Global conservation translocation perspectives: 2021

Case studies from around the globe

Edited by Pritpal S. Soorae

IUCN SSC Conservation Translocation Specialist Group

Conservation Translocation SPECIALIST GROUP

SSC Species Survival Commission

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Cover photo: Clockwise starting from top-left:
I. Darwin's rhea (*Rhea pennata pennata*) © Cristián Saucedo
II. Orinoco turtle (*Podocnemis expansa*)
III. Leopard cat (*Prionailurus bengalensis*) © Mei-Ting Chen
IV. White saxaul (*Haloxylon persicum*) © EAD
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Conservation Translocation Specialist Group

The IUCN SSC Conservation Translocation Specialist Group (CTSG) aims to ‘empower responsible conservation translocations that save species, strengthen ecosystems, and benefit humanity’ for a vision of ‘a world where courageous action repairs nature’s past damage and secures against threats of the future’. CTSG collaborates with others to plan, conduct, or evaluate any conservation programs that involve translocations in the wild, or releases arising from breeding, propagation, or headstarting. Through science, policy, guidance, training, action, and outreach, CTSG can help enable effective reintroductions, reinforcements, assisted colonization, or ecological replacements spanning all terrestrial, freshwater, or marine ecosystems.

www.iucn-ctsg.org/

Environment Agency - Abu Dhabi

Established in 1996, the Environment Agency - Abu Dhabi (EAD) is committed to protecting and enhancing air quality, groundwater as well as the biodiversity of our desert and marine ecosystem. By partnering with other government entities, the private sector, NGOs and global environmental agencies, we embrace international best practice, innovation and hard work to institute effective policy measures. We seek to raise environmental awareness, facilitate sustainable development and ensure environmental issues remain one of the top priorities of our national agenda.

www.ead.ae/

Calgary Zoo

The Calgary Zoo’s vision is to be Canada’s leader in wildlife conservation. In close alignment with IUCN, this vision is pursued through a mix of Canadian and global conservation initiatives regarding two strategic pillars: 1) conservation translocations, such as reintroductions, to avert species extinction and strengthen ecosystem function; and 2) community conservation to bring mutual and sustainable benefits for local livelihoods and biodiversity. The Calgary Zoo engages in collaborative partnerships around the world to develop the innovation and application of science-based solutions to achieve long-term benefits for conservation.

www.calgaryzoo.com/

Wildlife Reserves Singapore

Wildlife Reserves Singapore (WRS) is dedicated to the management of world-leading zoological institutions - Jurong Bird Park, Night Safari, River Safari and Singapore Zoo - that aim to inspire people to value and conserve biodiversity by providing meaningful and memorable wildlife experiences. A self-funded organization, WRS focuses on protecting biodiversity in Singapore and Southeast Asia through collaborations with like-minded partners, organizations and institutions. Each year, the four attractions welcome five million visitors.

www.wrs.com.sg/

The Aspinall Foundation

The Aspinall Foundation is a UK-based charity devoted to the conservation of endangered species and returning them to wild protected areas. Major achievements include the reintroduction of gorillas to the Batéké Plateau, the reinforcement of small isolated gibbon and langur populations in Java, the translocation of captive-born Eastern black rhinos and Southern cheetahs from the UK to protected reserves in Africa, and the implementation of a community-based species survival program for the Critically Endangered Greater bamboo lemur in Madagascar.

www.aspinallfoundation.org/

IUCN Species Survival Commission (SSC)

With over 8,000 members, the Species Survival Commission (SSC) is the largest of the six expert commissions of IUCN and enables IUCN to influence, encourage and assist societies to conserve biodiversity by building knowledge on the status and threats to species, providing advice, developing policies and guidelines, facilitating conservation planning, and catalyzing conservation action.

Members of SSC belong to one or more of the 140 Specialist Groups, Red List Authorities and Task Forces, each focusing on a taxonomic group (plants, fungi, mammals, birds, reptiles, amphibians, fishes and invertebrates), or a disciplinary issue, such as sustainable use and livelihoods, reintroduction of species, wildlife health, climate change and conservation planning.

www.iucn.org/theme/species/about/species-survival-commission

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Dr. Shaikha Al Dhaheri
Secretary General, Environment Agency - Abu Dhabi & IUCN Global Councillor for West Asia

Two years have passed, and I once again would like to present the 7th issue of the *Global conservation translocation perspectives* which has a total of 69 case studies covering a total of 78 species worldwide. The case studies are as varied as ever covering crayfish in Australia, desert fish in restricted waterbodies in Las Vegas, deer in China to large-scale rainforest restorations in Brazil. The Reintroduction Specialist Group has now had a name change to the Conservation Translocation Specialist Group which was timely as there are many new conservation actions which now fit under the "conservation translocations" umbrella.

In this issue plants cover 35% of the case studies, and I am glad to report that we have an example of a plant restoration project from the region specifically from Abu Dhabi, UAE. The White saxual is a species living on the extreme edge of its distribution range and is challenged by many factors such as increased aridity, and the Environment Agency - Abu Dhabi's (EAD) efforts have resulted in restoration of this species in its native habitat.

The EAD has also been working diligently on the iconic Scimitar-horned oryx reintroduction project in Chad which was comprehensively covered in the 2018 issue with currently 350 individuals in the wild. We have also released 50 Addax antelope and with Dama gazelle and Red-necked ostrich planned for the future. This will be a large-scale restoration of ecosystem function with these releases in Chad in the Sahel region of Africa.

Finally I would like to thank Pritpal Soorae for compiling these case studies and the support of partners such as the Calgary Zoo, Wildlife Reserves Singapore and The Aspinall Foundation who have come together to ensure best practices in species and ecosystem restoration are available for the wider conservation community.

Dr. Axel Moehrenschlager
Chair, Conservation Translocation Specialist Group, Calgary Zoo

This is a book about action. It is about saving species and ecosystems to yield profound benefits for nature and humanity. This book is also about courage. It is about amazing teams working together, sometimes against all odds, to make a difference. I am so grateful to my friend Pritpal for once again investing his passion, skill, and time to bring these stories into the limelight. Overarching analyses of previous case studies in leading scientific journals illustrate the collective power and potential of our approaches. Once again, I hope global efforts and successes on the pages to come inspire you as much as they inspire me.

Despite a 30-fold increase in the last 30 years, future projections call for even greater use of our techniques. Why? Because more species need help in a changing world, other conservation approaches are often insufficient on their own, and our science-based actions are increasingly effective. Building on a proud 30-year history as the Reintroduction Specialist Group, the recent Renaissance as the Conservation Translocation Specialist Group (CTSG) escalates our role to meet future needs and opportunities through….

- **Our Vision:** A world where courageous action repairs nature's past damage and secures against threats of the future.
- **Our Mission:** To empower responsible conservation translocations that save species, strengthen ecosystems, and benefit humanity.

Our new 10-year plan strives for even greater impacts felt in the oceans, deserts, grasslands, lakes, forests, and mountains of our world. With deep gratitude to the Calgary Zoo, Environment Agency of Abu Dhabi, Wildlife Reserves Singapore, The Aspinall Foundation, and the Species Survival Commission, I also invite others to come join this cause! Everyone can help. We need your help. Come share the joy of making a difference together…

Dr. Sonja Luz
Director, Conservation, Research and Veterinary, Wildlife Reserves Singapore

As modern zoos, conservation is what we do. Our work in the areas of animal care, education, public engagement, research and conservation give us the social mandate to operate. Hence, we believe that Wildlife Reserves Singapore (WRS) has a major responsibility as a world leading zoological institution to contribute towards species conservation, particularly in the region we operate and where incredible biodiversity exists.

With that, we are a proud supporter and partner to the IUCN SSC and the Conservation Translocation Specialist Group. Conservation translocations are key components of many conservation efforts and we congratulate the CTSG and all the contributors to this newest edition of the *Global conservation translocation perspectives*, for their valuable and impactful conservation contributions. WRS focuses its conservation efforts on the Southeast Asia region. We see an increasing need for conservation translocations, with many threatened species being displaced by human-wildlife conflict, habitat loss and over exploitation. We are particularly grateful for the CTSG who have provided guidelines and tools that have helped to address these pertinent issues in a structured and scientific manner. We hope the fantastic stories in this book will inspire many more conservation translocation activities and encourage new approaches to conservation translocations globally.

As threats to species show no sign of abating, zoo-based conservation institutions like WRS play an important role in protecting their future existence, especially under the concept of the “One Plan Approach”. Saving species cannot be done in isolation, and we are proud to be part of this community, and be able to contribute to the publication of this book. We look forward to more close collaborations with the CTSG. Together, we can protect wildlife and actively make a difference to the future of biodiversity and the preservation of animal species.

Tony King
Conservation and Reintroduction Coordinator, The Aspinall Foundation

It is a great pleasure to be involved in the seventh issue of the *Global conservation translocation perspectives* series, compiled with such dedication by Pritpal Soorae with contributions from conservationists from all around the world. I am very pleased that our own projects are included in this series.

We contributed one case study to the very first edition of the series back in 2008, concerning the reintroduction of Western gorillas to the Batéké Plateau region of Gabon and Congo in Central Africa. The project has continued to develop and flourish and has now seen over 70 gorillas released and over 30 births within the two re-established populations. In this edition we summarize similar projects for three primate species in Java, Indonesia, totaling 159 released primates in five population reinforcement projects. These highlight the benefits that the release of primates rescued from the illegal pet trade can have on isolated or locally-extirpated populations. They also demonstrate that zoo-born primates can be incorporated into such projects, but that zoo-born individuals may have lower survival and reproduction rates than rescued primates. Another case study highlights the successful welfare release in South Africa of a single Brown hyaena, born in a European zoo, illustrating that it can be possible to release zoo-born carnivores. Our final contributed case study showcases the return of Europe's largest land mammal, the European bison, to Romania. The European bison remains one of the surprisingly few examples of how zoo populations can bring species back from extinction in the wild.

The case studies in this series show us that habitats and populations can be restored when sufficient will and resources are put to the task. They offer hope and inspiration at a time when our planet needs action more urgently than ever before.

Dr. Jon Paul Rodriguez
Chair, Species Survival Commission (SSC)

Once again, our colleagues of the Conservation Translocations Specialist Group (CTSG) deliver a new volume of *Global conservation translocation perspectives*. Edited by Pritpal S. Soorae, the seventh edition increases the tally of case studies to 418. This issue includes 4 invertebrates, 6 fishes, 5 amphibians, 3 reptiles, 12 birds, 21 mammals and 27 plants, which includes multiple species in some case-studies. Jointly, the 69 studies cover all of IUCN's eight statutory regions, while an examination of their success rate indicates that only 4% of them are classified as failures, 24% were highly successful, 50% successful and 22% successful.

Since the publication of volume 6 in 2018, the group changed its name from Reintroduction Specialist Group, to Conservation Translocation Specialist Group, in recognition that reintroductions were a special case of a broader set of interventions, better characterized as conservation translocations. They span "any human-mediated translocation for conservation purposes," including conservation breeding, propagation, or head starting for release, reinforcement, ecological replacement, assisted colonization, rewilding and de-extinction. The case studies contained in volume 7 reflect this diversity of interventions, and include habitat restoration or protection, threat mitigation, partnerships with local communities, landowners, researchers and governments, and adaptive management. A complex portfolio or tools that seek to "to return species that have been lost regionally or globally from the wild."

What are the major lessons learned from this massive compilation? Are there common elements to successful projects? What must the conservation community avoid to prevent failure? How long does it take a project to begin generating positive results? Are there taxa that are comparatively "easier" than others? These are the kinds of questions that we can explore with the wealth of knowledge gathered by Soorae and his colleagues over the years. I would encourage a young and enthusiastic member of the CTSG to pursue such an analysis. There is enough

information for a Master's thesis or independent research project. I can imagine an influential publication in a major conservation journal, as an early step in the career of an emerging CTSG leader. Analysis of these data, combined with mining the knowledge of the SSC network of experts is sure to generate important results.

SSC is about evidence-based conservation, which is exactly what the seven volumes of *Global conservation translocation perspectives* are about. In two years or so, I would expect to see the next round of case studies to be ready for release. This is roughly the same amount of time required for the analysis of data already available. I look forward to the publication of a synthesis of past studies and the new cases at the same time.

There are very few examples of such a sustained research effort, and at least part of the explanation is the historical support of Environment Agency - Abu Dhabi, the strategic engagement of Calgary Zoo, and the participation of Singapore Zoo and The Aspinall Foundation, among others. My deepest gratitude to all for helping the SSC network advance species conservation around the world.

An overview and analysis of the reintroduction project case studies

Pritpal S. Soorae, Editor

Introduction

This is the 7th issue in the *Global conservation translocation perspectives* series and has been produced in the same standardized format as the previous six to maintain style and quality. The case studies are arranged in the following order: a) Introduction, b) Goals, c) Success Indicators, d) Project Summary, e) Major Difficulties Faced, f) Major Lessons Learned and g) Project outcome - with reasons for success or failure.

Case studies per issue

The following numbers of case studies have been collated for the last seven issues: 1) 2008 issue - 62 case studies, 2) 2010 issue - 72 case studies, 3) 2011 issue - 50 case studies, 4) 2013 issue - 52 case studies, 5) 2016 issue - 54 case studies, 6) 2018 issue - 59 case studies and 7) 2021 (this issue) - 69 case studies. This is a total of 418 case studies in all seven issues.

IUCN Statutory Regions

The IUCN Statutes have established a total of eight global regions for the purposes of its representation in council. The IUCN's "statutory regions" are a list of States by Region, as per article 16 and 17 of the Statutes and Regulation 36 of the Regulations.

All eight global regions are represented within these case studies and the numbers of case studies in the regions are as follows:

1. North America & Caribbean - 9 case studies
2. West Europe - 14 case studies
3. South & East Asia - 10 case studies
4. Oceania - 15 case studies
5. West Asia - 1 case studies
6. Africa - 3 case studies
7. Meso & South America - 18 case studies
8. East Europe, North & Central Asia - 8 case studies

There are 69 case studies with a total of 78 species as some case studies have multiple species.

Success/Failure of projects

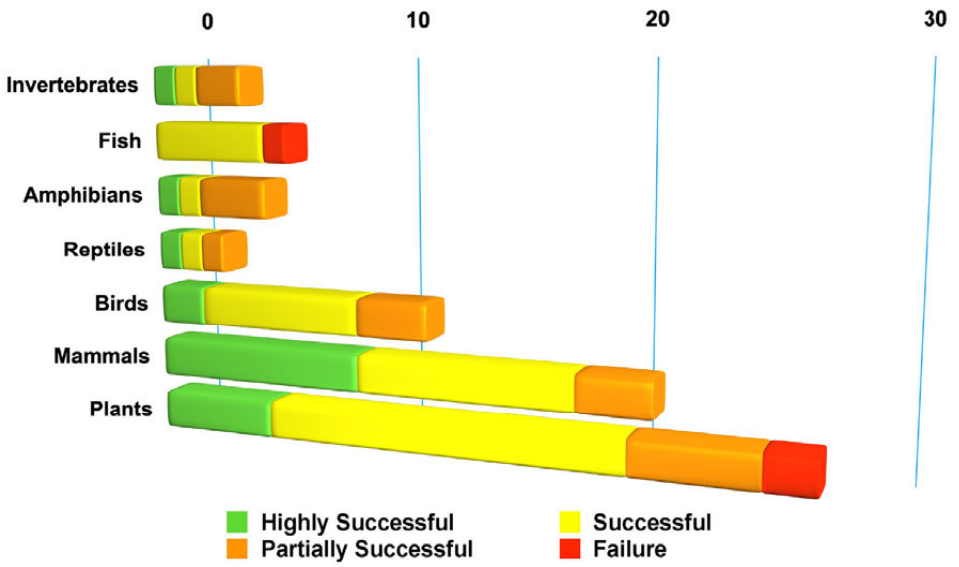
The projects presented here were ranked as Highly Successful, Successful, Partially Successful and Failure. Out of the 69 case studies, there were some cases of multiple rankings, as some projects had multi-species restorations. A total of 19 projects were Highly Successful (24%), 39 were Successful (50%), 17 were Partially Successful (22%) and 3 were listed as Failures (4%).

Success according to the taxa

An analysis was done to gauge the three different levels of success (highly successful, successful and partially successful) and failure against the seven major taxa i.e. invertebrates, fish, amphibians, reptiles, birds, mammals and plants as can be seen in figure 1.

As can be seen below the majority of case studies were covered in the following order - plants, mammals, birds, fish, amphibians, invertebrates and reptiles. Out of the seven major taxa only fish did not have a project ranked as Highly Successful. Successful projects were ranked in all 7 taxa. Only fish did not have a Partially Successful project. Only fish and plants had case studies ranked as a Failure.

Figure 1. Success / failure of projects according to major taxa



Translocation and habitat restoration to increase the field cricket population in Southern England

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Introduction

In the UK the Field cricket (*Gryllus campestris)*, is Vulnerable (Sutton, 2015) and fully protected under Schedule 5 of the Wildlife and Countryside Act 1981. It formerly occupied grassy heaths and chalk downland within Surrey, Sussex and Hampshire in an area bounded by the River Arun to the east, the Solent to the west, the River Wey to the north and the Isle of Wight to the south. During the 20th century it disappeared from most of its historic range, due to loss of heathland and downland resulting from changes in land use, including afforestation. Lack of disturbance by livestock and increased rates of succession on the remaining fragmented heaths reduced their suitability for Field crickets. By 1991, the UK population was confined to one site in West Sussex with fewer than 100 individuals and was expected to go extinct.

In 1991, the species was placed on English Nature's Species Recovery Program (SRP), and by 2007 it had been restored to four additional locations across Surrey, Sussex and Hampshire thanks to a program of captive-breeding and reintroductions (Pearce-Kelly, 2008). Here we report on work since 2008, including the current National Lottery Heritage funded Back from the Brink project.

Field cricket in burrow © Alex Hyde

Goals

- Maintain secure and self-sustaining populations on existing sites.
- Encourage population expansion through heathland restoration and habitat management.
- Establish new population of Field crickets through further reintroductions.
- Increase the robustness of isolated populations of Field crickets through translocations to adjacent areas, where required.

Success Indicators

- An expanding population at the indigenous site.
- Area of suitable habitat expanded on four sites and natural colonization supported through appropriate habitat management.
- Six additional populations established through reintroductions.
- New sub-population established at one site through within-site translocation.

Project Summary

Feasibility: The project was established in 1991 through English Nature's Species Recovery Program, once it was clear that the Field cricket was at risk of going extinct in the UK. The early stages of the project are detailed in Edwards *et al.*, 1996 and Pearce-Kelly, 2008. They involved instigating suitable habitat management at its indigenous site and establishing an *ex situ* breeding program and health screening protocol at ZSL. Nymphs were taken from the indigenous site and between1992 and 2007 the breeding program provided in excess of 17,000 late-instar nymphs for reintroductions. Ten sites in the Field cricket's former range were identified and managed appropriately and captive-bred nymphs were released at seven of these. Four of the seven field colonies established with the *ex situ* bred crickets were still extant in 2007, the longest of which was shown to have persisted to the 8th generation.

Catching Field crickets at Lord's Piece in April 2018
© Jane Sears, RSPB

The presence of gregarine parasites in the captive population was confirmed in 1996 and 1997, and releases of captive-bred Field crickets ceased. (Subsequently the parasites were found to be present in wild populations and are not considered harmful). By this time both the indigenous population and some of the reintroduced

populations were considered sufficiently robust to withstand small numbers of nymphs being taken for translocations, under license from Natural England. Further sites have been identified including two RSPB reserves on which conifer plantation was being removed to restore grassy heathland.

Field cricket habitat © Alex Hyde

Implementation: Releases to new sites commenced in 2010 with a protocol of releasing around 12 late instar nymphs of equal sex rations in April of each year. Donor sites are chosen with the aim of being as local to the release sites as possible, depending on the size of the available population. Three years of releases was found to be most successful although a crash in numbers at the donor sites in 2012 led to some sites only having two years of releases. Crickets are caught by hand by trained staff and volunteers (under license) and transported individually in plastic bags with some moss and grass for cover. They are held in the bags for no longer than one hour.

Arable reversion to heathland has been undertaken by a landowner on a private estate adjacent to the indigenous site, providing an extensive area of suitable habitat for a meta-population to establish. Field crickets have naturally colonized this, by dispersing from the indigenous site, and have managed to walk across a small road. At another location the crickets have expanded from a release site on private land onto an adjacent golf course which is now being managed appropriately.

At one location where a population was established on an isolated part of the reserve, the Field crickets have been assisted in their colonization of land to the north of the release site through in-population translocation. Given the presence of barriers such as trees, houses and a busy road it was considered unlikely that they would naturally colonize this area of the reserve.

Post-release monitoring: Monitoring of adult calling males is carried out annually at both the indigenous and reintroduction sites in May through to early July depending on the season. Although whole colony counts can be attempted during the initial stages of reintroduction, once the population has become

established, these are generally replaced with fixed transect counts. Transects aim to cover as much of the suitable habitat as possible and map all the singing male crickets heard.

At least two visits are made to each site:

1. Early visit at the start of the season to ascertain if crickets are singing and where they are likely to be.
2. Main visit at peak season. This figure is usually the maxima for the year.
3. In some cases a third visit is made to ascertain the occupied range.

Visits are made during the afternoon or evening on still, warm and dry days of at least 17^{o}C. Monitoring is aborted if the weather becomes adverse, and singing crickets are marked on maps/aerial photographs showing locations of trees and paths. Most of the monitoring is carried out by a paid specialist but increasingly volunteers are being trained to assist, under the Back from the Brink project. Reports are produced annually (Edwards, 2018).

Major difficulties faced

- Shortage of donor stock in some years due to poor weather and population crash.
- Boom and bust cycles of colonization.
- Lack of management of some sites leading to bracken dominance and loss of Field crickets.
- Inability to graze a nature reserve which is a Common due to objections to fencing.
- Lack of funding for specialist surveyors and problems with accurate monitoring.

Major lessons learned

- Value of having multiple reintroduction sites so if some fail the population can persist.
- Value of including some protected sites in nature conservation management where long-term habitat management is more assured.
- Value of long-term standardized monitoring to track broad population trends.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Strength and endurance of the long-term partnership between Natural England, NGO conservation organizations, entomological specialists and private landowners.
- Continuity of the Species Recovery Program (which has been much reduced over the years and could be threatened by future government funding cuts).
- Willingness of some large private estates to engage in heathland restoration and the conservation of this rare and threatened species.
- The awareness raising generated through Back from the Brink and the support of volunteers and members of the public.

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Reintroduction to re-establish locally extirpated populations of the Murray crayfish - second largest freshwater crayfish in the world - in S.E. Australia

Nick S. Whiterod[1], Martin Asmus[2], Sylvia Zukowski[1], Dean Gilligan[3] & Trevor Daly[3]

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Introduction

The Murray crayfish (*Euastacus armatus*) is a large (maximum documented occipital carapace length ~174 mm), long-lived (up to 25 years), late maturing (~8 - 9 years) freshwater crayfish endemic to the Murray-Darling Basin in south-eastern Australia. The species was once broadly distributed across ~12,500 km of lotic waterways; but has experienced substantial decline in range and abundance over the past 50 years due to river regulation, pesticides and pollutants, habitat degradation and harvest pressure and blackwater disturbance. Most recently over 2010 - 2011, the species was significantly impacted by extreme blackwater disturbance, with an 81% population loss in an affected reach of the Murray River. The magnitude of the impact prompted a threatened species listing in New South Wales and substantial amendment to the recreational harvest of the species, including area closures, implementation of a slot limit and a reduction in bag and daily limits.

Murray crayfish © Nick Whiterod

The species is listed internationally as Data Deficient (under International Union for Conservation of Nature Red List of Threatened Species 2010) and whilst not

nationally listed (under the Australian Environment Protection and Biodiversity Conservation Act 1999), it has threatened status under regional jurisdictions, so proactive management actions, such as conservation translocations, are prudent.

Goals

- Develop a feasible translocation strategy for the species.
- Demonstrate a positive trajectory of recovery (based on short-, medium- and long-term indicators of success) for the reintroduced population.
- Re-establish a self-sustaining and resilient reintroduced population.

Success Indicators

- A robust translocation strategy developed.
- *Short-term:* Detection of crayfish and presence of wild reproduction in the reintroduced population.
- *Medium-term:* Increasing relative abundance and presence of juveniles over time in the reintroduced population.
- *Long-term:* Demonstrate reintroduced population is genetically robust and maintain broad population structure.

Project Summary

Feasibility: Previous stocking for fisheries purposes (in the early 1900s) led to the establishment of populations with low levels of genetic diversity outside its natural range. To avoid a similar outcome, the present translocation relied on a robust multi-faceted strategy informed by contemporary research and monitoring. An important initial decision was reached to reintroduce into areas that supported the species immediately prior to the blackwater disturbance as minimal recovery had been observed. The rationale being that these areas would support the reintroduced population, but due to restricted dispersal and population fragmentation, natural recolonization was unlikely. As captive-breeding or head-starting has not been established, wild-to-wild reintroduction was selected. Both the potential reintroduction site and source populations were contained within a large interbreeding population revealed by genetic analyses - thus the risk of outbreeding depression was deemed minimal.

Population modelling was used to assess the optimal number and elements of the population (e.g. juveniles, females with eggs or all collected individuals) to be released. It was concluded, in part for pragmatic reasons, that releasing 200 individuals, annually for five years would not only be feasible but also maximize the likelihood of achieving the reintroduction goals and minimize impacts to source populations. The modelling also revealed that successful establishment of the reintroduced population would take time (e.g. decades), due to the life history of the species, which was important to convey to project stakeholders. The support of these stakeholders - including fisheries managers and compliance officers along with recreational fishers and environmental managers - was

another aspect that made the reintroduction feasible. Without this support, it was unlikely that there would be adequate funding and resources or protection of the reintroduced population.

Implementation: Annual reintroductions commenced in 2017 and will continue until 2021. Within the area that previously supported the species, a 2 km reintroduction site was selected on the basis of targeted surveys (to confirm absence) and assessment of prevailing habitat characteristics, water quality as well as logistical considerations (e.g. ability to access the site for releases). It was necessary to obtain permits to translocate the species, which assess the benefits of the reintroduction against any likely risks. Prior to reintroductions each year, an appropriate source population was selected based on knowledge of population status and proximity to the reintroduction site - the location of the source populations has changed each year so as to minimize risks. From source populations each year, 200 individuals were collected using standard sampling methods (see below) with the specific information recorded (weight, length, sex and for females the stage of maturity and presence of eggs) and a genetic sample obtained, from all collected individuals.

