicate a large scale decline occurred between 1968 and 1970, likely due to the amphibian chytrid fungus (*Batrachochytrium dendrobatidis* - Bd). By the mid-1990s it was apparent that this species had declined to a point that required active management in order to persist. In 2000, surveys were initiated to understand the population status and identify remaining populations. To date, nine populations have been found, occupying less than 1 km of stream habitat, with all but three populations containing less than 20 adults. Disease screening revealed all populations to be positive for Bd. Mitochondrial and microsatellite analyses show that substantial population structure is evident. This data suggests a high degree of historical isolation within and between mountain ranges and that each mountain range in southern California should be managed separately to protect unique evolutionary lineages of the mountain yellow-legged frog (Schoville *et al.*, in press). As part of an emergency salvage effort in 2006, 86 tadpoles were collected from Dark Canyon, San Jacinto Mountains, Riverside County, CA, USA, to prevent desiccation. These tadpoles were placed in a captive husbandry program at the San Diego Zoo Institute for Conservation and Research and raised to adults for captive breeding. In 2009, 106 additional tadpoles were collected from Devils Canyon, Los Angeles County, CA, USA, as an emergency salvage following a wildfire that burned the occupied watershed. These tadpoles were placed in a captive husbandry program at the Fresno

Typical habitat of the mountain yellow-legged frog © Adam Backlin

Chaffee Zoo with plans to raise these animals to adults for captive breeding. To obtain approval for a location to release mountain yellow -legged frogs, several permit and regulatory processes were required. Both federal and state permits were obtained to collect, relocate, breed, and release mountain yellow-legged frogs. A Memorandum of Agreement was developed and signed by all relevant partners to facilitate and approve releases of mountain yellow-legged frogs.

Preparing for release © Adam Backlin

Implementation: 2010 marked the first successful captive breeding of the mountain yellow-legged frog at the San Diego Zoo Institute for Conservation and Research. Two releases were conducted in April and in August 2010. The April release consisted of three egg masses (approximately 600 eggs) placed in cages in the stream. The August release consisted of 36 tadpoles head-started from the San Diego Zoo that were placed in cages in the stream. All releases were conducted in Indian Creek, Riverside County, California, USA. The breeding in 2010 produced approximately 1,200 eggs. Unfortunately, only 46 of the eggs released were fertilized and 36 tadpoles survived in the head-starting program to be released, totaling 80 released mountain yellow-legged frogs in 2010.

Post-release monitoring: Following the egg mass and tadpole releases, surveys were conducted to monitor the success of this effort. Eggs were monitored bi-weekly in their cages until they hatched. After hatching, weekly surveys were conducted. No tadpoles were detected in the creek following hatching. This is likely due to the small number and size of the newly hatched tadpoles and their cryptic coloration and behavior. All 36 head-started tadpoles were released into four cages at two locations within the stream. At each location, nine tadpoles were placed in each cage. Cages were monitored bi-weekly for the first two weeks then bi-monthly until the onset of winter. After the first week, nine tadpoles were released into the creek at each location. All 18 tadpoles appeared healthy when released. The remaining 18 tadpoles remained in the cages for monitoring until winter, approximately three months. With the first winter storm approaching in November 2010, the remaining 18 tadpoles were released. The bi-monthly monitoring also failed to detect tadpoles within the stream.

Major difficulties faced

- Problem obtaining the appropriate permits for all partners. This required approval by all partners which is challenging due to the complex logistics required for regulatory agencies and land managers to approve sites for reestablishments in southern California.
- Securing long term funding is difficult and requires actively seeking and applying for grants.
- Low fertility encountered in the first year breeding effort.

Major lessons learned

- Initiate restoration and conservation actions before species reaches critical stages.
- Develop comprehensive working group with representation from all required partners at the early stages of restoration.
- Develop long term adaptive recovery planning at early stages of project.
- Species level restoration requires long term commitments from multiple partners.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- The partial success of this project was the accomplishment of releasing a captively breed endangered species into the wild in southern California.
- The success of the frogs re-establishing their new site will require at least five years to evaluate.

References

Schoville, S. D., T. S. Tustall, V. Vredenburg, A. R. Backlin, E. Gallegos, D. W. Wood & R. N. Fisher (in press). Conservation of evolutionary lineages of the endangered mountain yellow-legged frog, *Rana muscosa* (Amphibia: Ranidae), in southern California. 23 pp.

Vredenburg, V. T., R. Bingham, R. Knapp, J. A. T. Morgan, C. Motitz & D. Wake. 2007. Concordant molecular and phenotypic data delineate new taxonomy and conservation priorities for the endangered mountain yellow-legged frog. Journal of Zoology 271: 361 - 374

Re-introduction program for the common midwife toad and Iberian frog in the Natural Park of Peñalara in Madrid, Spain: can we defeat chytridiomycosis and trout introductions?

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Introduction

The common midwife toad (*Alytes obstetricans*) is a broadly distributed toad in Western Europe cataloged as a near threatened species in the National Red List of Spain (Pleguezuelos *et al*., 2002), although in Madrid it is considered as endangered. In the Natural Park of Peñalara, a rocky montane area with around 250 ponds, the toad population was very abundant in the past, but declined during the late 90's due to the disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bosch *et al*., 2001). The Iberian frog (*Rana iberica*) is endemic of the Iberian Peninsula and is distributed mostly in the northwest, with few fragmented populations in the center and north of Spain. Its populations have been cataloged as Vulnerable in the National Red List of Spain, being threatened by habitat deforestation and alien species introduction. In Peñalara, due to past introductions of brook trout (*Salvelinus fontinalis*) and translocations of common trout (*Salmo* trutta), the Iberian frog disappeared from vast areas and is now confined to breed in suboptimal ponds where the trout were not present (Bosch *et al*., 2006)

Goals

- <u>Goal 1</u>: Maintain a captive population of *Alytes obstetricans,* preserving genetic identity, and develop a successful husbandry method.
- <u>Goal 2</u>: Rear *Rana iberica* larvae.
- <u>Goal 3</u>: Reinforce existing populations

Midwife toad (*Alytes obstetricans*) © J. Bosch and establish new ones for both species, with individuals reared in the Rearing Center.

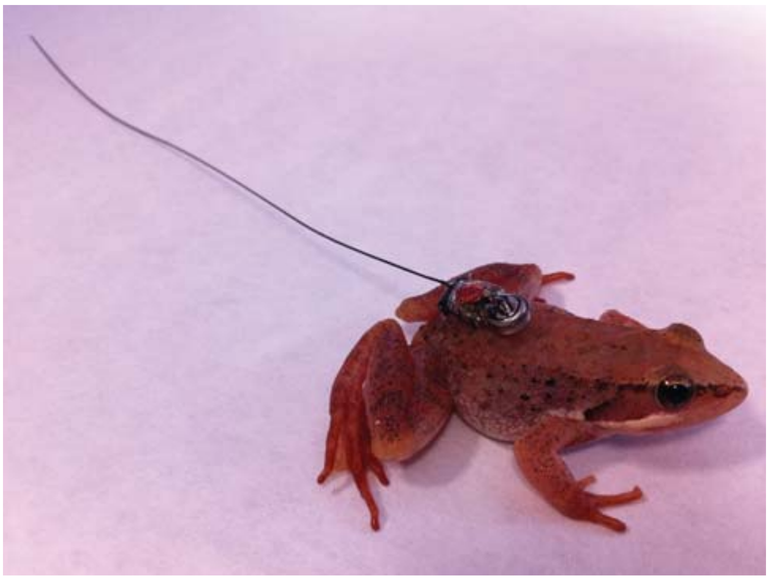
- Goal 4: Develop effective treatment methods against the fungus infection for a successful re-introduction of *Alytes obstetricans*.
- Goal 5: Eliminate all introduced trout within the Natural Park.

Success Indicators

- Indicator 1: Successful reproduction of *A. obstetricans* in the Rearing Center.
- Indicator 2: High survival rates of metamorphs of both species in the Rearing Center.
- Indicator 3: Increased number of adults of both species found in the field during monitoring programs, and number of reproductive events.
- Indicator 4: Higher rates of uninfected individuals of *A. obstetricans* in the field.
- Indicator 5: Smaller numbers of non-native trout found in the streams from year to year.

Project Summary

Feasibility: The common midwife toad was the most abundant amphibian in the Park before the outbreak of the disease. The male carries the eggs in their limbs for several weeks and then releases them in ponds, where tadpoles can remain in the water for several years before completing the metamorphosis. Such extended larval period increases the probability of contact with the waterborne zoospores of *B. dendrobatidis*. The low number of eggs in the clutches and the high rates of metamorphic mortality due to the disease drove the population almost to collapse within a few years. Two factors hinder the success of re-introductions. Even though the animals are treated before release, they become infected when they come into contact with the fungus. To avoid this problem, the first releases are being conducted in temporary ponds, where there is no overwintering larvae and, therefore, the probability of infection is lower. On the other hand, the genetic variability of the population is now reduced after a bottleneck. Therefore, to ensure the viability of re-introductions a microsatellite study has been carried out, and now we are sorting the crossbreedings to keep the maximum available genetic variability.



Iberian frog (*Rana iberica*) © J. Bosch

The Iberian frog's decline was not so dramatic. The high number of visitants and specially the trout introduction reduced the breeding sites of the species to only a few. The efforts of the Regional Government to recover


Overview of the habitat © J. Bosch

natural conditions began in the 1990s, and included the brook trout eradication in the original pond where the species was introduced by using bottom nets. Unfortunately, brook trout colonized the outlet of the original pond and, additionally, local anglers moved common brown trout from nearby downstream sites further upstream. Therefore, we have been electrofishing for trout during the last 9 years until the complete eradication of introduced trout in the Park. Obviously, the feasibility of re-introductions also depends on environmental awareness leading to the abandonment of these practices.

Implementation: In the case of *A. obstetricans*, since 2006 we have been capturing tadpoles from every location in the Park. These larvae were treated against the fungus using elevated temperature and antifungal drugs. We reared them in aquariums indoors matching environmental conditions to the park and using the same water source until they achieved juvenile or adult size. Most of them were then released in the same places where they were captured, while only some individuals were kept to establish a captive colony. A big effort has been directed to establish a new population in one pond which often dries out at the end of the summer season, keeping a lower chytrid fungus level than the surrounding area. In the case of *R. iberica*, we collected egg masses or tadpoles from a stream which dries out at the beginning of the summer, avoiding the complete development of the larvae. We head-started them in aquariums of 80 liters with up to 50 tadpoles in the Rearing Center, at the same Natural Park of Peñalara, and released them in the field, in streams where fish have been removed. This year, for the first time, we have released not only juveniles but also tadpoles and adults in several locations, in order to compare potential different survival rates across live stages.

Post-release monitoring: We search for active individuals of common midwife toad and Iberian frog two times per week in the summer season. For identification, we previously mark them with VIE (Visible Implant Elastomer tags) or take individual photos. At the moment we have found two males carrying eggs, one gravid female and some tadpoles of *A. obstetricans*. This year we have found, for the first time, some individuals of *R. iberica* that were released last year, and some adults released earlier this year. Additionally, this season we have followed 20 adult animals (15 *Alytes* and 5 *Rana*) by using radio-tracking technology. Additionally, two automatic recording devices (frogloggers) were installed a few years ago to count calling males.

Major difficulties faced

- Length of time between collected tadpoles for head-starting and F1 captive bred toads in *A. obstetricans*.
- The high difficulty to eliminate introduced trout from streams.
- Larval stages are not suitable for re-introduction in both species because they are highly susceptible to both fish predation and fungus infection.

Major lessons learned

- The common midwife toad is easy to maintain in captivity, while the Iberian frog gets easily stressed.
- The mortality of metamorphs in the field during the winter seems to be high, so adult re-introduction at the beginning of the season could be the best choice.
- Indoor rearing of *A. obstetricans* metamorphs under elevated temperatures (around 20º C) is effective, while keeping breeding adults outdoor, under semi-captivity conditions, is the best option to achieve mating.
- Trout eradication from montane streams by using electrofishing requires a great effort but is possible, and recolonization of native amphibian species is considerably quick afterwards.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- In the Rearing Center we have produced over 180 tadpoles of *Alytes obstetricans* this year.
- Metamorphs of *A. obstetricans* and *Rana iberica* have survived almost one winter after its re-introduction.
- Completely successful reproduction (from calling males to tadpoles) of *A. obstetricans* has been recorded this year from released animals.

References

Bosch, J., Rincón, P., Boyero L. & Martinez-Solano I. 2006. Effects of Introduced Salmonids on a Montane Population of Iberian Frogs. Conservation Biology, Volume 20 No. 1: 180 - 189

Pleguezuelos, J. M, Márquez, R. & Lizana, M. 2002. Atlas y libro rojo de los reptiles y anfibios de España. Dirección General de la Conservación de la Naturaleza-Asociación Herpetológica Española. Madrid. 584 pp.

Bosch, J., Fernández-Beaskoetxea, S. & Martín-Beyer, B. 2010. Time for chytridiomycosis mitigation in Spain. Alien: the invasive species bulletin 30: 54 - 58

Bosch, J., Martínez-Solano, I. & García-París, M. 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of Central Spain. Biological Conservation 97: 331 - 337

Re-introductions of Chiricahua leopard frogs in southwestern USA show promise, but highlight problematic threats and knowledge gaps

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Introduction

Chiricahua leopard frogs (*Lithobates* [*Rana*] *chiricahuensis*) inhabit a diversity of aquatic habitats at elevations between 1,000 and 2,710 m in Arizona, New Mexico, and Mexico (Sredl & Jennings, 2005). The species requires permanent or semi-permanent pools and may be excluded where *Batrachochytrium dendrobatidis* (*Bd*) or introduced predators are present. Additional threats include predation by non-natives, drought, floods, habitat degradation and loss, disruption of metapopulation dynamics, demographic effects of small populations in dynamic environments, and pollutants (U.S. Fish and Wildlife Service, 2007).

Lithobates chiricahuensis is listed as “threatened” in the USA under the Endangered Species Act (67 FR 40790) and “vulnerable” on the IUCN Red List of Threatened Species (Santos-Barrera *et al.*, 2004). The Chiricahua Leopard Frog Recovery Team finalized a recovery plan in 2007. This plan outlines a framework for delisting that, if implemented, will achieve the following recovery criteria: 1) establish at least 16 meta-populations and 8 isolated robust populations

Adult Chiricahua leopard frog, Pima Co., Arizona, USA
© A. King

rangewide, 2) restore breeding habitats and 3) dispersal corridors, and 4) reduce threats so it no longer needs the protection of the Endangered Species Act. This plan also identified management areas (MAs), which are large landscapes with great recovery potential (U.S. Fish and Wildlife Service, 2007).

Goals

- Goal 1: Reduce or eliminate threats in occupied and unoccupied areas needed for recovery.
- Goal 2: Identify sites for population re-establishment and augmentation.
- Goal 3: Develop and operate head-starting facilities.
- Goal 4: Develop and implement release techniques and protocols (collection > pre-release treatment for *Bd* > transport > release).
- Goal 5: Develop and conduct monitoring at re-introduced and other extant sites.

Success Indicators

- Indicator 1: Recovery of natural populations and metapopulations when threats are reduced or eliminated.
- Indicator 2: Successful rearing and release of *L. chiricahuensis.*
- Indicator 3: Establishment of sustainable populations.
- Indicator 4: Dispersal of released frogs to adjacent, unoccupied aquatic sites.
- Indicator 5: Success at creating refugia (assurance populations) when necessary.

Project Summary

We review three case studies that provide insight into key elements of successful *L. chiricahuensis* re-introductions in Arizona and make special mention of the problematic impact of chytridiomycosis in New Mexico.

Case Study 1 - Upper East Verde River Management Area (MA), Arizona, USA: The Upper East Verde River MA is located in north-central Arizona in the westernmost portion of the historical range of *L. chiricahuensis*. The upper Verde River drains approximately 6,500 km^2 and comprises most of the MA. Between 1995 - 2007, 38 surveys found fewer than 16 frogs at three sites and moderate threats. In 2009 and 2010, captively-reared frogs were released into four perennial tributaries of the East Verde River. A total of 3,542 metamorph frogs and late-stage tadpoles have been released to 13 sites throughout the watershed. Three of which were recipients of 3½ egg masses produced in the wild by released frogs. Post-release monitoring in 2010 - 2011 documented breeding at four of 13 release sites in as soon as 10 months post-release. Released individuals have dispersed and reproduced at four new localities. To date, 32 egg masses have been observed.

Case Study 2 - Pajarita Wilderness and Alamo-Pena Blañca-Peck Canyon MAs, Arizona, USA and Mexico: The Pajarita Wilderness and Alamo-Peña Blanca-Peck Canyon MAs are located in extreme southern Arizona and adjacent Mexico. In the 1930's, the Atascosa-Pajarito mountains supported three native ranid frogs: *L. chiricahuensis*, lowland leopard frog (*L. yavapaiensis*), and Tarahumara frog (*L. tarahumarae*). By the late-1970's, populations of *L. chiricahuensis and L. yavapaiensis* dramatically declined and *L. tarahumarae* was extirpated, likely due in part to chytridiomycosis. In addition, over the last few decades, *L. chiricahuensis* and *L. yavapaiensis* have slowly been displaced by invasive, introduced bullfrogs (*L. catesbeianus*). In fall 2008, efforts to eradicate *L. catesbeianus* were initiated. By 2010, post-removal monitoring confirmed that *L. catesbeianus* had been eradicated. Monitoring indicated immediate changes to *L. chiricahuensis* and *L. yavapaiensis* distributions. Surveys from 2010 - 2011, revealed *L. chiricahuensis* and *L. yavapaiensis* had dispersed into eight and three sites, respectively, that were previously unsuitable due to presence of *L. catesbeianus. Lithobates chiricahuensis* dispersed overland and through ephemeral drainages at least 7.9 km, occupying a site farther north than the species has recently been documented in the region. The results of this project indicate that re-introduction of native amphibians is not always necessary if a key threat is removed. Although *Bd* is still present throughout the mountain range, populations of native frogs are now persisting with the disease, and elimination of bullfrogs has created a landscape where both *L. chiricahuensis* and *L. yavapaiensis* can potentially thrive with minimal management.

Seining to reduce numbers of tadpoles from an earthen stock tank © AGFD

Case Study 3 - Black River MA, Arizona-New Mexico, USA: The Black River MA is located in central Arizona and adjacent New Mexico. This area contains the most mesic habitats and the highest elevation

Juvenile frog being released into a historical site © A. King

historically occupied site and still contains apparently suitable lotic and lentic habitats. Historically, the frog was known from numerous sites throughout the MA, but by the late-1980's it was known from only five sites. In 1996, wild frogs were collected for breeding and head-starting. Although, presence of non-native sportfish and crayfish has made habitat selection for re-introduction of frogs challenging, we considered that the overall complexity and connectivity of the sites in this area would allow for establishment and persistence of frogs. Since 1996, three sites were augmented, but by 2000, fewer and fewer frogs were being detected. Over the past 11 years, re-introduction of captive reared frogs has continued at four historical sites. Although frogs were released multiple times to some sites over several years, most releases comprised fewer than 100 individuals. Generally, post-release monitoring has included surveys shortly after release, followed by subsequent surveys two to three times a year. Survey results show releases have not been successful and *L. chiricahuensis* has not been detected in the Black River MA since 2009. Reasons for failure are not entirely clear; however, we have not detected *Bd* at any of these sites.