Due to the agonistic nature of the species, rubber rings were temporarily placed on the chelae of collected crayfish. The crayfish were transported in large insulated tanks (with wetted hessian provided as moisture and structure) before being released in groups across the reintroduction site. To date, 600 crayfish have been released (200 crayfish annually over 2017 - 2019), with collected individuals ranging from 36 - 154 mm OCL (29 - 1,480 g) and including 87 females with eggs, which represents the release of ~88,000 eggs (based on fecundity estimates), which may contribute additional individuals to the reintroduced population. There has been no mortality observed during the collection, transport and release.

Post-release monitoring: In addition to immediately prior to the reintroduction (one month before), the reintroduction site has been monitored four times (3, 12, 15, 24 and 27 months after the initial reintroduction) during the Austral winter or early spring, when the species is known to be most active. The monitoring follows a standardized protocol that is employed for other monitoring of the species with demographic information obtained

Catching a recapture Murray crayfish
© Nick Whiterod

Murray crayfish river habitat © Nick Whiterod

from all sampled Murray crayfish, including weight, length and sex, and for females the stage of maturity and presence of eggs.

In total, 61 Murray crayfish, ranging between 63 - 125 mm (106 - 1,042 g), have been detected (10.1% recapture rate). From one month before to three months after, the relative abundance increased from 0 - 0.03 ± 0.02 crayfish net^{-1} h^{-1} and remaining consistent (0.03 ± 0.02 crayfish net^{-1} h^{-1}) after 12 months. Following the second release event, relative abundance increased (0.18 ± 0.04 crayfish net^{-1} h^{-1} at 12 months after; 0.23 ± 0.05 crayfish net^{-1} h^{-1} at 24 months after) before dropping to 0.11 ± 0.03 crayfish net^{-1} h^{-1} at 27 months after the initial release event. This monitoring will continue regularly (i.e. 36, 39, 48 and 51 months after) over the duration of the reintroductions. Long-term goals will be assessed (funding dependent) following the five year reintroduction period using mark-recapture sampling to estimate population size and genetic analyses (funding dependent) to investigate of levels of genetic diversity and effective population size to provide long-term reintroduction goals.

Major difficulties faced

- It was important to have a sound, defensible framework to implement and assess the reintroduction.
- Strong support by multiple stakeholders has been imperative.
- Engagement of recreational fishers was useful to achieve broader support for the reintroduction.
- Ongoing funding to assess the long-term reintroduction goals may be a difficulty.

Major lessons learned

- There was an initial challenge to determine the most appropriate strategy as the species has not been subject to any form of assisted movement (e.g. fisheries stocking) for almost 100 years.
- As wild-to-wild reintroductions were implemented caution was required in the selection of sites for source individuals.
- Balancing (potentially) incongruent conservation and fisheries objectives.

- Ensuring ongoing funding and resources for the reintroduction.

Success of project

Highly Successful	Successful	Partially Successful	Failure

At this stage, the project is tracking along against the reintroduction goals but is considered a partial success given the long-term nature of the project. Following completion of the five years of reintroductions, it is hoped the project will be evaluated as a success.

Reason(s) for success:

- It was important to have a sound, defensible framework to implement and assess the reintroduction.
- Strong collaboration and support by multiple partners and stakeholders was imperative.
- Engagement of recreational fishers was useful to achieve broader support for the reintroduction.

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The restoration of the red-spotted apollo butterfly to Samcheok City, Gangwon-do, Republic of Korea

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Introduction

The Red-spotted apollo butterfly (*Parnassius bremeri*), is a high-altitude butterfly which is found in Russia, Korea and China. It is a member of the Snow apollo, genus *Parnassius* of the Swallowtail (Papilionidae) family (Omoto *et al.*, 2004). The butterfly in genus *Parnassius* has been designated as the most endangered insect species around the world and in this regard Korean government also designated *P. bremeri* as an Endangered Species Level I since 1989 (www.nibr.go.kr). Furthermore, genus *Parnassius* was classified as Vulnerable taxon to be extinct by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List (www.iucnredlist.org), and listed on Appendix II of the Convention on International Trade in Endangered Species (CITES). The restoration plan to bring Red-spotted apollo butterflies from Holoce Ecological Conservation Research Institute (HECRI), *ex situ* Insect Conservation Institute, to Samcheok started in 2011. Samcheok City is located in Gangwon Province, Korea, which is mainly a highland mountain area and the original habitat of the Red-spotted Apollo butterfly. Until 2010, numbers of Red-spotted Apollo butterflies in Samcheok have rapidly decreased by 10% to 32 individuals. This restoration project was carried out by releasing

Red-spotted apollo butterfly

Recording butterfly numbers in the field

240 individuals from the captive-breeding program of HECRI.

Goals

- Identifying Red-spotted apollo butterfly's life cycle exactly for successful restoration.
- Restoring Red-spotted apollo butterfly to the Samcheok wild habitats.
- Establishing suitable and sustainable habitat in Samcheok for Red-spotted apollo butterfly by planting host plants and clearing timber to make the canopy open.
- Promoting public access to an endangered species and Red-spotted apollo butterfly through newspaper columns, frequent broadcasting and social networking services such as YouTube, Blogs and Facebook, etc.
- Expanding the project to encompass all endangered species and conservation biology in Korea.

Success Indicators

- Exact identification of Red-spotted apollo butterfly's ecology and physiology which is quite different from well-known knowledge.
- Development of captive-breeding to rear large numbers of Red-spotted apollo butterfly through their exact physiology.
- Greater awareness of Red-spotted apollo butterfly and their ecological and economic importance.
- Populations disperse beyond release sites to additional available habitat.
- Manipulate suitable and sustainable habitat of the Red-spotted apollo butterfly.

Project Summary

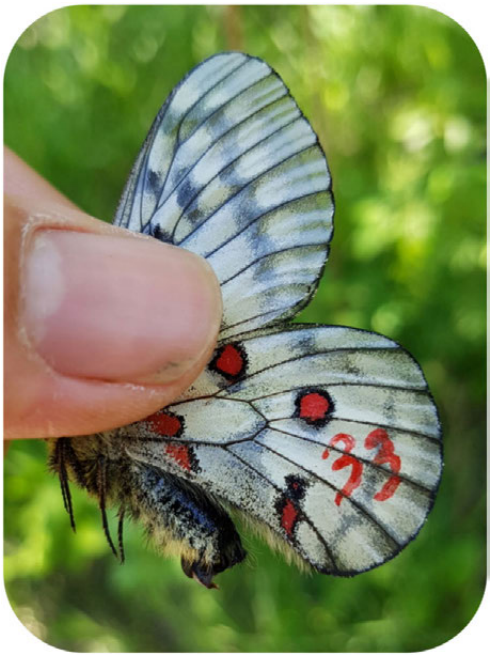
Feasibility: Red-spotted apollo butterfly is a representative Endangered Species Level I in Korea. Ecological research by Wonju Local Environmental Administration and HECRI revealed that the Red-spotted apollo butterfly occurred in small populations across the Korean peninsula and in most of its former habitats has become totally extinct in the wild. The Samcheok population also has been under threat of extinction. Despite of the vulnerability of *P. bremeri*, there is no any exact biological information around the world. We observed all stages of the life cycle of *P. bremeri* under natural conditions after the establishment of a rearing system. The total developmental period for a generation was

Overview of butterfly release site

approximately one year. It has a unique summer diapause period of more than six months (~194 days) extending from summer to winter. Moreover, the egg contained the pharate 1st instar stage (live in the egg), and the period of embryogenesis through the pharate 1st instar was ~15.7 days. Additionally, the 1st instar underwent development at low temperatures, ranging from -20.5 - 5.2°C (-4.3°C on average). The unique, biological characteristics of *P. bremeri* provide valuable information for understanding the mechanism of adaptation to low temperatures and for the conservation of this endangered species. Understanding its biology and physiology are essential for establishing an appropriate conservation system. The Red-spotted apollo butterfly require very specific conditions such as host plant and geographic characteristics, open landscapes on forest steppe as well as slopes up to the alpine zone (1,500 m). Samcheok which used to be original habitat is mainly is composed of over 800 m highland mountain area and was the best site to release Red-spotted apollo butterfly.

Implementation: Four pairs of adults were initially collected by HECRI, *ex situ* Conservation Institute, from Samcheok under the permission of Wonju Local Environmental Administration in June 2005. A whole rearing procedure of *P. bremeri* was performed under field conditions at HECRI. Briefly, eggs laid were collected manually attached to the fallen leaves and kept in field conditions for 180 days in a double net (0.1 x 0.3 mm mesh) to protect from parasitoids and/or predator attacks. The newly hatched larvae were then transferred to a young larval cage (acrylic cages, 40 x 50 x 70 cm) and supplemented with the host plant, *Sedum kamtschaticum*. At 4th instar (late April), the larvae were separated into 30 individuals and kept in a metal cage (71 x 51 x 88 cm covered with 1 x 1 mm metal mesh). Emerged adults were transferred to mating cages (270 x 190 x 220 cm covered with 1 x 1 mm metal mesh) and provided *Cirisium japonicum* as a feed source for *P. bremeri*. According to this process we identified that eggs of Red-spotted apollo butterfly hatch during December and young larvae grow during winter and early spring.

A few sites in Samcheok were designed and manipulated as sustainable habitat for Red-spotted apollo butterfly by planting host plant and clearing timber to receive more sunlight continuously. A total of 240 individuals of *P. bremeri* have been released in Samcheok from 2011 - 2015 (20, 40, 60, 60, and 60 individuals, respectively). More precise knowledge on how to maintain populations and



Marking of captured individuals

sustainable habitat could be used as a basis to establish effective conservation strategies. However more important key to the success of restoration program to date has been the harmonious collaboration of scientists, government and local residents. Considerable publicity through newspaper column, frequent broadcasting and social networking service has played a major role.

Post-release monitoring: Monitoring was carried out to find the estimation of the population size of Red-spotted apollo butterfly by using Mark-Release-Recapture (MRR) that has a 95% reliability level. Ten times (26th May, 27th May, 28th May, 29th May, 30th May, 31st May, 1st June, 2nd June, 3rd June, and 5th June 2016) collection was carried between 09:00 - 15:00 hours by five HECRI researchers. During the monitoring period, the picture of every captured species was taken by the smart phone camera embedded GPS system. Patches were classified by marking fore-wings or hind-wings with different colors mainly red and blue. During the monitoring period, 421 individuals (female: 188, male: 233) were captured and 177 individuals (female: 89, male: 88) were recaptured. The results showed that the meta-population of the release site is from 125 individuals (minimum) to 1,844 individuals (maximum) and recapture rates were 42%. Average of survival day was 3.59 and maximum survival day 11.

This species moved outside of the patch but came back inside of the patch during the monitoring period. The migration tendency seems to be sedentary but one individual was recaptured 5.6 km from the first collected site. This suggests that even though they have a sedentary tendency, they try to go out to better or new habitat. According to MRR estimation, restoration seemed to work successfully so far and the population size is stable. The heterozygous diversity showed almost 99% that means no inbreeding depression. Colonies have spread naturally to other sites and become established. This indicates that dispersal, habitat use and abundance has been successful over time.

Major difficulties faced

- Lack of budgeting for sustaining staff and captive-breeding system.
- Captive breeding of Red-spotted apollo butterfly is a labour-intensive

project, which may lead to no co-workers.

- An anthropogenic or natural removal of local habitat patches may prevent seasonal migration of Red-Spotted apollo butterfly according to host plants, which may result in collapse of local populations.
- A global warming due to climate change may accelerate the reduction of *P. bremeri*.

Major lessons learned

- Identification of the species' life cycle exactly including ecology and physiology led to the ultimate success of the restoration plan.
- Governmental budget should be sufficient for sustainable management. Successful start of rearing system is important, but more important is to keep greater numbers of individuals for future releases.
- Practical, executive, professional conservation institute and passionate researchers should be combined with local volunteers to make progress. Accessible technical volunteers can support restoration program.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Identification of the species' ecology and physiology lead to a high success of the restoration plan. Eggs of the Red-spotted apollo butterfly hatch during December and immature caterpillars grow during winter and early spring.
- Preserving method to manually attach collected eggs of pharate 1st instar to the sterilized oak tree leaves was carried successfully for 190 days.
- Collected eggs attached to the fallen leaves were kept in double net (0.1 × 0.3 mm mesh) cages to protect from any attacks of parasitoids and/or predators. By using a double net cage, we could increase the survival rate.
- Developed adults were transferred to mating cages (270 × 190 × 220 cm, covered with 1 ×1 mm metal mesh) for oviposition. Mating cages ensured all eggs laid were secure.
- Annual inter-breeding between cages and field individuals for biodiversity was carried out.

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Reintroduction of the scarce fritillary butterfly in the Czech Republic

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Introduction

The Scarce fritillary butterfly (*Euphydryas maturna*) is an endangered butterfly species which is classified as a Data Deficient species according to the IUCN Red List and as a vulnerable species in the European Red List of Butterflies. Its situation in the Czech Republic is even worse hence it is classified as Critically Endangered in the Red List of Invertebrates in the Czech Republic. The Action Plan for the Scarce fritillary in the Czech Republic has been implemented since 2010. As there is only one surviving population of this species in the Czech Republic and this species vanished from >95% of formerly occupied area, the return of the Scarce fritillary to some historical localities is one of the main goals in the Action Plan. Currently the Libický luh National Nature Reserve, where the species occurred until 1990s, has been chosen as the most suitable place and the reintroduction attempts started there in 2018. Although the preliminary results seem to be good it is yet too early for proper evaluation.

Goals

- To establish a captive-breeding program for the Scarce fritillary butterfly.
- *Medium-term goal:* To reintroduce the Scarce fritillary to the Libický luh National Nature Reserve.
- *Long-term goal:* To restore 3 - 5 former localities in the Polabí lowland region.

Success Indicators

- Confirmed successful breeding by monitoring the number of silken nests, which are

Scarce fritillary butterfly

made by the gregarious caterpillars.

- Increasing the reintroduced population to >500 butterflies.

Project Summary

Feasibility: The Scarce fritillary is an endangered forest butterfly whose host plant is the European ash (*Fraxinus excelsior*). As its host plant is very common, it is endangered because it inhabits only woodlands with a low canopy cover, a type of habitat which nearly disappeared in Central Europe. After the disappearance of old growth forest, traditional types of forest management, such as coppicing, were able to provide a large area of suitable habitat. In the first half of the 20th century the Scarce fritillary still occurred in many localities in Central Bohemia (Polabí lowland), Central and South Moravia. However coppicing was abandoned in the second half of 20th century and since then the Scarce fritillary has disappeared from almost all localities. Currently only the population in the Dománovický les Nature Reserve in the Polabí area persists.

To achieve a successful reintroduction it is essential to choose the most suitable localities. These localities are mentioned in the species Action Plan and they must include sufficient number of young Ash trees (the females do not lay egg on old trees) and suitable vegetation. All these localities are now too dark, so the reintroduction cannot start without previous habitat management. At first it is necessary to lower the canopy cover (0.4 - 0.6 is optimum) or to create as many small forest clearings as possible. It is also necessary to have enough nectar sources for the imagoes.

Implementation: The easiest way of reintroduction is to transfer sufficient number of individuals from another locality. However we were not able to use this strategy, because the number of imagoes at the Dománovický les Nature Reserve was still too low and we did not want to endanger the only surviving population. Therefore, captive-breeding attempts started in 2016. Although the breeding of this species in captivity is very difficult it was finally successful in 2017. The major advantage is that we needed only four egg clutches (~2% from the donor population) for starting the breeding in 2016 and now we can obtain large number of caterpillars each year. Under *in situ* conditions only about two caterpillars from each 200 - 400 are able to become imago (~1% success rate), but in optimal *ex situ* conditions we are able to reach >80% success rate.

The reintroduction of the Scarce fritillary started in 2018 in the Libický luh Nature Reserve. In total, 200 caterpillars were released in April 2018, and 3,000 were released in July 2018 and another 750 in April 2019. We chose two strategies - releasing the larvae in July (before hibernation) and releasing the larvae in April (after hibernations, shortly before pupal stage). It is not worth to release imagoes because they have bindings to the place were they hatched and released imagoes can be confused and leave the locality. In the summer of 2019, 26 egg clutches were found, which proved successful breeding at the locality and confirmed successful reintroduction attempts. The supplementations will continue in 2020 and 2021.

Post-release monitoring: After releasing the larvae the locality is visited several times during the end of May and June to confirm the presence of imagoes, and in June - July to monitor number of larvae nests. The monitoring was unsuccessful in 2018 which either meant there were no imagoes and reproduction in 2018 or the number was so low that we failed to confirm their presence. The monitoring scheme is derived from the monitoring which takes place in the Dománovický les Nature Reserve. Probably the most useful method is the calculating of larvae nests, because skilled person can find them quite easily and there is only very small chance that a significant number can be overlooked. Simple monitoring of the imagoes is problematic, because they can spend a lot of time in treetops and can be quite elusive. Every 2 - 4 years we use the mark-recapture method to calculate the exact number of butterflies. This method has not been adapted in the Libický luh Nature Reserve yet, but it is planned in the coming years.

Butterfly habitat in Domanovice forest
© Antonín Krása

Finally, we confirmed the presence of imagoes and reproduction both in 2019. It means that conditions in the Libický luh Nature Reserve for these species are good enough for successful reproduction, however it is too early for a proper evaluation.

Major difficulties faced

- Spreading of *Hymenoscyphus fraxineus* fungus which kills Ash trees.
- Ensuring finances for proper management and monitoring at new localities in the future.
- Strong resistance of foresters to coppicing, the most effective method of habitat management.

Major lessons learned

- Captive-breeding is absolutely essential for ongoing reintroductions.
- Even though coppicing is probably the best method there are more ways to achieve suitable habitat. It is only necessary to obtain sufficient area of forest with low canopy cover, enough young ash trees and suitable vegetation.
- It is better to release larvae than imagoes.

- There must be a long-term management conception for the chosen locality.

Success of project

Highly Successful	Successful	Partially Successful*	Failure

*** - Successful breeding was confirmed but numbers of egg clutches and larvae nest is too small yet.**

Reason(s) for success:

- The reintroduction has just started, so impossible to evaluate it properly. Maybe the results are successful or even highly successful in fact, but we will know it in the following years.

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Conservation introductions of the malanda rainbowfish in the wet tropics bioregion, Australia

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Introduction

The Malanda rainbowfish (*Melanotaenia* sp.) is a recently identified species from the upper reaches of the North Johnstone River in the Wet Tropics Bioregion, northern Australia (Unmack *et al.*, 2016). Before European settlement this area consisted of dense rainforest, but now consists primarily of exotic grassland used for dairy grazing with fragments of remnant rainforest. This land use change has resulted in most stream habitats changing from narrow, cooler, fast flowing, rocky-bottomed creeks (still found in some of the rainforest remnants) to wide, exposed, slow flowing, mud bottomed creeks choked with Para grass. Recent intensive sampling has documented a decline in the distribution of the species, and identified hybridization with Eastern rainbowfish (*Melanotaenia splendida splendida*) as a key threatening process (facilitated by habitat and climate change). As a recently identified unique species, the Malanda rainbowfish is yet to be assigned a formal Australian federal conservation listing. However, the Australian Society for Fish Biology listed the species as Critically Endangered in 2016, following IUCN criteria. To guard against

Malanda rainbowfish (male) © Keith Martin

Malanda rainbowfish habitat © Keith Martin

extinction several planned introductions occurred into two tributaries of the North Johnstone River and several local farm dams within the catchment. The tributaries, Ithaca River and Mungalli Creek each have high downstream waterfalls which prevent the upstream migration of Eastern rainbowfish.

Goals

- Establish several populations in farm dams as artificial refuges which separately conserve the genetic diversity of remaining natural populations.
- Establish a translocated riverine population protected from introgressive hybridization with Eastern rainbowfish.
- Ensure the allelic diversity of new populations represents the allelic diversity of the founding populations.
- Demonstrate that conservation actions for smaller species do not necessarily require large budgets and that interest groups in the aquarium hobby can substantially contribute to successful conservation actions.
- Demonstrate that without basic monitoring of species with restricted ranges some species will be lost before decline can be detected and prevented.

Success Indicators

- Released fish survive long enough to reproduce.
- Progeny recruitment in the new habitat.
- Introduced populations increase in abundance.
- Riverine population spreads to occupy the full extent of its accessible range.
- Genetic monitoring detects good allelic representation of source populations.

Project Summary

Feasibility: The objective was to prevent the extinction of the rapidly declining Malanda rainbowfish. The species is not commercially important but is becoming appreciated by aquarists around the world (e.g. Allen, 1995). A larger growing generalist species, the Eastern rainbowfish is gradually progressing upstream, invading the range of Malanda rainbowfish and replacing them via introgressive hybridization (Unmack *et al.*, 2016). This is the primary immediate threat to

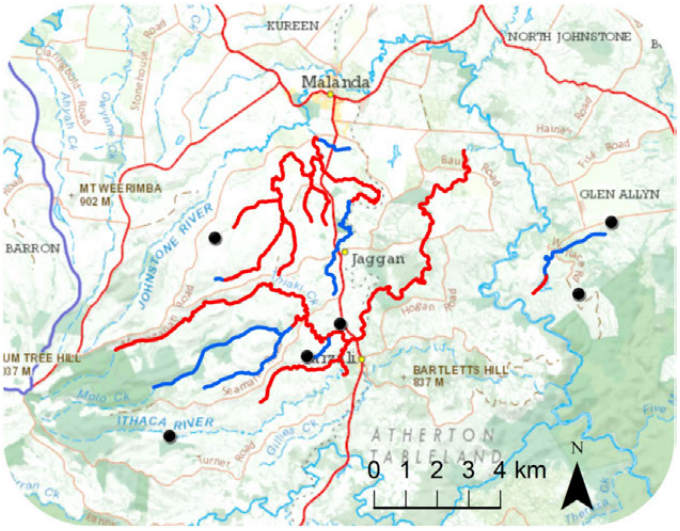
Malanda rainbowfish. Consequently, translocating Malanda rainbowfish into discrete waterbodies devoid of Eastern rainbowfish continues to be a priority since eradicating Eastern rainbowfish (and preventing introgression) is typically not feasible. Fortunately, the Ithaca River - a tributary of the North Johnstone River has two waterfalls which prevented the upstream dispersal of Eastern rainbowfish. In addition, farm dams are common in the area and few contain rainbowfish thus they were available as translocation sites, with this refuge approach being shown as an important part of the conservation efforts for species in a similar critical situation (Hammer *et al.*, 2013). All of this work took place on private land so we were fortunate that landholders were willing to facilitate the conservation of this species.

Implementation: Intensive sampling undertaken between 2014 and 2018 identified six remaining populations of Malanda rainbowfish, three in various parts of Williams Creek, an unnamed tributary to Molo Creek, in several instream dams on an unnamed tributary of Thiaki Creek and an unnamed creek (referred to as Wallace Road). One Williams Creek population is on the verge of extirpation while a major population from the mid Ithaca River and its tributary Thiaki Creek were largely extirpated by 2014.

In November 2016, 780 Malanda rainbowfish were moved to six new homes in each of the three catchments identified at that time. The upper Ithaca River received 300 fish, consisting of an equal number of fish from all three wild populations identified at the time (upper Molo Creek tributary, two Williams Creek populations and Wallace Road). The upper Ithaca River was deemed the best release site due to the presence of two large waterfalls which have prevented rainbowfish from moving upstream. Five dams were stocked, each with fish from their local catchment. Two dams on the creeks immediately south (unnamed) and north (Brodie Creek) of Wallace Road were stocked with 50 fish each from the Wallace Road population. One dam on a tributary of Williams Creek West Branch received 100 fish from upper Williams Creek West Branch. Two dams by Thiaki Creek were also stocked.

One dam received 100 fish sourced from upper Molo Creek tributary, the second much larger dam received 180 fish, 100 from Molo Creek tributary and 80 from the small creek immediately below the dam wall. In May 2019, 400 fish, consisting of 100 fish from each of four populations at Molo Creek tributary, Thiaki Creek tributary, Williams

Collection of Malanda rainbowfish



Known distribution of rainbowfish populations 1) red lines indicate likely former distribution, 2) blue lines indicate known distribution as of May 2019 & 3) black dots represent November 2016 release sites
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Creek East Branch and Wallace Road were translocated to Mungalli Creek above a major waterfall.

Post-release monitoring: Since translocation, there have been two monitoring events for the Ithaca River and farm dam populations. Fish were sampled using a seine net and box traps. Four months after release in March 2017 juvenile Malanda rainbowfish were detected in Ithaca River. Fish had moved approximately 100 m downstream since their release. By May 2019 Malanda rainbowfish were detected up to 1.3 km downstream. Releases into farm dams have been difficult to assess as they are difficult to sample due to dense aquatic vegetation and reduced water clarity. In May 2019, fish were found in three of the dams (one of which had an existing population that we had been unable to catch in our first attempts), one dam had no evidence of rainbowfish and one dam remains to be sampled. To some degree, substantial depth and soft benthos compromises access and thorough sampling to definitely conclude absence of rainbowfish from these dams.

Major difficulties faced

- Initial confusion regarding taxonomy and identification due to increasing introgression throughout the range of the species.
- It was necessary to DNA sequence all populations prior to translocation to ensure that only pure fish were translocated.
- All remnant populations and translocation sites only occur on private property.
- Lack of a formal description for the species and lack of any formal conservation listing precluded it from Government funding, so the project relied on a small amount of crowd funding and opportunism.
- Limited suitable translocation sites hindered conservation options.

Major lessons learned

- Replacement through species invasion and introgression can occur rapidly.
- Conservation of small-bodied fishes can be done on a small budget, such as one provided by crowd funding (but relies on time and availability of committed individuals and groups).
- Because there is no monitoring undertaken for many range restricted Australian freshwater fishes, the ongoing decline of Malanda rainbowfish was only noticed fortuitously.
- On ground actions are critical in the face of rapid environmental change.
- Action and involvement by community groups can set the example for monitoring and conservation, and pave the way for improved government funding and response.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Ithaca population is abundant and gradually expanding downstream.
- Some farm dam populations have persisted.
- Difficult to determine how well farm dam populations are doing.
- Land holders have been enthusiastically supportive of Malanda rainbowfish conservation.
- Various aquarium clubs and individuals from around the world have supported crowd funding efforts, without which the conservation work would not have been possible.

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Conservation breeding and reintroduction of pygmy perches in the lower Murray-Darling Basin, Australia: two similar species, two contrasting outcomes

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Introduction

The Southern pygmy perch (SPP) (*Nannoperca australis*) and the Yarra pygmy perch (YPP) (*N. obscura*) are freshwater fishes of the family Percichthyidae found in southeastern Australia, including in the Murray-Darling Basin (MDB). These small (up to 85 mm) fishes live in small groups on the vegetated margins of slow flowing habitat, such as streams and wetlands. Extended droughts and human-induced environmental degradation have caused unprecedented decline of aquatic biodiversity in the MDB. This is epitomized in the current conservation status of pygmy perches in the Basin. The SPP lineage has experienced large-scale extinctions from most of the middle MDB, and survives now only in very small population fragments mainly in tributaries of the upper Murray River and in pockets of the lowermost reaches of the basin. The MDB YPP lineage, originally restricted to the lower reaches of the basin, has not been detected in the wild since 2015 and in 2019 it was considered extinct. The SPP is listed as critically endangered in South Australia, threatened in Victoria and endangered in New South Wales. The YPP is listed as Endangered by the IUCN and Vulnerable nationally in Australia.

Goals

- Implement conservation breeding and reintroduction programs for the two pygmy perch species.
- Use genetic approaches to establish breeding groups that prevent inbreeding in captivity.
- Reintroduce the first generation of captive-born pygmy perches in the lower MDB.
- Assess the survival and breeding of released individuals in the wild (short-term) and carry out targeted monitoring (long-term).
- Implement novel conservation genomic programs to assess adaptive potential of pygmy perches and inform conservation management.

Yarra pygmy perch (*left*) & Southern pygmy perch (*right*) © Michael Hammer

Success Indicators

- Robust conservation breeding and reintroduction programs implemented.
- Minimized inbreeding and maximized the maintenance of genetic diversity in captivity for both SPP and YPP.
- Inform the successful reintroduction of SPP and YPP into former habitats of the lower MDB between 2011 and 2014.
- Genetic monitoring of recaptured individuals demonstrating survival and continuing recruitment.
- Maintain similar levels of genetic diversity in reintroduced populations as was present originally.

Project Summary

Feasibility: Freshwater fishes are globally recognized as being highly susceptible to decline following anthropogenic disturbance. The highly-altered MDB ecosystem typifies the extinction crisis faced by freshwater fish populations. This is the most important agricultural region of Australia, with water being both the life blood and most critical resource of this inland basin. However, allocation of water for agriculture and extended drought (possibly heralding a changed climate regime), has gripped southeastern Australia in the last two decades. The environmental impacts have been strongest at the terminus of the MDB, which relies on upstream water management to sustain important habitats. The lower reaches of the Basin suffered rapid and dramatic lowering in water levels in 2006 (detailed in Hammer *et al.*, 2013). By 2008 much of the lower MDB was dry, leading to extensive and unprecedented loss of freshwater habitats and aquatic biodiversity. This was especially the case for poor dispersive fish species such as pygmy perches that rely on off-channel, tributary stream and wetland environments. The SPP found in the lower MDB are geographically isolated, locally adapted and genetically divergent from other regional populations (Brauer *et al.*, 2016). The YPP of the lower MDB are an endemic lineage classified as an evolutionarily significant unit (ESU) isolated since the Pleistocene (Brauer *et al.*, 2013). Most of the focus for fish conservation programs in the MDB has previously been on large-bodied fishes.

Implementation: In response to the crisis, a collaboration of government agencies, a University genetics lab, a NGO and the community was formed to

Representative YPP habitat (*left*) & SPP habitat (*right*) © Nick Whiterod

undertake urgent conservation actions for small-bodied fishes under very high risk of extinction (Hammer *et al.*, 2013). The team implemented *in situ* habitat monitoring and response (e.g. watering), fish rescue, captive-breeding programs, reintroductions and population monitoring. Pygmy perches were rescued from Lake Alexandrina in the lower MDB (catchment area of ~1.061 million km²) before their habitat dried out. The YPP were sourced from three discrete locations and the SPP from two locations. A total of 84 adult YPP and 65 adult SPP were available for genetic-based breeding programs aimed at minimizing inbreeding and preserving genetic variation. For each species, the putative breeders were fin -clipped for DNA extractions, and genetic diversity and relatedness were estimated using microsatellite DNA markers (Attard *et al.*, 2016). Little information on captive husbandry was available for these species, but pond spawning had been previously achieved for SPP. Eleven breeding groups per species were established at Flinders University, Adelaide. Each group was kept in a separate 2,000 L tank exposed to natural light and ambient temperature. Breeding groups were selected based on genetic analyses aimed at ensuring low estimated pairwise relatedness between individuals within each group. Genetic analyses also identified inbred brooders, a likely result of the marked population declines experienced in the wild. These individuals were removed from the pool of breeders. Each SPP breeding group consisted of two females and two males, and each YPP breeding group consisted of three females and two males. Inferred relatedness between individuals of the same sex in a breeding group was also minimized to increase the power of parentage assignment of fish subsequently recaptured during post-release monitoring.

The number of generations in captivity prior to reintroduction was kept to the smallest possible (one generation) to minimize adaptations to captivity and, given the small size of captive populations, avoid loss of genetic diversity (Attard *et al.*, 2016b). A contingency plan was also put in place in the event that self-sustaining wild populations were not established; this consisted of maintaining individuals from multiple family groups in captive breeding, both at Flinders University and in artificial refuges monitored by government agencies. Reintroductions of the two species began in the Lake Alexandrina region during spring 2011 using equal numbers from each family group.

Post-release monitoring: Post-release monitoring at the reintroduction sites

demonstrated persistence and recruitment of SPP in subsequent years. YPP on the other hand failed to respond to the reintroductions and are now considered extinct in the MDB. This was observed despite higher numbers of reintroduced YPP (~5,850) compared to SPP (~1,350). Genetic data from 71 SPP captured during the three years after reintroduction confirmed that 19 of those were born in captivity, with the remaining 52 assigned as wild-born offspring of the genetic-based captive bred fish. Subsequent monitoring (as of December 2019) demonstrated persistence and recruitment of SPP, with 100s of individuals captured in and near the released sites. For YPP, only 13 individuals were captured during the first three years following release. These were classified as putative offspring from the genetic-based breeding program. Despite broader monitoring across its former range, including an occupancy study where triplicate surveys of 32 former and reintroduction sites were carried out in November and December 2018, the species has not been detected in the Basin since 2015.

Major difficulties faced

- The unprecedented temporal and spatial extent of environmental perturbation in the lower MDB, especially habitat desiccation, a) hindered habitat recovery for suitable reintroduction sites, b) provided ongoing pressure on fish establishment (e.g. difficult to manage threats from invasive fishes), and c) led to a partial mismatch in the capacity of the reintroduction project and the required scale of actions in a large system.
- Limited information about species life-history, husbandry and habitat requirements for both SPP and YPP, against critical time-frames for implementing a captive program.
- Low levels of genetic diversity of both species made relatedness and parentage analyses challenging.
- Lack of necessary governmental commitment and long-term continuing funding for planning and implementation in terms of fish surveys, rescue and post-release monitoring, as well as for large-scale hatchery production and fish husbandry.
- Broader uncertainty in water management relating to habitat availability and

SPP captured in the field © Scotte Wedderburn

recovery (although environmental water is increasingly seeking to create habitats that would be suitable for both species).

Major lessons learned

- The application of fundamental science about the genetics of small populations into an emergency community-driven restoration project enabled this project to be competitive in securing funding from a federal grant agency - the Australian Research Council.
- Collaboration by multiple stakeholders was essential to realise the outcomes of the project.
- Minimising time in captivity is key as maintaining genetic diversity and avoiding inbreeding becomes more difficult with every generation.
- Rescue for recovery is not a silver bullet, and should not be seen as a easy key management solution, but rather as a supplement to long-term habitat protection and restoration.
- There is a broad need (government managers, scientists and the community) to recognise that successful reintroduction of some species requires considerable effort, energy and time.

Success of project

H 1 - Success of reintroduction project for SPP:

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Rapid submission and subsequent funding of a multi-institutional grant proposal to Australia's research funding agency enabled the conservation breeding program to be implemented before putative breeders rescued from the wild reached senescence.
- Development and implementation of a novel genetic-based framework for captive breeding and restoration (described in Attard *et al.*, 2016) enabled both the genetic diversity of the wild population to be retained in the first generation of captivity and the genetic monitoring to assess recovery in the wild.
- The production of captive offspring for reintroductions coincided with the end of a major drought period in SE Australia and the availability of adequate aquatic habitat in the lower MDB.
- Commitment and passion of those involved to persist with efforts regardless of funding and resources.
- Continuous communication amongst the multiple stakeholders involved with the various stages and sections of the project.

H 2 - Success of reintroduction project for YPP:

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for failure:

- Both YPP and SPP are thought to have similar life-history and ecological requirements, which makes it hard to pinpoint to reasons accounting for the failure of YPP reintroductions; clearly the success of last-chance recovery efforts have a degree of chance in amongst habitat suitability and threats.
- One possibility is the long-term evolutionarily smaller size and lower genetic diversity of the YPP lineage in the MDB compared to SPP (Attard *et al.*, 2016; Brauer *et al.*, 2013).
- It is hypothesized that the human-driven collapse of the YPP population caused inbreeding depression, loss of fitness and lowered adaptive resilience hindering re-establishment success.
- A lack of ongoing funding and resources to ensure that reintroductions could be sustained.
- Conservation programs that improve adaptive resilience, such as crossing the remaining YPP MDB currently kept in captivity with wild YPP from coastal lineages (known as 'genetic rescue') are recommended options for subsequent reintroductions.

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Conservation of the bullhead by re-introduction in Flanders, North Belgium

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Introduction

Bullheads are small, bottom-dwelling freshwater cottid fishes. In Belgium, two indigenous species occur: *Cottus perifretum* in the Scheldt River drainage (including Demer and Dyle basin) and *Cottus rhenanus* in the Meuse River drainage (Volckaert *et al.*, 2002). Formerly bullhead was common in Flanders (North Belgium). Due to water pollution, habitat degradation and fragmentation however, cottid populations and distributions declined dramatically. Consequently bullhead is listed as IUCN Vulnerable in the national Red List (Verreycken *et al.*, 2014). Until the discovery of a relict population in the Dorpbronbeek (2003), *Cottus perifretum* was thought to be extinct from the entire Demer River basin. The status of this population was extremely precarious because of the small population size, restricted habitat area, and many external stressors. In 2008, Research Institute for Nature and Forest (INBO) and Agency of Nature and Forest (ANB) initiated an *ex situ* culture and a reintroduction program to prevent the loss of this unique population. In 2015, after successful introductions in the Demer basin, we shifted the focus towards the Dyle basin where no bullhead populations were present in Flanders (only in Wallonia, south part of Belgium). Bullhead is listed as an Annex II species. The main targets for the bullhead in Flanders are: to contain the current range of the species and to increase the number of populations. Moreover, specific goals were set for the Special Area of Conservation of the Dyle (Natura, 2000 BE2400011). In this regard, the IJse (a tributary brook of the Dyle River) and its headwaters (e.g. the Nellebeek) were given the highest priority to gain a self-sustaining population.

Bullheads © Chris Van Liefferinge

Goals

- *In situ* conservation of the bullhead population in the Dorpbronbeek.
- Reintroduce cultured progeny from the relict population in the Demer basin to other suitable locations within the same river basin in order to preserve the gene pool *ex situ*.
- Thrive for a favorable conservation status of the bullhead population (based on abundance and population structure). The reintroduced populations have to evolve toward healthy, self-sustaining ones.
- Meet the goals set by the Habitat Directive: an increase of the number of populations in Flanders and to gain a self-sustaining population in the IJse and the Nellebeek.

Success Indicators

- Develop captive-breeding techniques to allow the reliable production of bullhead for reintroduction purposes.
- Good survival, growth and natural reproduction of the released animals in brooks that, based on macro- and micro-habitat assessments, showed a high potential for success.
- Establishment of a viable, self-sustaining population with a length-frequency distribution indicating well balanced year classes and a high proportion of young of the year (YOY). A high contribution of juveniles to the population indicates successful recruitment, leading to a favorable local conservation status of the reintroduced populations.
- Further expansion of the species' range due to gradual colonization from the initial release sites.

Project Summary

The survival of the population in the Dorpbronbeek is at risk because of local habitat deterioration, its small population size, and restricted habitat size. Measures were taken to conserve the unique bullhead population in the Dorpbronbeek *in situ*. The population was locally protected by the restoration of its habitat e.g. reforestation of river banks, land use change, and both temporal and permanent measures to decrease erosion from adjacent agricultural sites. However, a local watercress farm still poses a high risk. The farm adds fertilizers at the instream and taps water from the headstream by blocking the upper part of the brook with a dam. This activity threatens the long-term survival of the entire bullhead population despite all previous conservation measures taken. Therefore INBO and ANB launched a restoration project by reintroducing cultured juveniles originating from the relict population to other suitable habitats in the same river basin. In 2015, after successful introductions in the Demer basin, we shifted the focus towards the Dyle basin. Because of different genetic strains between the Demer and the Dyle basin, we started another program using the same approach, but with different genetic lines.

Feasibility: The feasibility study consisted of a genetic study, a captive-breeding program, a habitat suitability study and a qualitative electrofishing survey to make

sure that no remnant bullhead populations were present (which was not the case). A genetic study using microsatellites revealed that the Dorpbronbeek population is indeed a relic of the Demer River basin population, with a high similarity to other bullhead populations in the Scheldt River basin but also with five unique alleles. Previous genetic research showed that the genetic diversity of bullhead in Flanders is low and the different populations are extremely fragmented (Knapen *et al.*, 2003). To prevent substantial loss of genetic variability of bullheads in Belgium, management should aim to protect and conserve as many of these populations as possible.

A captive-breeding program was developed at the aquaculture facilities of INBO to spawn and rear bullhead in captivity in order to provide sufficient individuals for a possible reintroduction. The broodstock for the Demer basin was collected from the source population in the Dorpbronbeek. The program started in 2004 and has become increasingly successful over time. Because of different genetic lines between Demer and Dyle basin, we started a new program in 2015 in cooperation with the angler Service of the Walloon Authority of Nature and Forest (DNF), with bullheads originating from the Blanc Ry, a tributary headwater of the Dyle located in the Walloon part of Belgium. This population was also genetically screened and found suitable as broodstock.

Habitat suitability of potential headstreams within the Demer and Dyle river basin was assessed on a macrohabitat and microhabitat scale prior to reintroduction. On a macrohabitat scale, water quality, habitat structure, food availability, and the present fish community were screened in nine and five brooks of the Demer and Dyle basins, respectively (see figure 1). Where the macrohabitat seemed suitable, the microhabitat suitability was assessed. Three variables were measured: water depth, water velocity near the substratum and substratum composition (including woody debris and the presence of roots in the river bank or stone-filled gabions as bank type reinforcement) (Van Liefferinge *et al.*, 2019). The results were extrapolated to the entire brook to determine the indicative population size. The bullhead microhabitat suitability model was used to predict the viability of the population.

In the Demer basin the Zevenbronnenbeek, Schoorbroekbeek, Waarbeek-Deesbeek and the Sint-Annabeek were chosen as potential reintroduction sites. For the Dyle river basin the Nellebeek, IJse, Bruelbeek, Mollendaalbeek and Molenbeek showed high potential. The reintroduction strategy of the Dyle basin will focus on the headstream of the Mollendaalbeek and the Bruelbeek, two tributaries of the Molenbeek. We expect that due to larval drift and downstream dispersion of juveniles and adults the entire sub-basin of the Molenbeek will get colonized. Similarly, in the Nellebeek, a fast population growth is also expected, and downstream colonization of the IJse will most likely occur.

Implementation: In 2008 and 2009, respectively 1,220 and 345 cultured young of the year bullhead were released into the headwater of the Zevenbronnenbeek. To enhance the habitat and to increase the breeding success at the release site even more, 68 ceramic tiles were added to the stream as additional artificial spawning substrates and shelter (Knaepkens *et al.*, 2004) before the first introduction in the Zevenbronnenbeek took place. In the Schoorbroekbeek, 1,285 YOY were released in 2010, 5,291 in 2011 and 420 in 2012. In the Waarbeek-Deesbeek 641 YOY bullhead were released in 2011, 385 in 2012, 724 in 2014 and another 519 in 2015. Worth mentioning is the fact that in the Waarbeek-Deesbeek in 2014 habitat amelioration was performed downstream of the release sites. The introduction cycle in 2014 was therefore focusing on this section. In 2015, due to a miscommunication, a thorough sediment clearing was carried out, impacting the entire population. Therefore, after taking restoration measures (restoring sinuosity and re-entering woody debris, boulders and stones), we decided to prolong our introduction strategy for another year.

Schoorbroekbeek © Van Liefferinge

In 2016, 50 adults were released in the Sint-Annabeek, and 354 YOY were released in the Dorpbronbeek in 2017 to reinforce the original population.

In the Dyle basin, 1,006 YOY were released in the Bruelbeek in 2015, 602 in 2016 and 208 in 2017. In the Nellebeek 849 YOY were released in 2015 and 600 in 2016. In the Mollendaalbeek 600 YOY were released in 2016 and 1,177 YOY in 2017.

Post-release monitoring: The conservation status of bullheads is assessed based on habitat quality and demographic population parameters (Coeck *et al.*, 2008). All habitat quality indicators of the release locations are in favorable conditions. For the demographic population parameters 'unfavorable' conditions are defined as degraded populations (C) when fish abundance is <0.1 bullheads/m² and population structure is showing only one age-class. Favorable conditions are defined as 'sufficient' (B) or 'good' (A) when bullhead abundance is between 0.1 - 0.3 bullheads/m² or >0.3 bullheads/m² respectively, and the age-distribution

Monitoring Deesbeek © Jules Robijns

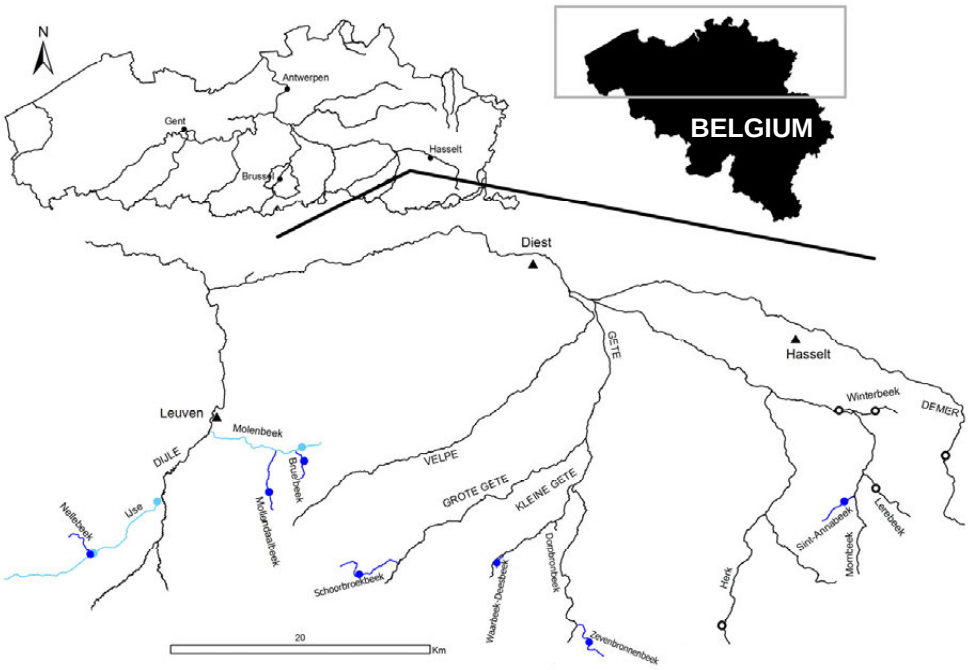
histogram is showing the presence of different age-classes (B), or is having a proportion of >50% YOY (A).

Reproductive success in the first introduced population in the Zevenbronnenbeek was assessed during the first year by visual inspection of the presence of territorial males, gravid females or egg clutches underneath the ceramic tiles. Field observations showed that a high proportion of the cultured fish were sexually mature in the year after the release. Throughout the natural breeding season about 40% of the tiles were occupied by territorial bullhead, but also natural substrates were used as nesting places. Notwithstanding the fact that these artificial nesting places could easily be monitored, we decided not to add ceramic tiles at the other brooks because sufficient natural nesting places (e.g. wooden debris, rocks and boulders) were present at the release sites.

Until 2019, we monitored the introduced populations every year by means of electrofishing, not only at the release sites but we also included downstream stretches and rivers. The reintroduction was a success at all release sites, except for the Sint-Annabeek. The populations show good survival, growth and breeding of the released animals, establishing a viable, self-sustaining population with a high proportion of YOY. Because of the rapid growth and fast maturation, new populations could be founded immediately one year after release and the populations can expand rapidly. Post-release monitoring showed that the reintroduced populations are in favorable condition and gradual colonization from the initial releasing sites is evident leading towards a gradual expansion of the species' range.

Moreover, favorable conditions of demographic parameters were encountered at the release sites (Table 1). For the Zevenbronnenbeek, Schoorbroekbeek, Bruelbeek, Mollendaalbeek and Nellebeek, favorable demographic parameters were reached even after the first introduction year. For the Waarbeek-Deesbeek favorable conditions were reached after two introductions. When taking into account the release sites and all downstream monitoring sites, covering the entire river systems, the Zevenbronnenbeek, Schoorbroekbeek, Bruelbeek and Nellebeek do show favorable conditions even after the initial introductions, while for the Mollendaalbeek and Waarbeek-Deesbeek it took two introduction rounds (Table 1). As expected, downstream colonization originating from the Bruelbeek and the Nellebeek has lead towards the colonization of the Molenbeek and IJse respectively. It is expected that within a few years favorable conditions will occur

Figure 1. Surveyed headstreams in the Demer and Dyle River Basin with their major tributaries in Flanders. Assessment of suitability for bullhead, based solely on macrohabitat characteristics (open circles) or also on microhabitat characteristics (full circles)



in both streams, which is already the case for the initial selected brooks.

In the Sint-Annabeek the reintroduction was unsuccessful despite of the initial habitat assessments due to severe droughts in the summers of 2017 and 2018 leading to a very low base flow and eventually the watercourse dried up.

Major difficulties faced

- *In situ* protection measures proved to be difficult to implement in the Dorpbronbeek. Despite all the conservation measures taken, the population is still facing problems since the agricultural plant continues to tap large quantities of water from the brook.
- Securing adequate funding for all the phases of the reintroduction program, including a long-term post-release monitoring.
- Instream measures were taken in the Waarbeek-Deesbeek in favor of bullheads. These were executed in a partnership with the local authorities and communicated within the proper formal consultation structure, the committee on integrated water policy of the Demer basin, and was even incorporated in the river catchment management plans. However, a third party was also authorized for the local water management and performed a thorough sediment clearing in the entire brook one year after the initial

project, claiming not to be informed properly, with severe impact on the entire bullhead population and their habitat.

- The impact of beaver activity and climate change on the reintroduction projects is hard to predict. We have already seen in two case studies that alteration in the stream characteristics and changes in base flow due to severe droughts and extreme water captation are detrimental to a bullhead population. These problems are most likely to increase because of global warming.
- Bullhead populations in high productive streams do show rapid growth and have a fast turn-over. Consequently, populations can be seriously threatened by two consecutive unsuccessful breeding seasons resulting in a local extinction.

Major lessons learned

- Overall, the ecological quality of several headstreams in Flanders remains insufficient.
- With respect to captive-breeding strategies for bullheads, two elements are crucial: the interior layout of the microcosm and larval nutrition. Infection and mortality rate of breeding fish is significantly lower in more natural microcosms having dark substrata (with sufficient coverts) compared to bare pale or light-bottomed fish tanks (with sufficient coverts). Secondly, larvae need to be fed with decapsulated artemia, when fed with artemia

Table 1. Local conservation status of bullhead in the Demer River Basin (1) and Dyle River Basin (2). Monitoring results of the entire brook, downstream from the release sites, for population structure (PS) and abundance (AB), with favorable conditions shown as A (good) and B (sufficient), unfavorable conditions shown as C (degraded population) and 0 when no bullheads were found. Local conservation status of the species at the release sites is shown as superscript. Light blue shadings are showing the years of bullhead reintroduction

	2008		2009		2010		2011		2012		2013	
			PS	AB	PS	AB	PS	AB	PS	AB	PS	AB
Zevenbronnenbeek[1]			B^b	B^a	B^a	A^a	B^a	A^a	A^a	A^a	B^a	A^a
Schoorbroekbeek[1]							B^b	B^a	B^a	A^a	B^a	A^a
Waarbeek-Deesbeek[1]									B^b	C^c	C^c	C^c

	2014		2015		2016		2017		2018		2019	
	PS	AB	PS	AB	PS	AB	PS	AB	PS	AB	PS	AB
Zevenbronnenbeek[1]	B^a	A^a	B^b	B^a	B^a	A^a	B^a	A^a	B^b	A^a		
Schoorbroekbeek[1]	B^b	A^a	B^a	A^a	B^a	A^a	B^a	A^a	B^a	A^a		
Waarbeek-Deesbeek[1]	B^b	A^a	B^a	A^a	B^a	A^a	A^a	A^a	B^b	A^a		
Sint-Annabeek[1]							C^c	C^c	0	0	0	0
Bruelbeek[2]					A^a	A^b	B^a	A^a	B^a	A^a	B^b	A^a
Mollendaalbeek[2]							B^b	C^b	B^b	B^a	B^b	B^a
Molenbeek[2]					0	0	0	0	C	C	B	C
Nellebeek[2]					B^b	A^a	B^b	A^a	B^b	A^a	B^b	A^a
IJse[2]					C	C	C	C	B	C	B	C

nauplii as direct food (otherwise their digestive tract will be blocked and mortality rate is 100%).