Recovery in New Mexico, USA: Re-introductions in New Mexico have not taken place as frequently as in Arizona, partially because the frogs appear to be particularly susceptible to chytridiomycosis. This sensitivity has caused annual population extirpations and has necessitated a different initial recovery strategy focusing on creating off-site refugia to safeguard genetics. To create refugia, wild eggs, tadpoles, or metamorphs are collected, brought into captivity, reared, tested for disease, treated if necessary, and released to confined steel rim tanks. These tanks not only serve as refugia, but in time will also serve as sources for re-introduction efforts. To date, 8 lineages have been established in refugia. Two of the source populations for the eight refugia have since experienced die-offs and are believed extirpated (U.S. Fish and Wildlife Service, 2011). Now that sufficient refugia have been established, the focus of recovery in New Mexico has shifted to augmentations and re-introductions.

Major difficulties faced

- Presence and impact of nonnative predators and pathogens.
- Lack of suitable habitat.

- Poor understanding of habitat requirements.
- Poor understanding of *L. chiricahuensis* metapopulation dynamics.
- Lack of resources for effective post-release monitoring.

Major lessons learned

- Removal of non-natives is possible if done by using a systematic landscape-level approach.
- Disease appears to be a major impediment to success in some portions of the range.
- The success of *L. chiricahuensis* re-introductions is enhanced by multiple releases of late-stage larvae and metamorph frogs (n=100 - 400) to multiple sites within a watershed.
- Egg mass transplants can be successful.
- Close coordination among partners in re-introduction projects is essential.

Success of projects

Overall success summary of all case studies:

Highly Successful	**Successful**	Partially Successful	**Failure**
1	**1**		**1**

Case Study 1 - Upper East Verde MA:

Highly Successful	Successful	Partially Successful	Failure
√			

Reason(s) for success/failure:

- Large number of frogs released to the watershed.
- Lack of non-natives present at release sites.
- Adequate post-release monitoring.
- Documented successful reproduction and dispersal after releases.
- Determination of success is based on two years of post-release data.

Case Study 2 - Pajarita Wilderness and Alamo-Peña Blanca-Peck Canyon MAs:

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- Implemented a systematic, landscape-level approach to remove *L. catesbeianus* from all possible sites.
- Focused on complete removal, not reduction or control.
- Removal of *L. catesbeianus* allowed for re-colonization of *L. chiricahuensis* and *L. yavapaiensis.*

- *Bd*, although widespread in the region, currently does not appear to significantly affect native leopard frog populations.
- Continued monitoring for *L. catesbeianus.*

Case Study 3 - Black River MA:

Highly Successful	Successful	Partially Successful	**Failure**
			√

Reason(s) for success/failure:
- Small numbers of frogs available for release.
- Insufficient post-release monitoring to determine success of releases.
- Presence on nonnative predators.
- Potentially unknown reason for failure (e.g. low genetic variability, extreme susceptibility to disease, etc.).

References

(67 FR 40790) Endangered and Threatened Wildlife and Plants; Listing of the Chiricahua Leopard Frog (*Rana chiricahuensis*); Final Rule; Federal Register 67:114. 2002. p. 40790 - 40811. <http://www.fws.gov/policy/library>.

Santos-Barrera, G., G. Hammerson, & M. Sredl. 2004. *Rana chiricahuensis* in: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. <www.iucnredlist.org>.

Sredl M. J. & R. D. Jennings. 2005. *Rana chiricahuensis* (Platz and Mecham, 1979) Chiricahua leopard frogs. Pages 546 - 549 in: M. J. Lannoo (ed). Amphibian declines: the conservation of United States species. University of California Press, Berkeley, California.

U.S. Fish and Wildlife Service. 2007. Chiricahua leopard frog (*Rana chiricahuensis*) recovery plan. Region 2, U.S. Fish and Wildlife Service, Albuquerque. New Mexico.

U.S. Fish and Wildlife Service. 2011. Chiricahua leopard frog (*Lithobates* [=*Rana*] *chiricahuensis*) 5-year status review: Summary and evaluation. Region 2, U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

Conservation introduction of the Cape platanna within the Western Cape, South Africa

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Introduction

The Cape platanna (*Xenopus gilli*) has a disjunct distribution in the winter rainfall region of the south-westernmost part of the African continent. Records for this frog span a distance of around 160 km from the Cape Peninsula towards Cape Agulhas (de Villiers, 2004). It has been listed as Endangered (B1ab(i,iii)+2ab(i,iii)) in view of its declining extent of occurrence (currently 1,450 km^2) and area of occupancy, and a continuing decline in the extent and quality of its habitat (SA-FRoG & IUCN SSC-ASG 2009). The majority of its recorded acid blackwater localities have been destroyed or degraded through development and associated threats (Picker & de Villiers, 1989). By the late 1980s the species could no longer be found at 60% of known localities, including one of the originally described localities in the Silvermine River, and virtually the entire western population was effectively confined to Cape of Good Hope Nature Reserve (CoGHNR), at the tip of the Cape Peninsula (de Villiers, 2004). Few acid blackwater pools remained in this region, but several were identified in the protected upper catchment area of the Silvermine River. It was thus decided to introduce individuals from CoGHNR, over about 25 km, to this Silvermine area.

Goals

- Goal 1: To establish a new *Xenopus gilli* population in appropriate blackwater habitats in Silvermine.
- Goal 2: To seed the Silvermine River with individuals which might spread onto the Cape flats and surrounds
- Goal 3: To safeguard the genetic integrity of the Cape platanna away from the invasive common platanna (*X. laevis*) and to reduce disease threat.

Freeze brand visible on ventral surface of a recapture after ten years (inset) © G. J. Measey

Success Indicators

- Indicator 1: Sustainable populations of *Xenopus gilli* in acid blackwater ponds in Silvermine.
- Indicator 2: Absence of genetic introgression with common platannas.
- Indicator 3: Spread of *Xenopus gilli* into surrounding water bodies.

Project Summary

Feasibility: A survey of the acid blackwaters in Silvermine Nature Reserve took place in 1987 when waterbodies were also trapped to make sure that none contained existing populations of *Xenopus gilli* or *X. laevis*. Thereafter, larger waterbodies in different geographic areas within Silvermine were chosen as release sites. It was hoped that the released frogs would lead to the colonization of smaller surrounding waterbodies.

Implementation: On 23rd April 1988, 154 newly metamorphosed *X. gilli*, were translocated from the genetically pure Gilli Dam population in the Cape Point area to Silvermine. Metamorphs were released into four water bodies: Nellie's Pool, Hennie's Pool, Silvermine Reservoir and Dammetjie. Most of the froglets (69) were released in Nellie's Pool as it appeared to have the most suitable habitat.

Post-release monitoring: Monitoring was conducted from 1989 to 1990. On April 3rd, 1989 1 male *X. gilli* was captured at Nellie's Pool (by R. Rau, pers. comm.), and on 2nd November 1989 2 males and 4 females were trapped at the same site (AdV). No *X. gilli* were found during a 5th October 1990 visit. Further monitoring was left to reserve staff, but no records exist. In 1998 when we returned to one site (Nellie's Pool) to determine whether individuals were still present. Six females were captured, of which three were marked by freeze-branding (Measey, 2001). A hiatus of 10 more years passed before in June 2008 we again visited all sites where *X. gilli* had been released. Baited funnel traps were placed into each of the release points to ascertain presence of *X. gilli*. Only Nellie's Pool was found to have individuals present. Amongst those captured were two which were still marked with freeze brands from 1998.

In August 2011, we trapped on two occasions at Nellie's Pool catching a single female (and sighting one more individual). The belly pattern on this individual corresponded unambiguously to a female caught in June 2008 and had a freeze brand from 1998. Our results are of interest as we demonstrate the extreme longevity of this species in its natural habitat (>13 years). The individuals that were marked in 1998 were adult and it is not infeasible that these were the same individuals which were released in 1988.

Major difficulties faced

- Finding suitable acid black water release sites.
- Lack of suitable lowland habitat restoration.
- Insufficient monitoring to detect recruitment and dispersal of released population.

Major lessons learned

- No funding or capacity was available to systematically monitor translocated frogs in this study in the short or long term. Approval of such projects should be dependent on such provisions being demonstrated.
- Little is known about the distribution densities of this species in upland areas, with all known populations being in lowland sites. It may be that if upland sites are suitable but they occur at low densities.
- Survival of the frogs in Nellie's Pool could be because it is artificially dammed and thus contains an increased volume of suitable blackwater habitat for this species.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- Unexpected longevity of individuals may have facilitated successful breeding spanning unfavorable years with low winter rainfall.
- One of the release sites has remained stable throughout the study and facilitated at least occasional breeding of this frog.
- Other sites either contained predatory fish lacked suitable habitat to maintain viable populations.

References

de Villiers, A. L. 2004. Species account: *Xenopus gilli* Rose and Hewitt, 1927. In L. R. Minter, M. Burger, J. A. Harrison, P. J. Bishop, & H. Braack (Eds.), Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland (pp. 260 - 263). Washington DC: Smithsonian Institution Press.

Picker, M. D. & de Villiers, A. L. 1989. The distribution and conservation status of *Xenopus gilli* (Anura, Pipidae). Biological Conservation, 49(3): 169 - 183.

South African Frog Re-assessment Group (SA-FRoG) & IUCN SSC Amphibian Specialist Group 2009. *Xenopus gilli*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 22nd May 2011.

Measey, G. J. 2001. Growth and ageing of feral *Xenopus laevis* (Daudin) in South Wales, UK. Journal of Zoology, London. 254, 547 - 555

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Global Re-introduction Perspectives: 2013

Further case-studies from around the globe

Edited by Pritpal S. Soorae

IUCN/SSC Re-introduction Specialist Group (RSG)

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Re-introduction of European tree frog in Latvia

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Introduction

European tree frog was considered as extinct in Latvia since last decades of the 20th century. Data on the former distribution of this species are rather incomplete. Several faunists of German origin (Fischer, Seidlitz and Schweder) have mentioned the species as being present in Latvia in the 18th - 19th centuries (Silins & Lamsters,1934). Several reports have even been received in the 1980s (Zvirgzds *et al.*, 1995). Intensive agriculture, rapid deterioration in total area covered mainly by wetlands, and extinction of beaver (*Castor fiber*) in Latvia in the end of 19th century, could be the main factors, which could cause the vanishing of *Hyla arborea* from Latvia. The re-introduction program was started by Riga Zoo in 1987, and a total of 4,110 juveniles in total were released in SW Latvia (Liepaja district), where protected area with total area of 350 ha was established in 1999. The area accommodates a large number of ponds, considerably changed by beavers. Before the re-introduction started, the European tree frog was listed in Red Data Book of Latvia under Category I (endangered species) (Latvijas PSR Sarkana gramata, 1985), at meantime Category II (vulnerable species) (Latvijas Sarkana gramata, 2003). The European tree frog is included in Appendix II of the Bern Convention.

Goals

- Goal 1: Creating sustainable populations of European tree frog in Latvia.
- Goal 2: Proving that creating sustainable populations of amphibians in nature is possible by releasing of specimens, bred under laboratory conditions.
- Goal 3: Proving that *Hyla arborea* can survive Latvia climatic conditions, therefore this species most likely was a natural part of Latvia nature during past centuries.

Success Indicators

- Indicator 1: Self-sustaining population established at re-introduction site, with more than 10 generations developed naturally.

European tree frog © Sergey Cicagov

Typical pond habitat © Andris Eglitis

- Indicator 2: The distribution of the population around the re-introduction site, as far as the suitable environment is available.

Project Summary

Feasibility: Laboratory of Ecology (Amphibian Department since 2006) was founded in Riga Zoo in 1987 with its main task to re-introduce the European tree frog in Latvia. The re-introduction was planned with captive-bred tree frog youngsters in their first year of life.

The considerations were as follows:

- The translocation of a larger amount of adult specimens from other natural populations could place the donor population at risk, even if the population is considerably stable.
- The youngsters would have a considerably higher ability to adapt to wild conditions than adults, if captive bred specimens are released into wild (Dunce & Zvirgzds, 2005).
- The adult specimens for captive breeding were caught in Southern Belarus, near the confluence of Goryn and Pripyat rivers, what is geographically closest stable population (there is also small population in Lithuania).

Implementation: The adults were kept in outdoor terrariums and fed with artificially bred insects as well as meadow sweeps. At the end of October and early November the frogs were placed in wooden boxes, filled with sphagnum, and boxes were kept in refrigerator for hibernation (average temperature 5°C) till the end of January and early February. Later it was found out that an old cellar as a hibernation place is better for the amphibians welfare, despite greater fluctuations of temperatures (from 1°C - 7°C). After hibernation the temperature was raised gradually, and the artificial daylight period gradually lengthened, imitating the day length of the breeding period. The frogs were fed intensively and breeding was stimulated with hormone injections, using Surphagon, a synthetic analogue of Luliberin (produced by Bapex Co., Latvia). During the first year of breeding effort the hormone treatment was given in the beginning of May, in other years during the beginning of March. In both cases the results were virtually identical.

Two males and one female were usually placed in a 35 liter aquarium with a water level of about 5 cm and several plants. Each female produced 200 - 1,000

Amphibian experts at a potential release site in Latvia during 2004 © Elvira Hrscenovica

or even more eggs. Hatching usually started on the 8th - 10th day of development. The larvae were placed in aquariums with aerated water; temperature was maintained 24°C - 27°C at day, 20°C - 23°C at night. The density of tadpoles never exceeded 2 - 3 larvae per liter. Tadpoles were fed with dried and boiled nettles, meat, aquarium fish food (Tetra) and pollen. The natural photoperiod was simulated using luminescent lamps. The average amount of animals that metamorphosed was 60% - 70% of the initial larvae; in some cases it even exceeded 90% (Zvirgzds *et al.*, 1995). The metamorphosis took 30 - 60 days (in the wild it usually takes 90 days). Froglets were fed with meadow sweeps and captive bred insects. About 2 - 6 weeks after metamorphosis the froglets were taken to the re-introduction site.

During 1988 - 1992 a total number of 4,110 juveniles, progeny from 14 - 17 breeding pairs, were released. All releases were conducted in one locality, enabling accurate further monitoring of population dispersal.

Post-release monitoring: The release site was chosen in SW Latvia (Liepaja district, ca. 56°30' N 21°42'E) where a protected area was established with total area of 350 ha. The first vocalizations of adult tree frog male in the re-introduction site were recorded in 1990 - two years since the start of the re-introduction program. This confirms that under particular conditions males can reach sexual maturity in 2 years. The first tadpoles in the wild were found in 1991, at the release site. The first calling males outside the release site were recorded in 1993. Further distribution progressed even faster and up to 2002, tree frogs were recorded already in 110 localities.

The distribution of the newly created population was monitored mainly on the basis of the spring mating calls. All new-recorded localities were registered by GPS and mapped till 2005. The local communities were informed about the project by dispersing booklets, giving lectures in schools, as well as cooperating with media (TV, radio). In later years the area of the population reached the size what made it practically impossible for accurate monitoring and further dispersal of tree frogs is followed up by reports of local people.

Major difficulties faced

- It is difficult to estimate the present size of population because of extended area. Despite of informational work with local people the reports about tree frogs are occasional and do not show the full picture of species occurrence.

Major lessons learned

- Under laboratory conditions the breeding can be effected to happen earlier than in the wild, and the larvae develop faster. Thus, the released froglets have more time to adapt to natural conditions as well as for feeding and growing. We hypothesize that it could result in a much higher survival rate during the first winter.
- Despite that the breeding of tree frogs was stimulated by hormonal injections in all cases, we did not face any problems regarding tadpole or froglet survival or growing rates.

Success of project

Highly Successful	Successful	Partially Successful	Failure
√			

Reason(s) for success/failure:

- After 14 of initiating the re-introduction program, monitoring data showed that total area of population dispersal covered 800 - 900 km^2 (Dunce & Zvirgzds, 2005). As it could be inferred from later reports, it continues to expand.

References

Dunce, I. & Zvirgzds, J. (2005) The European Tree Frog re-introduction in Latvia. Herpetologia Petropolitana 138-139.

Latvijas PSR Sarkana gramata (1985) Zinatne, Riga.

Latvijas Sarkana gramata (2003) LU Biologijas instituts, Riga.

Silins V. & Lamsters V. (1934) Latvijas rapuli un abinieki, A/S Valters un Rapa, Riga, 72-73.

Zvirgzds J., Stasuls M. & Vilnitis V. (1995) Re-introduction of the European Tree Frog (*Hyla arborea*) in Latvia. Memoranda Soc.Fauna Flora fennica 71:139-142.

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Global Re-introduction Perspectives: 2016

Case-studies from around the globe

Edited by Pritpal S. Soorae






IUCN/SSC Re-introduction Specialist Group (RSG)

RSG

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Cover photo: Clockwise starting from top-left:
i. Bolson's tortoise, USA @ Turner Endangered Species Fund
ii. Wetapunga, New Zealand @ Richard Gibson
iii. Morelos minnow, Mexico @ Topiltzin Contreras-MacBeath
iv. *Silene cambessedesii*, Spain @ Emilio Laguna
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vi. Agile frog, Jersey @ States of Jersey Department of the Environment

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Re-introduction of the northern corroboree frog in the Northern Brindabella Mountains, New South Wales, Australia

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Introduction

The northern corroboree frog (*Pseudophryne pengilleyi*) is a small Myobatrachid frog native to the Brindabella and Fiery Ranges of New South Wales and the Australian Capital Territory in south-eastern Australia. The species has suffered dramatic declines over the last 30 years and has disappeared from the majority of its former range. It is estimated that populations within the Northern and Southern Brindabella mountains, which are two of the three recognized distinct genetic populations or evolutionary significant units (ESUs), have less than 200 mature individuals remaining.

Northern corroboree frog

The decline of this species has been primarily due to the introduced fungal pathogen, amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), though other factors may have contributed on a lesser scale, including climate change, exotic weeds and habitat degradation due to introduced fauna species (Hunter *et al*., 2010; Scheele *et al*., 2012). The species is listed as Critically Endangered in


Release of 1 year old frogs

NSW under the *Threatened Species Conservation Act 1995* and Federally under the *Environment Protection and Biodiversity Act 1999*. It is also listed as Endangered by the IUCN and in the ACT under *Nature Conservation Act 1980*.

Goals

- Goal 1: Establish a sustainable *ex-situ* colony of the *P. pengilleyi* Northern Brindabella ESU and maintain as a genetically-viable insurance colony.
- Goal 2: Ensure the persistence of *P. pengilleyi* in the Northern Brindabella mountains by supplementing wild populations with captive-bred stock.
- Goal 3: Develop efficient and reliable re-introduction protocols by assessing the effectiveness of releasing different life-stages.

Success Indicators

- Indicator 1: Have developed successful captive husbandry and reproduction techniques.
- Indicator 2: Sufficient numbers of offspring to facilitate re-introduction efforts have been produced.
- Indicator 3: Post-release survival to sexual maturity of individuals released at different life-stages has been quantified.
- Indicator 4: Breeding populations of *P. pengilleyi* in the Northern Brindabella mountains continue to persist.

Project Summary

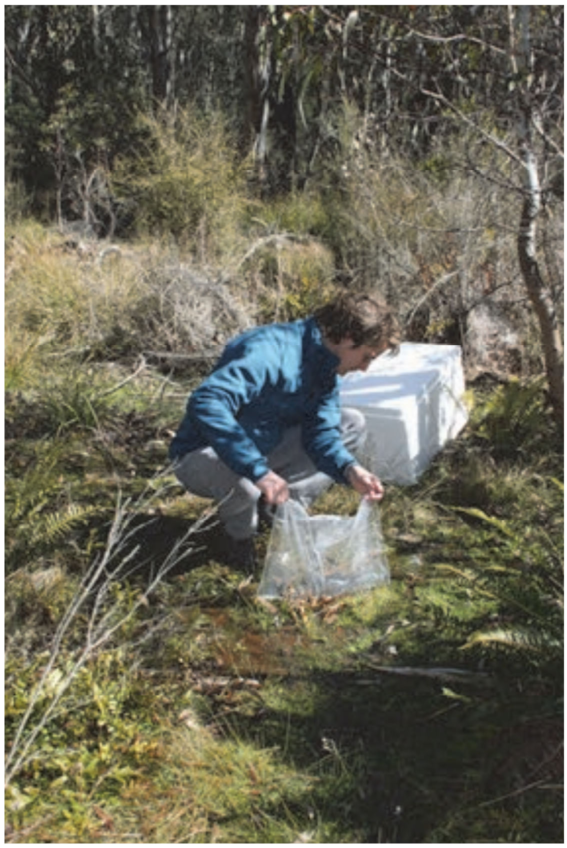
Feasibility: The Northern Brindabella ESU of *P. pengilleyi* has been in continual decline since the arrival of chytrid fungus over three decades ago. In 2010, annual surveys indicated that the number of mature calling males had dropped to 66 calling males. By 2012, only three calling males were located throughout breeding sites within the ESU. These results suggest that population numbers at existing sites are at critically low levels and are at risk of extinction. Between 2003 and 2005, eggs were collected from a number of wild nests and taken to Tidbinbilla Nature Reserve to establish an insurance colony for this population. During 2010 and 2011, most of this captive colony was transferred to Taronga Zoo, Sydney. Successful breeding protocols have been established for this species at both institutions.

Within the Northern Brindabella mountains, the habitat of the species remains largely intact, with numerous suitable breeding sites. As far as can be discerned, chytrid fungus is present at all suitable release sites available to the species. However, despite the presence of the fungus, the species rate of decline has been relatively gradual over the past three decades. This indicates that it may be feasible to maintain wild populations of the species in the presence of the pathogen with supplementation from an *ex-situ* colony.

Ensuring the persistence of *P. pengilleyi* in the Northern Brindabella Ranges will assist the broader recovery program through maintaining the species existing genetic variation, and allowing ongoing field research into techniques to mitigate the impact of the chytrid fungus. Additionally, enabling the population to persist in the presence of the chytrid fungus may allow the possibility of continued selection for resistance to disease caused by this pathogen.

Implementation: Two release sites were selected in the Northern Brindabella Mountains that until recently maintained significant populations of *P. pengilleyi* and were reasonably resilient to pool drying during the period of tadpole development. Eggs and tadpoles were released in 2010 (179), 2011 (146), 2013 (167) and 2014 (293), evenly divided between the two sites. All releases were undertaken between July and September, coinciding with when wild tadpoles would be at a similar stage of development.

In December 2014, 160 one-year old frogs and 49 five-year old frogs were released, with numbers of each cohort also divided evenly between the two sites. Sex ratios of the adult frogs were split evenly between the two sites. The juveniles frogs could not be sexed so were randomly assigned to each site. Undertaking releases at various life stages has been conducted to assess the most effective re-introduction technique to establish populations of this species, taking into account the cost implications of rearing individuals to a later stage of development in captivity. Just prior to release, each of the frogs was weighed, measured and had photographs taken of their ventral and dorsal surfaces to permit individual identification upon recapture using pattern recognition.



Releasing tadpoles in the Northern Brindabella Mountains

Post-release monitoring: Annual monitoring has been conducted at each of the two release sites since 1999, during the peak breeding season from late February to early March. Monitoring is conducted using a shout-response technique that has a high confidence of detecting mature calling males (Scheele *et al.*, 2012). The number of mature females is estimated based on the number of clutches within male nests. Due to their cryptic nature, there are no techniques to monitor immature individuals.

Surveys in March 2014 detected 7 males at each of the two release sites, though no eggs were laid in any of their nests. Due to the low number of adults at release sites between 2009 and 2011, and the lack of detection of frogs since 2011, it is suspected that these individuals were likely from the first tadpole releases in 2010. This is supported by length of time to maturity, with males typically maturing at 3 years in the wild, whilst females mature at 4 years. Thus in 2014, males from the 2010 tadpole release would be mature at just over 3 years of age, whilst the females may not, resulting in the perceived sexual bias.

In March 2015, seven males were detected at one site, whilst 13 were detected at the second site. At the end of the breeding season, the nests were inspected to identify and photograph males and assess their size. From the 20 nests, 12 males were still present upon inspection, of which four were identified by markings as being released 3 months earlier. At the latter release site, eggs were detected within 4 nests representing between 12 - 15 clutches of eggs.

Major difficulties faced

- The inability to detect frogs prior to maturity due to their small size and cryptic nature prevents the tracking of released young (eggs, tadpoles & juvenile frogs) animals for up to 4 years after their release.
- No practical technique to track females (because they do not call), reliance on limited data from opportunistic sightings in nests.
- Limited ability to directly link breeding adults with cohorts of released eggs. With additional funding it may be possible to do this using genetic techniques.
- The small size of the captive population and the low number of eggs produced by this species limits the number of offspring available for re-introduction.

Major lessons learned

- Survivorship to maturity can be achieved despite the persistence of chytrid fungus. Hence, it should be possible to maintain wild populations via a captive breeding and supplementation program.
- Presence of the chytrid fungus should not be a factor preventing re-introduction attempts as this will reduce the ability to gain increased knowledge of the disease dynamics in *P. pengilleyi* and prevent any possibility of selection for resistance to the disease.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- Successful captive reproduction has been achieved in each year attempts were undertaken, facilitating the provision of offspring for re-introduction efforts.
- Survivorship of a small proportion of released tadpoles to maturity at the two sites has been attained from the first cohorts of eggs and tadpoles released.
- It is too early in the program to declare this project to be a success or failure, as this will require at least another 5 years of post-release monitoring.

References

Hunter, D.A., Speare, R., Marantelli, G., Mendez, D., Pietsch, R. & Osborne, W. (2010) Presence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in threatened corroboree frog populations in the Australian Alps. Diseases of Aquatic Organisms, 92: 209-216.

Scheele, B.C., Driscoll, D.A., Fischer, J. & Hunter, D.A. (2012) Decline of an endangered amphibian during an extreme climatic event. Ecosphere, 3(11): 101.

Head-starting, re-introduction and conservation management of the agile frog on Jersey, British Channel Isles

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Introduction

Agile frogs (*Rana dalmatina*), found throughout much of Europe and northern Turkey, are listed on Appendix II of the Bern Convention, Appendix IV of the EU Habitats Directive, and as Least Concern in the IUCN Red List. The Channel Island of Jersey (117 km^2) is towards the northern edge of the species' range, and hosts the only agile frog population in the British Isles. In Jersey, population declines occurred throughout the 1900s, with animals becoming restricted to a single 10 ha dune heathland site (L'Ouaisné Common) by 1988. Causes of decline are thought to include habitat loss and fragmentation due to development, pollution of groundwater, water shortages and the loss of breeding ponds (Racca, 2002), and an increased predation pressure due to the introduction of non-natives (States of Jersey, 2006). The agile frog is therefore regarded as locally Critically Endangered within Jersey, and is protected under the Conservation of Wildlife (Jersey) Law 2000. Furthermore, Jersey's agile frogs show lower genetic variability than other European populations (Racca, 2004). The population has been the subject of a Species Action Plan since 2001, with captive husbandry undertaken by Durrell Wildlife Conservation Trust (DWCT).

Agile frog © Jersey States Department of the Environment

Goals

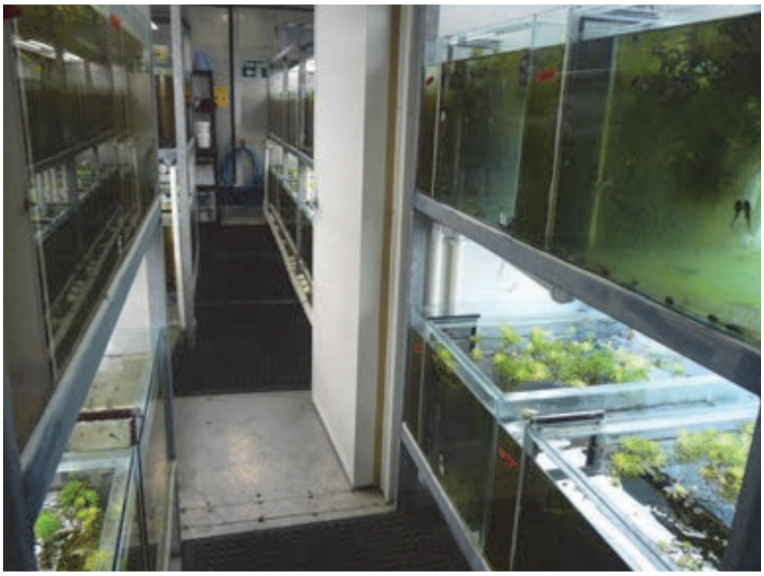
- Goal 1: To ensure that there is protection of, and a conservation management program for, all existing natural sites, introduction sites or re-introduction sites.
- Goal 2: To increase the number of populations and widen the species' distribution through introductions/re-introductions.
- Goal 3: To maintain a viable breeding population of frogs through head-starting and translocation with a minimum of 20 adult animals at a minimum of three locations (a minimum of 60 adults in total).
- Goal 4: To have annual monitoring of spawning in all populations.
- Goal 5: To further investigate the threats to, and applied ecology of this species in Jersey.

Success Indicators

- Indicator 1: Protection of all sites where the species occurs, and where it will be introduced/re-introduced.
- Indicator 2: Restoration of wild, naturally spawning populations at more than one site.
- Indicator 3: Wild frog populations of at least 20 adults breed successfully at a minimum of three locations.
- Indicator 4: Populations are monitored annually allowing detection of annual variation in spawning.
- Indicator 5: Research carried out to determine ecological requirements.

Project Summary

Feasibility: This project aimed to restore the population to the point where it is self-sustaining at multiple sites. The European habitat for the agile frog comprises slow-flowing or stagnant water bodies of 30 - 80 cm depth for breeding, and woodland for their terrestrial phase. Jersey's population shows some differences in habitat use compared to its mainland counterparts, by their use of coastal habitats (States of Jersey, 2006). Survival of eggs to metamorphosis in Jersey is higher than the expected rate of 1.0% - 2.0% for wild anurans, at 2.4% - 17.1% per year when spawn is protected or head-started (Racca, 2004). The agile frog population in Jersey declined in both range and numbers from the early 1900s until the 1990s. In the 1970's frogs were known from seven localities, and by the mid-1980s this had fallen to two sites; Noirmont and L'Ouaisné. A pesticide spill in 1987 decimated the Noirmont population, prompting the first intervention for the population. Declines are attributed to poor water quality and quantity through intensive agriculture and water extraction leading to a shortened hydroperiod and earlier pond desiccation; disturbance and loss of habitat; and an increase in both native and introduced predators (States of Jersey, 2006). Frogs migrate between terrestrial and breeding habitat, requiring identification of suitable habitat and engagement with stakeholders to encourage sympathetic management. Further obstacles include road mortality during migration, water pollution from agricultural sources, and limited available habitat with poor connectivity. The partner organisations working on this project provide a strong knowledge-base for the various actions requiring implementation, increasing the likelihood of success of this project. Consideration must be made for biosecurity both *in-* and *ex-situ* as


Agile frog head-starting container © Matt Goetz

captive management carried out by Durrell Wildlife Conservation Trust (DWCT) has to ensure strict separation between its captive population of exotics and the agile frogs. Re-introduction sites can be identified through historical distribution, habitat suitability and connectivity to the existing population.

Implementation: Interventions to arrest the declines began in 1987. A collaboration between the States of Jersey Department of the Environment (DoE), DWCT, the Société Jersiaise and a number of private stakeholders created the Jersey Agile Frog Group (now the Jersey Amphibian and Reptile Group). This group has worked to implement a head-starting, re-introduction and habitat management program (Racca, 2002). This has resulted in deepening of slacks to lengthen the period that water is held, regular water quality monitoring, and localised habitat management in order to improve habitat suitability (Racca, 2004). Protection of spawn clumps *in-situ*, and removal of spawn clumps for head -starting has taken place, with tadpole rearing undertaken by the herpetology department at DWCT since 1986, and the use of a dedicated biosecure unit since 2008. Head-started individuals achieve greater mass and survival than those left *in-situ* (Jameson, 2009), and have enabled the translocation of tadpoles to new sites. In 2000 tadpoles were re-introduced back to Noirmont following work to improve water quality, and by 2012 re-introductions had taken place at a further two sites, resulting in a total of four sites receiving monitoring and management. Both principal agile frog breeding areas at L'Ouaisné and Noirmont were designated as ecological Sites of Special Interest (SSI) in 2007. Furthermore, management plans for L'Ouaisné and Noirmont SSI's have been prepared by the DoE to ensure appropriate management for amphibian populations. Further work with local stakeholders to encourage sympathetic habitat management outside of protected areas could result in improvement in the future. Press coverage, involvement of and visits to educational institutions, and printing of educational materials have all attempted to raise public awareness of the issues surrounding the conservation of Jersey’s amphibians.

Post-release monitoring: Night surveys are made to each site during the breeding season to count breeding adults and spawn clumps. This monitoring has detected an increase in the number of clumps per year and the number of sites at which spawning occurs; from 12 in 1987 at a single site, to 134 spawn in 2014 at three sites, with no spawning in some years (Ward & Griffiths, 2015). Daytime visits are also made to each site to check the condition of spawn clumps and provide spawn protection where needed. Ongoing monitoring and research has allowed identification of effective methods for maintaining a population increase, which in this case is head-starting of individuals from egg to tadpole (Ward & Griffiths, 2015). It has also enabled intervention to take place when reductions in numbers of spawn or individuals have occurred, as well as improved our knowledge of the species ecology and threats. Water quality has also been monitored at all potential wild breeding sites.

Major difficulties faced

- Determining suitable release sites due to lack of appropriate sites isolated from external threats such as agricultural runoff as well as poor connectivity in a densely populated island.
- Understanding the differences in ecology between agile frog populations in Jersey and mainland Europe, particularly the terrestrial phase.
- Unpredictable recruitment due to annual variation in water levels.
- Impacts on the population from human disturbance, including road mortality.
- Difficulties in securing staff time and funding for head-starting.

Major lessons learned

- With assistance (head-starting and spawn protection), the frog population was able to maintain a steady increase in population size, and has led to the recovery of the population at L'Ouaisné.
- Restoration to previous population levels may be difficult due to habitat availability and connectivity, and the time taken for populations to establish.
- Habitat management has probably played an important role in sustaining the population.
- Biosecurity measures put in place to reduce the threat of diseases (e.g. *B. dendrobatidis*) may have played an important role, as did monitoring of sites to mitigate unexpected threats to the habitat in the way of invasive freshwater plants (*Crassula helmsii*). This highlights the importance of being cautious, and that external factors otherwise unrecognised could play a role in the success or failure of conservation programs.
- Captive-breeding enclosures had mixed success and required a large amount of

Agile frog release into a re-introduction site

© Rob Ward

resources, whereas head-starting wild clumps proved to be more cost effective.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- Intervention with spawn protection and head-starting avoided complete population loss.
- Both principal breeding sites given protection, being designated as ecological Sites of Special Interest, with habitat management programs implemented.
- Agile frog numbers are increasing at L'Ouaisné, with some wild breeding also occurring at Noirmont, Woodbine corner and Beauport, following re-introduction.
- Research into the ecology of Jersey's agile frog population has been carried out by a PhD student (Racca, 2004), as well as further research undertaken by other students to assess the success of different conservation strategies and methods applied to the population.
- There are a limited number of potential release sites, with little data on which to base their selection. Furthermore connectivity between sites further afield is likely to be poor.

References

Jameson, A. (2009) An assessment of the relative success of different conservation strategies for the Jersey agile frog (*Rana dalmatina*). MSc Thesis, Durrell Institute of Conservation and Ecology, University of Kent.

Racca, L. (2002) The conservation of the agile frog *Rana dalmatina* in Jersey (Channel Islands). Biota 3: 141-147.

Racca, L. (2004) Ecology and conservation of the agile frog (*Rana dalmatina*) in Jersey. PhD. Thesis, Durrell Institute of Conservation and Ecology, University of Kent.

States of Jersey (2006) Agile frog (*Rana dalmatina*) Biodiversity Action Plan. Available from http://www.gov.je/sitecollectiondocuments/environment%20and%20greener%20living/id%20biodiversitybookletamphibians%20%20dm.pdf.

Ward, R.J. & Griffiths, R.A. (2015) Agile frog data analysis 1997 - 2014. Report to the Department of the Environment, States of Jersey. Available from http://www.gov.je/SiteCollectionDocuments/Government%20and%20administration/R%20Agile%20Frog%20Data%20Anlaysis%20Research%20Project%202015%20DM%2015092015.pdf.

Re-introduction of the northern leopard frog in British Columbia and Alberta, Canada

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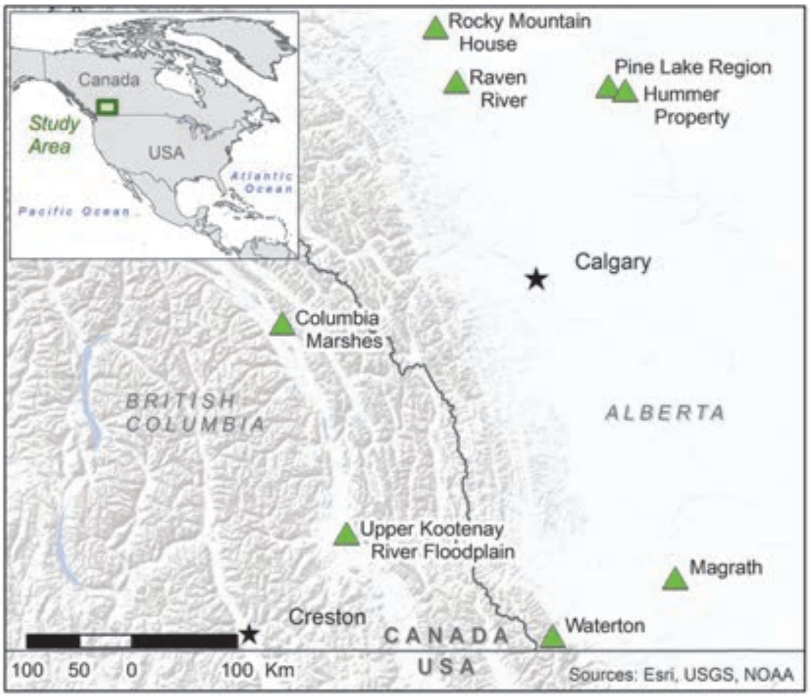
Introduction

The northern leopard frog (NLF) (*Lithobates pipiens*) was once widespread and numerous across much of North America. Reductions in range, number of populations, and abundance have led to the designation of 'Endangered' for the Rocky Mountain population in British Columbia (BC) and 'Special Concern' for the Western Boreal/Prairie populations (COSEWIC, 2009). In BC, there is a single extant population of NLFs located in the Creston Valley Wildlife Management Area (CVWMA) (BCNLFRT, 2012). The NLF is 'threatened' in Alberta (AB), and remaining populations are isolated resulting in reduced gene flow and hampering re-colonization (AESRD, 2012). Habitat loss and fragmentation, reduced water quality and quantity, introduced fish, and disease have been implicated as possible causes of declines (COSEWIC, 2009).


Northern leopard frog in BC wetland

Chytridiomycosis is thought to have been a primary cause for population declines in BC and may have contributed to declines in AB (BCNLFRT, 2012; AESRD, 2012). Re-introduction is identified as a key strategy to recover NLFs in both provinces

Figure 1. Map of select re-introduction sites covered in the document (green triangles) in BC and AB



(BCNLFRT, 2012; AESRD, 2012). Recovery efforts in BC are led by the BC NLF recovery team. Most of the AB re-introductions described were directed by the Alberta Environment and Parks (AEP) led advisory group and by Parks Canada in collaboration with AEP in Waterton Lakes National Park (WLNP). Additional re-introductions not covered in this document have occurred in AB between 2007 - 2015.