- Raising awareness and informing land owners and water managers creates the necessary support for a successful reintroduction. Incorporation of the reintroduction programs as part of the realization of integrated water management plans, within the formal consultation and communication structures and submitted and approved by the committee on integrated water policy (e.g. for the Demer and Dyle basin) was not always sufficient.
- A governmental drought committee should be installed to prevent captation of surface water from vulnerable headstreams during periods of (severe) drought. In Flanders, in the province of Vlaams-Brabant, a governmental decree ensured that captation of surface water was directed towards (small) river systems in order to protect the ecological fragile headstreams from being dried up.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Successful and reliable captive-breeding.
- When the habitat suitability model for bullheads predicts suitable habitat, the success rate of the re-introduction is high. Except for the Sint-Annabeek, the introduced fish formed the basis for the development of a new and sustainable local population in a status assessed as favorable. Moreover, downstream colonization is observed for all the released populations and most reintroduced populations have colonized the entire brook. Often bullheads are even colonizing the adjacent downstream river systems.
- The captured fish show good growth and are visually in good condition.
- Natural recruitment was successful.
- A broad setup of collaboration and partnership with the implementation of an integrated water management project is crucial for a successful water-related conservation program. In our case study of the bullhead, a partnership was set up between local (municipalities) and governmental authorities (Agency of Nature and Forest and DNF - Service de la Pêche), local water managers (Province of Vlaams-Brabant and Province of Limburg and the Watering De Kleine Gete), VMM integrated water management of the Demer basin and the basin of Dyle-Zenne), research Institutes (INBO) and the local nature associations.

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A fish out of water: rewilding the Pahrump poolfish 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 Las Vegas dace (*Rhinichthys deaconi*), an extinct species described from museum specimens (Miller, 1984). 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 federally endangered Pahrump poolfish (*Empetrichthys latos*), a species considered critically endangered by the IUCN. This endemic fish was extirpated in 1975 from Manse Spring in Pahrump Valley, Nye County, Nevada. Although Manse Spring was lost to groundwater pumping for agriculture, some fish were translocated proactively to establish three refugia populations (Minckley & Deacon, 1968). Recently, two of these refugia were decimated by the illegal introduction of non-native species, in particular Goldfish (*Carassius auratus*), Western mosquitofish (*Gambusia affinis*), and Red swamp crayfish (*Procambarus clarkii*). The establishment of a population at the Preserve further protects the species from stochastic events that can lead

Pahrump poolfish adult (above) and fry (bottom)
© Aaron Ambos

to extinction.

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 Pahrump poolfish.
- Establish a self-sustaining population of Pahrump poolfish.
- Increase the geographic distribution and total population count to increase species resilience to stochastic events.
- Educate public about the plight of the Pahrump poolfish and foster community support.

Success Indicators

- Ratification of federal Pahrump poolfish Safe Harbor Agreement.
- Establishment of pond mesocosm at the designated site.
- Obtain and translocate Pahrump poolfish.
- Pahrump poolfish population becomes self-sustaining.
- Implement public education programing on conservation efforts.

Project Summary

Feasibility: To assist with conservation of the critically endangered Pahrump poolfish, 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 is only about 65 km from Manse Spring. 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 an endangered Pahrump poolfish population, a 15-year Safe Harbor Agreement was ratified in 2017 by LVVWD and the US Fish and Wildlife Service (USFWS). The legally-binding document spelled out the rights, responsibilities, and obligations of both parties (LVVWD & USFWS, 2017).

Implementation: The design and construction of a pond mesocosm suitable for Pahrump poolfish was potentially the most challenging part of the project. At the Preserve, 10 previously-built ponds were evaluated for their potential suitability for Pahrump poolfish and imperiled Relict leopard frogs (*Rana onca*). Three ponds were selected because of their larger sizes and/or ease of access for future public education, although none had supplemental aeration or filtration. The suitability of these ponds was tested for Pahrump poolfish by first introducing approximately 50 Moapa White River springfish (*Crenichthys baileyi moapae*) into each pond in August 2013. Although the fish thrived initially, the death of large quantities of

green algae (*Chara* sp.) in the fall of 2013 led to anaerobic water conditions in the test ponds. Trapping surveys in November 2013, revealed that only two of the fish had survived in a single pond. Supplemental aeration and filtration was necessary in order to establish refugia populations at the Preserve.

Mark-recapture survey at pool habitat
© Aaron Ambos

In order to move forward with the Pahrump poolfish refugium at the Preserve, two other existing ponds were chosen because of the availability of power for aeration and filtration systems. These ponds had not been selected previously because of the effects of decomposing leaves from overhead Cottonwood trees (*Populus fremontii*) on water quality. Once additional funding and approvals were secured, a new low-maintenance pond mesocosm was designed in August 2016. The new 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). By May 2018, the system was working as designed and plans for the translocation of a Pahrump poolfish population were made. In late May 2018, the Nevada Department of Wildlife (NDOW) translocated a total of 290 Pahrump poolfish from the Shoshone Ponds refugia to the Preserve.

Post-release monitoring: Since the ponds can be visited regularly by staff, post-release monitoring has occurred almost daily. Following the translocation of 290 adult Pahrump poolfish on 29th May 2018, the first fry were observed on 18th June 2018. Fry continued to be observed throughout the summer. Between 3rd - 9th October 2018, a mark-recapture survey was conducted using standard fisheries methods. The survey revealed that an estimated 386 (95% CI: 278 - 605) Pahrump poolfish inhabited the ponds, an increase of 25% (N=96 fish) in a little over four months. From 8th October to 27th November 2018, at least 5.5% (n=22) of the population died from a virulent attack by a flavobacterium and secondary fungal infection. A federal fish pathology laboratory concluded that “immunosuppressed mature fish were succumbing to opportunistic aquatic bacteria and fungi.” The pathologists speculated that the pathogens were the result of two confounding stressors: 1) *Environmental:* Water temperature dropped by 7°C (i.e., from 22°C to 15°C between the 1st and 2nd capture sessions); and, 2) *Anthropogenic:* trapping, handling, and marking during a mark-recapture survey.

Pahrump poolfish pond habitat
© Raymond A. Saumure

Mark-recapture surveys in June and September 2019 documented an estimated 173 (95% CI: 131 - 232) and 164 (95% CI: 120 - 232) Pahrump poolfish in the ponds. Although the population size stabilized in 2019, it represents a 58% decline from the October 2018 survey. This may be partially explained by the presence of hundreds of imperiled Relict leopard frog (*Rana onca*) tadpoles in 2019, which may have altered the primary productivity, and thus carrying capacity, of the system. As of 27th November 2019, there has been no observed recurrence of mortalities as a result of immunosuppressed Pahrump poolfish. Recruitment is still occurring, as schools of Pahrump poolfish fry were observed from 14th May until 3rd October 2019.

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.
- Mature fish were succumbing to opportunistic aquatic bacteria and fungi. Fish pathologists speculated that mortalities were likely the result of the synergistic effects of anthropogenic (i.e. mark-recapture survey) and environmental (i.e. drastic temperature drop) stressors.
- Manse Spring was a thermal system with a constant spring pool temperature of 24°C; however, translocated fish were known to survive temperatures as low as 4°C under ice (Selby, 1977). The two Preserve ponds are not heated and dropped to 0.5 and 1.5°C during the unusually cold winter of 2018 - 2019.
- 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.
- Mark-recapture surveys must occur before precipitous seasonal declines in water temperature, which can compromise the immune system of Pahrump

poolfish. This recommendation was implemented in 2019 and no further post-handling mortalities were documented.

- The rewilding of the Preserve generated a surprising amount of positive local media coverage. This media coverage was leveraged to educate the public about the plight of endangered species and the damage caused by the illegal introduction of non-native species to native fauna.

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.
- Managed the outbreak of a virulent pathogen and adapted procedures and protocols to decrease the likelihood of a recurrence in subsequent years.
- 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.

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Restoration to historic and artificial habitat of a rare desert fish, the Moapa dace in Nevada, USA

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Introduction

The Moapa dace (*Moapa coriacea*) is a Red List Critically Endangered species, a United States Fish and Wildlife Service (USFWS) Endangered Species, and a State of Nevada Endangered species. This species is endemic to the springs and headwater streams of the Muddy River, Clark County, Nevada, USA about 70 km northeast of Las Vegas, Nevada. This thermal endemic species was historically found in approximately 25 warm springs (30 - 32°C) and the upper 16 km of stream forming the Muddy River. Currently, Moapa dace inhabit approximately 38% (9.5 km) of historic stream habitat, including four spring systems and the mainstream Muddy River. Major threats include invasive non-native fishes, habitat alteration for agriculture and recreation, and municipal water development. Conservation measures for the Moapa dace have been a collaborative effort between federal and state agencies, and the Southern Nevada Water Authority (SNWA). Efforts have included the acquisition of private land for the creation of the Moapa Valley National Wildlife Refuge (Refuge) and Warm Springs Natural Area (WSNA). These areas protect 541 ha, including the headwaters of four of five major springs and 7.9 km of fluvial habitat. Active recovery efforts occur in these areas to ensure

Moapa dace © Aaron Ambos

the preservation of the species.

Moapa dace survey © Aaron Ambos

Goals

- Protect and restore Moapa dace habitat.
- Minimize impacts of non-native fishes.
- Monitor abundance of Moapa dace.
- Protect instream flows.
- Re-establish Moapa dace in five spring systems, using translocation if necessary.

Success Indicators

- Protect Moapa dace habitat and instream flows in three of five occupied spring systems for reclassification from Federally “Endangered” to “Threatened” and in all five spring systems for five consecutive years for removal from the Federal Endangered Species list.
- Confirm that non-native fishes and parasites no longer adversely impact the long-term survival of Moapa dace.
- Document recruitment of Moapa dace via evidence of three age-classes (larval, juvenile, adult) occurring in three spring systems.
- Document that 75% of historical habitat across the five spring systems and upper Muddy River provide spawning, nursery, cover, and foraging.
- Demonstrate an annual abundance of 4,500 fish for reclassification from “Endangered” to “Threatened”, and to 6,000 fish (across five spring systems) for five consecutive years for removal from the Federal Endangered Species list.

Project Summary

Feasibility: Although heavily impacted by habitat modification and invasive species, the geographic range of thermophilic habitat historically and potentially available to Moapa dace is small (16 km). As such, the logistical concerns oftentimes encountered in large river systems such as piscicide effectiveness, uninstallation of concrete structures, and restoring connectivity are financially tractable. The USFWS ranks the Moapa dace within its highest category of recovery potential. The restricted geographic range of this species also limits the number of managing government agencies, municipalities and private landowners, each with overlapping, but varied missions. The small number of

Pederson Channel construction © David Syzdek

parties involved makes it much easier to meet regularly for discussion and coordinate recovery actions. Using a combination of land purchases and agency cooperation, historical challenges associated with private lands have largely been resolved.

Implementation: Private land in the Moapa Valley was purchased for the creation of the Refuge and adjacent WSNA. The Refuge was established by purchasing individual parcels of land between the 1970s and 2010s. Overall, the Refuge protects three of the five major springs on 43 ha of land and approximately 6% of historical Moapa dace habitat. The establishment of the WSNA by the SNWA in 2007 protected the majority of Moapa dace stream habitat (~76%), as well as one additional springhead. These adjacent properties make it possible for the USFWS, the Nevada Department of Wildlife (NDOW), the SNWA, the Moapa Band of Paiutes, Coyote Springs Investments, and the Moapa Valley Water District to work together in an active collaborative planning and restoration process for the Moapa dace and its habitat.

Significant restoration projects include the creation of the Lower Pederson Stream segment in 2008. This project is a 400 m long stream that redirects water from a historic irrigation ditch to an artificial channel designed to recreate optimal Moapa dace habitat with pool-riffle-run complexes and drift feeding areas. This stream was naturally recolonized by Moapa dace and now consistently supports 25% of the entire Moapa dace population (200 - 600 individuals). Habitat restoration was conducted on the nearby 170 m Apcar Stream in 2011. The stream was narrowed so water velocities would increase and benefit the Moapa dace. As a result, the population in this restored stream increased abundance from an average of only three Moapa dace between 2008 and 2011, to an average of 226 Moapa dace between 2012 to 2019.

The removal of non-native Blue tilapia (*Oreochromis aureus*) by the NDOW between 2009 and 2012 and the subsequent removal of fish barriers that once excluded tilapia invasion from much of the core Moapa dace habitat, allowed natural recolonization in areas previously extirpated. For example, Moapa dace were extirpated from Big Muddy Creek in 2003. Big Muddy Creek transects a 29 ha private inholding and is one of the few remaining swimming recreation facilities in the area. One dace was seen there in August 2011 and again in February 2012. No Moapa dace were seen there again until February 2015 when several fish began to recolonize this reach. Since February 2018, the 868 m Big Muddy

Moapa dace © PhotoArk.com

Creek reach has supported an average of 32 Moapa dace.

Concerned with the absence of natural recolonization after piscicide treatment in the North Fork (544 m) and the South Fork (1,138 m), biologists from USFWS, NDOW and SNWA translocated 59 Moapa dace adults into the South Fork in early 2019. Surveys in August 2019 found 30 adult and 10 juvenile Moapa dace in this reach, indicating successful reproduction occurred in 2019. More translocations are planned in the South Fork in early 2020 and subsequently in the North Fork after restoration.

Post-release monitoring: Due to species-specific life-history attributes and efforts to reduce harm, biologists estimate population size via intensive snorkel surveys with 100% coverage of the historic range of Moapa dace. Surveys are conducted with biologists swimming and crawling upstream to count individual fish. These surveys have been confirmed to be accurate using mark/recapture studies. Snorkel surveys were periodically conducted beginning in 1985.

Since 2008, surveys have been conducted biannually in the spring and fall seasons to inform both winter survival and document annual reproduction, respectively. Historical habitat has been split into 18 reaches for management. Each reach is snorkeled by trained biologists familiar with fish species in the system. In addition, life-stage is estimated by total length as adult (>45 mm), juvenile (20 - 45 mm), and larvae (<20 mm). Total numbers are grouped by reach and counts are compared year-to-year. Biologists from SNWA, USFWS, NDOW, along with other stakeholders, generally meet monthly to plan fish counts, research, restoration actions, and/or outreach.

Major difficulties faced

- Pervasive invasive species, such as tilapia, are difficult to remove and require sustained effort and complex planning for successful eradication.
- Natural and artificial barriers to fish passage, while sometimes useful in blocking the movement of invasive species, also prevent recolonization by target species.
- Some private landowners restrict access, highlighting the need for coordination.
- Wildfire carried by non-native invasive plants can cause severe habitat damage but can improve access for piscicide treatments by temporarily removing vegetation.
- Property acquisition, habitat restoration, and engineering projects such as fish barriers are extremely expensive.

Major lessons learned

- Successful protection of core Moapa dace habitat from tilapia invasion using a gabion fish barrier, prevented Moapa dace extinction for 16 years and provided time for planning, property purchases, and restoration projects.
- Complete removal of tilapia with piscicides from historical Moapa dace habitat allowed natural and artificial reintroduction of fish into historical habitat and a subsequent increase in Moapa dace populations.
- Properly engineered artificial habitat can support large numbers of Moapa dace.
- Multi-agency collaboration is key to success; it allows for constant progress despite changes in conditions, funding, and personnel.
- Property purchases funded by grants made access and restoration possible by purchasing the majority of the species' historic range.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Multi-agency collaboration allowed for long-term planning and adaptive management of various aspects of the reintroduction project.
- Property acquisition with grant money and continued funding of conservation and restoration experts by SNWA allowed for long-term project management and planning.
- Protection of in-stream flows for Moapa dace is critical for their survival. Legal agreements and state and federal protections from excessive groundwater withdrawals have ensured aquatic species survival by maintaining adequate in-stream flows.
- Stream gages, culverts, and other infrastructure that block fish passage can be difficult or impossible to remove or mitigate due to landowner

access restrictions, physical limitations, and legal considerations. However, projects such as waterfall removals, culvert improvements, and irrigation dam removals have restored fish passage and allowed for recolonization and restoration of natural gene flow between subpopulations.

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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.

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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).

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

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Introduction

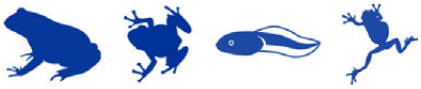
The Mountain yellow-legged frog (*Rana muscosa*) is an amphibian species endemic to the San Bernardino, San Gabriel, San Jacinto, and southern Sierra Nevada mountains of California. Formerly abundant at high-elevation streams and lakes, populations of Mountain yellow-legged frogs in the southern California 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

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.

Researchers collecting field data
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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.

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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

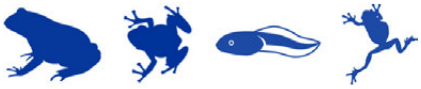
models showed that chytrid reduced monthly frog survival at Experimental Site One. Comparative surveys between Site One and wild KI populations, indicated that chytrid levels were lower in wild frogs. At both experimental sites, frogs readily used salted and unsalted ponds, but avoided salinities over 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.

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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.

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Resetting-translocation to the release point promotes reinforcement success in the Hermann's tortoise

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Introduction

The Hermann's tortoise (*Testudo hermanni*, Gmelin, 1789) is exposed to multiple threats in Europe (Nikolić *et al.*, 2018). Populations of both western (*T. h. hermanni*) and eastern sub-species (*T. h. boettgerri*) are fading throughout their geographic range (Livoreil, 2009). Listed on the Appendix II (A) of CITES, it is classified as "Near Threatened" on the IUCN World Red List, but the western sub-species is considered "Vulnerable" on the French National Red List. The situation of the western sub-species is particularly worrying. Continuous declines since the beginning of the 20th century in Italy, France and Spain resulted in strongly fragmented and reduced populations. In continental France, only one population located in and nearby the Maures Mountains (Var district, South east) persists. Classified as "Endangered" (IUCN regional status) this population is particularly vulnerable due to habitat loss, forest fire, and illegal harvesting.

Conservation projects involve sensitization, habitat management and translocation. We tested the efficiency of using rescued individuals to re-enforce the most weakened populations. Following preliminary experiments, we present the results from a second monitoring of successful translocation.

Hermann's tortoise © Franck Bonin

Goals

- Checking sanitary and genetic profiles of the rescued candidates.
- Selecting suitable releasing sites and monitoring resident host populations.
- Releasing adults, sub-adults and juveniles from a pool of rescued animals.
- Radio-tracking of translocated and

resident tortoises during two years to assess survival, settling rate and body condition.

- Testing resetting-translocation to the release point in case of over dispersal.

Overview of release site © Sébastien Caron

Success Indicators

- Obtaining official agreements and permits.
- Accurate monitoring of juveniles, sub-adults and adults of both translocated and resident tortoises.
- High survival rate (>80%) and stable body condition of translocated individuals.
- High settlement rate (>50%) of translocated tortoises following initial release and possible resetting-translocation to the release point.
- Evidence of reinforcements, e.g. sexual behaviors between translocated and resident tortoises.

Project Summary

Feasibility: The Hermann's tortoise exemplifies the worrying impact of habitat loss in the Mediterranean areas that threatens many endemic species. Thanks to strict protection, wild specimens must be displaced before habitat destruction under the framework of the Avoid-Reduce-Compensate plan (ERC). This strategy aims at ensuring that economical development does not result in any net loss of biodiversity. Rescued specimens collected over time represent excellent candidates for population restoration (i.e. reinforcement translocation). Nevertheless, translocation of reptiles requires robust feedback before being generalized. For example, homing or persistent dispersal may compromise the establishment and the survival of the released tortoises.

A project to build a college planned on a site where an isolated population of tortoises prompted ERC mitigation procedures. The SOPTOM was mandated to conduct a rescue operation, and to perform translocations and scientific monitoring of released individuals.

Within the framework of a conservation Life+ program (2010 - 2014), a global feasibility study was undertaken. Then, tortoises were hard-released in spring and autumn. A three year monitoring of translocated tortoises provided encouraging results (~70% survival) and suggested that this approach is a suitable tool to

Post-release monitoring © Magalie Aferiat

reinforce eroded populations. Yet, high dispersal of several individuals compromised their survival while effective settlement sometimes required two years, and thus occurred far away from the release point. Moreover, only adults were involved, other age-classes were neglected. Here, we paid attention to the origin of individuals (e.g. discarding hybrids), site selection, and we included different age classes in the program. To minimize possible negative effect of persistent dispersal we used resetting-translocation to the release point: over-dispersing individuals were put back to the initial point of release. Expectedly, individuals prone to over dispersal may surrender and eventually decide to settle into the targeted area.

Implementation: In fall 2015 and spring 2016, tortoises rescued and maintained temporarily in the SOPTOM center were selected. This sample includes four adults (>11 years old), four sub-adults (6 - 11 years old), and five juveniles (3 - 5 years old). To our knowledge, this study is the first to involve the monitoring of young individuals. It is often assumed that the survival of juveniles is low; based on this criterion they are usually discarded from translocation projects. Robust field data are required to test this assumption. A translocated plan was granted in 2016. The wild native origin of candidates was assessed with genetic analyses. Because *Mycoplasma agassizii* and tortoise herpesvirus are important emerging pathogens, candidates underwent a strict health screening program to ensure that the selected tortoises were clean.

The release site was large (>40 ha), located in the historical range of the species but 30 km away from the SOPTOM center and from the native site of tortoises to prevent homing. Habitats were favorable (e.g. mosaic of open and forest patches) while land management was under control. Previous census of the resident host population indicated that density was low (<1 individual/ha), probably due to the impact of recurrent forestry work over years. We hard released individuals in spring 2017, a technique already validated in this species.

In addition to the translocated individuals, four resident adults from the host population and six from a nearby control population were also radio-tracked. Each tortoise was located 3 - 5 times per week during 15 months after release enabling us to precisely describe movement patterns, notably the timing and location of settlement.

Post-release monitoring: Most (85%) of the released individuals did not settle, showing over dispersal and moving beyond that limit of 1 km targeted in this project. Therefore they did not remain within the boundaries of the protected host area. Our previous studies showed that tortoises are more likely to experience mortality during prolonged dispersal, especially when they cross unfavorable habitats or dangerous obstacles. Only two juveniles settled under the 1 km limit the first year. Therefore, we put over-dispersing individuals back to the initial release point. A single resetting-translocation was successful for 50% of the over dispersing tortoises; further resetting-translocations (1 - 2) were successful for 21% more tortoises. Overall, following (1 - 3) resetting-translocations, only ~25% of the released individuals did not settle in the targeted area.

After 15 months, high survival rate (100%) and stable body condition of the individuals suggested that translocation procedures were successful. Importantly, this included supposedly highly vulnerable juveniles. Our results show that individuals adapted well to their novel environment, finding enough trophic resources to maintain their body condition within a normal range (i.e. not different compared to resident host tortoises). Resident individuals did not display any sign of perturbation due to the introduction of exogenous individuals. Resetting-translocation to the release point were essential to promote settlement into the targeted area, and thus likely to enhance survival of the released individuals.

Major difficulties faced

- Reaching a consensus on site selection amongst French authorities, land owner and site manager.
- Finding a releasing site as large as possible to limit the problems associated with over-dispersal.
- Limiting over-dispersal of released tortoises via intensive radio-tracking and resetting-translocations.

Major lessons learned

- Post-release dispersal was inevitable; many individuals did not stay in the (small) favorable host site.
- Resetting-translocation to the release point promoted settlement into the targeted area, and represent a valuable alternative to expensive acclimation pens.
- Monitoring and possible resetting-translocation must be conducted on the long term; at least more than 1 year.
- Juveniles (2 - 5 years old) are suitable candidates for translocation.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Candidates were correctly selected (high body condition, genetic and health profiles).
- The host area selected provided diverse and favorable microhabitats, notably abundant refuges for the young tortoises.
- Tenacity to perform radio-tracking and resetting-translocations payed off.

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Population reinforcement for the recovery of the Orinoco crocodile in Venezuela

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Introduction

The Orinoco crocodile (*Crocodylus intermedius*) takes its common name from the river basin where it is an endemic species. The river basin extends for 1,123 million km^2 covering almost 75% of Venezuela's territory and nearly 35% of Colombia's (Seijas, 2011). This crocodile is classified as Critically Endangered (A2bcd; C2a(i)) by the World Conservation Union-IUCN (Balaguera-Reina *et al.*, 2018). As a result of this intense overexploitation, the range of *C. intermedius* in Venezuela was reduced to a small fraction of its historical extent, and only two ecologically viable sub-populations currently survive in the country: 1) one located in the Capanaparo River in Apure state and, 2) the other in the Sarare-Cojedes River System, Portuguesa and Cojedes states (Seijas, 2011).

The captive-breeding of the Orinoco crocodile in Venezuela starting in 1978 with captive adults and achieving the first brood of 15 newborn crocodiles the next year. Since then, 10 breeding facilities have been active in Venezuela, although to this day, only four are operating at minimum capacity due to severe financial constraints. The National Strategy for the Conservation of the Orinoco crocodile in Venezuela and its Action Plan (GECV, 2007) establish comprehensive strategies to achieve the recovery of this

Orinoco crocodile

species, including regular monitoring, conservation driven research, emphasis about *in situ* conservation, captive-breeding to reinforce and reintroduce populations and encourage public awareness.

Nevertheless, through the last three decades, the only action maintained along the years is the reinforcement of the wild populations with captive-bred young crocodiles, so the present account will be restricted to analyze the results of this population reinforcement.

Goals

- Reinforce extant wild populations of *C. intermedius* and reintroduce new ones by releasing captive-bred young crocodiles.
- Enhance the genetic diversity of reinforced populations releasing wild born individuals collected from various localities.
- Increase the annual number of released crocodiles adding the breeding of captive newborn individuals with the collection and rearing (ranching) of wild born hatchlings.
- Train national park rangers, zookeepers, university students and government officers to join the population reinforcement program.
- Integrate national zoos with Orinoco crocodiles in its inventory to the captive-breeding and reinforcement activities.
- Develop tolerance and responsible attitudes of local inhabitants toward crocodiles and explain the ecological importance of the restoration and preservation of this species.

Success indicators

- Increased abundance of extant populations of *C. intermedius* and reintroduce new populations in suitable habitats.
- Good adaptation of released individuals in their new wild localities.
- Increased the number of captive reared young crocodiles released annually.
- Zoos breeding *C. intermedius* manage to release young to the wild.
- Professionals and students qualified to contribute to the *ex situ* conservation of the species.
- Reduced killing of crocodiles and nest poaching by local inhabitants.

Capture of hatchlings © Ernesto O. Boede

Project summary

From 1990 to 2019, the Orinoco crocodile reinforcement program released into wild habitats of the Orinoco basin 10,696 young captive-reared crocodiles, mostly yearlings. About 47.5% of these individuals were released in national parks, 25% in wildlife refugees, 10.4% in cattle ranches, 4.8% in wildlife reserves and the remaining 12.3% in other areas such as rivers and dams without legal protection.