Goals

- Goal 1: Ensure well-distributed, self-sustaining populations of NLFs throughout their historical range in BC and AB.
- Goal 2: Re-introduce NLFs to at least two major river basins in both BC and AB.

Success Indicators

- Indicator 1: Re-introduced eggs hatch and some tadpoles complete their metamorphosis (includes head-starting of eggs and/or tadpoles).
- Indicator 2: Frogs overwinter successfully.
- Indicator 3: Frogs survive to sexual maturity and there is evidence of breeding activity as indicated by calling, wild-bred eggs, tadpoles, or frogs.
- Indicator 4: Some or all life-stages are detected at least 3 years post-release.
- Indicator 5: Evidence of colonization of nearby breeding habitat.

Project Summary

Feasibility: Northern leopard frogs require well-connected and proximate habitats for breeding, foraging, and overwintering. Habitat fragmentation, disease and invasive fish may hamper re-introduction efforts (BCNLFRT, 2012; AESRD, 2012). There are several wild populations that can be a source of eggs for translocation in AB; in contrast, the only sources in BC are from the CVWMA and a captive assurance population at the Vancouver Aquarium. Chytrid fungus (*Batrachochytrium dendrobatidis), or Bd*, has been detected at multiple sites in AB and BC but evidence of chytridiomycosis-caused mortality is rare (BCNLFRT, 2012; AESRD, 2012). Currently, no disease testing is done prior to release as translocations are of eggs or early-stage tadpoles which have a low probability of

Researcher working in the wetlands
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harboring *Bd* (Kendell *et al.*, 2007). However, every effort is made to minimize transfer of disease, parasites and invasive species.

Implementation: Biological and habitat connectivity assessments are required prior to selecting a re-introduction site, and consultation is required with landowners (private and governmental agencies), and any relevant First Nations aboriginal groups. In BC, there are two re-introduction sites: 1) Upper Kootenay River Floodplain (UKF) and 2) Columbia Marshes (CM) (Fig. 1). The first phase of re-introduction to UKF was between 2003 - 2005, when a total of 493 tadpoles and 3,639 head-started young-of-year (YOY) were translocated from the CVWMA (Fig. 1) (BCNLFRT, 2012).

No animals were translocated between 2005 - 2010 but between 2011 - 2015, approximately 7,500 tadpoles per year were translocated from the CVWMA for a total of approximately 34,000 (unpublished data). At CM approximately 2,000 captive bred tadpoles from the Vancouver Aquarium were released in 2013 and 2014. To increase the chance of success, these numbers were bolstered in 2015 with tadpoles from CVWMA (approximately 3,000) and Vancouver Aquarium (621) (unpublished data).

Re-introductions have occurred in AB for almost 35 years. NLFs were first re-introduced at two sites in the Pine Lake region in the 1980's (Kendell *et al.*, 2007). Between 1999 - 2004, eggs were collected from source sites in southern AB. Approximately 70,000 tadpoles were reared in two outdoor ponds at the Raven Brood Trout Station, near Caroline. This resulted in the survival of about 14,000 head-started YOY that were released at the Raven River (10,000+), a site near Rocky Mountain House (2,845), and Hummer Property (1,310) (a Ducks Unlimited property near Red Deer). Between 2002 - 2004, eggs were collected from source sites in southern AB and 8,500 tadpoles were released at a pond near Magrath. Between 2007 - 2010, eggs were collected from several sites in southern AB and over 75,000 tadpoles were released at three ponds in WLNP (Johnston, 2013).

Post-release monitoring: To measure success, we conducted call surveys as well as visual encounter surveys for all age classes of frogs. Success has been documented at the UKF sites both in Phase 1 and 2 (Table 1). Successful *in-situ* breeding, as indicated by calling adult frogs and YOY, was detected post-phase 1 in 2007, 2008, & 2010 (BCNLFRT, 2012). Success of phase 2 has been confirmed by breeding call surveys and by detection of eggs in 2014. Frogs have been detected by call surveys at nearby breeding sites although breeding has not been confirmed. While the re-introduction effort at the UKF site is considered successful, populations are still too small to ensure persistence. It is too soon to expect breeding at the CM site (initiated 2013) but the first indicator of success has been met. Although YOY were detected, the small numbers released makes the detection probability of overwintered frogs extremely low.

In AB, the Pine Lake re-introduction sites reported successful metamorphosis, overwintering and reproduction for several years before one site failed due to a winter kill event and the status of the other population is currently unknown (Kendell *et al.*, 2007). Despite a successful head-starting program at the Raven Brood Trout Station, there were no confirmed observations of NLFs at the Rocky Mountain House or Hummer Property release sites between 2001 - 2006 (Kendell *et al.*, 2007). The Raven River site experienced initial success (i.e. there was evidence of successful overwintering 2001 - 2004 and evidence of breeding in 2002) but there were no observations in 2005 or 2006 (Kendell *et al.*, 2007). The Magrath re-introduction has been the most successful of the AB re-introductions, with evidence of successful overwintering and reproduction each year since 2005 (unpublished data).

Table 1. Measures of success at BC and AB re-introduction sites

Site	Years of re-introduction	Success Indicators				
		1	2	3	4	5
British Columbia (BC)						
UKF Phase 1	2003 - 2005	√	√	√	√	UK
UKF Phase 2	2011 - 2015	√	√	√	√	√
CM	2013 - 2018*	√	TBD	TBD	TBD	TBD
Alberta						
Pine Lake	1980s	√	√	√	-	UK
Raven River	1999 - 2004	√**	√	√	-	UK
Rocky Mountain House	2001 - 2003	√**	-	-	-	UK
Hummer Property	2002 - 2003	√**	-	-	-	UK
Magrath	2002 - 2004	√	√	√	√	UK
Waterton	2007 - 2010	√	√	-	-	UK

Key:
TBD - To be determined; UK - unknown due to lack of survey effort
***Anticipated assessment date to continue or terminate effort**
****Eggs hatched and tadpoles captive-reared (head started) to YOY, then released.**

Researcher releasing tadpoles at reintroduction site
© Audrey Gagné-Delorme

Many YOY were observed at two of the WLNP re-introduction sites in the years when releases occurred, indicating initial re-introduction success at these sites (Johnston, 2013). No YOYs were observed at the third site possibly because of the presence of introduced brook trout (*Salvelinus fontinalis*) (Johnston, 2013). One adult NLF was observed in the area in 2008, and another in 2009, indicating limited intermediate success (Johnston, 2013). Disease testing later revealed *Bd* in the region (Johnston, 2013). New release and egg source sites have been selected for re-introductions beginning in 2015 in the WLNP.

Major difficulties faced

- In BC, the limited number of NLFs available to serve as founder stock has resulted in low numbers of individuals released.
- In AB, sources of eggs for translocation were readily available but suitable release habitat was more difficult to find.
- *Bd* was present at some source and release sites. Other health and parasite problems have also been documented but the population level impacts remains unknown.
- It was difficult to detect NLFs post-release because of the complexity of the habitat, the extensive search areas and inaccessibility of some sites.

Major lessons learned

- In BC, annual re-introductions spanning five years may be required to ensure even modest success. Continued releases may be necessary until *in-situ* reproduction is sufficient to sustain the population. Because of the effort required and the limited founder stock available, few translocation projects can be run simultaneously.
- Long-term monitoring is required to assess the success of the re-introduction (>5 years).
- The presence of *Bd* may influence probability of success but does not guarantee failure (e.g. UKF re-introduction site in BC).
- Head-starting and release of YOY was used in the early stages of re-introduction efforts in both provinces but release of eggs or tadpoles was

speculated to encourage site fidelity, was more cost-effective, and presented a lower risk of transmitting pathogens and parasites.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- We repeated re-introductions over several years, which likely contributed to success at some sites.
- The presence of disease and introduced fish may have led to the failure of some re-introduction sites.
- We suspect that other species of amphibians (e.g., Columbia spotted frog (*Rana luteiventris*)) may have served as reservoirs and vectors for disease.
- Although every effort was made to select good release habitat, we speculate that frogs may not have been able to locate suitable habitat, or there may have been inadequate connectivity between habitats, which may have led to failure at some sites.

References

COSEWIC (2009) COSEWIC assessment and update status report on the Northern Leopard Frog *Lithobates pipiens*, Rocky Mountain population, Western Boreal/Prairie populations in Canada. Ottawa, Ontario: Committee on the Status of Endangered Wildlife in Canada. pp. 69.

BCNLFRT (2012) Recovery Plan for the Northern Leopard Frog (*Lithobates pipiens*) in British Columbia Victoria, BC: B.C. Ministry of Environment.

AESRD (2012) Alberta Northern Leopard Frog Recovery Plan, 2010-2015. Edmonton, Alberta: Alberta Environment and Sustainable Resource Development. pp. 34.

Johnston, B. (2013) Northern Leopard Frog Re-introduction Efforts in Waterton Lakes National Park, 2007-2012, Waterton Lakes National Park. pp. 15.

Kendell, K. & Prescott, D.R.C. (2007) Northern Leopard Frog re-introduction strategy for Alberta. Sherwood Park, Alberta: Alberta Conservation Association. pp. 34.

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Global Reintroduction Perspectives: 2018

Case studies from around the globe

Edited by Pritpal S. Soorae

IUCN/SSC Reintroduction Specialist Group (RSG)

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Trial reintroduction of the endangered yellow spotted mountain newt in western Iran

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Introduction

The yellow-spotted mountain newt (YSMN) (*Neurergus microspilotus*) (Caudata: Salamandridae) is listed as a Critically Endangered by IUCN because of its very small area of occupancy in its breeding streams (<10 km^2), fragmented habitats, continuing decline in the extent and quality of aquatic habitats, habitat degradation, drought, and the pet trade (Sharifi *et al.*, 2009). YSMN has been recorded from 42 highland streams in the mid-Zagros Range in western Iran and eastern Iraq. Most localities inhabited by YSMN are located in the southern portion of the geographic range with 81% of localities in Iran and 19% in Iraq and over 50% of localities are located at border areas between the two countries. In aquatic habitats, the YSMN is a high predator of diverse benthic macroinvertebrate communities (Farassat & Sharifi, 2014). This newt lives long with reported a 14 years' longevity and reaches sexual maturity at about 3 - 4 years. Surveys in 32 of the 42 localities within the Iranian range have yielded in 1,379 visual counts of adult, juveniles, and larvae in 5.5 km of stream reaches. Most of the observed newts (51%) were found in just two localities, 44% in 14 streams, and the remaining 5% were scattered among 16 streams (Afroosheh *et al.*, 2016).

Goals

- Goal 1: To demonstrate that YSMN can live, grow, mate and reproduce successfully in captivity.
- Goal 2: To apply a multi-criteria decision analysis alongside with a geospatial analysis for the selection of streams which are located in general distribution area of YSMN but do not have YSMN.
- Goal 3: To demonstrate that post-metamorph juveniles of YSMN bred and raised in the breeding facility can overwinter in a selected stream with a reasonable survival rate.

Success Indicators

- Indicator 1: To have developed a successful captive husbandry and reproduction leading to high rate of hatching, low mortality of larvae and post-metamorphs and stable growth rate in the YSMN rearing in the captive-breeding facility.
- Indicator 2: Have established viable stocks of mealworms (*Tenebrio molitr*), *Artemia* sp. and earthworms (*Lumbricus terrestris*) needed for different life stages of the YSMN living in the captive-breeding facility.


Yellow-spotted mountain newt

- Indicator 3: Demonstrated by a trial reintroduction that post-metamorph captive-bred released into the wild can survive to the second growing season, and provides a choice of life-stage for a reintroduction program.

Project Summary

Feasibility: The YSMN has been in continual decline in recent decades as a result of increased human population and extensive land-use alteration. Diversion of water from highland streams to orchard and agricultural lands in conjunction with disturbing impact of climate change have caused many springs and small streams to be completely dehydrated. Various diseases including *Batrachochytrium dendrobatidis* have been reported for this newt presumably as a result of poor water quality and quantity (Sharifi *et al.*, 2014). In 2010, the Mohamed bin Zayed Species Conservation Fund helped to develop and implement a conservation management plan for YSMN. Part of this plan included the development of a captive-breeding facility at Razi University, Kermanshah, Iran. The ultimate goal of the captive-breeding program was to provide stock and increase the species' population size across different breeding streams to ensure their long-term survival and release of captive-raised YSMN to their habitat. In establishing the captive-breeding facility and performing the subsequent trial reintroduction, individuals from different breeding streams were kept separate in order to avoid genetic interaction. The reintroduction site identified by application of a multi-criteria decision analysis (MCDA) in a GIS format.

Implementation: The captive-breeding facility for reintroduction of YSMN began with allocating a 5 m long × 2.5 m wide × 3 m high room at Razi University. Additional space was available for eggs and larvae. The CBF was ventilated by an air-conditioner that recirculated the indoor air and each aquarium included terrestrial habitat in the form of small pebbles collected from the wild. The aquaria contained some aquatic plants for egg attachments and hiding opportunities. The suitability of different potential reintroduction sites was assessed against several criteria, i.e. degree of isolation from human settlements, proximity to a benthic macroinvertebrate community, submerged vegetation cover, water temperature, altitude, and land use along the stream. We examined the morphology of springs and streams, as well as their vegetative composition and structure. Among five sites investigated, the Mivan Spring was selected for a trial reintroduction of YSMN (Sharifi & Vaissi, 2014). This spring immediately joins Mivan Stream, which contains a well developed submerged periphyton vegetation. Along the stream there are also well-established emergent and marginal plant communities. For the reintroduction, the largest individuals of similar age (5 - 7 mm) newts were considered to be of sufficient size to withstand predation by crabs (*Potamon bilobatum*), toads (*Bufo bufo*) and water snakes (*Natrix natrix*).

Captive-breeding facility

This trial reintroduction was an intentional release of captive-bred individuals inside their indigenous range. Our ultimate objective was to determine not only an optimum choice of life stage for a reintroduction program but also an optimal size and age based on a cost-effective evaluation of the reintroduction to the wild. The present trial reintroduction demonstrated that young-of-the-year captive-bred YSMN released into the wild can survive to the second growing season and may be a choice for a reintroduction plan. Observed post-overwintering visual counts gave an estimated average survival rate of 20.5% of the total number reintroduced. This preliminary result suggests that an expensive control of predator populations before large-scale releases may not be required. The experiment also demonstrates that it may be more effective to release post-metamorph rather than adult newts. The slow growth rate of YSMN means that newts would have to be maintained in captivity for a longer period. Moreover, maturation at age three or four slows down the build-up of stock available for a reintroduction and increases the expenditure per released newt. Additionally, in the case of a very long captive period, especially if individuals become mature in the captivity, adaptation to the captive life may cause negative impacts on the fitness of the reintroduced individuals.

Post-release monitoring: For identification purposes, each individual was photographed using a fixed tripod in order to use the photographic identification procedure used for this species. Post-metamorphic juveniles were released in the spring on four occasions (Sharifi & Vaissi, 2014). The newts selected for the trial reintroduction were given a visual health screening (skin slough and wound) and behavioral examination (viability and responsiveness to stimulus) to ensure they were healthy. The probability of released newts contracting an infection was considered very low because the release was planned for a site that no longer contained free-ranging newts. In 12 visits to the site before and after overwintering, a total of 31 individuals were identified. Based on an average diurnal detection probability for this newt (0.61 ±0.19 SD), the observed newts during the pre-overwintering period gave a survival rate of 20.5 of the reintroduced newts (Sharifi & Vaissi, 2014).

Monitoring of newts at release site

Major difficulties faced

- The YSMN is a poorly know species and the captive-breeding facility provided opportunities to gather information on reproductive biology of the species but there are still many important questions that should be answered.
- Sexual maturity at age 3 - 4 years, low number of eggs per female (up to a hundred), slow development and low rates of growth are major inherent difficulties encountered in a captive-breeding and reintroduction program for YSMN.
- High cost of infrastructures for a good husbandry for very long time before a captive-breeding facility begins producing adequate number of eggs, larvae, juveniles or adults. Such infrastructures are not available in zoos in developing countries and universities and other agencies are not willing to invest.
- Academic research is essential, but not adequate, to demonstrate that a proposed management action plan can work.

Major lessons learned

- We learned and published about various aspects of reproduction biology, food habits, cannibalism, effect of temperature, density, spatial diversity, water level and food quantity on growth of YSMN, spot ontogeny, disease, complete spatial randomness (CSR) in spots, life table dynamics, genetic diversity, life cycle choices for reintroduction (under investigation) and trial reintroduction in YSMN.
- Success depends on close cooperation among diverse agencies and stakeholders, who agree on common goals. Such cooperation develops slowly and depends on individuals from different agencies and groups to make sure it works.
- An efficient captive-breeding able to reintroduce significant number of offspring regularly is likely many years away because of the difficulties of dealing with many diverse factors influencing YSMN.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- Completion of reproductive cycle of YSMN in the captive-breeding facility.
- Learning more about disease in this species and reporting such as *Batrachochytrium dendrobatidis* (Sharifi *et al.*, 2014).
- Applying suitability analysis for identifying potential reintroduction streams for reintroduction of YSMN using GIS-based sitting procedure.
- Witnessing how post-metamorph captive-bred YSMN when released to a selected site were able to withstand the harsh winter in the area with a good survival rate.

References

Afroosheh, M., Akmali, V., Esmaeili Rineh, S. & Sharifi, M. (2016) Distribution and abundance of the endangered yellow spotted mountain newt *Neurergus microspilotus* (Caudata: Salamandridae) in western Iran. *Herpetological Conservation and Biology* 11(1): 52-60.

Farassat, H. & Sharifi, M. (2014) Food habit of the endangered yellow-spotted newt *Neurergus microspilotus* (Caudata, Salamandridae) in Kavat Stream, western Iran. *Zoological Studies* 53: 61. https://doi.org/10.1186/s40555-014-0061-z

Sharifi, M., Bafti, S.S., Papenfuss, T., Anderson, S., Kuzmin, S. & Rastegar-Pouyani, N. (2009) *Neurergus microspilotus*. ***In***: IUCN 2012: IUCN Red List of Threatened Species, version 2012.2, www.iucnredlist.org (accessed 28 April 2017).

Sharifi, M. & Vaissi, S. (2014) Captive breeding and trial reintroduction of the endangered yellow spotted mountain newt *Neurergus microspilotus* (Caudata: Salamandridae) in western Iran. *Endangered Species Research* 23: 159-166. https://doi.org/10.3354/esr00552

Sharifi, M., Farassat, H., Vaissi, S., Parto, P. & Siavosh Haghighi, Z.M. (2014) Prevalence of the Amphibian Pathogen *Batrachochytrium dendrobatidis* in Endangered *Neurergus microspilotus* (Caudata: Salamandridae) in Kavat Stream, Western Iran. *Global Veterinaria* 12(1): 45-52.

Lessons learned from the reintroduction of the Chinese giant salamander

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Introduction

The Chinese giant salamander (*Andrias davidianus*) is the world's largest amphibian and is endemic to China. The species was once widely distributed in all three major river systems in central and southern China and has been found in various water bodies including streams, rivers, and underground waterways in karst caves (Wang *et al.*, 2004). Due to habitat destruction, water pollution and over-exploitation for its flesh, the species has suffered an 80% population decline since the 1950s (Liang *et al.*, 2004). In 2004, it was listed as Critically Endangered by the IUCN Red List, making it one of the most threatened amphibians in the world. In China, it was listed as a Class II Protected Species in 1989, which prohibits by law the collection of wild salamanders. However, wild populations do not appear to be rebounding due to continued threats and without restocking efforts the recovery of salamander populations might be slow, given their rarity and long generation intervals (sexual maturity occurs at 6 - 8 years or longer). Thus, captive-breeding and reintroduction are possible conservation strategies for restoration and recovery of wild populations and down-listing from its current threat level. To test this theory, our team released 31 salamanders into two head-water streams in Shaanxi Province in central China and monitored their survival and movement for one year using radio-

Chinese giant salamander

telemetry and passive integrated transponder (PIT) tags. The goals of this project are listed below.

Goals

- Goal 1: Evaluate survivorship and compare morphometric variables of post-release animals, following capture-recapture, to wild-caught conspecifics.
- Goal 2: Identify environmental variables and habitats that are selected by released giant salamanders.
- Goal 3: Assess post-release migration distances, linear home range sizes, activity, and compare seasonal movement patterns.
- Goal 4: Raise local awareness of giant salamander conservation through releasing ceremonies and local field assistant training.

Success indicators

- Indicator 1: Radio transmitters would work normally for at least one year, enabling data collection on the salamander's reintroduction.
- Indicator 2: More than 50% of released individuals survived the first year and experienced growth similar to conspecifics.
- Indicator 3: Animals that survived had a period of settlement and chose habitat similar to wild animals.
- Indicator 4: Salamanders established territory and followed seasonal movement patterns similar to wild animals.
- Indicator 5: Increased local awareness of giant salamander conservation, such that no poaching happened during the reintroduction study.

Project summary

Feasibility: Over the past 20 years, the high market price of giant salamander meat has invoked a rapid development of a salamander farming industry. Approved by provincial fisheries bureau, these farms are expected to help generate income for rural families and support local villages. Some farms have gained sufficient experience rearing these salamanders that reproduction has become very successful in recent years (Cunningham *et al.*, 2016). Thus, salamander farms could provide a large and stable source population for reintroduction programs throughout the country if managed correctly.

In 2009, a partnership was established between Shaanxi Institute of Zoology, Memphis Zoo and Mississippi State University to conduct a reintroduction project of captive-reared Chinese giant salamanders into the wild in Shaanxi Province. This project represents a positive model for the conservation of China's aquatic ecosystems that works with local industry, which is perhaps the only hope for biodiversity in many cases. We are hopeful that this project will serve as a positive example to inspire other conservation initiatives across China, especially those dealing with threatened aquatic species.

Implementation: The two head-water rivers selected for reintroduction were the Heihe and the Donghe rivers in the Qinling Mountains. The Heihe River, on the north slope of the mountains, belongs to the Weihe River watershed, which is the largest branch of the Yellow River. The Donghe River, on the south slope of the Mountains, belongs to the Hanjiang River watershed, which is the largest branch of the Yangtze River. Wild Chinese giant salamanders were abundant in these


Recapturing giant salamanders

two rivers in the past according to local villagers; however, they have rarely been observed in recent years.

Thirty-two juvenile giant salamanders were purchased from two farms within the Qinling Mountains for this reintroduction study. Half the animals were collected as larvae from the wild and head-started in captivity; whereas, the other half were born in captivity from stock that was collected from our release site. The Heihe group of released salamanders were about three years old with body mass that ranged from 0.36 - 1.14 kg; whereas, the Donghe group of salamanders were about five years old at release with body mass ranging from 1.10 - 2.34 kg. In March 2013, all salamanders were surgically implanted with VHF radio transmitters and PIT tags for identification and tracking (Marcec *et al.*, 2016). Half the salamanders were released six weeks post-surgery into the Heihe River, while the Donghe River group were release 16 weeks post-surgery. One salamander from the Heihe group died before release because of dehiscence of suture and several more cases were observed afterwards in the river, prompting the later release of animals into the Donghe River so they could fully recover.

Post-release monitoring: Two field assistants from local communities were trained to monitor the reintroduced salamanders at both sites. Animals were located every day through radio telemetry and presence/absence checked using an under-water inspection camera occasionally. Monitoring continued until the battery life of transmitters died (the last radio signal was collected in September 2014). Near the end of the study, recapture of all living individuals was attempted before the radio signals disappeared. We recorded body mass, snout-vent length, total body length, any abnormalities and external parasites for all recaptured salamanders to compare to their pre-release morphometric data, and compared to wild caught conspecifics. Once all measurements were completed, salamanders were released at the same location where they were caught.

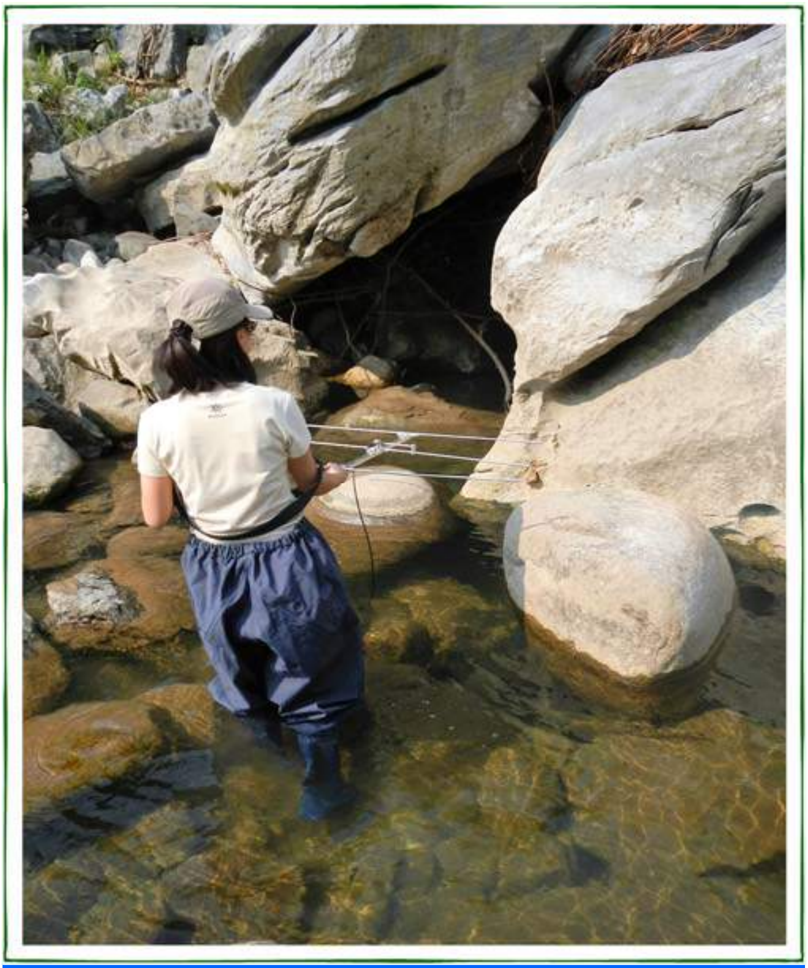
Survival rates of the two groups of salamanders were calculated and we also identified the most influential factors on their survival. The Donghe group had an annual survival rate of 0.7 in their first year in the wild, which was comparable to wild and reintroduced hellbenders (Bodinof *et al.*, 2012). However, the younger group of animals at Heihe River had a much lower survival rate of 0.4, largely because of the dehiscence of suture sites following release and several large floods that washed the animals downstream beyond detection. Salamanders would have had a higher survival rate if they had a longer recovery time from surgery. For those salamanders that survived and were recaptured, they all increased in body mass and total length after a year in the wild and they were only 7% lighter than wild animals of the same length (Zhang *et al.*, 2016). Habitat selection analyses confirmed that large boulders were the most important environmental variable to post-release settlement for reintroduced salamanders (Zhang *et al.*, 2017). Salamanders were able to move long distances, up to 880 m/in a single day; however, they usually made short-distance movements of ~10 m/day. They moved more frequently than hellbenders, with an overall sedentariness smaller than 0.3. The annual linear home range of these salamanders were about 300 m. Salamanders showed different movement patterns across seasons, such that they had a higher sedentariness, shorter daily movement, and smaller linear home range in winter than in summer (Zhang *et al.*, in prep).

Major difficulties faced

- The surgically implanted radio transmitters worked well on giant salamanders; however, it took too much time for salamanders to recover from surgery (need almost four months to fully recover). If not given enough time to recover, salamanders may experience dehiscence of suture sites after release and die soon thereafter. Furthermore, internal transmitters only last for about one year and it is difficult to replace expired transmitters with new units, thus longer monitoring plans could not be applied.
- Flooding shortly after the first release negatively impacted our smaller animals such that many of them were injured or moved beyond our ability to locate them.
- The two rivers chosen as release sites by the Provincial Fisheries Bureau were outside of any protected areas such that poaching could be a threat to our released animals now that the study has concluded.

Major lessons learned

- Captive-reared Chinese giant salamanders, even though they were raised for commercial use, could survive over a year following release with an annual



Radio-tracking giant salamanders

survival rate comparable to wild or captive-reared hellbenders reintroduced to the wild.
- For juvenile giant salamanders, older individuals may survive better than younger animals, considering their better recovery from surgery and higher resistance to floods.
- Newly released salamanders are susceptible to floods, especially younger individuals. Floods may cause injuries or long-distance movements downstream away from suitable release sites; thus, reducing salamander survivorship. It is better to release salamanders in autumn, when the rainy season is over.
- Large boulders are the most important variable selected by salamanders for settlement; thus, habitat structure providing appropriate cover should be carefully considered when selecting release sties.
- Captive-reared juvenile giant salamanders have a relatively high fidelity to release sites and are tolerant of conspecifics, which may contribute to the re-establishment of a population in the wild.
- Release sites outside of protected areas can support reintroduced giant salamander populations for short time periods; however, they remain at a high risk of poaching. It will be difficult and impractical to apply longer conservation plans outside of protected areas, considering logistics, manpower, funds, and poaching pressure. Soliciting permission to release salamanders in protected areas should be a future goal of the reintroduction program.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:
- Chinese giant salamanders are long-lived amphibians and mature at 6 - 8 years old. To establish a self-sustaining wild population this needs to be a long-term project with continued reintroductions, monitoring, funding and support from both government and the private farming industry. Our project was the first step to show that captive-reared giant salamanders are suitable for reintroduction, but we are still far from claiming that this reintroduction was successful as viewed through the lens of a self-sustaining and reproducing wild population. We do not have any data to support this with the limited number of animals released and limited monitoring period.
- The two rivers selected as release sites had good water quality, abundant fish and invertebrates for salamanders to prey on, and plenty of large boulders for them to hide beneath. In addition, natural predators were probably extirpated from our two sites, such that there was very little threat to them outside of poaching. Hence, quality of the habitat helped with the success of the project.
- The two field assistants trained to monitor salamanders were leaders of the local communities. Villages near our release sites were fully aware that we released salamanders into the rivers and that they were being monitored by community leaders; thus, poaching was minimized during this project. Hence, community buy-in to the project helped with the success of the project.

- The final fate of released salamanders could not be determined because funding was limited to continue monitoring work past a year once the radio signal failed. Regular funding, e.g. support from related governmental agencies (Federal, Provincial and County), should be acquired for when new reintroduction projects are planned, such that more animals can be released and long-term action plans toward monitoring can be established.

References

Bodinof, C.M., Briggler, J.T., Junge, R.E., Mong, T., Beringer, J., Wanner, M.D., Schuette, C.D., Ettling, J. & Millspaugh, J.J. (2012) Survival and body condition of captive-reared juvenile Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) following translocation to the wild. *Copeia* 150-159. https://doi.org/10.1643/CH-11-024

Cunningham, A.A., Turvey, S.T., Zhou, F., Meredith, H.M., Guan, W., Liu, X., Sun, C., Wang, Z. & Wu, M. (2016) Development of the Chinese giant salamander *Andrias davidianus* farming industry in Shaanxi Province, China: conservation threats and opportunities. *Oryx* 50: 265-273. https://doi.org/10.1017/S0030605314000842

Liang, G., Gengm, B. & Zhao, E. *Andrias davidianus*. Version 2014.3. The IUCN Red List of Threatened Species. 2004. Available: www.iucnredlist.org. Accessed May 11, 2015.

Marcec, R., Kouba, A., Zhang, L., Zhang, H., Wang, Q., Zhao, H., Jiang, W. & Willard S. (2015) Surgical implantation of coelomic radiotransmitter and post-operative survival of Chinese giant salamanders (*Andrias davidianus*) following reintroduction. *Journal of Zoo and Wildlife Medicine* 47: 187-195. https://doi.org/10.1638/2015-0044.1

Wang, X., Zhang, K., Wang, Z., Ding, Y., Wu, W. & Huang, S. (2004) The decline of the Chinese giant salamander *Andrias davidianus* and implications for its conservation. *Oryx* 38: 197-202. https://doi.org/10.1017/S0030605304000341

Zhang, L., Jiang, W., Wang, Q., Zhao, H., Zhang, H., Marcec, R., Willard, S. & Kouba, A. (2016) Reintroduction and post-release survival of a living fossil: the Chinese giant salamander. *PLOS ONE* 11(6): https://doi.org/10.1017/S0030605304000341

Zhang, L., Wang, Q., Willard, S., Jiang, W., Zhang, H., Zhao, H. & Kouba, A. (2017) Environmental characteristics associated with settlement of reintroduced Chinese giant salamanders. *Journal of Herpetology* 51: 417-424. https://doi.org/10.1670/16-106

Zhang, L., Willard, S., Zhao, H., Wang, Q., Jiang, W., Zhang, H. & Kouba, A. Spatial ecology of reintroduced Chinese giant salamanders (*Andrias davidianus*). In preparation.

Reintroduction of the pool frog to the United Kingdom

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Introduction

The pool frog (*Pelophylax lessonae*) is found through much of central and northern continental Europe. Its global IUCN Red List category is Least Concern. However, some populations in the far north of the range have been found to be genetically and phenotypically distinct, representing a northern clade. This form was once found in the United Kingdom but was generally considered to be an introduction. It was only in the late 20th century that its status was investigated thoroughly, and in the early 2000s compelling evidence emerged to demonstrate that the species was in fact native. By this time the last known population had gone extinct. The reintroduction was planned for a confidential location in the county of Norfolk, in the east of England, the same region where the last native population occurred, using northern clade stock from Sweden. At the time of reintroduction planning, the species was listed as a national biodiversity priority and remains so. It now has a high degree of legal protection, but it was not protected at the time of reintroduction.

Goals

- Goal 1: To establish a viable population of northern pool frogs in the UK at a suitable site within their UK historical range.
- Goal 2: To assess the effectiveness of amphibian reintroduction using wild-to-wild translocation.
- Goal 3: To assess the impacts of reintroducing pool frogs on other co-existing species and habitats.

Success indicators

- Indicator 1: *Early indicators* - Survival of eggs/larvae through to metamorphosis, survival of adults, and breeding activity.
- Indicator 2: *Long-term indicators* - Adult population size of at least 50 and ideally at least 100; mixed population structure in terms of demography; progressive colonization of multiple ponds by dispersing frogs.
- Indicator 3: Co-existing species and habitats are not negatively impacted, and ideally are enhanced, by the reintroduction of pool frogs.

Female pool frog © Jim Foster/ARC

Project Summary

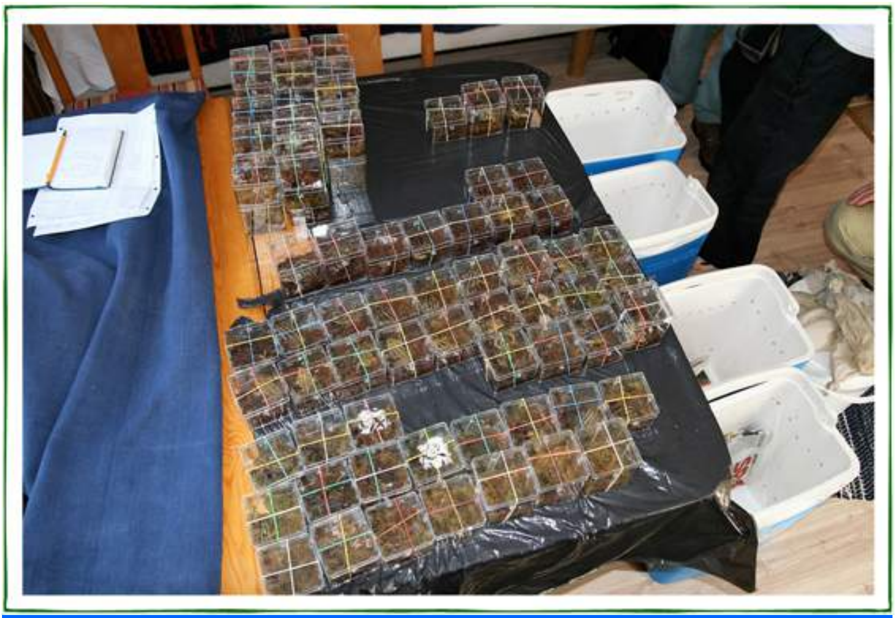
Feasibility: The northern pool frog was only recognized as a UK native species in 2005 after its national extinction, having been generally regarded an introduction from other parts of Europe. Research in the 1990s and 2000s confirmed its native status, reversing its position from an unwelcome alien species to one of high conservation concern. An investigation into the desirability and feasibility of reintroduction concluded that establishing a population in the UK would represent a significant gain for national biodiversity, as well as a contribution to its European status, given that the northern populations are scarce and often imperiled. The main reasons for decline and extinction were thought to be a reduction in water levels due to abstraction, and substantial deterioration in habitat condition. The species was listed as a biodiversity priority, though it was not yet legally protected because of the earlier confusion over its status. All of these issues were thoroughly investigated and a reintroduction strategy was produced following consultation with experts in amphibians and reintroduction methods (Buckley & Foster, 2005). Goals and indicators of success were set out in that document, and further developed in documentation supporting the releases, in particular to ensure compliance with IUCN reintroduction guidance. Much effort was put into early liaison with site managers and regulatory authorities to ensure that the more complex challenges were considered and addressed well before the releases were due to occur. Efforts to restore habitat for a receptor site involved examination of habitat characteristics at historic pool frog sites in the UK and existing sites in Sweden. It was decided to keep the precise location of the receptor site confidential to reduce the chance of collection of frogs, for what would be the rarest UK amphibian after reintroduction.

Implementation: The reintroduction was achieved by wild-to-wild translocation, using founders from Sweden (a close genetic match and where populations were robust enough to tolerate some removals). Early discussions with the Swedish authorities were important, because of the need to carefully assess potential impacts, and legal issues relating to capture, export from Sweden and import to the UK. Frogs were caught during four annual visits from 2005 to 2008, flown to the UK and released at a specially prepared receptor site. Following a population viability analysis, a mix of adults, juveniles, spawn and larvae was imported. Mortality during import was minimal, with a loss of <5 larvae per year, and no mortality of post-metamorphic animals. Head-starting was used in addition to hard release in some years, with mixed success. Early discussions with veterinary experts (the Institute of Zoology) were important, to ensure that we implemented a full disease risk assessment, disease risk management, and post-release health surveillance (Sainsbury *et al.*, 2016). An advisory group, comprising species experts, landowners and regulatory authorities, assessed progress by reviewing monitoring reports, undertaking site visits and providing additional advice on methods.

Post-release monitoring: Monitoring comprised three main strands: 1) monitoring of released pool frogs via individual identification and counts of all detectable life stages; 2) monitoring of co-existing amphibians, reptiles and habitat condition; 3) monitoring of health status of pool frogs and other amphibians. In summary, we found: a) a breeding population of pool frogs has been established, with an estimated adult population size of 67 (95% CI = 64-76) [as at end of 2016]; there is a good demographic profile, with regular breeding, though in some years counts of metamorphs or juveniles have been low; pool frogs have colonized and breed in multiple ponds; b) common frogs (*Rana temporaria*) appear to have increased substantially, while the status of newts has not noticeably changed (there are issues with detectability, but no decline is evident); habitats appear to be providing excellent conditions for a range of other wildlife, including aquatic beetles, reptiles and mammals; c) pool frogs and other amphibians appear to be in good health condition and there is no evidence of co-introduction of serious infectious disease. Ecological monitoring has been undertaken by a contractor working to a specification provided by the project leaders, and health monitoring has been undertaken by the Institute of Zoology. Annual reviews ensure that monitoring goals and methods remain appropriate and take account of changing constraints.