Released crocodiles visible on sand bank

These crocodiles come from a stock of captive reproductive adults combined with the ranching of wild populations in order to capture wild born hatchlings. These wild animals are reared jointly with those born in captivity, for at least three year and later released preferably in protected areas with proper kind of habitat to assure its adaptation and future contribution to the reproductive segment of the reinforced populations. The idea of rearing and releasing individuals after a year is that during this initial period the mortality rates on wild born crocodiles are extremely high, so we increase the survival probabilities spending this critical period in captive conditions.

However, this *ex situ* conservation strategy has not been supported by the necessary *in situ* conservation actions, especially an effective protection and law enforcement to mitigate the threats such as illegal hunting and nest poaching, impeding the recovery of the species. Nonetheless, the population reinforcement with animals raised in captivity is regarded as the main factor that prevented the local extinction of this species in Venezuela, artificially increasing the net fecundity rate of the endangered populations and increasing survival during the critical first year of life (Babarro, 2017).

During the last decade, three locally reintroduced populations have been confirmed (Velasco *et al.*, in press). All of them paradoxically are located in cattle ranches (El Frío, El Cedral and Santa Rosa) with no suitable habitat for the development of large crocodile populations, mainly composed by small streams and savanna lagoons without proper reproductive conditions such as deep rivers and sand banks, but with strict protection of its borders. This apparent contradiction clearly reveals that the main factor affecting the success of the reinforcement and reintroductions is the awareness and law enforcement that prevents the unlawful killing of released crocodiles, as happens in Capanaparo and Cojedes rivers. The main concern is that the main populations that throughout the last 30 years were reinforced with thousands of captive-bred

Juvenile crocodiles prior to release

individuals are not increasing, although in the case of Capanaparo river a slight increase in nests numbers was detected, perhaps as a result of the release 10 years ago of 30 semi-adult female individuals (Hernández *et al.*, 2017).

These facts support the reality that the recovery of the Orinoco crocodile in Venezuela will not be achieved only with the reinforcement strategy without the effective protection of wild populations and the conservation of its habitats, as well as strict law enforcement, educational policies and socio-economic alternatives directed to local inhabitants in order to induct tolerant attitudes towards this big predator.

Through the years, there has been a sharp decline in the number of breeding centers in Venezuela, from 10 facilities fully operating a decade ago to currently just two. Moreover, these remaining facilities operate with increasing financial constraints, affecting the supply of food, maintenance of infrastructure and logistic needs to perform the releasing activities in remote areas. This state of affairs is mainly due to the lack of financial resources and the fact that the government expropriated cattle ranches where three important facilities were located, which are now inactive or operating at minimum capacity. As the result of these circumstances, form an all-time record of 795 crocodiles released in 2009 is expected a number lower than 100 in 2020.

A strategy to improve the efficiency of this *ex situ* conservation scheme is based on collection of wild born hatchlings in order to rear them during at least one year. Reproductive populations in Capanaparo, Cojedes and Manapire rivers, as well as in El Frío and El Cedral ranches, provide the wild hatchlings that will reinforce these and other areas together with crocodiles born from captive parents. The intention is to increase the number of individuals annually released and enhance the genetic variability between physically isolated populations, preventing inbreeding and genetic drift. Regarding to the contribution of national zoos, efforts to motivate administrative and operative personnel to manage their captive Orinoco crocodiles have been ineffective.

Since 2005, 11 courses have been carried out as an effort to train people in techniques for *in situ* and *ex situ* conservation of Venezuelan crocodiles. Eight courses trained 155 students in biology, veterinary medicine and related fields at the final stage of their careers, which have the intention to pursue professional careers in crocodilian conservation. Four undergraduate thesis on wild

populations and four in captive breeding resulted from these courses. In addition, three courses were focused to National Park Rangers and Zoo Managers, related with crocodile management and conservation, training 77 people, including National Guard members and government officers.

Major difficulties faced

- The hyper-inflation suffered by the country constantly raises costs of crocodile feed (meat and fish), vitamins and minerals, needed for the feeding of adult reproductive stock and the rearing of newborn. Additionally to the constant raise in operating costs, scarcity of resources is common.
- Negative attitudes of indigenous people and local inhabitants resulting in the unlawful hunting of released crocodiles that combined with extended nest poaching, are hindering the recovery of the species.
- Lack of financial resources, widespread insecurity in remote country areas, difficulties to obtain logistic elements such as gasoline and absence of official support has delayed the full extent monitoring needed to assess if the reinforcement effort have been successful or not in the national scale, based on population abundance, number of wild nests and recapture of released individuals to evaluate demographic variables, such as survival, migration, etc..

Major lessons learned

- With the reintroduction of three new populations in cattle ranches with limited suitable habitat for the species, we confirmed that law enforcement and effective *in situ* protection are the critical factors to assure the survival of released individuals, avoiding poaching and allowing the crocodiles to grow and reproduce. Intensively reinforced but unprotected populations have not increased its numbers because of this crucial reason.
- In order to change the negative perception of local inhabitants about the Orinoco crocodile, we need educational policies and economic alternatives for local inhabitants, to achieve real benefits from the presence of this big predator, using strategies based on ecotourism, alimentary alternatives other than the wild fauna (that includes crocodiles) and the communal vigilance of nests in order to annually sell hatchlings to rearing facilities.
- All Venezuelan governments, since the start of the reinforcement program to the present day, seem to believe that the captive breeding is the only needed activity to recover the populations of the Orinoco crocodile, therefore neglecting law enforcement and protection of areas where crocodile populations exist and rendering unimportant vital needs such as research, education for sustainability, population management and socio-economic improvements for local inhabitants.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reasons for success:

- The captive-breeding and releasing activities have been maintained without interruption for 30 years in spite of growing difficulties.
- The reinforcement with thousands of captive reared crocodiles have stabilized endangered populations by compensating the human related mortality.
- The confirmed reintroduction of three new populations (the first achieved for this species) confirms that this is a feasible option given the right conditions.
- The diverse origins of released crocodiles will contribute to enhance the genetic diversity of the reinforced populations.

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Population reinforcement for the recovery of the Orinoco turtle in Venezuela

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Introduction

Podocnemis expansa, is known in Venezuela as "arrau turtle", “Orinoco turtle” or simply "turtle" by the inhabitants of the banks of the Orinoco, is a species intensively exploited before the arrival of the Spaniards to America; however, the indigenous population seems not to have been big enough to deplete this resource. From the time of the Spanish colony to the present day, there has been a steady population decline due to the consumption of its hatchlings and eggs, but mainly because of the hunting of reproductive females on the nesting beaches (Pritchard & Trebbau, 1984). The Red Book of Venezuelan Wildlife considers it “Critically Endangered” by criteria A2abd (Hernández & Marín, 2008).

When in 1800 Alejandro Von Humboldt arrived to the Orinoco region, he found records that between La Urbana and Pararuma, 5,000 oil jars were produced that year, (each jar equivalent to 25 bottles), estimating that 200 eggs were required to produce an oil bottle. So based on the average number of 75 eggs per nest, he projected that during that year at least 330,000 turtle nests were exploited on these islands alone. Based on these figures, Humboldt estimated the presence of at least two million turtles in the Orinoco River. For the year 2019, only 794 nests, were recovered in this same region.

Goals

- Reinforce extant wild populations of *P. expansa* and reintroduce new ones by releasing captive-bred young turtles.
- Raise awareness among local people about the ecological and economic importance of restoring and preserving this

Orinoco turtle

species, particularly in those places where it was released.

Success indicators

- Increase the population size of *P. expansa* in the Middle Orinoco river, specifically in the Arrau Turtle Wildlife Refuge and establish new populations in the historical range of the species.
- Obtain evidence of the increase in the size classes corresponding to young and sub-adult specimens as a reflection of the growth of the released specimens.
- In the long-term, observe an increase in the number of nesting females in localities where records of their population trends are made.
- Reduction of turtle illegal consumption and nest poaching by local inhabitants of the Arrau Turtle Wildlife Refuge.

Project Summary

In 1989, the Arrau Turtle Wildlife Refuge (RFSTA) was created to protect the main nesting beaches in the Middle Orinoco. From that year until 2018, the Ministry of Environment (MINEC) carried out a conservation program in this refuge with the law enforcing support of the National Guard. It included *in situ* (transplantation of nests at risk for flood, vigilance of nesting areas, rescue of hatchlings, release of young turtles and population monitoring) and *ex situ* management (captive rearing of hatchlings for 12 months each year and research on captive growth), additionally to an environmental education program (Hernández & Marín, 2008).

By 1992, the MINEC started the captive rearing of wild born hatchlings with the purpose of increasing their survival during the first year of life and then releasing them to reinforce population. Throughout the years at least 15 turtle farms participated in this activity, pioneered by the Foundation for the Development of Physical, Mathematical and Natural Sciences (FUDECI).

Orinoco turtle release

The first release of young turtles raised in captivity was in 1993 and until 2019, 733,334 captive reared *P. expansa* yearlings have been released, according to official figures. However, these figures do not fully coincide when comparing different publications and reports prepared by this Ministry and other sources. The vast majority of these turtles

(89%) have been released in the middle Orinoco River inside the limits of the Arrau Turtle Wildlife Refuge, but also in other localities, such as two National Parks, two Wildlife Reserves, a Wildlife Refuge, a Biosphere Reserve, a Forest Reserve, several localities of the Orinoco and three cattle ranches.

Post-release monitoring of turtles

It is estimated that females take between 15 and 28 years to mature (Mogollones, 2005; Hernández & Espín, 2006), so the turtles released at the beginning of the captive-rearing program should already be breeding adults, but so far there has been no evidence of a significant increase in the number of nesting females in the Arrau Turtle Wildlife Refugee, where the number of nests is monitored year after year. It's expected that their recovery will only be possible after many decades of effective protection to avoid the illegal hunting of nesting females. However, sampling conducted in the years 1998, 2000, 2001 and 2004 showed an increase in the proportion of young females, suggesting that the specimens released in previous years survived and grew up, so every year there is a certain level of recruitment of new specimens of the bigger size classes (Mogollones, 2005).

However, the species is relentless hunted by the riverside inhabitants, fisherman and farmers, for consumption and sale. Assessments made in 2000, 2001, 2002 and 2008 in localities near the Arrau Turtle Wildlife Refuge, determined that of the three species of turtles consumed, *P. expansa* represented between 72 - 78%, and about 95% of these recorded specimens belonged to individuals in the juvenile and sub-adult classes, indicating that part of these consumed individuals belong to the released specimens raised in captivity (Hernández & Espín, 2003; Peñaloza *et al.*, 2013). If this persistent illegal consumption of young and adult specimens continues, the recovery of the population by means of reinforcement is not guaranteed.

Currently and since the last two years (2018 and 2019) the Arrau Turtle Wildlife Refuge have very limited personnel presence from the Ministry of Environment (MINEC), so the vigilance and law enforcement are not being carried out and it is assumed that the hunting pressure on the species should be increasing. The monitoring of population abundance and its consumption has not been addressed since 2008, therefore the current state of the population is mainly unknown.

Major difficulties faced

- In the last decade, there was an implicit policy from the government to exclude Environmental Civil Society Organizations from this conservation program, but the government itself is not carrying out the functions that these organizations previously accomplished, therefore, an information vacuum has been created on the current situation of the populations of this species in Venezuela.
- The lack of financial support has been another great difficulty to keep the *in situ* and *ex situ* conservation activities for population reinforcement, monitoring and environmental education.
- The absence of environmental vigilance and law enforcement has surely caused an increase in the illegal hunting of turtles of all sizes, as well as the poaching of nests by river communities, so the recovery of the species in Venezuela is not guaranteed.

Major lessons learned

- The field studies carried out until 2008, proved that the population reinforcement was successful, because over the years there was an increase in turtles of size-classes corresponding to the older stage previous to adultness.
- Captive-breeding of this species is easy activity perform successfully, and under adequate hygiene conditions and enough food supply, is a speedy way to increase the population numbers.
- *P. expansa*, with a late sexual maturity and under intense and continued hunting pressure, needs many years to achieve an increase in the adult population by reinforcement strategies, taking for granted an adequate protection of nesting females and law enforcement, without which the captive breeding effort will be useless.
- The different governments of Venezuela between 1990 and 2019 seem to believe that population reinforcement is the only activity necessary to recover the species, so the lack of vigilance, law enforcement, education for sustainability, applied research, *in situ* species management and socioeconomic initiatives to improve the economical condition of local inhabitants.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reasons for success:

- The captive-breeding and releasing activities have been maintained for 30 years agonists all odds, although for the moment it is suspended because of financial constraints.
- The reinforcement with hundreds of thousands of captive reared turtles have created an input of enhanced survival recruits to the young and adult

segments of the population, although no visible increase in the abundance of breeding females has been evidenced.

- The confirmed increase in higher-size young individuals confirms that reinforcement is an efficient strategy to increase threatened populations, granted the reduction of illegal hunting.

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Translocation of Newell's shearwaters and Hawaiian petrels to create new colonies on Kaua`i, Hawai`i, USA

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Introduction

The critically endangered Newell's shearwater (*Puffinus newelli*) and the endangered Hawaiian petrel (*Pterodroma sandwichensis*) are Hawaii's only endemic seabirds. Both species are rapidly declining (Raine *et al.*, 2017) due to collisions with power lines, light attraction, predation by invasive feral mammals and introduced Barn owls and habitat degradation by feral ungulates and invasive plants. Given the challenges in protecting nesting birds in their rugged, montane habitat, it has long been desirable to create protected populations of both species in more accessible locations. Translocation has been part of recovery planning since 1983 and translocation to predator exclusion fences was ranked as the highest priority action in the interagency five year Action Plan for Newell's shearwater and Hawaiian petrel (Holmes *et al.*, 2015). In 2012, funding became available to construct a predator-proof fence and conduct translocations to create new colonies at Kīlauea Point National Wildlife Refuge (KPNWR) on Kaua'i. The translocation site already supports one of the largest seabird colonies in the main Hawaiian Islands, is close to the coast, away from artificial lighting, and under

Newell's shearwater (left) & Hawaiian petrel (right)
© Lindsay C. Young (left) & Andre Raine (right)

federal ownership in perpetuity. The goal of this project was to create the first predator free colonies of Newell's shearwater and Hawaiian petrel.

Goals

- Establish the first fully protected colonies of Hawaiian petrel and Newell's shearwater to reduce extinction risk for both species.
- Create a predator-free translocation site for Hawaiian petrel and Newell's shearwater.
- Locate and monitor suitable burrows from which to translocate birds.
- Translocate and rear until fledging up to 100 individuals of each species over a five year period.
- Use social attraction to attract translocated and wild birds to the site.

Success Indicators

- Proportion of translocated chicks that fledge ≥70% in year one; ≥80% afterwards.
- Wing and mass measurements of translocated chick are larger than those of wild chicks at fledging, indicating good health.
- Proportion of translocated chicks that return to KPNWR is expected to be 15 - 40%, which is the estimated return rates in existing colonies.
- Some wild birds from other colonies also visit the translocation site.
- Number of birds (both translocated and fledged from other sites) that recruit to the new colony is about ≥15% (estimated return rate of existing KPNWR colony) and 33% (rate of survival in unprotected colonies from Greisemer & Holmes, 2011) and by year six.

Project Summary

Feasibility: Three years of planning and scoping were done prior to commencement of translocation. The translocation protocols were based on methods developed in previous projects and the translocation team visited similar projects in New Zealand to learn translocation, predator exclusion and habitat restoration techniques. From 2012 - 2019, potential source colonies of Newell's shearwater and Hawaiian petrel were located with visual, auditory, and ground searching methods in montane areas of Kaua`i. The source sites chosen had high call rates, high burrow densities to provide an adequate number of chicks for translocation, and had active predator control operations in place to offset any potential impacts of the monitoring. Finally, several community meetings and multiple government consultations were conducted to keep stakeholders informed on the project and allow the public the opportunity to comment.

Implementation: In preparation for the translocation, as well as for the predator exclusion fence, a total of 17 permits or consultations were completed. A complete list of the permits and processes followed can be found in Young *et al.* (2018). In 2014, a 650 m long predator fence was constructed enclosing 2.8 ha and all mammalian predators were eradicated. Approximately 70% of the fenced

Nesting habitat © Andre Raine

area was cleared of non-native vegetation using heavy machinery and herbicide application. A water catchment and irrigation system were installed and over 18,000 native plants of 37 species were out-planted in the restoration area. Fifty artificial burrows were installed in the restored area to house and facilitate the feeding and care of the chicks.

From 2015 - 2019, a total of 90 Hawaiian petrel and 67 Newell's shearwater were translocated from source colonies to the translocation site. Upon arrival at the translocation site, chicks were placed in artificial burrows and hand-fed until they fledged 2 - 6 weeks later. Chicks were fed a mixture of fish, squid, fish oil, and vitamins that was developed by previous successful translocation projects. Morphometric monitoring was done daily to measure chick growth and health and the majority of birds appeared to be in excellent health. All (100%) of Newell's shearwater survived to fledge over the four years of translocations and 98% of Hawaiian petrel survived to fledge during five years of translocation.

Post-release monitoring: The translocation site is monitored with 10 - 12 camera traps and in-person twice monthly during the breeding season to determine if birds are visiting the translocation site. However, no monitoring is done at the collection site to determine if birds are returning to their natal location. Two acoustic attraction systems are set up at the release site playing calls of each species 24 hours a day. The artificial burrows are checked for seabird sign (digging, guano, feathers, and scent) twice monthly. Visual and nocturnal acoustic surveys are done monthly to watch and listen for prospecting birds. While multiple individuals of both Newell's shearwaters and Hawaiian petrels have been observed flying overhead, only Hawaiian petrels have landed at the site today, and at the time of writing, it is still considered too early for the species to have begun breeding at the site. Wedge-tailed shearwaters, Bulwer's petrel, Kermadec petrel and Red-tailed tropicbirds have all been observed on the ground at the site.

Major difficulties faced

- Locating enough accessible burrows to establish suitable translocation cohorts each year.
- Restoring habitat within the translocation site from predominantly invasive species to native species and to a habitat structure suitable for burrow

nesting seabirds was labor intensive and significantly increased the cost and scope of the project.

- Maintaining optimal temperatures and drainage inside the artificial burrows.
- Ensuring continuity of the volume and quality of calls on the acoustic attraction system.

Translocating Hawaiian petrel via helicopter
© Lindsay Young

Major lessons learned

- When applying for permits, ask for the maximum number of sites and individual animals to ensure you have the flexibility to expand or modify the scope if needed.
- Artificial burrow designs are every site (and climate) specific, and multiple designs should be trialed prior to installation to determine the best design.
- Post-translocation monitoring of the source colonies should be undertaken to determine where the chicks return as adults.
- The primary driver of habitat restoration efforts must be needs of the seabirds.

Success of project

Highly Successful	Successful	Partially Successful*	Failure

*** - ranking for both Newell's shearwater and Hawaiian petrel**

Reason(s) for success:

- The translocation aspect (removal of chicks from natal burrows and hand rearing until fledging) was highly successful, with fledging rates of 100% for Newell's shearwater (N=67/67) and 98% for Hawaiian petrel (N=87/90). This was due to strong coordination between partners and extensive avian husbandry training of staff involved in captive rearing.
- Social attraction results have been encouraging, with both translocated species observed flying over the speakers along with Wedge-tailed shearwaters, Red-tailed tropicbirds, Bulwer's petrel, Kermadec petrels as well and Hawaiian petrels all observed on the ground at the site.
- It is still too early to determine ultimate success, which would entail recruitment of breeding individuals to the release site.

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Reintroduction of the red-billed chough in Jersey, British Channel Islands

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Introduction

Red-billed choughs (*Pyrrhocorax pyrrhocorax*) are specialist invertebrate feeders found in mountain or coastal regions of Europe, North and East Africa, and Asia. The species is listed as Least Concern by IUCN. However, in the British Isles, the population has become fragmented with less than 500 breeding pairs. Choughs died out on Jersey (117 km^2), and the other British Channel Islands, at the turn of the 20th century. Changes in agricultural practices and, to a lesser extent, human persecution (egg collecting) led to their demise. *Birds On The Edge*, a multi-partner project, was established in 2010 to restore Jersey's depleted coastland bird populations through management of coastal farmland, and to reintroduce chough, which, as a very visible flagship, will help drive habitat restoration. Durrell Wildlife Conservation Trust manages the reintroduction with a captive-breeding program at Jersey Zoo. Paradise Park (Cornwall, UK) provided the donor stock for breeding (which were originally of wild UK origin) and most of the birds used in the release.

Goals

- Establish a free-ranging population of Red-billed choughs on Jersey, Channel Islands.
- Enhance the survival of the declining north-west European chough population, by re-establishing Jersey's population.
- Use the reintroduction to drive coastal farmland habitat restoration in Jersey.
- Develop the techniques of releasing and managing choughs and other social birds.
- Promote public awareness of coastal restoration and farmland ecology.

Red-billed chough © Elizabeth Corry

Success Indicators

- 30 - 50 chough released over five years.
- 33% survival from release to adulthood (two years).
- A population of at least 15 individuals, including at least one breeding pair, established by end of Year 5.
- In the medium-term (20 years), coastal farmland habitat is restored enough to support a free-living population of choughs with minimal need for supplemental feed.
- Chough ecology data disseminated to stakeholders and practitioners.
- Supportive landowners and residents actively engaged in the conservation of Jersey's coastal biodiversity.

Project Summary

Feasibility: Red-billed chough are sedentary, very poor dispersers and the likelihood of a natural recolonization on Jersey is remote. Historic population sizes in the Channel Islands are unknown. Their demise was in large part due to the removal of sheep in the 19th century which resulted in bracken invading coastal farmland making their main food, soil and dung invertebrates, inaccessible. Conservation grazing began on the north coast using Manx loaghtan sheep in 2009. Feasibility studies concluded this grazing site was the most suitable for the choughs. Durrell's conservation experience, and lessons from a chough reintroduction attempt in the UK, informed this restoration. This was the first licensed reintroduction in Jersey. Staff liaised with the States of Jersey and stakeholders. Perceived negative impacts to farmland crops were assuaged following staff consultation.

Implementation: Jersey Zoo started captive-breeding in 2010. Soft-releases were conducted between 2013 and 2018; where 43 choughs were reintroduced. Birds were released in eight cohorts of 3 - 8 (mean 5.4) replicating normal family group size. The intention was to release chicks shortly after fledging. Low productivity at Jersey Zoo resulted in Paradise Park having to provide sub-adults (1 - 2 year olds, and a four year old) for the initial release. Subsequent releases used juveniles (<6 months old) (60 - 159 days, mean 103 days). There were no releases in 2017. In 2018, three 1 year old males were released to address the sex ratio imbalance. Jersey Zoo developed artificial incubation and hand-rearing techniques, resulting in eight hand-reared chicks released (58 - 71 days old at release).

These birds were held in the release aviary for 15 - 150 days. The aviary was 18 x 8.5 x 3.6 m high, made of semi-circular poly-tunnel hoops covered with nylon netting. At one end a smaller covered area (3.6 x 8.5 x 3.6 m) served as a shelter and service area. The flight was divided in two, with interconnecting doors and hatches, so the birds could be separated if necessary. The birds left and entered the flight via hatches (0.6 x 0.4 m) high up at one end of the flight. They were trained to associate a whistle with food, enabling staff to call birds into the aviary if they need to be re-captured.

Staff in field tracking chough © Elizabeth Corry

The birds first bred in the wild in 2015 in buildings within a working quarry <1 km from the release site. The year before, five nest boxes had been installed along the cliffs at the release site. The birds chose not to use them until 2019 when three were occupied; none fledged chicks. An additional two boxes were placed in quarry buildings in 2017 to discourage nesting on machinery. One was used and fledged five chicks from three breeding attempts. The other was not used until 2019 and fledged three chicks. Future efforts will focus on understanding feeding ecology, allowing supplemental feeding to be minimized, shifting the birds from a current mixed diet to a pellet diet, and protection of wild nests. Further release, of unrelated birds may be attempted to increase genetic diversity.

Post-release monitoring: Monitoring was undertaken by a Durrell staff member (EC) and volunteers. The public reported sightings to www.BirdsOnTheEdge.org, via social media, or direct to the team. Birds were fitted with colored leg rings and a permanent numbered metal ring. Tail-mounted VHF transmitters were attached for six months, or until first molt. Daily fixes were taken to determine survival, dispersal and foraging habits. Supplemental feed was given daily at the release site. Health, behavior, and life events were recorded into Species360 Zoological Information Management System (ZIMS). Pre-and post-release fecal screening monitored parasite load. Birds with high loads were treated by a veterinarian. In two cases of injuries requiring anesthesia (foot injury caused by ring and lameness), the individuals were transported to Jersey Zoo's Vet Centre for treatment and released that day. Cause of death where known has been starvation (n = 3), aspergillosis (n = 2), and predation/mortality by Peregrine falcon (*Falco peregrinus*) and Lesser black-backed gulls (*Larus fuscus*) (n = 2). In 63% of cases (n = 19) cause of disappearance was unknown.

Nesting attempts were monitored. Chicks were ringed at 3 weeks old for those nests that were accessible. Between 2015 and 2019, 18 different pairings, and 36 pair years, produced 36 nesting attempts where eggs are believed to have been laid; 19 (53%) fledged at least one young. In total, 36 young fledged. Excluding chicks hatched in 2019, 70% of the 20 chicks that fledged survived to one year. Clutch size was four (n = 3), and mean brood size at fledging of those fledging young 1.9. In 2019, a chick fledged from a natural nest site 5 km from the release aviary. Once the birds survived their first year at liberty survival is high. From 2015 to 2018, during 95 bird years, annual survival was 93.7%. By July 2019,

Jersey's chough population was 48, of which 24 were hatched and reared in the wild, including 13 fledged that year.

Major difficulties faced

- Limited success with captive breeding at Jersey Zoo meant sub-adults were used in the first year which relied on imported birds.
- The design of the aviary was not optimal for socializing birds imported from the UK, since it was difficult to adequately separate and mix birds. Planning laws and landowner prerequisites meant that the aviary had to be a temporary structure restricted in square footage. The operational design and materials used for the release hatches led to management problems. Salt air corrosion and exposure to adverse weather resulted in regular repairs. The choughs learnt how the hatches operated and avoided entering if staff were present.
- Health and Safety regulations at the working quarry created problems during initial releases. A bird refusing to leave the quarry might be in an inaccessible location for staff to provide supplemental food. In two cases this resulted in death by starvation.
- Birds imported from the UK have always tested positive for *Syngamus trachea* parasites. Stress levels due to aviary confinement pre-release elevate the problem. Treatment was successful in all cases pre-release. Once released, treatment depends on the ability to recapture an individual. One individual that tested positive, and evaded capture, was predated by a Peregrine falcon. In 2017, all four wild-reared chicks tested positive at or soon after fledging. Two were trapped in the aviary, treated, and survived. Two evaded capture and died.