Major difficulties faced

- Given that population establishment takes many years and there is a background of fluctuating reproductive success, establishing meaningful short-term indicators is difficult.
- Understanding patterns and causes of mortality in reintroduced frogs and, especially, their progeny.



Frogs prepared for export in boxes © Jim Foster/ARC

- Uncertainty over interpreting the significance of potential threats such as shifting habitat condition or increase in predator abundance.
- Deciding how to balance resources available for pool frog conservation between: 1) ensuring activity at the first reintroduction site progressed adequately, and 2)

establishing additional populations to ensure a more resilient national population of pool frogs (releases for the second reintroduction site started in 2015).
- Securing continuity of funding for implementing reintroduction activity.

Pool frog habitat © Jim Foster/ARC

Major lessons learned

- Given the inherent uncertainty in the outcomes of reintroduction activity, flexibility in implementation was crucial, based on monitoring and adaptive management of the reintroduction program.
- Detailed ecological knowledge of the target species was key to planning the reintroduction.
- Setting a clear objective and indicators of success helped to plan monitoring.
- Planning the reintroduction required substantial lead-in time and consultation with a range of authorities, and this effort required significant co-ordination and funding.
- Project management takes time and needs clear governance, especially where there are risks relating to legal and procedural issues, and where implementation requires flexibility to deviate from agreed plans.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- Careful planning, implementation, documentation and resourcing of the reintroduction.
- Selection of an appropriate receptor site with resources reasonably guaranteed for long-term management.
- Development of a thorough evidence base on which to plan the reintroduction, notably on pool frog status, monitoring methods, ecological requirements and decline factors.
- Advice from an inclusive partnership of researchers, practitioners, site managers and government agencies.

References

Beebee, T.J.C, Buckley, J., Evans, I., Foster, J.P., Gent, A.H., Gleed-Owen, C.P., Kelly, G., Rowe, G., Snell, C., Wycherley, J.T. & Zeisset, I. (2005) Neglected native or undesirable alien? Resolution of a conservation dilemma concerning the pool frog *Rana lessonae*. *Biodiversity and Conservation* 14: 1607-1626. https://doi.org/10.1007/s10531-004-0532-3

Buckley, J. & Foster, J. (2005) Reintroduction strategy for the pool frog *Rana lessonae* in England. English Nature Research Report 642. *English Nature*, Peterborough.

Sainsbury, A.W., Yu-Mei, R., Ågren, E., Vaughan-Higgins, R.J., Mcgill, I.S., Molenaar, F., Peniche, G. & Foster, J. (2016) Disease Risk Analysis and Post-Release Health Surveillance for a Reintroduction Programme: the Pool frog *Pelophylax lessonae*. *Transboundary and Emerging Diseases*. https://doi.org/10.1111/tbed.12545

Williams, C. & Griffiths, R.A. (2004) A Population Viability Analysis for the reintroduction of the pool frog (*Rana lessonae*) in Britain. English Nature Research Report number 585. *English Nature*, Peterborough.

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Global conservation translocation perspectives: 2021

Case studies from around the globe

Edited by Pritpal S. Soorae





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Restocking of the Apennine yellow-bellied toad in Central Italy

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Introduction

The Apennine yellow-bellied toad (*Bombina pachypus*) is an anuran species endemic to Italy, where it is unevenly distributed between central Liguria and Calabria. *Bombina pachypus* is listed as Endangered in the IUCN Red List (Andreone *et al.*, 2009). The species was formerly common in suitable habitat. However, it has declined in most of its range (with the exception of Calabria, where several populations remain stable) over the last 20 years. The species occurs in ephemeral shallow, unshaded pools where spawning and larval development takes place. Threats to this species were identified in the loss and fragmentation of wetlands to drainage for agricultural purposes. However, many populations appear to have declined or gone extinct in areas of presumably intact habitat. In most places the population are reduced to 6 - 20 individuals, thus being highly prone to stochastic extinctions. This species might also be threatened by chytridiomycosis. The very small size of most populations suggests restocking with captive-bred animals as the main conservation measure after removing the possible causes of decline. We report a pilot restocking project in two demes in central Italy that underwent dramatic decline with population size <10 individuals.

Apennine yellow-bellied toad © L. Vignoli

Goals

- Mitigate the main threats for the selected demes prior restocking: early drought of pools and alteration of wetlands by Wild boars (*Sus scrofa*).
- Produce a suitable captive-bred population of one year old metamorph individuals from wild caught eggs from the same place

selected for restocking.

- Release of one year old captive-bred individuals into the wild in four yearly restocking events from 2014 - 2017.
- Double (at least) the pristine population (i.e. N>20) after the fourth year of restocking.
- Create two long-term self-sustainable populations of *Bombina pachypus*.


Release site © A. Pieroni

Success Indicators

- Significant reduction or elimination of the main threats to the selected populations.
- Total captive bred individuals released during the four years of restocking and recaptured at the end of the fifth year of the project.
- Reproduction achieved of the released captive bred individuals after one year from restocking.

Project Summary

Feasibility: *Bombina pachypus* was declining all over its central and northern range. The species may be declining due to the loss of wetland habitat as a result of agricultural damage but also it faces a threat from Chytrid fungus (Canestrelli *et al.*, 2013). Neometamorph *B. pachypus* can experience high mortality, dying within 1 - 2 weeks from collection and a few days after experiencing symptoms. In captivity the infection was nearly always fatal for newly metamorphosed *B. pachypus* froglets, but only sometimes for sub-adults and adults. Two small populations (N<10) from a protected area (Natural Reserve Monti Cervia and Navegna, Latium region - Lat: 42.235435°; Long: 12.980531°) inhabiting unshaded pools along two hilly ridges were selected for a conservation program aimed at increasing the population size to reduce the risk of extinction from stochastic events. Epidemiological screening revealed no presence of chytrid fungus. The observed threats for the species at the study site were the high risk of pool desiccation at the early phase of reproductive season (i.e., June) and the alteration of the pools by Wild boars.

Pre-Action monitoring: The two populations were monitored from 2005 to 2013. The population size (i.e. number of distinct contacted animals) was 18 individuals (nine per site) and remained stable with just three new individuals entering the population in nine years. Each site consisted of one or two small ephemeral pools

Release at the recipient site © C. Maragoni

where toads started to breed in late March and stopped at the end of September.

Concrete actions: In 2012, two main conservation actions were performed to mitigate the main threats: 1) four additional pools per site were built and fed by perennial springs to prolong the hydroperiod from March to October; 2) each pool was fenced to prevent Wild boars from using the pools for drinking and bathing. After two years from the concrete conservation actions (2014), no population growth was observed.

Implementation: Since no population increase was observed after two years from the fulfilment of concrete actions, in 2014, we started a four year project aimed at increasing the population size through restocking of individuals collected from the selected demes at the egg stage and raised in *ex situ* facilities until one year from metamorphosis. The release of metamorph individuals close to age maturity allowed the individual recognition by means of ventral coloration pattern and was supposed to significantly decrease the mortality rate that has a peak at the egg and larval stages (Mirabile *et al.*, 2009). Overall, a total of 67 unsexed individuals were released (20 in 2014, 19 in 2015, 16 in 2016, and 12 in 2017).

Post-release monitoring: The post-release monitoring revealed that toads re-capture rate was highly variable across years of release. For instance, toads released in 2014 were 100% re-captured in 2015 and 50% in 2016 - 2018, whereas for the toads released in 2015, just two out of 19 were re-contacted in the following years. In 2018, we re-contacted a total of 21 restocked individuals (10 released in 2014, two in 2015, four in 2016, and five in 2017). The pristine population remained stable (13 individuals out 19 re-captured in 2018) with a few new recruited animals and a few losses. At the end of 2018, a net increment of 21 released individuals plus some from natural recruitment allowed to double the original population size. Moreover, restocked toads bred repeatedly over the years and captive-bred individual were ready to breed just after 13 months, well before the reported age at maturity for wild animals (three years). Considering the positive outcome of the restocking of the captive bred population, the release of further individuals in the considered demes was stopped but the monitoring is still ongoing. Given that further suitable sites where the species presence is not reported are available in the protected area, the reintroduction of the species in one or a few new sites has been proposed as a further action within the project of *B. pachypus* conservation.

Major difficulties faced

- Production of one year-old individuals from the egg stage requires proper facilities and high personnel effort.
- Identify the real causes behind the high inter-annual variability in individual recapture rate.
- Identify the real causes of failure in recapture released animals (i.e. death or dispersion).

Major lessons learned

- *Ex situ* captive bred toads can be used for restocking *B. pachypus* declining populations.
- Restocking should be performed by releasing individuals in distinct phases along a multi-year project to overcome the possible failure due to stochastic or unpredictable events.
- The success of the project can be achieved by coupling restocking to concrete actions (habitat implementation and protection).

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reasons for Success:

- Restocking conducted in distinct repeated phases along four years.
- Threat mitigation before individuals are released through habitat implementation and protection.
- Monitoring with high frequency before and after release.

References

Andreone, F., Corti, C., Sindaco, R., Romano, A., Giachi, F., Vanni, S. & Delfino, G. (2009) *Bombina pachypus* (errata version published in 2016). The IUCN Red List of Threatened Species 2009: e.T54450A86629977. http://dx.doi.org/10.2305/IUCN.UK.2009.RLTS.T54450A11147957.en.Downloaded on 23 September 2019.

Canestrelli, D., Zampiglia, M. & Nascetti, G. (2013) Widespread occurrence of *Batrachochytrium dendrobatidis* in contemporary and historical samples of the endangered *Bombina pachypus* along the Italian peninsula. PloS one, 8(5): e63349.

Mirabile, M., Melletti, M., Venchi, A. & Bologna, M. (2009) The reproduction of the Apennine yellow-bellied toad (*Bombina pachypus*) in central Italy. Amphibia-Reptilia, 30(3): 303-312.

Review of two translocations used as a conservation tool for an endemic terrestrial frog, *Leiopelma archeyi*, in New Zealand

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Introduction

Leiopelma archeyi Turbott 1942 (Anura: Leiopelmatidae) is a Critically Endangered amphibian that currently occurs in three areas of the North Island, New Zealand: Coromandel Peninsula, Whareorino Forest and Pureora Forest (Easton, 2018). Male parental care of eggs (1 - 2 clutches, each with 2 - 13 eggs) are laid on land (e.g. under stones or inside dead tree-fern trunks). The tadpole stage is bypassed within the eggs, and upon hatching, froglets complete their metamorphosis on an adult's back. The total duration of development is approximately three months and has been observed between October and February. Longevity of the species is 25 - 35 years and maturity is reached between 3 - 5 years of age. The combination of the biology and ecology of *L. archeyi*, and the current threats

Leiopelma archeyi © Phil Bishop

reported for this species (e.g. predation by introduced rat species and the presence of chytrid fungus in wild populations) make *L. archeyi* a prime candidate for translocation (IUCN/SSC, 2013). Here we summarize available information for the conservation translocation (*sensu* IUCN/SSC, 2013) of *L. archeyi* frogs from Whareorino Forest to Pureora Forest in 2006 and 2016, review the context that triggered the decision to translocate, and provide the most up to date demographic estimates for the population in Pureora Forest.

Goals

2006 Goals:

- Establish a new wild population of *Leiopelma archeyi* in Pureora Forest.
- Establish a chytrid free population of *Leiopelma archeyi*.

2016 Goals:

- Enhance the genetic and demographic profile of *Leiopelma archeyi* in Pureora Forest.
- Improve the likelihood and rate of establishment of a long-term viable population of *Leiopelma archeyi* in Pureora Forest.

Success Indicators

2006 Indicators:

- High and long-term survival rate of frogs in the release site with at least 60% survival during the first year.
- Recruitment is recorded at the release site within three years of transfer (i.e. 2009).
- The first generation of offspring from the release site successfully breed and the second generation of offspring survive.

2016 Indicators:

- 100% survival during transfers and less than 5% mortality during quarantine in captivity.
- Recapture of 20% or more of release frogs during any subsequent monitoring, and an increase in the number of new frogs at the release site.

Project Summary

Feasibility: In New Zealand, native frogs (*Leiopelma* spp.) are treasured species (taonga) for indigenous Māori people, such that native frog translocations are culturally sensitive processes (Cisternas *et al.*, 2019). In the Māori worldview (te ao Māori), translocations affect the genealogical interconnectedness of all elements from the natural and supernatural realms (Māori concept of 'whakapapa'), as well as traditional Māori guardianship responsibilities (kaitiakitanga). Thus, during translocations, representatives from the local Māori community at donor sites are required to transfer guardianship responsibilities for

Release site in Pureora Forest © Javiera Cisternas

these treasured species to representatives of the local Māori community at release sites.

Additionally, three biological components should be considered to maximize the chances of a successful translocation: 1) genetic viability of the translocated population, 2) habitat quality/ availability of release sites, and 3) knowledge of species' biology, thereby reducing stress during translocations. However, the 2006 translocation was performed during a biosecurity emergency (the presence of Chytrid fungus on frogs in Whareorino was expected to cause a significant population decline, as observed on the Coromandel Peninsula between 1999 - 2001), and under these circumstances, a rapid response was prioritized over the additional time necessary for quantitative translocation assessments or *in situ* habitat measurements. Habitat at the release site and the population's genetic diversity were only subjectively considered during the 2006 translocation because no detailed information was available (see Appendix A in Cisternas, 2019). Implementation of a genetic assessment associated with the 2016 translocation failed due to sampling problems.

Implementation: Below is a summary of the procedures used in the translocations of *L. archeyi* from Whareorino Forest to Pureora Forest in 2006 and 2016 (for details see Cisternas, 2019). The main focus during capture and transport of the frogs was to avoid rapid fluctuations in temperature and humidity, thereby preventing physiological stress in the translocated frogs. This species is nocturnal, therefore emerged frogs were caught by hand at night for both translocations. However, in 2006, frogs were also collected from inside their retreat sites during the day. In 2006, 48 frogs were collected from areas with high densities of frogs and 52 from low-density areas in Whareorino Forest at the beginning of the breeding season (September). In 2016, 80 frogs were collected after the breeding season (April) in four sites (~100 m apart) from an area with a high density of frogs. Frogs were transported inside chilly bins by hand inside the forest and by car between sites (collection-quarantine-release).

To reduce the likelihood of releasing chytrid positive frogs, they were kept in quarantine and screened for disease. In 2006, frogs were kept at Hamilton Zoo for three months (mortality of frogs - 2%). In 2016, frogs were kept at Auckland Zoo for six months (mortality of frogs - 4%). Despite the increased mortality in 2016, the captive husbandry procedures were greatly improved by the provision of UV light, and a varied diet high in 'natural' prey items enriched with calcium. In addition, the sex of the frogs to be released was determined by measuring urine hormone metabolites, hence the extended quarantine duration.

In 2006, 28 frogs were retained by institutions for a captive-breeding program at Auckland Zoo and chytrid studies at the University of Otago, while the remaining 70 frogs were released around midday (during the oviposition/parental care period) in a 10 x 10 m grid. In contrast, in 2016, 17 frogs were retained at Auckland Zoo for the captive colony and 60 frogs (28 males, 17 females, 15 of undetermined sex) were released after dusk, during the early breeding season. To enhance the habitat quality of the release site, frogs were released into an area enclosed by a herbivore-resistant fence (enlarged in 2016), and predator control for rats has been carried out since 2006. In 2016, a trial was set up to test the effect of fern coverage on the post-release dispersal of the frogs.

Post-release monitoring: A capture-recapture post-release monitoring program was initiated at the release site, Pureora Forest, in April 2007. The site was searched for frogs once or twice a year, during four consecutive nights, inside the 10 x 10 m release grid. Identification of individual frogs was carried out manually using photographs of natural markings in individuals.

Multiple changes were made in the monitoring program between 2013 and 2017 in order to improve its design and increase the recapture rate of frogs (e.g. the search area was increased from 100 to 280 m^2) (Cisternas, 2019). As of 2018, the apparent bi-annual survival of the translocated frogs was estimated as 0.49 (CI= 0.15 - 0.69, using Cormack-Jolly-Seber models), and the abundance of frogs was estimated as 132 frogs (CI = 91 - 199, using Jolly-Seber Schwarz-Arnason models). The model selection criteria used in these capture-recapture analyses are adapted from Cisternas (2019).

Major difficulties faced

- *Low recapture rate of frogs and infrequent analyses of monitoring data:* Monitoring (*sensu* IUCN/SSC, 2013) provides essential information for determining translocation success or failure. In addition, monitoring results inform adaptive management to improve translocation outcomes. In these translocations, formal capture-recapture monitoring analyses could only be performed 12 years after the first release of frogs at Pureora Forest by Cisternas (2019). This delay was due to a low recapture rate of frogs and technical limitations (e.g. insufficient funding, lack of staff capacity or time delays in identifying individual frogs). In the absence of capture-recapture analyses, management decisions were based on descriptive statistical summaries of accumulated counts of frogs captured during monitoring (e.g. capture counts, mean, range). Thus, prior to 2018, the absence of probabilistic statistical analyses made it impossible to include error associated with frog detectability (e.g. due to weather conditions) and spatial variation (e.g. due to misrepresentation of the sampling area) in estimates of population size. Recent coordinated work of practitioners, stakeholders, and researchers has improved the situation by testing different monitoring methods. Regression analyses concluded that the monitoring method applied in 2017, with 4 - 6 people searching for frogs at night inside a fixed 280 m^2 grid, should obtain enough data for robust capture-recapture models (Cisternas, 2019). Additionally, the translocation team is working on automated individual identification systems and the development of R code that will enable analysis of new monitoring data.

- *Limited information on habitat quality at the release site:* Habitat (the sum of resources needed by an organism to persist in a given area) and the condition of a species' habitat (habitat quality) are critical determinants of translocation success or failure (IUCN/SSC, 2013). Regrettably, the emergency situation in which the 2006 translocation was carried out prevented a thorough quantitative assessment of habitat quality at the release site. Based on demographic results we concluded that conditions at the release site enable *L. archeyi* frogs to survive and breed, including frogs captured as juvenile and recaptured as adult (75% of 56 frogs captured within juvenile size [snout-to-vent length [SVL] < 24 mm] reached adult size [SVL > 24 mm]). Vegetation regeneration inside the fenced area, coupled with the predator control program carried out at the release site, should improve the future habitat quality and therefore the translocation outcomes. Nonetheless, robust experimentation is required to corroborate these hypotheses.
- *Unknown genetic diversity:* While obtaining sufficient DNA from skin swabs of the 80 frogs collected in 2016 proved problematic, the preliminary analysis of existing specimens held in storage suggested that the Whareorino population suffers from low genetic diversity relative to other populations of *Leiopelma* spp. Genetically depauperate populations have a poorer ability to adapt to environmental changes, are more susceptible to novel diseases, and are typically associated with inbreeding depression. However, in an attempt to select a genetically diverse group of individuals for release at Pureora Forest, the translocation in 2006 involved the collection of frogs from three sites located at least 10 km apart, while the 2016 translocation involved collecting frogs from four sites spaced approximately 100 m apart. Assessing the genetic diversity of the source and translocated populations is thus important to determine how genetically viable (and thus adaptable) these populations are, especially given the unprecedented environmental changes that will likely occur in the future (Easton, 2018).
- *Lack of opportunity to build experimentation in translocation procedures (especially in 2006):* A translocation should be designed as a management learning process, thus translocations need to be planned as experiments (or trials) to test the effectiveness of different translocation procedures. In these frog translocations, there were no experimental designs associated with the processes of capture, transport, captivity or release. The only exception being a release trial tested in 2016 which, in time, may offer learning outcomes about sex differences on post-release dispersal of frogs released in different microhabitat conditions (Cisternas, 2019). We acknowledge that often translocations cannot be designed as 'ideal' experiments because of limitations in sample size or lack of replicates or a control group. Easton (2018) and Cisternas (2019) offer a baseline of procedures that could be used as a reference point for the design of future translocations with this species.