Major lessons learned

- Greater success is achieved by releasing choughs under six months of age. The one year post-release survival for birds released <1 year old was 25 out of 30 (83%) with the loss of three occurring after living free for more than six months. Survival of birds released at ≥1 year old was 8 out of 13 (63%) with four losses occurring less than three weeks post-release. The oldest bird released, a four year old male, flew away from the group upon release and was never seen again.

Chough aviary © Elizabeth Corry

- Hand-reared birds, because they do not fear their manager, are easier to look after, are orientated to the release

aviary, and act as anchor for the flock at the release site.

- Supplemental feeding supports the population during times of limited wild food availability. The three birds that died of starvation were recently released birds that became stranded in the quarry and could not access supplemental feed.
- Socialization with siblings or other young choughs is important prior to release for them to learn social and foraging skills; individuals reared alone without siblings are more likely to fail in the wild even if parent-reared in captivity.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- A year-round hands-on approach to daily management taking adaptive measures when necessary.
- A mix of hand-reared and parent-reared birds in the release cohorts creates a more amenable flock. The hand-reared birds easier to train to return for supplemental food and the parent-reared birds are better socialized to other choughs.
- Supplemental feeding is crucial during the initial release phase and during periods when natural food availability is low and/or food competition is high, e.g. over winter.
- Veterinary support to monitor and treat choughs for Gapeworm (*Syngamus trachea*) and injuries, has increased survival.
- Without stakeholder support we would not have been able to rescue birds trapped in buildings, retrieve bodies for *post-mortem*, monitor roost and nest sites, and access nests to collect data and fit leg rings.

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Conservation of the Andean Condor in Chile: *ex situ* and *in situ* actions

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Introduction

The Andean condor (*Vultur gryphus*), the largest obligate terrestrial scavenging bird, inhabits the Andean range from Venezuela to Cabo de Hornos. Globally, it is considered as a Near Threatened and decreasing species, and CITES Appendix I. Andean condor populations have decreased critically in the northern part of their range, while southern populations are healthier, however they are showing signs of retraction. The population in Chile has declined due to persecution and reduction of food resources, except in the southernmost tip of the country, where it may be stable. Central Chile's Andean condor population is declining due to habitat loss, hunting, and reduction of food resources. This species was classified in Chile as Vulnerable by the Chilean law. However, it is classified nowadays by the Ministry of Environment as Near Threatened, according to the IUCN classification criteria. In addition to its ecological importance as scavenger, the Andean condor has been important to Andean cultures during centuries as a biocultural element.

The Chilean Ornithologists Union (UNORCH) manages a Center for Condors and other Birds of Prey, promoting knowledge about these species and their population health, including, among other areas, field research, rehabilitation, captive-breeding, releases and educational work with the communities.

Andean condor in flight © Eduardo Pavez

Goals

- Implement a conservation program including captive management, rescue, rehabilitation and release of Andean condors in Chile.
- Generate useful information for conservation strategies of the Andean condor, by means of casuistic of the cases received in the Center and satellite monitoring after releasing.

- Spread a message on nature conservation, by means of explaining to the public the work of rehabilitation and showing release cases of charismatic species, such as the Andean condor.
- Implement an educational program in rural areas, before the condor's release, in order to change the false image of them being livestock predator.

Andean condors at release site © Caroline Moreno

Success Indicators

- Number of condors which are managed (n = 169, 100%) and received from the wild (n = 144, 85%).
- Number of released condors (n = 75, 44%).
- Number of condors which are born in captivity (n = 25, 15%).
- Number of condors which are sent to other conservation programs in South America (n = 7, 4%, 1 to Bolivia, 3 pairs to Colombia).
- Mortality in the center (n = 28, 17%) and after its release (n = 2, 1%).

Project Summary

Feasibility: Once dependent on the wild camelids and other large mammal predated by pumas throughout the Andes, the Andean condor is a carrion-eating species that now relies mostly on livestock. In the northern part of its range it is rare and Chile and Argentina host the largest proportion of the world's population. The Andean condor has an exceptionally low natural mortality and reproductive output, having one of the highest survival rates among birds. Condors may breed every two years or even longer, depending on food supply, having their first fertile egg-laying only over eight years of age. Therefore, any increase in their mortality due to human persecution, may jeopardize the population's persistence.

Among demographic factors of the population regulation of raptors, mortality is probably the most difficult factor to assess because most deaths are not easily detectable. This makes it particularly difficult to accurately assess mortality over large areas and long periods of time. The study of raptors received in rehabilitation centers provides information on mortality issues faced by their population, helping to identify risk factors to which they are exposed. Quantitative assessment of these risk factors might allow managers to improve their

Condor at release site © Francisco Croxatto

approaches to the conservation of wildlife. On the other hand, benefits of rehabilitation and captive-breeding programs are evident, including the return of individuals to wild population, and significant educational effect on people.

Implementation: In 1991, the Center for Condors and other Birds of Prey began to operate. Statistics of the cases admitted to the Center are an important source of information on the threats that the Andean condor faces currently in Chile. A total of 144 wild Andean condors were admitted in the Center between 1992 - 2019. They were obtained from the following areas of Chile - northern (1), central (112) and southern (31). The sample includes areas with human population density which is contrasting: low in Southern Chile and high in Central Chile.

Southern Chile: Most commonly admitted birds were young condors captured by humans shortly after leaving the nest (68%). Some other condors had unspecific trauma and symptoms of starvation and 25% of radiographed birds had ammunition in their bodies. Condors were admitted with no discernible peaks in temporal distribution and there were not cases of poisoning.

Central Chile: Most frequent causes of admission were poisonings (52%) and collisions with power lines. There were also cases of starvation, and a few birds were admitted with evident gunshot wounds. Among birds for which X-rays were taken, 72% had ammunition in their bodies, mainly adults, indicating they were shot during their life. Most condors (95%) were found in the wintering period for livestock, when they move to lowlands and, therefore, are more exposed to people and infrastructure. Results point out a strong effect of anthropogenic factors on mortality in central Chile.

Condor poisonings in central Chile occur in two contexts. Firstly, birds may ingest poisoned carcasses placed in the field by ranchers, mainly to control feral dogs, particularly during the calving season for cattle. Secondly, condors may be poisoned by ingesting toxic products in landfills. The proportion of adult/non-adult birds received from central Chile was significantly higher than in the south, suggesting therefore, that the causes for admission of condors from central Chile are related to age-independent factors (e.g. poisoning, collisions with power lines and hunting), which tend to affect adults and juveniles equally. In long-lived species, high mortality among adults may result in catastrophic effects, due to its negative effect on reproduction.

Post-release monitoring: Twenty-seven condors (36% of total released condors) were equipped with radio telemetry and/or satellite tags, jointly with intense field work, allowing close monitoring of released birds. Two condors with transmitters died shortly after their release (7%), due to poisoning and shooting; one and eight months after being released, respectively.

Released condors © Francisco Croxatto

Satellite monitoring allows us to study several important parameters for conservation. For instance, the same individuals use both, Chilean and Argentinean slopes of the Andes, with a home range between 14,169 and 266,624 km^2, extending it in summer whereas reducing it in winter and spring. In central Chile, condors occupied higher areas in summer and lower areas in winter, what was probably related to distribution dynamics of extensive livestock. Condors using landfill regularly for feeding have much shorter home range.

The wide area where condors move, including Chile and Argentina, reveals the need to coordinate bi-national conservation efforts, and to consider strategies that include large territories with extensive human uses. For this reason, Fundación Bioandina Argentina and UNORCH work coordinately since 2001. All releases have been covered by media and accompanied with education activities for rural communities located nearby liberation areas, emphasizing identified threat factors to mitigate.

Major difficulties faced

- To get permanent funding, allowing the project work in the long-term.
- Scarcity of commitment and Government's contribution.
- Complexity of the Andean topography and climate, which imposes hard logistic challenges for field monitoring.

Major lessons learned

- Importance to work with rural communities in the release areas, because people usually perceive condor as an enemy of cattle.
- Effectiveness of using the charismatic image of Andean condor as umbrella species, spreading the message of nature conservation in a broad sense.
- Importance of cooperation with others foundations that are interested in releasing condors in its own territories, regarding to spread the message on

nature conservation by mean of the condors image, for instance, the Tompkins Conservation in Chilean Patagonia and Caserta Foundation in Central Chile.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Persistence over time of the Project and Center.
- Charismatic image of the Andean condor.

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Rewilding Patagonia: population augmentation of the Darwin's rhea in Patagonia National Park, Chile

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Introduction

The Darwin's rhea (*Rhea pennata pennata*) is a large flightless bird that inhabits the steppes of Patagonia in Chile and Argentina. The species is categorized as Least Concern showing a wide distribution, but the population trend is declining in most of its range due to intensive farming, hunting and egg harvesting. All of these impacts have been identified as primary limiting factors for Darwin's rheas (IUCN, 2019). Recently, the northern Patagonia population of this ratite species has been listed in Chile as endangered, due to both the small population size and population fragmentation (MMA, 2018). Internationally, the species is listed in CITES Appendix II.

The Darwin's rhea recovery and conservation program in Patagonia National Park in southern Chile began in 2015, beginning with a remnant population of less than 15 birds that remained in the wild.
The long-term objective of this species conservation project has been to ensure the persistence of a viable wild population of rheas in the only protected area of the region. This is the first effort to rewild the species in suitable habitats in Chile, along with the Guanaco (*Lama guanicoe*), the endemic Darwin's rhea represents a significant proportion of native animal biomass on the Patagonia steppe.

Darwin's rhea © Cristián Saucedo

Goals

- To avoid local extinction and reaching a minimum population of 100 mature

rheas in the wild.

- To double the range distribution of the species in the new National Park.
- To diversify the genetic pool of the local wild population through the addition of individuals from other wild populations.
- To manage and breed a captive rhea population in a wild environment.
- To document social integration and subsequent reproduction of individuals released into the wild.

Success Indicators

- Wild rhea population surveys have consistently documented a small population of <30 birds (2008 - 2015). Recently, an increase to 45 - 50 birds (2018 - 2019) has been documented.
- Expand the current distribution of rheas in the national park by at least 30% (4,000 ha in total) of their former range.
- Use of at least three geographically distinct rhea populations as genetic sources for breeding and eggs in the management plan.
- Annual breeding success in captivity and employing artificial incubation during four subsequent seasons.
- Release 24 rheas into the wild population as these birds were hatched and raised at the breeding center and maintain an annual mortality rate of <10%. Rhea individuals demonstrate social integration from 2016 - 2019 and display reproductive behavior with wild birds (2019).

Project Summary

Feasibility: The early conservation actions developed focused on converting the traditional land use of the area from a fenced ranch to a proposed national park through the removal of 30,000 domestic sheep and associated dogs and fences which have been historically responsible for population fragmentation. In 2014, the first park wardens were created for rhea monitoring, and to control of threats such as poaching and egg collection. A study of the available rhea habitat for the local area was conducted by experts from the University of Chile who concluded that large tracts of land with suitable rhea habitat remained (Estades, 2013). This provides a unique conservation and recovery opportunity that is enhanced by the proposal for an official designation of the area as a national park by the Chilean government. The local rhea population was also extremely small and isolated. Technical meetings and field work with species experts, Daniel Sarasqueta (Argentina) and Jurgen Rottmann (Chilean Ornithologists Union), were organized to discuss the feasibility of establishing a rhea breeding center. One major difficulty identified was access to adult and mature breeding birds in captivity.

At the end of 2014, two orphaned rhea chicks were rescued by the Chilean border police. This triggered the Chilean wildlife authority (SAG) to authorize the creation of the breeding center for reintroduction purposes, the first wildlife facility of its kind in the district.

In order to maximize the success of the breeding program, a unique special aerial

transport of 12 rhea chicks from commercial breeders up north to Patagonia (covering 1,000 km in a single day) was organized by Fauna Andina and Tompkins Conservation. The genetic origin of the chicks was from two Patagonia populations and they were tested for possible diseases by the health authorities.

Darwin's rhea at release site

Implementation: One major challenge has been the operation of the breeding facilities in a very remote and isolated mountain area in the park, which is very close to the to the Argentina border and is exposed to severe wind storms and heavy snow. Currently, the program infrastructure is highlighted by three features components: 1) Reproductive pens: 3,000 m^2, 2) Management and acclimation pens: 1,400 m^2 and 3) Pre-release pens: 72 ha. The first two areas have both perimeter fences and are electrified. They were constructed in rhea habitat. Native vegetation was utilized which allowed an important nutritional contribution for rheas grazing inside the pens, incorporating food-based supplements, pellet food, alfalfa hay, vitamin and mineral intake (Saucedo *et al.*, 2019). Information about the care, management and design of facilities for rheas with commercial purposes were available, but only under controlled conditions to maximize profitability. In this case, we made adaptations for the management of a captive population in a natural harsh environment that is exposed to natural wild predators and we used native rhea food complemented with food pellets.

During the first breeding season, artificial incubation and chick adoption by breeding males were attempted by taking advantage of excess eggs that were available. We also identified one wild healthy population of rheas located 300 km north on private land that served as an egg donor and established a partnership with the local land administrator. This type of wildlife management that yields successful results maximizes the chances of adding eggs and subsequent rhea chicks to wild populations. In captivity we decided to evaluate some methods for marking individual rheas, which was not an easy task. We initially used a simple Multi-Loc system and combined that with VHF transmitters fitted on the neck with acceptable results, but with a high rate of losses (Saucedo *et al.*, 2018).

The wise involvement and education efforts for neighbors and local communities has been a permanent component since the early days of this initiative. This provided permanent local support by establishing a network of collaborators and partners.

Post-release monitoring: Park wardens were established in the area to develop regular patrols employing direct point counts to monitor the local rhea population in the wild. Population counts recorded less than 30 birds for 2008 - 2015, showing an increase to 45 - 50 birds during 2018 - 2019. As a consequence of fence removal and the release of 24 rheas during 2016 - 2018, the area of habitat used by the species increased to 4,000 ha, representing an increase of 30%. All released birds were marked with plastic collars, but less than 10% retained the mark for over three months, which affected the record of detailed individual data. Marking rheas for monitoring purposes represents an ongoing challenge. A new system of neck and leg rings is in place for 2020.

After three months in an acclimation enclosure of 72 ha, the released rheas in all cases were able to explore greater distances (3 - 6 km) into new habitats and interact with wild individuals. Potential positive breeding and mating behaviors have also been recorded in the wilds of Chile for first time in August 2019.

Major difficulties faced

- Limited access to incorporate adult captive rhea individuals into the breeding program.
- Remote environmental and access conditions which complicate the maintenance of operational infrastructure and optimal size.
- The access to a reliable marking and tracking system for released birds has been especially challenging.
- Unexpected impact of a puma predation event that killed 22 rheas in pre-acclimation pens during the second reproductive season (2018). It forced the change in the design and height of electric perimeter fences (from 2.5 to 3.1 m).
- Difficulty to predict the expected annual results given the variability in number and sex ratios of breeders and chicks due to losses by trauma associated with climate events (storms).

Major lessons learned

- Artificial incubation and easy adoption by male rheas allow the diversification of genetic sources using eggs from other populations.
- Reduction of acclimation time for rheas in small pens at early ages (<4 months) reduces the risk of predation inside the pens and promote the development of skills to avoid predators.
- It is preferable to use soft release protocols covering a period of 2 - 3 months, to reduce habituation behaviors in captivity.
- Food supplementation is not necessary after release, since rheas prefer wild food over pellets.
- To encourage group associations over individual relationships in all rhea development stages (birthing, adoption, transportation, translocation and release).

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Released rheas show positive social interactions with wild rheas and guanacos, which is a natural anti-predatory strategy between species (more vigilance and increase in escape distances). A significant restoration indicator of the ancestral relationship and an indicator of ground-breaking rewilding success.
- Increase of rhea population from less than 30 to over 50 in Patagonia National Park.
- An increase in over 30% of the habitat range where the species is now present.
- The use of three geographically different population sources for breeders and eggs involved in the management and augmentation of the species to ensure that the wild rhea population has increased its genetic diversity.
- The improvement in local conditions for the species due to removal of fences, threat reduction, increase in patrolling by park wardens and more awareness of the species by neighbors and the local community.

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Lessons learnt from the reintroduction of long-lived, cooperatively breeding southern ground-hornbills in South Africa

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Introduction

The Southern ground-hornbill (*Bucorvus leadbeateri*), which is the largest cooperatively breeding bird, is listed as Vulnerable globally and Endangered in South Africa. It occurs from southern Kenya, to the Eastern Cape province in South Africa. Reintroduction protocols used in South Africa can be applied throughout the species range, and to the species congener, the Northern/ Abyssinian ground-hornbill (*Bucorvus abyssinicus*). A specialized captive rearing center has been built to ensure that the redundant, second-hatched, wild chicks harvested for the reintroduction program have optimal physiological attributes and behavioral skills to survive and reproduce post-reintroduction. Juvenile males identified for reintroduction must go through a minimum of three years in a wild group, called a 'bush-school' where wild-experienced group members mentor them in survival skills. They are then paired with a naive female mate to form a new breeding group, and additional helpers are added to the group annually for mentoring until the group successfully begins to breed.

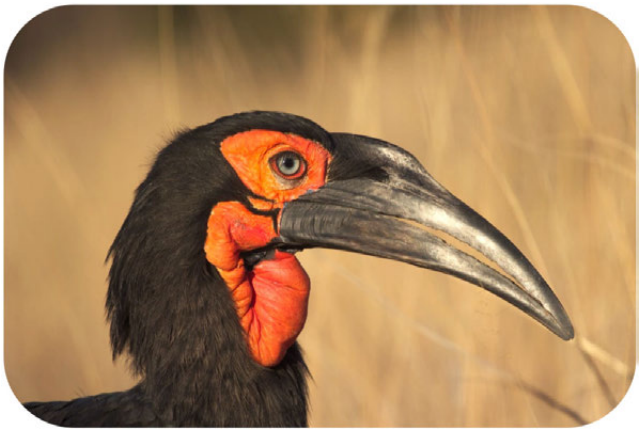
Goals

- Restore populations by reintroducing founder groups into areas where local extinction has occurred.
- Link fragmented populations to connect and encourage natural gene flow.
- Use reintroductions as a powerful awareness tool with rural communities and local landowners.
- Engage in active threat mitigation to stimulate growth in the wild population and to enhance security for the reintroduced population.

Success Indicators

- Three new founder groups are reintroduced per annum.
- As many groups as possible, both wild and reintroduced, are supported with the provision of artificial nests.
- The rearing center provides stock for the captive-breeding and rearing program, removing the need for any further wild offtake.
- Slow and ultimately reverse the decline of the population.
- Enhanced conservation effort and policy supported by government

agencies, landowners and local communities.



Southern ground-hornbill © Heinrich Nel

Project Summary

Feasibility: The species is long-lived, with low reproductive rates, and is the largest avian obligate cooperative breeder. Individuals live in socially complex groups comprising an alpha breeding pair, with additional individuals, predominantly male offspring as non-breeding helpers. Their role is territorial defense, predator vigilance, feeding of the nest-bound female during incubation and early brooding, nestling feeding, and post-fledging care; essentially allo-parenting. Each group is highly territorial, with low dispersal rates between groups. Given the species extensive territorial requirements, ranging from 80 - 200 km^2, groups outside of protected areas often span several privately-owned properties, which complicates implementation of census or conservation actions. The species is a savanna and grassland habitat generalist, primarily favoring short grass, open foraging areas, tall roost trees, and a nest (tree cavity, cliff crevice, or earth bank burrow). The species is culturally revered and lives relatively unharmed and in association with human settlements in many parts of its range, moving comfortably through villages and farms. This provides more opportunity for reintroduction sites, if the known threats of electrocution, poisoning, lead toxicosis, trade for rituals, medicine, or aviculture can be mitigated or managed.

Socio-political: With the human population increasing in density, affluence and consumption, the conservation status of ground-hornbills can change swiftly at the whim of a new development, fashion, or fad (e.g. the Helmeted hornbill (*Rhinoplax vigil*), another k-selected species that recently suffered sudden massive population declines as new trade threats emerged). Where known threats can be mitigated and managed, reintroduction of founder groups is the only way of rebuilding populations, and without it, captive populations would be ecologically meaningless. One of the primary ways this is being tackled is through working with traditional leadership structures to strengthen or reignite the profound cultural protection that used to exist for this species across its range.

Economic: There is sufficient global support for this program at present to start growing the scale of the reintroduction efforts. All efforts are made to ensure this support continues. The species, when reintroduced offers an ecotourism potential, with weekly tours ongoing for one of the bush-school sites. The species is a top-order predator feeding on rodents and snakes, much to the joy of rural

DNA sampling on ground hornbills

communities, and also forages on agricultural invertebrate pest species which can reduce crop damage.

Implementation: The use of redundant, second-hatched, chicks as source stock for reintroductions is viable, supporting the 'insurance' hypothesis in obligate brood reduction. Despite these chicks being artificially reared, they can effectively function as founder and mentor stock, breed successfully, and rear a new generation, provided that behavioral factors are adequately addressed. Their wild harvest has no known negative implications for the dynamics of the wild population, thus granting more experimental leeway than removing individuals from an already small and endangered population.

Initial reintroductions have shown that naive stock had a low chance of survival without constant management and that without managing habituation to humans, behaviors of stock birds were too aberrant. To negate this a specialized rearing facility that maximally enhances socialization of rearing stock chicks has been constructed. The use of wild-experienced alpha males to mentor a naive mate and helpers in ‘bush-schools has been replicated successfully. As a technique for ensuring correct behavior in artificially reared individuals, this best supports complex social learning requirements. All reintroduced birds are released only if a custodianship agreement between the reintroduction project and the landowner is in place. The rearing center can also be used to produce stock, harvested intentionally to fill gaps in the captive global insurance population(s), offset demands for wild ground-hornbills and retain maximum genetic diversity with no further harvest from the wild. Establishment of neighboring groups to the existing 'bush schools' becomes the next challenge as any inherent territorial aggression must be managed.

Post-release monitoring: Ground-hornbills are extremely difficult to recapture and so unlike other programs where birds can be recaptured annually, e.g. Californian condors (*Gymnogyps californianus*), for lead chelation, to replace transmitters, and monitor physical condition, it is not always possible to maintain these levels of monitoring. However, it is only necessary to track a single individual to locate the group to check their daily status. Monitoring has been varied over time but, where possible, a daily visual check is the minimum requirement. Currently, the released birds are not habituated to humans and will not allow a monitor within 100 m of them before they fly away. This increased flight distance made monitoring of these birds for foraging and social behavior

more difficult, but ensures they are unlikely to encounter direct harm from humans (though they are still vulnerable to indirect threats such as poisoning and electrocution). Where needed, tail-mounted transmitter are used and have up to a 24 month attachment time.

Raising public awareness with school children

Major difficulties faced

- Complete removal of threats, particularly of secondary and lead poisoning from the environment.
- Logistically challenging as each group requires a minimum of 100 km^2 and a 30 km buffer to allow for post-release movements.
- Isolated groups will not survive into perpetuity, so a core of at least 10 adjacent groups must be released.
- The species is slow-breeding and long-lived thus management and funding efforts must be committed for the long-term.

Major lessons learned

- Alpha males will accept non-kin males into their groups, until the group has reached an optimal size, but will not accept a second female.
- Reintroductions are significantly impacted by two variables:
    I. The reintroductions must take place during the rainy season to give inexperienced captive reared foragers the best chance of survival.
    II. Reintroduction stock must gain wild experience in a 'bush-school' where they are mentored and protected by experienced birds and accepted as group members.
- An alpha male cannot be moved to a new site after he has established his territory as he will simply attempt to return to his old territory, however wild sub-adult males may be moved without this risk.
- There is no evidence of reduced fitness in second-hatched chicks that form the basis of the reintroduction stock.
- Experimental reintroductions helped us discover and understand existing and unexpected and/or unrecorded threats to the remaining wild population, providing insights and data for population models that are otherwise hard to acquire for such a low-density species.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Second-hatched chicks that would naturally die in the nest provide a viable source of birds for reintroduction stock with no risk to the existing population.
- Lack of nests in viable habitat is not a constraint because the groups readily accept and occupy provided artificial nests.
- Alpha males will accept non-kin, which allows for the bush-school model to be successful.
- Management interventions such as vaccination against Newcastle Disease Virus, and chelation for lead toxicosis improve survival success.
- The program has developed successful protocols to increase the scale of reintroductions to three founder groups per annum.

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Reintroduction of Laysan teal on the remote atolls of the Hawaiian Islands, USA

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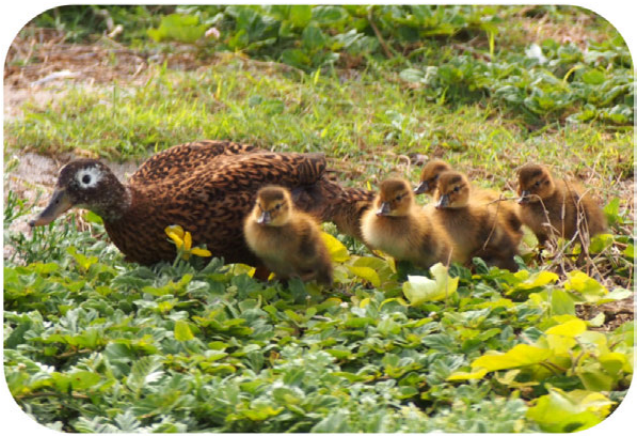
Introduction

Previously widespread in the Hawaiian Archipelago, Laysan teal (Laysan duck (*Anas laysanensis*)) went extinct on the main islands approximately 800 years ago primarily due to invasive predators. By 1850, the Laysan teal existed only on two remote atolls; Laysan and Lisianksi Islands. By 1870, a single island population existed. Although these dabbling ducks can fly, today they do not migrate or disperse from Laysan Island, where there are no mammalian predators. The species was listed as state and federally endangered and Critically Endangered by the IUCN because of its small population and restricted range (<4 km^2). Laysan teal are vulnerable to anthropogenic and natural disasters, including climate change. To lessen the risk of extinction from random disasters, a population was reintroduced via wild-to-wild translocation from Laysan Island to Midway Atoll in 2004 - 2005, and from Midway to Kure Atoll in 2014 (Reynolds *et al.*, 2008). Both reintroduction sites eradicated invasive rats before the translocations. These reintroductions to additional small low-lying isolated islands (total range now 11 km^2) will not eliminate its extinction risks; however, the probability of continued species existence increased since the number of island populations greatly influences a species' extinction likelihood from random catastrophes (Melbourne & Hastings, 2008).

Goals

- Provide “insurance” for the single remaining isolated population.
- Reduce extinction risk by restoring populations to additional islands.
- Restore and increase the range of the species.
- Increase abundance of Laysan teal as potential source birds for future reintroduction to a



Duckling brood at Kure Atoll
© Naomi Worcester - DLNR

larger higher elevation island, after invasive predators removals.

Success Indicators

- Translocation and aviary survival >70%.
- Post-release survival first year >60%.
- Successful breeding by year 3 and 25 founding breeding pairs.
- Births exceed deaths by year 3 (lambda >1.0).
- Population growth and persistence by year 10.

Project Summary

Feasibility: Prior to reintroduction attempts on other islands with different habitats, research was conducted to determine if the range of resources used on Laysan Island was broad enough to include prey and habitat available on other islands. Translocation feasibility and site assessments were made for all Hawaiian Islands. Next a population viability analysis and a structured decision ranking with the US Fish and Wildlife Service ranked translocation site suitability (USFWS, 2009). The absence of mammalian predators, abundant invertebrate prey, fresh water sources for ducklings, and dense vegetative cover are habitat requirements. Removal of invasive species was recommended prior to translocations, however Midway Atoll was selected as the most suitable site because it met habitat requirements and its protected status as National Wildlife Refuge, and easier logistic access by air for biologists to conduct post-release monitoring.

Kure Atoll, a State of Hawaii Wildlife Sanctuary was ranked the third best site for translocation and had prioritized restoration and habitat enhancements for Laysan teal. Lisianski Island was ranked second, but no translocations have been attempted there. Both Kure and Midway Atolls removed Black rats (*Rattus rattus*) prior to reintroduction of Laysan teal and restored fresh water wetlands and native sedges. Kure Atoll eliminated most weeds and attempted eradication of invasive Big-headed ants (*Pheidole megacephala*), before the translocation. Challenges included biosecurity and the remoteness of the population on Laysan Island (1,463 km from Honolulu), translocation sites (>1,930 km from Honolulu) and access to remote islands only by

Offloading of Laysan teal at Kure Atoll
© Matt Saunter - DLNR

boat. Availability of non-sibling independent juveniles in good condition as candidates for translocation was uncertain as the birds do not breed annually at Laysan Island. On Laysan, we monitored the population size and trend and the breeding success of the source population from March to September to target non-sibling juveniles as candidates for translocation in 2004 - 2005. Midway Atoll and Kure Atolls were ecosystems lacking the large hypersaline lake, fresh water seeps and mudflat wetland ecosystem dominating Laysan Island.

Wetland restoration at Kure Atoll
© Cynthia Vanderlip

Implementation: Thirty juvenile non-sibling ducks were fitted with radio-transmitters on Laysan to facilitate relocation and capture during 2004 and 2005. These young birds could fly and had not formed pair-bonds or attempted to breed on Laysan at the time of translocations. Several adult were also translocated mistakenly. At Midway, 42 founders were released. Translocation from Midway to Kure selected 28 juveniles of different ages from different parts of the atoll to maximize probability of selecting non-siblings for founders. Capture and selection of candidate founders was conducted at night with approximately 8 - 48 hours on islands while the transport ship waited. Parasite treatments (ivermectin) were given to prevent infestation of the nematode *Echinuria uncinata*, a significant cause of mortality on Laysan Island. Kure candidates were given a single vaccine (Botumink) for avian botulism, a significant cause of mortality on Midway. A soft release was implemented on Midway, and a quick release at Kure Atoll.

Post-release monitoring: All birds were given unique color band combinations and Midway founders were fitted radio transmitters. Bird locations were determined 1 - 6 times/week by homing with hand-held antennas. Kure Atoll birds were monitored with re-sighting of color banded birds. Early post-release, Midway Atoll showed high annual survival of translocated founders 0.92 (SE 0.019; N = 42) and their offspring 0.82 (SE 0.008; N = 67) (Reynolds *et al.*, 2008). These survival estimates, from the first two years post-release were similar to adult survival estimates from the Laysan Island population estimated 0.88 - 0.91 (SE 0.017 - 0.019) n = 355 during 1998 - 2004. Reproduction was faster than observed on Laysan Island, however, four years post release, the Midway population experienced its first epizootic of avian botulism type C, with over 180 carcasses collected during a three month period (Work *et al.*, 2010). Subsequent habitat changes (landscape scale herbicide application and weed removal),

catastrophic sudden flooding from the 2011 Tohoku Tsunami and chronic annual botulism outbreaks, were concurrent with increased carcass detection and Laysan teal declines. Recent population sizes were estimated around 600 post fledglings at Midway Atoll indicating a post tsunami recovery after eight years. The Kure Atoll founder cohort (N = 28) had 100% survival 18 months post release but survival dropped 39% during the first botulism outbreak. Today, 10 of 20 founders and a population of 50 - 70 birds persist on Kure Atoll in 2019.

Major difficulties faced

- Avian botulism (neurotoxin type C) outbreaks annually at Midway and Kure Atoll due in part to high bird carcass and fly densities.
- Sea level rise, tsunamis, and storm intensity due to climate change threaten all three small populations on low lying islands.
- *Management conflicts:* Invasive species eradications after, instead of before translocation of Laysan teal at Midway Atoll have caused conflicts. Weeds are used as nesting and foraging habitat by the species and delays occurred replacing habitat (replanting habitat with native vegetation). Next, after herbicide applications, de-vegetation and drought, invasive mice began attacking Laysan albatross.
- *Invasive species management conflicts:* Mouse eradication using aerial distribution of brodifacoum toxicant is planned at Midway Atoll in 2021. Laysan teal are highly susceptible to primary and secondary poisoning with brodifacoum. A plan proposed by the USFWS indicates up to 200 birds will be captured and held in captivity on the atoll for up to 22 weeks during the toxicant application. The USFWS has allowed take (mortality and injury) of more than 1,000 adult and juvenile endangered Laysan teal at Midway Atoll (USFWS, 2019).

Major lessons learned

- Wild juvenile birds were adaptive to novel foods and novel habitat; birds translocated as independent juveniles were most likely to breed successfully at translocation site compared to older birds.
- During the feasibility phase, emphatic emphasis on the potential management conflicts with the mangers and their successors if staff turnover is frequent (e.g. weed and mouse eradications to benefit seabirds require extensive and complex mitigation to reduce negative impacts to Laysan teal).
- Implement genetic management as soon as possible while the project has momentum, since the logistic complexity or project apparent success may lower priority to meet minimum number of founder or minimum immigration goals.
- Avian botulism vaccination and subsequent booster vaccination for translocated birds and annual vaccination for new offspring may substantially reduce the mortality.
- Shorter transport and holding times conserved body condition of birds and quick (hard) release was as effective as soft release.

- Sea level rise, severe storms and tsunamis threaten small low lying islands.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Populations reproduce successfully most years with adequate rainfall but survival of adults and juveniles are limited by avian botulism (type C) which may increase with climate change as botulism epizootics are associated with extreme temperatures, droughts and sudden flooding that mobilize spores, create carcasses, and other conditions favorable for toxigenesis.
- Duckling survival is highly variable at Kure Atoll. Poor brood survival is coincident with dry conditions.
- Laysan teal use novel habitats and prey, and during the early post release phase at Midway demonstrated breeding at younger ages and producing larger clutches under ideal habitat conditions and when below carrying capacity.
- Range and numbers of birds were approximately doubled by the reintroductions, genetic management to reduce loss of limited genetic variability is recommended.

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Reintroduction of the great green macaw to the South Caribbean coast of Costa Rica

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Introduction

The Great green macaw (*Ara ambiguus*) is considered globally endangered (IUCN Red List). This magnificent species has suffered dramatic declines across its range of six countries from Honduras to Ecuador. The wild population is estimated to be between 1,000 to 2,500 adults occurring in several isolated sub-populations. The loss of habitat and collection for the pet trade have driven the species' decline (Chassot & Monge, 2012; Snyder *et al.*, 2000). In Costa Rica, where this effort took place, the macaw is found in the Caribbean lowland rainforest. Of the 40 active nests currently monitored by the Macaw Recovery Network and partners 85% of nests are found in Mountain almond trees (*Dipteryx panamensis*). This emergent tree is a key food resource but is also highly desired by people for its durable wood and has undergone dramatic declines until it was protected in Costa Rica in the 1980s (Chassot & Monge, 2012). The impact of habitat loss has been compounded by collection of chicks for the pet trade and hunting (Snyder *et al.*, 2000) and consequently the macaw is locally extinct from areas of its natural range despite some remaining areas of suitable habitat. Once such area was the Southern Caribbean Coast (Fraixedas, 2014).

Goals

Goals and success indicators were not formally or explicitly established prior to project initiation. Retrospectively we can assert the following are relevant.

Great green macaw © Corey Raffel

- Reduce the risk of extinction.
- Establish a self-sustaining sub-population.
- Restore a flagship species to inspire conservation awareness.

Success Indicators

- Survival of released birds >70% in the first year.
- Breeding pairs within 10 years.
- Community engaged in conservation actions.

Project Summary

Feasibility: This reintroduction took place on privately owned land within the Gandoca Manzanillo Wildlife Refuge, which protects a total of over 4,856 ha of land. The protected status allows mixed use, including residential development. Large areas of primary and secondary rainforest are found in the reserve and these include mature Mountain almond trees. Conservation awareness in the region is generally high and community members were expected to be receptive to the reintroduction, having lost this species within their lifetimes. No formal biological or socio-cultural study was conducted prior to the reintroduction; however, a separate feasibility study was coincidentally conducted in 2010 using a neighboring reserve as the study area and published after the reintroduction (Fraixedas, 2014).

Implementation: Reintroduction candidates were bred in captivity in the San Jose area, from rescued or confiscated individuals that had been illegally collected. Outside of the macaw's natural range and at an altitude of nearly 1,000 m the breeding center was climatically quite different to the reintroduction site. Great green macaws are known to inhabit higher areas seasonally and this was not an issue. All released birds were ringed (banded) with closed or open stainless steel rings, and disease screening was conducted prior to moving birds to the reintroduction site. The birds were held in the release aviary (approximately: 18 x 6 x 3 m) prior to release and were fed a base diet including seeds, pulses and fruits with wild foods added as available including Mountain almond. The reintroduction took place prior to the author's involvement and no formal reporting was made, however the following information comes from anecdotes and has been gleaned from various informal articles. It is included here as the project achieved success despite a somewhat

Author climbing to nest box
© Sarah Williams

Macaws at a feeder © Tom Lewis

unconventional release methodology.

The first 10 birds reintroduced were between 4 - 7 years old, as were additional birds released subsequently. This first cohort included an equal mix of males/ females and parent/hand reared. The individuals were not human fearful and no human aversion training was conducted. They were held at least four months prior to release and then released individually or in pairs at intervals of up to one month. Over the following two years a further 35 birds were released. Released birds were encouraged to use feeders and supplemental food was provided twice daily. Food has ranged from sunflower seeds, fruits and pelleted diets to wild food including readily available non-native Beach almond (*Terminalia catappa*) nuts gathered by volunteers. Food was not restricted due to some fear from the land owners that the birds would roam near humans and be vulnerable if forced to forage independently beyond the immediate area.

Five wooden or metal nest box/barrels were provided in 2015 but only one pair investigated a box and no breeding took place. The author became involved with the project later in 2015 and subsequently the team installed 10 wood-lined plastic barrels and in 2016 one pair made a successful breeding attempt. In 2017 and again in 2018 nine pairs made successful breeding attempts.

Post-release monitoring: Immediately following releases observations were made of birds at the immediate reintroduction site area and of those that flew away from the site. The latter were greatly aided by anecdotal reports from the community, which were encouraged through local media and network. Occasionally birds in distress were reported and in at least 10 cases it was possible for those individuals to be rescued and returned to the site for rehabilitation and re-release. The primary method of monitoring involved observing individuals using the feeder. The availability of food at the reintroduction site presumably reduced any drive to disperse. The released birds continue to use/depend on the feeder although there is an apparent drop in visitation when the Mountain almond trees are fruiting. Whether the offspring will continue to use the supplemental food or forage more widely over time is yet to been seen. monitoring at the feeder presents a challenge. If numbers drop no information is available as to whether individuals have left the area and become independent, or died. Five birds have been confirmed dead with the project being alerted by the local community. Approximately 30 of the original founders continue to use the feeders daily, therefore survival has been high with at least

66% of birds accounted for eight years after reintroduction.

Major difficulties faced

- Monitoring of macaws presents a considerable challenge. Survival to one year has some value but really we need longer term tracking. Furthermore not being able to determine the fate of individuals that do not use the feeder leaves many questions.
- Parrots being considered for reintroduction that are not human fearful may have high survival at the reintroduction site. This could be useful as a strategy where an initial flock is established which supports a very-soft reintroduction, but it can also present a range of long-term challenges, particularly with macaws which are long-lived. Challenges include a commitment to that location, as well as the financial burden of staffing and the provision of food for those individuals.
- Dependency on feeders will require long-term provision of food. Such management may be acceptable in founders but if their offspring do not gain independence the value of the reintroduction is questionable.
- Private land owner involvement can be valuable but well thought through agreements are necessary prior to project implementation.

Major lessons learned

- Despite several unconventional approaches this reintroduction achieved some success. The long-term value for the species is yet to be seen.
- Macaws are complex social species and we may need to adjust post-release expectations. Some individuals may never achieve independence however if carefully managed their offspring might and as such those individuals make a contribution to the population.
- A technological solution is needed to enable long term monitoring in order to refine reintroduction methodology.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- The project has established a breeding cohort of Great green macaws in an area where they were previously extinct.
- Current management of the reintroduced birds assures their continued dependence on feeders.
- Feeders have enabled released birds to be productive. Whether their offspring are more exploratory and become independent remains to be seen.

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MACAW
RECOVERY NETWORK

Scarlet macaw reintroduction on the Nicoya Peninsula of Costa Rica

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Introduction

The Scarlet macaw (*Ara macao*) is one of the most iconic birds. With its huge range the species is considered Least Concern (IUCN) however, numerous local extirpations have occurred, particularly in Central America. In Costa Rica, there remain only two large wild populations (>250) of Scarlet macaws. A further two remnant wild and several reintroduced small populations (<50) are found across the country (Dear *et al.*, 2010). In the province of Guanacaste, which includes the Nicoya Peninsula where this project took place, extensive deforestation for cattle pasture occurred between 1950 and 1980. The cattle boom was short-lived which enabled forest regeneration after 1980 (Calvo-Alvarado *et al.*, 2009). Prized for centuries, the Scarlet macaw continues to suffer from the collection of chicks from nests for the international pet trade (Toft & Wright, 2015). Although legal protections were introduced in 2005 which prohibit keeping native species for pets, trade remains a problem and Scarlet macaw populations have not recovered. Prior to reintroduction efforts, only one wild population of 15 macaws remained on the Nicoya Peninsula. Elsewhere on the peninsula they had been extinct for over 60+ years.

Goals

- Establish a breeding population of Scarlet macaws on the Nicoya Peninsula.
- Maximize reproduction of the released macaws through the provision of nest boxes, monitoring and management.
- Inspire local support for the birds by using the reintroduction to benefit the local community and economy.
- Drive Scarlet macaw related tourism to fund ongoing conservation efforts.

Scarlet macaw © Macaw Recovery Network (MRN)

Success Indicators

- Post-release 1st year survival rate >50%; annual mortality <10%; and evidence of successful reproduction by 7th year of the project.
- Wild nests located and chick fatalities reduced through intervention when deemed necessary.
- Number of local people who directly benefit from the project to be greater than 10 per year.
- Income from reintroduction related tours, and merchandise to generate >US$ 20,000/year.

Project Summary

Feasibility: Following a decrease in the price of beef in the 1980s, extensive land-use change resulted in an increase in forest cover on the Nicoya Peninsula (Calvo-Alvarado *et al.*, 2009). Additionally, private reserves around the village of Punta Islita ensured further available forests, with a degree of protection. It was calculated that there was sufficient habitat to support Scarlet macaw reintroductions, with enough forest cover to provide adequate food for an increasing population. The Scarlet macaw has also shown a capacity to adapt to a somewhat degraded habitat. The secondary forest is lacking some ecological functions, with the most important being the deficiency of mature trees with cavities. However, limited nesting opportunities can be mitigated by providing artificial nest boxes until mature trees develop. Following community outreach and engagement initiative, the Punta Islita community has been shown to value the reintroduction of macaws. Many from the local community are employed by the nearby luxury hotel and are aware of the value the of the macaws as a driver of tourism. Local support for this project is high.

Implementation: The Macaw Recovery Network (formerly The Ara Project) acquired Scarlet macaws through rescues and confiscations. These individuals are unsuitable for reintroduction themselves, but are capable of producing healthy offspring. Previous releases in Punta Islita had been conducted using birds bred in the San Jose area prior to the authors' involvement. However, since then our breeding centre, which holds around 40 Scarlet macaws, has been moved to Punta Islita. Our current approach is to be hands-off when possible, but as we closely monitor breeding pairs we will facilitate parent rearing when it is needed. Fledglings remain with their parents until weaned, then they are housed in large social aviaries and prepared for release. Wild foods are increasingly provided prior to release.

Dry forest release site © Sam Williams

Local children with macaws © MRN

In 2017, we developed and conducted a pre-release assessment of social ability prior to releasing 13 individuals. The results of this pilot study indicated that individuals with poor social skills are less likely to make good release candidates. We conducted a soft release where food was placed on a shelf outside the specially constructed aviary and individuals were able to climb to the shelf or fly from the open aviary as and when they chose. We provided daily supplementary feeding to help support the macaws that gradually decreased from 100% to around 10% of their daily intake. We still provide 10% of their daily dietary requirements at a feeding station. We believe the cohort being reintroduced benefited from the established flock but lack data to confirm this.

Post release monitoring: The chicks were ringed with stainless steel rings prior to their fledging, however these do not enable identification of individuals unless in the hand. Individuals were also marked prior to release with a combination of non-toxic nail polish on their beaks and unique patterns cut into their outer tail feathers. We conducted on-site observations at the feeding stations, where food is provided twice daily. Additionally, our volunteer team worked alongside local staff members and consulted members of the public to track the newly released birds. We have monitored breeding activity since 2015. The first three breeding pairs appeared to prefer dead palm trees, but these were prone to falling over and had poor rates of success. In 2016, we installed and continue to monitor artificial nest boxes around the area. Although explored by the macaws, these were not widely adopted as nesting sites, possibly due to the young age of most of the flock. We have since added several free-standing wooden boxes, which attempt to replicate dead palms and additional standard boxes in trees. We climb to nests where possible or use a camera on a long pole to check the presence of eggs and the health of any chicks.

Major difficulties faced

- Low reproduction rates from captive birds in the breeding center due to low egg fertility, which is currently being investigated.
- Accurately determining post reintroduction survival for macaws and other parrots is challenging and expensive. Data can be collected on individuals using supplemental food however if they cease to use visit the feeder, or never visit the feeder their success is typically unknown.
- High mortality rates in wild chicks due to poor nest site selection.
- Determining success will require monitoring over multiple generations

which is an extremely long time for long-lived species like macaws.

Major lessons learned

- Social ability appears to be key factor in post-reintroduction survival/ success in this highly social species. Further research is needed.
- Despite familiarity with man-made structures, some pairs will not adapt to use nest boxes.
- In addition to supplemental food, supplemental water was readily consumed when provided in the dry season.
- It has been possible to generate important unrestricted funding for ongoing conservation work and to support local artisans through tourism. However, this must remain a secondary motivation in decision making.
- Monitoring and management post-release, particularly through interventions with chicks, can be worthwhile to ensure early recruitment and to help establish the reintroduced population. This will be important for slow life history species like macaws.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- A reintroduced population has been established in Punta Islita.
- Reintroduced Scarlet macaws have bred and produced chicks, in some cases independent of the supplemental food.
- Local community members are engaged and value our project often providing reports that greatly aid our monitoring efforts.
- The project is providing considerable economic benefit for both the organization and the local community.

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Reintroducing the mallee emu-wren to Ngarkat Conservation Park, South Australia

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Introduction

The endangered Mallee emu-wren (*Stipiturus mallee*), is a diminutive *Triodia* (spinifex*)* specialist, endemic to semi-arid mallee vegetation south of the Murray River in Victoria and South Australia. In recent decades the global population has declined within remaining habitat due to drought, and a series of catastrophic wildfires (Brown *et al.*, 2009). In 2014, the last remaining population in South Australia was destroyed by wildfire, and subsequently the global population of the species has been restricted to a small number of Victorian reserves. In today's fragmented landscape, Mallee emu-wren have little capacity to naturally re-colonize recovering habitat following extirpation from individual reserves (Brown *et al.*, 2013). Without management intervention there is a high likelihood that these remaining populations will be lost to successive wildfires (Verdon *et al.*, 2019). Translocation has been identified as a priority conservation strategy for the Mallee emu-wren (Boulton & Lau, 2015). Successful translocations would increase the global population of the species, provide insurance populations against the threat of catastrophic wildfire in currently occupied habitat

Mallee emu-wren (male)
© Thomas Hunt

and pave the way for larger-scale reintroductions. The reintroduction reported here was implemented within an adaptive management framework, with a focus on optimizing husbandry and release methods to inform future Mallee emu-wren reintroductions.

Goals

- Determine how timing of release and social bonds affect post-release, dispersal, persistence, and reproductive output at the release site.
- Optimize husbandry and capture methods.
- Monitor potential impacts of harvesting individuals from source populations.
- Assess alternative marking methods for post-release monitoring.
- Successfully address key questions regarding translocation feasibility and likelihood of success, to resolve whether this is a suitable conservation tool for this species that warrants implementation of larger-scale translocations.

Success Indicators

- More than 80% of birds survive to release, when caught and held overnight before release (i.e. held for a maximum of 24 hours) during each transfer season.
- Greater than 10% of removal sites are reoccupied within 12 months and >50% of removal sites are reoccupied within five years given suitable breeding conditions prevail (no long-term droughts).
- Successfully determine via re-sightings which release methods maximize site fidelity.
- During the first 14 days post-release >50% of Mallee emu-wren encounters involve ≥2 individuals found within 50 m of each other.
- At least 40% of the Mallee emu-wrens remaining at the conclusion of the short-term monitoring period go on to establish territories during the first spring following their release.

Project Summary

Feasibility: Mallee emu-wren have adapted to move rapidly through the complex tangle of spines that form a *Triodia* hummock and are, therefore, adept at avoiding and escaping mist-nets. Rate of capture was a major feasibility concern for the project. How Mallee emu-wren would respond to captivity was also unknown. Alternative capture methods and husbandry protocols were first trialed using the closely related Rufous-crowned emu-wren (*Stipiturus ruficeps*, IUCN Least Concern). In May 2017, eight birds were captured using a modified throw net technique initially developed by S. Brown, and successfully transported and housed. Due to their small size (~5 g), cryptic nature and complex habitat, leg bands and VHF transmitters presented a risk of entanglement for Mallee emu-wren. Several marking methods were trialed on captive Rufous-crowned emu-wren before a two-color nail-polish marking on the tail tip was selected for a field trial during the first Mallee emu-wren release. As identification proved difficult

Emu-wren capture site - Murray-Sunset NP © Thomas Hunt

using this method, two-color epoxy-coated Australian Bird and Bat Banding Scheme (ABBS) bands were subsequently trialed first in captivity then in the field during the second release.

The release site in Ngarkat Conservation Park (NCP) was selected due to the presence of suitable Triodia-heath habitat and because it was formerly occupied by Mallee emu-wren, prior to wildfires in 2006. Western Murray Sunset National Park (MSNP), Hattah-Kulkyne National Park (HKNP) and Nowingi State Forest (NSF) were chosen as suitable source sites due to expected high Mallee emu-wren abundance and road accessibility.

Implementation: In 2018, we released 78 wild-sourced Mallee emu-wren in NCP, South Australia. Releases were structured to test the effects of season and familiarity of release group (based on association at capture) on post-release persistence, dispersal and reproductive output. During autumn, Mallee emu-wren aggregate into social groups of up to eight birds and only loosely maintain territories. As spring approaches, groups split into smaller breeding units and territories become more defined. We were concerned that higher dispersal in autumn might reduce the probability that translocated emu-wrens would form a cohesive population. Release closer to the breeding season may reduce dispersal but may also delay the onset of breeding. Progeny of translocated individuals are expected to have greater fitness than their parents and will form the basis of any ongoing population. For this reason, maximizing reproductive output in the first breeding season following release was a high priority.

In April 2018, an ‘autumn’ cohort of 40 Mallee emu-wren, sourced from western MSNP and HKNP, were released in NCP. This cohort included seven ‘familiar’ groups and two ‘unfamiliar’ groups containing between three and eight individuals. In late August 2018, a ‘spring’ cohort of 38 Mallee emu-wren, sourced from western MSNP and NSF, included eight ‘familiar’ groups and nine ‘unfamiliar’ groups containing two to three birds. Unfortunately, two and five mortalities occurred during the autumn and spring releases respectively. Following necropsies, two mortalities during autumn and one during spring were attributed to physical injury while cause of death could not be determined for the remaining five. We speculated that elevated hormone levels associated with the

Tom Hurley (ZoosSA) releasing Emu-wrens into Triodia habitat © Thomas Hunt

imminent breeding season may have rendered individuals more susceptible to stress.

Post-release monitoring: Translocated Mallee emu-wren were monitored during two distinct phases. First, a team of skilled observers carried out daily area searches during the fortnight immediately following release and recorded the location and identity of all birds encountered. For subsequent monitoring, Mallee emu-wren occupancy was estimated using repeated call-playback surveys at 122 key habitat points, characterized by high *Triodia* abundance. Occupancy surveys were completed in July and October 2018; and April and August 2019 (two and 12 months after each release). Surveys were also carried out at source and control sites to assess the impact of removing birds for translocation, which will be repeated in the future to track recovery.