- *Uncertainty of the agent of decline:* To identify and neutralize an agent that causes a population decline, it is essential to use the scientific method with testable hypotheses that determine, and not assume, why and how a population has declined. It was assumed that Chytrid fungus was responsible for the declines in populations of *L. archeyi* on the Coromandel Peninsula during 1996 - 2001. Thus, it was presumed that the presence of chytrid-positive frogs in Whareorino Forest might result in a similar decline. Research carried out during and after the first translocation determined that chytrid was geographically widespread in New Zealand (Shaw, 2012). Furthermore, studies conducted between 2006 - 2010 revealed a chytrid prevalence of 16% and 6% in frogs swabbed in the Coromandel and in Whareorino, respectively (see Shaw, 2012 and references therein). Currently, monitored Coromandel populations are stable but at levels much less than before population declines and showing an apparent female bias (only big frogs survived). The Whareorino population has not exhibited any declines related to chytrid since monitoring began in 2005. Further research is required to fully understand the reasons for this species' decline in the Coromandel Peninsula. Furthermore, based on fossil evidence from the eastern and northernmost areas of the North Island, New Zealand, Easton (2018) inferred that the historical distribution of *L. archeyi* has dramatically contracted to its current state. Thus, this restricted distribution, together with poor genetic diversity within this species, could be the result of prolonged exposure to human-induced activities (e.g. introduction of mammalian pests, habitat destruction, etc.). However, even if the chytrid strain present in New Zealand is not the major agent of decline, biosecurity should be maintained as a precautionary action, considering the link between chytrids and worldwide declines of amphibian populations, and the potential impact of novel chytrid strains on this frog species.

Major lessons learned

- *Well planned translocations take time:* Planning translocations for *L. archeyi* are problematic due to a lack of basic biological knowledge about the species (Cisternas, 2019). We propose that future translocations include studies to fill these gaps. For instance, the sex ratio of this species is currently unknown in the wild. Sexual dimorphism in body length (measured as SVL) is the only external morphological sexual characteristic for *L. archeyi*. Therefore, size has been the base criteria for several demographic and behavioral studies targeting this species, although sex recognition based on body length can be inaccurate given the marked size range overlap in measurements for both sexes. Indeed, size was the criteria used in these translocations to determine the cohort of frogs collected. In 2016, a novel technique measuring hormone metabolite levels in frog urine was used to determine the sex of the frogs to be released in the second translocation while frogs were held in quarantine. This technique could again be used in future studies to determine sex ratio in the wild and as part of the collection procedures for any future translocation endeavors. Likewise, an optimal design should include the recording of temporal changes on the resources available for this species at the translocation sites (i.e. habitat temporal variation). As the target species of this translocation is a terrestrial anuran, we propose that, at the very least,

Frogs collected for translocation © Luke Easton

temporal variation of climatic conditions should be included to assess the suitability of the release site (i.e. monitor climatic conditions for greater than one year). Additionally, translocations in New Zealand require time to coordinate with relevant stakeholders, including governmental institutions (e.g. Department of Conservation) and local indigenous communities, due to The Treaty of Waitangi (New Zealand's founding document) (Cisternas *et al.*, 2019). Based on the experience acquired during these translocations, we suggest that an optimal translocation design for this species would require about three years to allow the development of: 1) habitat (including climatic) studies to select a suitable frog release site, 2) a relationship between interested parties (especially the government institutions and the local Māori communities), and 3) the criteria used to select the founding individuals (e.g. determine the number of founder frogs to be translocated based on population viability analyses [e.g. Easton, 2018]).

- Leiopelma archeyi *translocations require a long-term commitment: Leiopelma archeyi* is a long-lived species (25 - 35 years [B. Bell pers. comm. 6th October 2017]), with parental care of a small number of offspring and first reproduction estimated to occur five years after metamorphosis. Thus, post-release monitoring to assess the establishment of this species at a new site should continue for at least one generation length after translocation (i.e. 16 - 17 years [B. Bell pers. comm. 6th October 2017]). Similarly, the resources associated with monitoring and management actions at the release site (e.g. improve habitat quality, predator control) must be budgeted for the long-term (IUCN/SSC, 2013). Finally, only a long-term commitment with the translocation project would allow current and future generations of local Māori communities the opportunity to interact meaningfully with this treasured species (Cisternas *et al.*, 2019).
- *Interdisciplinary and intercultural teams improve translocation outcomes:* The use of interdisciplinary teams leads to better translocation processes if they utilize the expertise and knowledge of each of the members. In these translocations, there has been an increasing involvement of practitioners and stakeholders. In 2006, the Department of Conservation (DOC) initiated the translocation with participation of the local Māori community. Researchers also became involved to determine the impact of chytrid on *Leiopelma* species, and later they assisted with the capture-recapture program. Auckland Zoo retained the frogs collected from Whareorino Forest in 2006 and 2016 that were not released in Pureora Forest, to

supplement the captive program for this species. Researchers from the University of Otago and Auckland Zoo, and the local Maori community, continue to support DOC in their monitoring efforts to assess translocation outcomes.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Preliminary monitoring results showed apparent lower survival of frogs at the release site than the value estimated for apparent survival in the donor population (Cisternas, 2019). Nevertheless, there is not enough evidence to assess survival trends given the longevity of this species (see above). An estimate of survival could be obtained with capture-recapture studies using open population models (e.g. Cormack-Jolly-Seber models). However, given the monitoring constraints in this translocation (see above), we encourage the collection of monitoring data until ~2030 for robust estimates comparable with its donor population. In addition, 26% of the frogs translocated in 2006 were recaptured at least once during 14 monitoring nights 27 months after release. Only six individuals from this original cohort, however, were recaptured during monitoring after 10 years since release. In 2018, 42% of the frogs released in 2016 were recaptured at least once during 16 monitoring nights 25 months after release. Furthermore, an increase in body mass of recaptured individuals has been observed (e.g. Appendix F in Cisternas, 2019), which, in addition to other indicators (see below), may indicate competitive release at the release site.
- Evidence of reproduction at the release site. A newly metamorphosed frog (SVL=11.2 mm) was first found at the release site during monitoring in March 2008 (15 months after the first translocation). During monitoring in November 2016, two observations of a single adult-sized frog, sitting over eggs under a rotten tree-fern log, were also recorded. In October 2017, three frogs were found in one of these oviposition sites during the day, which may indicate the timing of amplexus in this species. As with survival, robust estimates of recruitment (e.g. using Jolly-Seber Schwarz-Arnason models) would only be feasible with more long-term monitoring data.
- Uncertain long-term viability of *L. archeyi* frogs in Pureora Forest. Further research is recommended to determine the genetic and demographic viability of this translocated population using, for example, single nucleotide polymorphisms (SNPs) and population viability analyses, respectively. Additionally, investigations of pedigrees will need to rely on genetic markers (e.g. SNPs) given that there is no other current method to reliably determine the relatedness of individuals.
- Pureora Forest is not chytrid free. To assess the presence of chytrid on frogs in the translocated population, all frogs captured during the fourth night of monitoring were swabbed and tested for chytrid. In 2016, chytrid was detected for the first time: two frogs tested positive with zoospore counts of 188 and 751 (i.e. a frog tested negative has zero zoospore

count). Since then, frogs have tested negative. A frog infected with chytrid fungus can develop the disease chytridiomycosis, although the relationship between immunity and the presence of Chytrid fungus in *L. archeyi* is currently unclear (Shaw, 2012). Given that all the frogs released in both translocations had tested negative for chytrid three times before release, the finding of chytrid in the translocated population demonstrates the difficulty in maintaining any wild frog population as chytrid free despite quarantine protocols (e.g. cleaning boots with disinfectant, changing gloves between handling frogs, etc.). It may also be possible that chytrid is prevalent in the environment and spread via other means (e.g. other wildlife).

References

Cisternas, J. (2019) Translocation management of *Leiopelma archeyi* (Amphibia, Anura: Leiopelmatidae) in the King country. PhD thesis, University of Otago, New Zealand.

Cisternas, J., Wehi, P.M., Haupokia, N., Hughes, F., Hughes, M., Germano, J.M., Longnecker, N. & Bishop, P.J. (2019) ‘Get together, work together, write together’: a novel framework for conservation of New Zealand frogs. New Zealand Journal of Ecology 43 (3): 3392.

Easton, L.J. (2018) Taxonomy and genetic management of New Zealand’s *Leiopelma* frogs. PhD thesis, University of Otago, New Zealand.

IUCN/SSC (2013). Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission, viiii + 57 pp.

Shaw, S.D. (2012) Diseases of New Zealand Native Frogs. PhD thesis, James Cook University, Australia.

Conservation breeding and reintroduction of the endangered mountain yellow-legged frog in Southern California, USA

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Introduction

The Mountain yellow-legged frog (*Rana muscosa*) is an amphibian species endemic to the San Bernardino, San Gabriel, San Jacinto, and southern Sierra Nevada mountains of California. Formerly abundant at high-elevation streams and lakes, populations of Mountain yellow-legged frogs in the southern California distinct population segment (comprising the San Bernardino, San Gabriel, and San Jacinto mountain ranges) began declining in the late 1960s, and now exist at precariously low numbers (<200 wild adult individuals; Backlin *et al.*, 2015). Factors that contributed to the decline of this species include introduced predators, infection with diseases (e.g. chytridiomycosis), habitat loss and degradation (development, pollution, etc.), climate change, and extreme climatic events (fires, droughts, and floods).

In 2002, this species was federally listed as endangered by the U.S. Fish and Wildlife Service and is also recognized as endangered by the IUCN and the state of California. Beginning in 2006, the San Diego Zoo Institute for Conservation Research (ICR) was tasked with developing a conservation breeding program for individuals from the San Bernardino and San Jacinto mountain ranges, with the goal of developing breeding methods and establishing a stable captive population with sufficient reproductive success to produce individuals for reintroduction into the wild.

Goals

- Prevent extirpations of Mountain yellow-legged frogs at sites in the San Bernardino and San Jacinto mountains of southern California by capturing remaining individuals for

Reintroduced juvenile frog © Talisin T. Hammond

preservation in captivity.
- Develop captive husbandry and breeding protocols that optimize survival and reproduction.
- Develop a reintroduction protocol for transporting and releasing captive-bred animals into the wild.
- Through reintroduction of hundreds of captive-bred animals each year, establish new populations in the wild at sites within historical range of this species.
- Develop successful surveying techniques for monitoring reintroduced animals.

Success Indicators

- Maximize survival of offspring within each life stage from a variety of pairs within each population range.
- Produce at least ~1,000 individuals annually for reintroductions and head-starting from at least two pairs per population.
- Detect at least 50 frogs per site for five consecutive years.
- Observe breeding in reintroduced animals in the wild.

Project Summary

Feasibility: While some of the threats that contributed to this species' decline still exist in the wild, others have been mitigated. Trout removal has taken place in many critical Mountain yellow-legged frog habitats, and several sites currently or formerly inhabited by this species fall in protected areas, including national parks and forests. Chytrid fungus is regularly detected at release sites, but the historical and current impact of chytrid on southern California populations is not well understood.

Implementation: In 2006, 86 tadpoles from the San Jacinto Mountains were collected as a salvage effort and transported to the San Diego Zoo Institute for Conservation Research (ICR) to serve as founders for the conservation breeding program. In 2015, an additional 20 tadpoles were collected and brought into captivity to increase the genetic diversity within the captive population. Between 2011 - 2015, six juveniles and eight adults were collected from the San Bernardino Mountains and transported to ICR to establish a captive San Bernardino mountain population for breeding and release to the wild.

Captive-breeding and husbandry: The adult breeding colony at ICR currently consists of nine adult individuals from the San Jacinto population and 13 adult individuals from the San Bernardino population, in addition to numerous tadpoles and juvenile frogs. A husbandry protocol was developed in which all individuals are monitored daily and water quality is assessed regularly. Animals are fed a variety of insect species to increase dietary diversity (e.g. crickets, fruit flies, horn worms, phoenix worms, flies). In 2010, a brumation experiment was conducted to determine whether exposing captive animals to winter temperatures would impact reproductive success. Results indicated that brumated frogs were significantly

Researchers collecting field data
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more likely to breed in the spring than un-hibernated frogs (Santana *et al.*, 2015). Since adopting a brumation protocol for all animals, the colony has produced an average of ~400 eggs per female per year, with ~30% fertilization success. To further improve captive reproduction, we have implemented research on assisted reproductive technologies (Calatayud *et al.*, 2019), mate choice, and genetic management.

Pre-release conditioning: Prior to release, all animals are weighed and measured. Sufficiently large individuals are tagged with 8 mm passive integrated transponder (PIT) tags. In some years animals that were insufficiently large for PIT tags were instead tagged/identified using visible implant elastomer, alpha tags, and/or photo identification based on unique spot patterns. Prior to release veterinarians conducted health assessments and a subset of animals were tested for chytridiomycosis (all tested negative). We used experimental approaches to test the utility of a variety of pre-release treatments, taking advantage of the fact that PIT tags enable assessment of individual fates. Research has examined ties between post-release survival/movement and the pre-release manipulations including brumation, vegetative cover availability, experience with water currents, soft vs. hard releases, and treatment with the anti-fungal probiotic *Janthinobacterium lividum*. Data are still being analyzed from these studies. Preliminary results suggest that exposure to naturalistic environmental conditions (e.g. brumation in captivity; matching vegetative cover in captivity to that available in the field) may increase post-release survival.

Release: Release sites within this species historical range were selected in the San Bernardino and San Jacinto mountains. Habitat assessments took place prior to release, and only locations that were uninhabited by introduced trout and bullfrogs were used. Releases of tadpoles (approximately two months old) and/or froglets (approximately one year old) took place at one or both mountain ranges between June - September of 2010 - 2019 (Table 1).

Post-release monitoring: Post-release monitoring surveys took place in all years but were more frequent in 2016 - 2019, when they occurred at least weekly for the first month after release, then at least monthly until October, and then at least annually thereafter. Within-year re-detection rates of froglets were variable across years and sites (~25 - 80%), but generally decreased with time since release.

Tadpole re-detection was also variable (~4 - 37%) but was generally lower than froglet re-detection. Reproduction of reintroduced animals was detected at one release site, though reproductive rates were low. A small number of individuals have been detected across multiple years at most of the reintroduction sites, though overall interannual apparent survival is low. However, surveys have revealed relatively high, upstream movement rates in many froglets (as far as 2.5 km in some individuals). This, in combination with the challenge of detecting this species in the wild, makes it difficult to distinguish between mortalities, false absences, and dispersal out of the survey area. Currently we are assessing new techniques to increase detection of frogs after release, including camera traps, scent detection dogs, PIT tag readers, and radio-telemetry transmitters. In 2019, a long-range PIT tag reader was deployed, which increased re-detection rates.

In 2016 - 2019, *Batrachochytrium dendrobatidis* skin swabs were collected from re-captured individuals. Thus far swabs have revealed relatively low infection prevalence (~14% during the summer sampling period) and intensity in the focal populations.

Major difficulties faced

- Due to private ownership, recreational use permits, regulatory delays and other conservation projects, it can be difficult to gain approval for new release sites for this species.
- The Mountain yellow-legged frog camouflages well in its habitat, does not produce audible vocalizations, is small and diurnal; these factors make detection of this species difficult in the wild.
- The fungal disease, chytridiomycosis, is still present at most potential release sites, and the extent to which it is currently or was formerly an issue is poorly understood.
- Because this project began as an emergency salvage effort rather than as a planned conservation program, relatedness of captive founders from one population was high, and thus, the genetic diversity of the population is low.
- Climate change and the drought in California continue to be a problem for

Table 1. Summary of ICR Mountain yellow-legged frog reintroductions: 2010 - 2019

Year	San Bernardino		San Jacinto	
	Tadpoles	Froglets	Tadpoles	Froglets
2010	-	-	36	-
2011	-	-	153	-
2012	-	-	-	-
2013	-	-	-	308
2014	-	-	-	49
2015	-	-	911	27
2016	150	-	1,121	165
2017	183	91	404	-
2018	-	259	309	-
2019	-	196	685	35

this species, and sites with permanent water have been difficult to permit.

Major lessons learned

- Adding a brumation period to captive husbandry protocols can be critical to improve reproductive success in amphibians that inhabit mountain streams and should be tested more broadly with other species.
- To improve survival after release to the wild, it is important to integrate the natural habitat conditions to which species are adapted into captive environments (e.g. cover, etc.).
- Designing and implementing conservation breeding and reintroduction programs in an adaptive management framework allows for meaningful modifications and improvements of husbandry protocols and means that results may be more generalizable to other systems.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Husbandry and breeding protocols were experimentally tested and improved from year to year, as determined through increased survival and reproduction in the assurance colony.
- Many hundreds of animals are consistently produced annually and released into the wild.
- Some individuals were recaptured years after initial release, indicating captive born animals can survive in the wild at the selected release sites.

References

Backlin, A.R., Hitchcock, C.J., Gallegos, E.A., Yee, J.L. & Fisher, R.N. (2015) The precarious persistence of the Endangered Sierra Madre yellow-legged frog *Rana muscosa* in southern California, USA. Oryx 49(1): 157-164.

Calatayud, N.E., Chai, N., Gardner, N.R., Curtis, M.J. and Stoops, M.A. (2019) Reproductive techniques for ovarian monitoring and control in amphibians. JoVE (Journal of Visualized Experiments), (147), p.e58675.

Santana, F.E., Swaisgood, R.R., Lemm, J.M., Fisher, R.N., & Clark, R.W. (2015) Chilled frogs are hot: hibernation and reproduction of the Endangered mountain yellow-legged frog *Rana muscosa*. Endangered Species Research 27(1): 43-51.

Reintroduction of green and golden bell frogs into created habitats on Kooragang Island, Australia

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Introduction

The Green and golden bell frog (*Litoria aurea*), is a semi-aquatic hylid native to south-eastern Australia. Despite being invasive in New Zealand and New Caledonia, *L. aurea* has disappeared from over 90% of its historical range (Mahony *et al.*, 2013), and now persists only as fragmented populations, predominately along the coast. Consequently, *L. aurea* is listed as Vulnerable under the IUCN Red List and Australian Commonwealth legislation, and endangered under NSW State legislation. The pathogenic Chytrid fungus (*Batrachochytrium dendrobatidis*) (Stockwell *et al.*, 2010), habitat loss, and predation by invasive mosquitofish, are the major threats. This reintroduction took place on Kooragang Island (KI) at the mouth of the Hunter River north of Newcastle, NSW, Australia. KI contains one of the largest remnant *L. aurea* populations in Australia.

Land use on KI includes industry (predominantly coal), exotic pasture, and natural freshwater and saltmarsh wetlands. *Litoria aurea* can be found in waterbodies within each of these land-use types, although its distribution is patchy. This reintroduction took place in two stages across four created habitats. First, we released *L. aurea* tadpoles into two small-scale experimental trial sites. Findings from these sites were then

Green & golden bell frog

incorporated into the creation of two large-scale compensatory wetlands.

Goals

- To assess the effectiveness of created habitat mosaics to support a reintroduced population of Green and golden bell frogs.
- To assess the efficacy of these habitat mosaics in reducing chytrid pathogen prevalence, through manipulation of environmental factors unfavorable for the fungus.
- Create large-scale habitats based on experimental findings to increase occupancy of sites on KI, bolster wild populations, and extend the meta-population distribution.

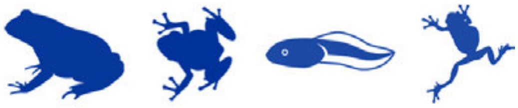
Success Indicators

- The persistence of a reintroduced population at experimental habitat mosaics for four years, including the detection of breeding within constructed ponds.
- Reduced severity and prevalence of chytrid infection within the reintroduced populations compared to wild sub-populations.
- The need for little active intervention to support frog populations within constructed habitats.
- No invasion of predatory mosquitofish.
- Extension of the distribution of the metapopulation on KI through persistence, colonization, and breeding in the large-scale compensatory habitats.

Project Summary

Feasibility: The reintroduction was funded by industry partners required to compensate for habitat loss. This involved creating habitat mosaics in areas not already occupied by the species. Two enclosed experimental habitat mosaics were constructed first, to test the efficacy of certain habitat features in supporting a *L. aurea* population. These habitat features included a mosaic of permanent and ephemeral ponds, increased salinity in a subset of ponds, fencing to exclude predators and prevent *L. aurea* dispersal, and rock piles for shelter. As chytrid is present in this landscape, and currently impossible to eradicate, our primary aim was to test if these features increased population survival in the presence of this pathogen. Outcomes from the experimental sites were then incorporated into the design and construction of two large compensatory wetland habitats.

Implementation: Released tadpoles were reared at the University of Newcastle’s outdoor breeding colony, established with *L. aurea* originally collected from KI. Before large-scale releases, we placed a subset of tadpoles into 1 m^3 mesh cages secured inside the constructed permanent ponds to ensure water quality suitability. These “soft releases” allowed easy and accurate monitoring of tadpole survival. With survival confirmed, over 10,000 tadpoles were released into half the permanent ponds at each experimental site in two stages. The staggered



Chad Beranek compensatory pond

reintroductions were to bolster population viability, as females are not sexually mature until two years of age.

Rock salt was added to half the experimental ponds to try and mitigate chytrid. We raised salinity levels from an average of 0.3 ppt to 2.5 - 3 ppt, a concentration known to reduce chytrid growth and motility (Stockwell, *et al.,* 2012 & Stockwell *et al*., 2015). Salt was incrementally applied over six weeks to allow salt to dissolve and to prevent over-dosing. Salt was added after tadpoles had metamorphosed and left the ponds at Experimental Site One and prior to tadpole reintroductions at Site Two.

After four years of monitoring the experimental sites, two large-scale compensatory habitats were constructed. Each compensatory site contained “clusters” of ponds, creating a mosaic of permanent, semi-permanent, and ephemeral ponds, with emergent and fringing vegetation. After a flood, invasive, predatory mosquitofish colonized some ponds within Compensatory Wetland One. In response, earthen walls (bunding) were constructed around the perimeter of most ponds to prevent future colonization of mosquitofish via the flow of water overland during high rainfall. Passive chytrid mitigation was achieved by constructing permanent ponds that intersected the groundwater, providing a permanent salinity profile. Ephemeral ponds were designed to periodically dry out to reduce the presence of the aquatic chytrid fungus. In Compensatory Wetland One (157 ha) 40,000 tadpoles were released to a subset of permanent ponds over a three year period (2015 - 2017). Approximately 1,800 of these tadpoles were marked with visible implant elastomers (VIE, Northwest Marine Technology, Shaw Island, WA, USA) (Bainbridge *et al.,* 2014) to record survival and movements of post-metamorphic frogs. Tadpoles were not released into Compensatory Wetland Two (2.6 ha), as natural colonization occurred rapidly after construction.

Post-release monitoring: We performed weekly mark recapture surveys at the experimental habitats over four years to determine frog growth and population size. Chytrid prevalence was monitored by skin swabs analyzed with qPCR. The reintroduced populations survived the four year monitoring period, however, the relative abundance of frogs declined each year. In Experimental Site One, no breeding was recorded, and mosquitofish colonized one of 10 ponds. Breeding was recorded in 2014 at Experimental Site Two, but mosquitofish entered 12 out of 16 ponds shortly after, and no further breeding was detected. Multi-state models showed that chytrid reduced monthly frog survival at Experimental Site One. Comparative surveys between Site One and wild KI populations, indicated that chytrid levels were lower in wild frogs. At both experimental sites, frogs readily used salted and unsalted ponds, but avoided salinities over 9 ppt (reached during drought). Chytrid did not impact monthly survival at Experimental Site Two, suggesting that the mosaic of salt levels might bestow a beneficial effect on the population through a complex interaction of frog movement, disease transmission and survival.

Experimental permanent pond

Mark recapture was also conducted across both compensatory habitats during breeding seasons (September - March between 2014 - 2015 and between 2019 - 2020). After metamorphosis, released animals dispersed to five out of seven constructed wetland clusters. Adults mostly dispersed from constructed ponds to brackish natural wetlands after significant rainfall recharged wetlands. Due to floods during construction, mosquitofish invaded 40% of ponds in Compensatory Wetland One, and 18% of ponds in Wetland Two. Bunding prevented further invasions, and fish were naturally lost from six ephemeral ponds after pond drying. Across four years, breeding has been detected six times in Compensatory Wetland One, and 27 times across eight ponds at Wetland Two. Breeding was detected only once in a pond containing low abundance of mosquitofish. Recruitment has been so successful at Compensatory Wetland Two, that population size has increased by 1,200% in three years.

Major difficulties faced

- The impact of chytrid on released populations at the experimental habitats significantly lowered survival and was not reduced by one of our habitat designs compared to wild populations.
- The addition of salt to waterbodies to mitigate the effects of chytrid required active intervention during extreme weather conditions.
- Juvenile dispersal into terrestrial habitats was explosive and random in orientation, making any assessment of terrestrial habitat suitability problematic.
- Uncoupling effects of tadpole predation and chytrid within the first compensatory site was difficult due to continued colonization of experimental ponds by predatory fish.

- There was difficulty in ensuring mosquitofish did not enter permanent ponds during their construction. Flooding, transportation via wading birds, or pre-existing occupancy of fish in the construction zone, may all have contributed to the presence of mosquitofish in compensatory ponds.

Major lessons learned

- Without recruitment into a released population, mortality due to chytrid will likely drive the loss of all individuals within three years.
- *Litoria aurea* readily used artificially salted ponds. Furthermore, constructed ponds set within a brackish saltmarsh habitat maintained higher frog abundance than ponds set within a pasture landscape, suggesting that higher salinities may be a useful tool to mitigate the effects of chytrid across the landscape.
- A habitat mosaic design was successful in supporting sub-populations at our constructed habitats as it provided year-round aquatic habitat (via permanent ponds, which act as refuge habitat during dry periods) plus a higher proportion of fish-free ponds for recruitment (via ephemeral ponds, which also exhibit good water quality after recharge). Bunding is a successful construction technique to prevent colonization by fish during floods.
- Tapping into the water table is an effective method for maintaining pond permanence and maintaining desired salinity levels.
- Viability of a released population is better supported when natural colonization is possible (compared to tadpole release alone). Building new habitat close to extant populations, and providing aquatic habitat corridors is effective for achieving colonization of this species.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- The created experimental sites were partially successful as they supported *L. aurea* populations for four years, but they were not able to lower chytrid infection levels and breeding was limited.
- We consider the use of experimental sites for testing created habitat conditions, a success, as the results they provided allowed for improved design of the larger compensatory projects, e.g. the need to tap into the water table to achieve true permanence and naturally regulate salinity; *L. aurea* readily occupied all pond types within a wetland mosaic; larger ponds are preferred for breeding; physical barriers (bunding) were needed to prevent fish colonization.
- Compensatory Wetland Two was likely successful due to high recruitment and high survival. A largely fish-free habitat mosaic supported breeding and provided year-round aquatic habitat. The permanent ponds with a saline influence may have supported survival in the presence of chytrid. This site

also benefited from its proximity to extant populations, which allowed natural colonization.

- Compensatory Wetland One experienced less recruitment than Wetland Two, potentially because it was further from extant populations and relied on the development of released tadpoles versus colonization of adults. This is problematic because post-metamorphic frogs exhibit high dispersal and low survival rates and can take two years before females reach sexual maturity. Furthermore, the large size of the habitat (157 ha) means monitoring is less intensive and breeding events are more likely to be missed.

References

Bainbridge, L., Stockwell, M.P., Valdez, J., Klop-Toker, K., Clulow, S., Clulow, J. & Mahony, M.J. (2014) Tagging tadpoles: retention rates and impacts of visible implant elastomer (VIE) tags from the larval to adult amphibian stages. Herpetological Journal 25: 133-140.

Mahony, M.J., Hamer, A.J., Pickett E.J., McKenzie D.J., Stockwell, M.P., Garnham, J.I., Keely, C.C., Deboo, M.L., O'Meara, J., Pollard, C.J., Clulow, S., Lemckert, F.L., Bower, D.S. & Clulow, J. (2013) Identifying conservation and research priorities in the face of uncertainty: a review of the threatened bell frog complex in eastern Australia. Herpetological Conservation Biology (8): 519-538.

Stockwell, M.J., Clulow, J. & Mahony, M. (2015) Evidence of a salt refuge: chytrid infection loads are suppressed in hosts exposed to salt. Oecologica 177: 901-910.

Stockwell, M.J., Clulow, J. & Mahony, M. (2012) Sodium chloride inhibits the growth and infective capacity of the amphibian chytrid fungus and increases host survival rates. PLoS One **7**:e36942.

Stockwell, M.J., Clulow, J. & Mahony, M. (2010) Host species determines whether infection load increases beyond disease-causing thresholds following exposure to the amphibian chytrid fungus. Animal Conservation 13:62-71.

Leaping from extinction: Rewilding the relict leopard frog in Las Vegas, Nevada, USA

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Introduction

The Springs Preserve (Preserve) is a 73 ha urban park known as the birthplace of Las Vegas, Nevada, USA. Historically, the Preserve contained three springs that flowed into riparian meadows. These spring systems were once inhabited by the Vegas Valley leopard frog (*Rana fisheri*), which was once presumed extinct but has persisted in central Arizona, USA. Today, the Preserve is privately-owned by the Las Vegas Valley Water District (LVVWD), the local municipal water purveyor. As part of ongoing restoration efforts, ponds were constructed at the Preserve to rewild the state-protected Relict leopard frog (*Rana onca*), a species considered Endangered by the IUCN. This frog species was once presumed extinct, but populations persisted along drainages of the Virgin and Colorado rivers in Arizona and Nevada, USA (Jaeger *et al.*, 2001).

Since then, eight natural populations have been documented and 13 refugia sites established. In spring 2018, surveys at all known sites documented a total of 1,125 frogs; although, the actual number was likely several times larger. The establishment of a population at the Preserve further protects the species from stochastic events that can lead to extinction.

Relict leopard frog © Aaron Ambos

Relict leopard frog tadpole © Aaron Ambos

Goals

- Obtain regulatory and legal agreements, permissions, and permits necessary for private land owners to conduct actions that may contribute to the recovery of species listed as endangered or threatened under the U.S. Endangered Species Act.
- Design and construct a pond mesocosm suitable for Relict leopard frogs.
- Establish a self-sustaining population of Relict leopard frogs.
- Increase geographic distribution and total population count to increase species resilience to stochastic events.
- Educate public about the plight of the Relict leopard frog and foster community support.

Success Indicators

- Ratification of Landowner Cooperative Agreement with Nevada Department of Wildlife (NDOW).
- Establishment of pond mesocosm at designated site.
- Obtain and translocate Relict leopard frogs.
- Relict leopard frog population becomes self-sustaining.
- Implement public education programing on conservation efforts.

Project Summary

Feasibility: To assist with conservation of the Relict leopard frog, additional public education and refugia populations are required. The Preserve was identified as a potential translocation site because: 1) it is a secure property that will reduce the likelihood of illegal introductions of non-native species, 2) it hosts two museums that promote conservation and public education, and 3) it was historically inhabited by the extirpated Vegas Valley leopard frog.

The Preserve, however, encompasses a 44 ha operational groundwater well-field that provides water to meet Las Vegas' peak municipal demands. In order to maintain operations of the active well-field, while ensuring the safety of a Relict leopard frog population, a 15-year Landowner Cooperative Agreement was ratified in 2017 by LVVWD and NDOW under a programmatic Candidate

Pond mesocosm in pond © Raymond A. Saumure

Conservation Agreement with Assurances between the U.S. Fish and Wildlife Service (USFWS) and NDOW. The legally-binding document spelled out the rights, responsibilities, and obligations of the parties (LVVWD & USFWS, 2017).

Implementation: The design and construction of a pond mesocosm suitable for Relict leopard frogs was potentially the most challenging part of the project. Two previously-built ponds at the Preserve had been negatively affected by decomposing leaves from overhead Cottonwood trees (*Populus fremontii*). Supplemental aeration and filtration was necessary in order to rectify water quality issues. Once funding and approvals were secured, a new low-maintenance pond mesocosm was designed in August 2016. This design included two interconnected concrete ponds with shared aeration systems (i.e., bubblers, waterfalls), and both natural filtration (i.e., emergent macrophytes) and mechanical filtration (i.e. high-capacity skimmer baskets, settling basin). The intricacies of the unique aeration and filtration systems were detailed in Wallace (2018).

Relict leopard frog eggs were collected in spring 2018 and 2019 from natural populations in Lake Mead National Recreation Area, Clark County, Nevada. Tadpoles were reared in a laboratory setting by biologists from the University of Nevada, Las Vegas. Once the ponds were working as designed in May 2018, 100 newly metamorphosed Relict leopard frogs were released into the ponds. An additional 101 tadpoles and 111 newly metamorphosed frogs were translocated from March to May 2019.

Post-release monitoring: Since the ponds can be visited regularly by staff, post-release monitoring has occurred almost daily. Upon the release of the initial 100 young frogs in May 2018, a female Mallard duck (*Anas platyrhynchos*) was observed consuming several frogs as they floated on the surface of one of the ponds. These laboratory-raised frogs appeared to have not developed effective flight response, which was compounded by a lack of dense cover in the newly-planted riparian areas. Few frogs were observed during subsequent diurnal visits.

A nocturnal visual encounter survey (VES) in July 2018 noted the presence of only six Relict leopard frogs. By October 2018, four (one male and three females) large adult-sized frogs were captured and PIT tagged during a nocturnal survey.

Although little is known about the overwintering habits of this species, dataloggers revealed that water temperatures in the two ponds decreased to 0.5°C and 1.5°C, respectively, over the winter of 2018 - 2019. All extant natural populations of Relict leopard frogs inhabit geothermally influenced systems, where water temperatures can reach 30 - 55ºC at sources (Bradford *et al.*, 2005). Nonetheless, refugia populations have been established at sites with colder water (Conservation Team, 2016).

In March 2019, a nocturnal survey revealed the presence of two adult Relict leopard frogs. A male was captured at that time and its identity confirmed via PIT tag. This male, released as a newly metamorphosed frog in May 2018, was calling prior to capture, and thus already sexually mature.

In April 2019, *in situ* reproduction was confirmed when hundreds of small tadpoles were observed in the ponds. Although no egg mass was observed, Relict leopard frog egg masses can contain up to 1,100 eggs (Conservation Team, 2016). Thereafter, tadpoles were observed regularly on sunny days resting on algae and vegetation, but were noticeably absent on overcast days. These tadpoles began to undergo metamorphosis in July 2019, and by August 2019, a VES documented 195 frogs and one tadpole in the ponds. Six of the observed frogs were of adult size.

In October - November 2019, 214 Relict leopard frogs were captured and marked in the ponds. Twelve of these frogs were of adult size, including a very large PIT tagged female from the 2018 cohort. A subsequent recapture revealed that an estimated 424 frogs inhabiting the ponds (with a 95% Confidence Interval=308 - 540). Although the vast majority of the frogs were young and had not yet overwintered, the presence of so many frogs is promising in terms of their potential contribution to the overall status of this species.

Major difficulties faced

- Prior to the addition of aeration and filtration systems, there was an unanticipated decline in water quality because of large quantities of decomposing leaves in the fall and winter. The 2012 International Swimming Pool and Spa code recently adopted by the City of Las Vegas requires any body of water built deeper than 46 cm to be surrounded by child-proof, unclimbable, security fences. After consultation with the City of Las Vegas, it was determined that the ponds met the code requirements of a man-made lake used for recreational, scenic, or landscape purposes; therefore, no pool fencing was required.
- In spring 2018, the density of native plants in the riparian zone did not provide the translocated frogs with sufficient cover from previously undocumented avian predators. Riparian plant growth by 2019 appeared sufficient to resolve this issue.
- In 2018, most of the lab-raised young Relict leopard frogs did not appear to exhibit a sufficient flight response upon release to avoid avian predation. The contrast in wariness was especially evident in 2019, as the young frogs that developed *in situ*, or from tadpoles released at the site, had

PIT tagging frogs © Raymond A. Saumure

pronounced flight responses.

- The mechanical aeration system (i.e., bubblers) had to be adjusted so that the bubbles did not prevent falling leaves from reaching two large skimmer baskets. Given the closed nature of the system, large quantities of decomposing leaves could still potentially lead to water quality issues.

Major lessons learned

- Small pond mesocosms require supplemental aeration and filtration.
- In 2019, modifications to the translocation protocol were implemented in an attempt to reduce the impact of diurnal avian predators: 1) all translocations were scheduled at dusk to allow animals to acclimate prior to experiencing potential diurnal avian predation, and 2) large tadpoles were released in addition to the newly metamorphosed frogs.
- Although plant cover was substantial by 2019, cover was further enhanced in 2019 by placing several partially-submerged large sandstone slabs in the riparian zone. These slabs were heavy enough that ducks could not dislodge them, with access only under the edges. Subsequent monitoring has documented numerous metamorphs and young frogs sheltered under these slabs.
- Survivorship of young frogs that developed from the eggs deposited *in situ* was probably higher than the translocated lab-reared young frogs.
- The rewilding of the Springs Preserve generated a surprising amount of positive local media coverage. This media coverage was leveraged to educate the public about the plight of imperiled amphibian species in the Mojave Desert.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- The initial buy-in and subsequent commitment from partner agencies to see the project through, despite temporary setbacks, was critical to the success of the project.

- The pond was redesigned to be a low-maintenance mesocosm that provided redundant natural and mechanical aeration and filtration systems.
- Enhanced riparian habitat with additional cover to mitigate for previously undocumented avian predation by ducks.
- The probability of success was increased by adjusting translocation protocols for the species.
- Public education followed a multifaceted approach, including interpretive panels, site tours, and public television. These activities resulted in additional reporting in local print and social media, generating even more public interest.

References

Bradford, D.F., Jennings, R.D. & Jaeger, J.R. (2005) *Rana onca* Cope 1875, Relict leopard frog. pp. 567-568. *In:* M.J. Lannoo (Ed.). Status and conservation of U.S. amphibians. University of California Press, Berkeley, CA.

Conservation Team (2016) Conservation agreement and conservation assessment and strategy for the relict leopard frog (*Rana onca* [=*Lithobates onca*]). Las Vegas, Nevada: Relict Leopard Frog Conservation Team. http://www.ndow.org/uploadedFiles/ndoworg/Content/Our_Agency/Divisions/Fisheries/Relict-Leopard-Frog-Conservation-Agreement.pdf

Jaeger, J.R., Riddle, B.R., Jennings, R.D. & Bradford, D.F. (2001) Rediscovering *Rana onca*: Evidence for phylogenetically distinct leopard frogs from the border region of Nevada, Utah, and Arizona. Copeia 2001: 339-354.

Las Vegas Valley Water District & U.S. Fish and Wildlife Service (2017) Safe Harbor Agreement for Pahrump poolfish (*Empetrichthys latos*) at the Spring Preserve, Las Vegas, Clark County, Nevada. 25 p.

Wallace, K. (2018) Fish-safe airlift systems for protecting endangered species. Pond Trade Magazine. February 26. https://www.pondtrademag.com/best-pond-practices-fish-safe-airlift-systems-protecting-endangered-species/

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