Mallee emu-wren released in spring were more likely to persist at the release site than those released in autumn and less likely to disperse in the fortnight following release. Amongst groups containing familiar birds, group cohesion remained high and there were fewer dispersive movements of both individuals and groups. However, familiarity of release group did not impact short-term persistence. Birds released in spring were more likely to successfully reproduce in the first breeding season following release than those released in autumn. In total, seven groups were confirmed to have produced either nestlings or fledglings (Hunt *et al.*, 2019), while another three groups exhibited behavior indicative of breeding. Mallee emu-wren occupancy declined following releases and by July 2019, no birds could be detected at the NCP release sites or surrounding habitat. This apparent decline could be attributed to poor detectability, dispersal or stochastic environmental conditions. During the translocation period NCP experienced below-average rainfall and Mallee emu-wren numbers declined in Victorian source populations. Future reintroduction attempts should aim for late winter or spring releases of socially cohesive groups.

Major difficulties faced

- Mallee emu-wren weigh ~5 g and are adapted to move rapidly through complex vegetation. This means externally attached trackers and bands pose an entanglement risk. Monitoring is a challenge without these

technological aids.

- Ecological traits make this species difficult to capture using standard techniques.
- No prior case studies on which to base captive husbandry.
- Little known about ecological requirements of Mallee emu-wren.
- Very low detectability, especially outside breeding season.

Major lessons learned

- Spring release improves indices of post-release success.
- Maintaining cohesive social groups during translocation is not essential for survival of individuals post-release.
- Capture, holding and release methods were refined and found to be viable.
- Painted tail markings are an unsuitable short-term substitute for colored leg -bands.
- Translocated Mallee emu-wrens can successfully reproduce soon after release (fledglings detected <8 weeks post-release).

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Vastly improved knowledge of species and translocation methods.
- This project was only possible through a collaborative effort and significant in-kind contributions, including support from the South Australian Murray-Darling Basin Natural Resources Management Board through the Australian Government's National Landcare Program and NRM levies funding, as well as contributions from all project partners including BirdLife Australia, Zoos SA, the Victorian Department of Environment, Land, Water and Planning, Parks Victoria, Rotary International, La Trobe University, Monash University and Zoos Victoria.
- An adaptive management approach was essential for overcoming many challenges, especially regarding marking protocols and monitoring approaches.
- Further research is required to more fully understand what caused the decline of the translocated population in Ngarkat Conservation Park.

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Reintroduction of the griffon vulture in Kresna Gorge, South-west Bulgaria

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Introduction

The Griffon vulture (*Gyps fulvus*) is one of the largest bird of prey in the Old world, distributed across Southern Europe, Middle East, North Africa and parts of Asia. Although listed as “Least Concern” in the IUCN Red List, the species suffered from dramatic decline in many parts of Europe, including Bulgaria, where it was almost brought to extinction in the 20th century and is now listed as “Endangered” in the national Red Data Book and protected by the National biodiversity law. It is also included in the Bern convention, CITES and Birds Directive. A small colony of the species re-established naturally in late 1970s and since then the number of pairs slowly increased in Eastern Rhodopes, but for decades it was unable to re-colonize other historical areas of the species in other parts of Bulgaria. Thus a plan for local reintroductions was put in motion along five sites in the country. Kresna Gorge in South-western Bulgaria was one of them and the first releases of birds started in 2010. The location was chosen after evaluation of multiple factors - habitats suitability, strategic geographical position, socio-economic and logistical specifics of the area. Meanwhile, the reintroduction activities also started in three more locations in the country along Balkan Mountains.

Goals

- Establishment of a breeding colony of the species in South-western Bulgaria.
- Establishment of vulture safe area in Kresna Gorge as a stepping stone for migrating vultures from Balkans.
- Providing a “bridge” and linking the existing colonies of the species in eastern Rhodopes, northern Greece

Griffon vulture © Hristo Peshev

Griffon vulture release © Hristo Peshev

and North Macedonia.

- Identification and neutralization of conservation risks for the vultures in the area.
- Boosting the national (Bulgarian) and Balkan Peninsula's populations of the species.

Success Indicators

- A year-round presence of a sufficient group of individuals (8 - 10) of the species.
- A regular presence of migrating birds from other parts of the region.
- A breeding colony producing at least 10 chicks/year established.
- Creating another source colony of Griffon vulture in the Balkans.
- Creating positive attitude for the species in local people and support for conservation and threats' mitigation measures.

Project Summary

Feasibility: Widespread in the past, the Griffon vulture was almost extinct in Bulgaria in the 20th century, mainly due to the use of poison baits against large carnivores and birds of prey - an already illegal, but still implemented practice in the country and in many parts of the species distribution areas. After the improvements of the local and the international biodiversity legislation, and the build capacity of conservation experts, an initiative for the species reintroduction in Bulgaria started in 2010. Kresna Gorge was selected for the implementation of the plan, combining data for the historical presence of the species with relatively easy access, suitable nesting conditions, food presence and low levels of disturbance. The gorge is situated along Struma River, between Pirin and Maleshevska mountains and consists of rocky slopes, cliffs, scrublands and pastures.

Implementation: The reintroduction activities in Kresna Gorge started with the import of Griffon vultures from Spain and France, which included both rehabilitated wild birds and birds hatched in captivity by the partnering zoological gardens. After the arrival, the vultures spent 1 - 6 months in acclimatization aviary and were released individually or in groups afterwards. The vultures fixing and survival in the reintroduction area was supported by the establishment of feeding station, where carcasses were provided intensively during the whole process. A large system of local contributors, mostly farmers, was made constantly during time, which provided actual information for any dead domestic and farmed animals in the region, which were fast delivered to the vultures by the Fund for Wild Flora and Fauna (FWFF) team. The estimated quantity of food delivered on

the feeding station per year was between 35,000 - 60,000 kgs.

Kresna Gorge © Hristo Peshev

Post-release monitoring: From the beginning of the reintroduction activities, an active year-round monitoring was conducted in the area. All released birds were marked with rings and wing-tags, visible from a large distance. The markings helped significantly to confirm the main goals of the reintroduction - if birds will remain in the target area and also will find and use the other species' main areas on the Balkans. As a result of the monitoring, the first breeding success attempt was identified in 2011, but the first success came in 2016 as increasing number of pairs was observed in the following years. In 2017 about 10 pairs were formed, but a large poisoning incident occurred in Kresna Gorge, which led to the dead of at least 18 (but probably more than 40) Griffon vultures and consequent dispersal of the established colony. Then few more birds were released in short time, to support the consolidation of a new group by the remaining few individuals and to recreate the colony, but also to intensify the conservation threats monitoring by using of GPS-GSM transmitters, attached to the newly released individuals. Soon, a local group of vultures was re-established, together with other migrating and roaming individuals, and this process led to the recovery of the local colony to such extent, that over 70 individuals, five pairs and three new nests were observed in the beginning of 2020.

The monitoring of transmitter-equipped vultures showed very promising results for the success of reintroduction, especially as a tool for early identification and addressing of poisoning incidents all along the Balkan Peninsula - the major threat for the species. Other threats as electrocution were also identified to act on large scale for the species and were locally addressed. Consequently, the number of installed transmitters was increased as much as possible in order to cover and monitor larger areas in the whole region and everyday intensive monitoring was planned. Three more poisoning incidents in other locations were timely detected and addressed using this method and a new concept for local conservation of the griffon vulture was proposed: The so-called vulture safe areas must combine: 1) Concentrated conservation measures against multiple and hardly controlled threats, and 2) Constant presence of as much individuals as possible in the area.

Major difficulties faced

- Although illegal practice, the use of poison baits against predators is a hardly controlled and unpredictable in time and space.
- Power lines with pylons dangerous for birds and related electrocution was underestimated threat.
- Lack (in the beginning) of concentration of the conservation measures and vultures in situation of limited resources.
- Lack (in the beginning) of intensive monitoring system for early detection and reaction to poisoning incidents.

Major lessons learned

- The Griffon vultures despite flying large daily distances tend to concentrate in areas with conspecifics, which at present are only six territories in mainland Balkans.
- Griffon vultures can be concentrated by establishment of well managed and strategically placed feeding sites, situated close to existing or newly created through assistance colonies of the species.
- Concentration of both - conservation measures and vultures in Vulture Safe Aras is a necessary tool to protect this group of species in the *Anthropocene*. Following the first lesson learned, the birds will spend most of the time in these areas or moving between them.
- New technologies, such as the more affordable and precise GPS-GSM transmitters provide for in time monitoring and addressing of otherwise hidden and hardly controlled threats, such as the wildlife poisoning.
- Establishment of early-warning system for wildlife poisoning and poaching based on intensive GPS-GSM tracking of vultures in marginal populations is an irreplaceable tool.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- The species is constantly present and restored as breeding in the area.
- The local people are aware of the vultures' importance and support their conservation.
- The Kresna Gorge reintroduction area serves as a "stepping stone" along the migration route of the species and is connecting the colonies in eastern Rhodopes and North Macedonia.
- The Vulture Safe Areas concept based on concentration of conservation efforts and the vultures was established, developed and proven working.
- The early-warning system for wildlife poisoning and poaching based on intensive GPS tracking of vultures was established, developed and proven working.

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Reintroduction of the great-billed seed-finch in the Brazilian Cerrado, Brazil

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Introduction

Seed finches are small-sized Neotropical granivorous birds characterized by extremely strong and thick beaks. The Great-billed seed finch (*Sporophila maximiliani*) has been intensively trapped due to their melodious song. As a consequence, it has experienced a fast and disastrous population decline, becoming one of the most endangered bird species in South America (Ubaid *et al.*, 2018). However, despite its virtual absence in the wild, the species reproduces well in captivity and a large number of captive birds are known. The species is classified as 'Endangered' by IUCN (BirdLife International, 2019) and as 'Critically Endangered' by the Red List of Brazil (MMA, 2018). In 2014 we started a series of studies aiming to implement an unprecedented reintroduction program of Great-billed seed finch in the Brazilian Cerrado. We conducted searches in more than 50 locations in Brazil, accumulating an effort of ~7,000 hours of fieldwork, yet only two small and isolated populations were located and studied. Our results supported the reintroduction program in a private reserve (RPPN Porto Cajueiro) in a Cerrado area of the Minas Gerais state, SE Brazil. This region is part of the Sertão Veredas-Peruaçu Mosaic, which houses a set of protected areas in the left bank of the São Francisco River.

Great-billed seed finch

Goals

- Establish genetic, morphological and sanitary protocols for the selection of pure (non-hybrid) specimens appropriate for

reintroduction.

- Design appropriate management methods for reintroducing the species, including evaluation of physical and psychological aptness of individuals.
- Evaluate the adaptation of reintroduced Great-billed seed finch to the natural environment.

Habitat at release site © Flávio Kulaif Ubaid

- Establish a free-living reproductive population from individuals born in captivity.
- Contribute to the species downlisting.

Success Indicators

- Number of individuals surviving in the wild one year post-release.
- Observation of natural feeding, avoidance of natural predators, and behavior similar to the natural populations.
- Number of individuals dispersed, occupying and defending territories.
- Breeding events (nest building and laying) recorded.
- Improved knowledge of the biology and ecology of the species.

Project Summary

Feasibility: Despite the agricultural expansion and habitat degradation in Cerrado regions, we were able to find some extremely preserved areas in conservation units where the species was extirpated. Habitat availability favors reintroductions, although trapping still stands as the most important threat to the Great-billed seed finch. Therefore, the success of the reintroduction program relies on the selection of a well-preserved place with restricted and controlled access by the people. The selected site for the reintroduction, a private reserve in the state of Minas Gerais (RPPN Porto Cajueiro), fulfills the requirements, thus reducing the chance of trapping. We estimate the global population of the Great-billed seed finch to be less than 1,000 mature individuals and, in Brazil, it should not reach more than 100 free-living birds. Although on the verge of extinction in the wild, there is a large captive population, accounting for more than 180,000 individuals in cages spread across the country (Machado *et al.*, 2019). Despite this large captive population, we found a great number of hybrids with other taxa, and the use of individuals for conservation purposes depends on rigorous genetic, morphologic, and morphometric protocols, as well as the severe protection of the areas (Machado *et al.*, 2019).

Implementation: Since 2018, our team has released 24 Great-billed seed finches (12 pairs) in the RPPN Porto Cajueiro. We have not detected any deaths from the released birds, although some have disappeared. Some birds have dispersed over great distances from the release point, making monitoring logistics unfeasible. Techniques for the reintroduction of passerine birds are poorly developed in the Neotropics, and monitoring individuals which good capacity of dispersion is challenging due to the lack of effective tracking devices compatible with the weight of the birds. In future stages, we will adopt techniques to avoid nest predation, which has been high until now. The implementation of the project was made possible with the sponsorship of the Fundação Grupo Boticário de Proteção à Natureza, Critical Ecosystem Partnership Fund (CEPF), Usina Coruripe, and a large network of employees that includes the State University of Maranhão (UEMA), University of São Paulo (USP), Federal University of São Carlos (UFSCar), Clube dos Criadores de Bicudo de Canto do Brasil (CCBCB), Instituto Ariramba, and Angá.

Post-release monitoring: Since the first release, our team has been monitoring the birds almost daily. Monitoring is carried out by biologists and park rangers. Thus, the monitoring protocol involves a combination of standardized and opportunistic methods. The environment occupied by the Great-billed seed finch occurs in a linear way along the paths and is surrounded by a predominant matrix of cerrado *stricto sensu*. The access to the paths is made by roads that follow a large part of its perimeter, through which biologists access the flooded areas. Each reintroduced bird received one or two rings with a unique color combination, allowing its individualization at a distance with the aid of binoculars. Our greatest indication of the success of the reintroductions and the effectiveness of the management methods adopted, was the verification of a couple's reproductive activity three months after the release. We located two nests with eggs that were unfortunately preyed upon. In general, Great-billed seed finches adapt in a few weeks to the natural environment, recognize the food available naturally and in a short time acquire great flight autonomy. We located birds reintroduced by our project more than 20 km away from the release point, outside the reserve. This proves that birds are naturally dispersed along the paths and can colonize adjacent areas.

Major difficulties faced

- Techniques for the reintroduction of passerine birds are poorly developed in the Neotropics, and monitoring individuals which good capacity of dispersion is challenging due to the lack of effective tracking devices compatible with the weight of the birds.
- Reduce nest predation.
- Increase the control of criminal fires in the vicinity of the protected areas.
- Complex bureaucracy and lack of communication and common protocols.
- Funding for long-term studies (>5 years) are hard to obtain in Brazil, compromising the monitoring of birds and the continuity of the project.

Major lessons learned

- The major limiting factor for the survival of the Great-billed seed finch in the

wild is the illegal trapping, not the availability of suitable habitats.

- Feed supplementation provided continuously post-release facilitated the monitoring and management (when necessary) of the birds.
- We learned that the reintroduction of individuals who are less skittish to human presence facilitated all stages of the program.
- The pre-release pairing of individuals significantly increased the chances of reproduction in the first months after release.
- The use of artificial nests induced laying and facilitated the monitoring of the nests.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Rapid adaptation of birds to the natural environment.
- Multiple breeding attempts few months post-released.
- Individuals monitored for more than 1 year.
- The birds performed long distance flights (>2 km), attesting to the effectiveness of pre-release training.
- Establishment of a wild population of the Great-billed seed finch in an area where it has become extinct.

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Reintroduction of Père David's deer into the Poyang Lake area, China

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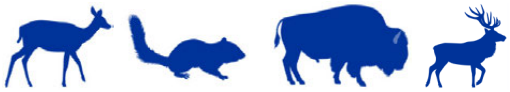
Introduction

Père David's deer (*Elaphurus davidianus*), also known as the milu, is endemic to China. They used to be widely distributed in the wetlands of the Yangzi River basin and Yellow River basin (Cao *et al*., 1990). Its historical distribution ranged from 18°N to 45°N, east of the 110°E in China. As a result of climate change and expansion of human activities, milu went extinct in the wild (Yang *et al*., 2003; Cao, 1985). And the last captive population was lost at Nanhaizi, Beijing in 1900 (Cao, 1985; Jones, 1951). Milu was listed on the IUCN Red List as Extinct in the Wild (EW) (IUCN, 2016), and the China Key List-I (Maddison *et al*., 2012). However, two founder populations, a total of 79 individuals, were reintroduced into Nanhaizi of Beijing and Dafeng City of Jiangsu Province in China from England from 1985 to 1987 (Thouless *et al.*, 1988; Maddison *et al.*, 2012). Even though the exact extinct time of milu in this area was unknown, Poyang Lake, the biggest freshwater lake of China, located in the middle of Yangzi River basin, was the historical distribution area of the species. This project involved a preliminary fenced reintroduction into a wetland park, then releasing to the wilderness, and also involved a continuous monitoring program. It is a milestone in the history of wildlife protection.

Père David's deer being released in Poyang Lake National Wetland Park



Goals

- Successfully release individuals into the fenced area of the Poyang Lake National Wetland Park (PLNWP) to have a wild population.
- Raise awareness of milu and wildlife conservation for local residents by eco -tourism and media campaigns.
- A steady population growth of milu in PLNWP.
- Reintroduce a second group into PLNWP after the first one adapts to the habitat and climate, and then release both to the wild.
- The released population adapts to the wild, utilizes suitable habitat, and reproduces.

Success Indicators

- Successfully reintroduced 10 individuals into PLNWP in 2013, an annual growth rate of 10% or above of this population.
- Reintroduced second group of 30 individuals to PLNWP in 2018. Release them together with the former group to the wild in Poyang Lake wetland area.
- The released population found their adaptive habitat in the wild, fawns were born and survived the flood period with adult individuals.
- Protection and propagation of this species nationwide.
- Introduce policies of ecological compensation and try to alleviate human-wildlife conflicts.

Project Summary

Feasibility: Immediately after National Forestry and Grassland Administration (NFGA) (former named SFA (State Forestry Administration)) proposed reintroduction of milu into Poyang Lake area, the scientists and managers of Beijing Milu Ecological Research Center (BMERC) of Beijing Academy of Science and Technology (BJAST) and Wildlife Protection and Administration Bureau of Jiangxi Province (WPABJP) mobilized experts to make assessment of habitat suitability for milu in 2012. The assessment included wetland plants, water, temperature and topography. The result showed that PLNWP of Jiangxi Province (N 39°46′33″, E 116°27′4″), located in the southeast of Poyang Lake, with an area of 362.85 km^2, including 351.16 km^2 wetlands, eligible for the reintroduction project. In addition, the area of Poyang Lake wetlands was, conterminously, 2,698 km^2, and located in the hilly and plain area, with an elevation of 30 - 300 m. Results showed that the Poyang Lake area was a suitable habitat for milu to survive and reproduce.

Implementation: An area about 20 ha surrounded by a fence was built for the preliminary reintroduction population in buffer zone of PLNWP in June 2013. Administration Committee of PLNWP was authorized a license to breed milu after an expert demonstration meeting organized by NFGA in July 2013. Then in December 2013, quarantine formalities and transport formalities were adopted. Finally, 10 individuals (four males & six females) of milu were transported to

PLNWP from BMERC on 25th December 2013. The transportation distance was 1,400 km and took about 30 hours.

These individuals readily adapted to the local habitat and climate and the first fawn was born on 20th April 2014. There were about 2 - 6 fawns born every year, and the maximum population number was 23 in 2017. In winter, they were fed with some carrots, formula feeds and local wetland grasses. NFGA proposed the second reintroduction of milu into PLNWP in March 2017 and 30 individuals (17 males & 13 females), of which five individuals with a GPS positioning collar, were transported from BMERC to PLNWP on 29th March 2018. They lived with the first group and after five days of adaptation observation, including behavior, physical status, appetite, etc. were observed. The 47 healthy individuals (23 males & 24 females) were released into the wild of Poyang Lake area on 3rd April 2018.

For the purpose of raising public's awareness of milu conservation, the national and local mainstream media had made lots of reports since 2013. Furthermore, right after the population was released to the wild in 2018, an announcement about the release event and protection initiatives were posted on notice-boards in every village and town of Poyang County. To solve the problem of human-wildlife conflict caused by milu, the local government had established an ecological compensation mechanism. In 2018, the loss caused by milu and water birds was given to the victims, 1.5 million to 2 million Yuan (~US$ 216,241 - 288,321) in total. On 3rd January 2019, Forestry Bureau of Jiangxi Province promulgated 'Regulation of Compensation Fund for Ecological Benefits of Poyang Lake National Wetland Park'.

Post-release monitoring: The reintroduced population was released into the wild at the fenced site and we monitored the released population regularly. In addition, the local wildlife protection department hired nine volunteers to do the job. The patroller consisted of researchers of BMERC, staff from the local wildlife protection department and nine volunteers. There were three dispersal groups. One group migrated northward to the wetland near Douli Mountain in Yaquehu Town one week later and in October 2018, 17 individuals were located there. In December 2018, they migrated to the wetland of Beihu in Yinbaohu Town a short distance away, and 15 individuals (3 males, 9 females & 3 fawns born in 2018) were found here on 11th March 2019. The first fawn born in 2019 of this group was found on 18th April and as of 8th May, four fawns

Monitoring Milu at Yinbaohu Town

were born in this population.

Monitoring team at Poyang Lake

Another group, 11 individuals (4 males & 7 females) came back to the fence zone on the release day, the fence was closed and they were left in the semi-free area. But on 26th September 2018, they escaped from the fence with their three fawns and migrated southwards. The third group migrated southward and nine individuals were found in the wetland of Lianhu Town on 16th May 2018. In July and August of 2018, 11 individuals (10 males & 1 female) and 15 individuals (3 males & 12 females) were seen respectively in the farmland inside the Poyang Lake dam of Shuanggang Town. In August 2018, nine male individuals were found in the wetland of Changshandao of Shuanggang Town. In the same month, nine individuals (1 fawn & 8 males), were spotted in the farmland in Lianhu Town. On 22nd September, 2018, eight individuals (6 females and 2 fawns) were found in Changshandao in Shuanggang Town.

Unfortunately, a three year old male individual was found dead in Waxueba village of Lianhu Town on 20th December 2018 and there was no evident injury and a post-mortem could not reveal the definite cause of death. Two male individuals were trapped by fishing nets in Dongtang Town of Yugan County, and Dalianzihu of Lianhu Town of Poyang County, they were rescued by police and patrolling volunteers respectively. Though a male milu was found dead in Shuanggang Town with an antler trapped by an abandoned fishing net on 3rd May 2019.

There were five individuals wearing GPS positioning collar in the released population, of which two females lost their collars, and another two had their collars either broken or with no signal. The last collar the signals showed that it had been moving southward along the river to the mountains of Yingtan County since 6th August 2018. But we did not receive any signal after 31st August 2018.

Currently the released population dispersed and distributed into four groups, including Beihu in Yinbaohu Town, Changshandao in Shuanggang Town, Hanchihu and Dalianzihu in Lianhu Town. The population number was 51 in total and during the flood season, they migrate to the farmlands, wetlands and hills around the lake area and after this period they went back to the middle area of the lake.

Major difficulties faced

- During the flood season, most of the suitable habitats, such as grasslands are inundated.
- Human-wildlife conflict - When the flood season comes the populations of milu are forced to move to areas where human activity is dense. Consequently, they destroy crops and bring economic losses to local residents.
- As a tradition, fishing is popular in Poyang Lake area, thus fishing nets are a potential danger to milu.
- The lake area is huge land full of swamps, making it difficult for us to monitor the reintroduced individuals in the long-term, especially when the GPS system was down.
- There is a shortage of funds for the local wildlife department and research institute to monitor and study the released population. There is also a shortage of funding for ecological compensation.

Major lessons learned

- Support of government and project funding are significant to the program.
- Ecological compensation is vital for achieving the final goal of rebuilding wild populations.
- Dealing with the relationship between community and milu, and raising the awareness of local residents to conserve the reintroduced populations is very important.
- It is very important to improve the economic status and living standards of local residents through ecotourism and other ways.
- The legal protection of the original wetland of Poyang Lake Area is as equally important as conserving milu itself.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- The Poyang lake area is the historical distribution area for milu and provides suitable habitat and climatic conditions.
- The project has had long-term support from the government and news media.
- Wild release protocol followed an adaptation and release stage.
- Close cooperation and long-term attention and follow-up monitoring by a research institute and local wildlife protection department.
- A dramatic increase in the number of people caring about wildlife.

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Southern pudu: captive-breeding, rehabilitation & reintroduction in Araucania, Villarrica-Chile

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Introduction

The Southern pudu (*Pudu puda*) is a small-sized deer with a height of 40 cm and 7 - 10 kg body weight. This species is “endemic” to South American temperate forest, the species occurs in southern Chile and southwestern Argentina from 35° 10’ to 46° 45’ S and is listed on CITES App. I. This species is considered Vulnerable by Chilean authorities and as Near Threatened by IUCN. The primary habitat of the Pudu is native forest from which it has dramatically declined, thus the species is nearly absent in the central valley lowlands in the reintroduction area (Vidal F., pers. obsv.) Even though population numbers are unknown, the perception is that numbers are decreasing due to an exotic predator, the Domestic dog (*Canis lupus familiaris*), being probably the main threat. Fauna Andina (S 39° 10’ 40”, W 072°10’ 06”), is a private reserve ~100 ha at an elevation of 300 m above sea level in the Araucania region. The reserve consists of native forests, riparian ecosystems, grasslands and bushlands as a result of a “recent restoration process”. This area is located at the transition zone of the central valley Into the Andes mountain, the reserve is connected with private and public lands (including Villarrica National Reserve) with a temperate forest available for the species.

Southern pudu

Goals

- Initiate a breeding and rehabilitation center for the Southern pudu.
- Convert a farm into a private reserve and construct a biological corridor for the Southern pudu and several other

threatened native species.

- Reintroduce the species into the reserve and lowlands temperate forest.
- Generate information about the pudu such as biology, ecology and management.

Success Indicators

- Continue captive-breeding and a successful rehabilitation of pudu which have been attacked by Domestic dogs.
- Create a reserve (including a corridor) out of a “highly impacted farm” with good quality temperate forest and ecosystem suitable for pudu.
- Adult individuals surviving among predators and breeding after being released into the wild.
- Threatened species, other than pudu, making use of the restored habitat.
- Generate practical experience and knowledge on global management for the target species.

Project Summary

Feasibility: Considering that most of the land in the southern shore of Villarrica Lake has been deforested and urbanized, the conversion of a “farm” into a reserve on the northern side, with pudu habitat, would create a corridor amongst the few remaining forested lands with public and private properties of good quality habitat. Since social attitude toward pudu is mostly positive, the project could indirectly protect other threatened species (including diverse ecosystems) such as Guiñas cat / Kodkod cat (*Leopardus guigna*), Monito del monte (*Dromiciops glyroides*) and Puma (*Puma concolor puma*) which are continuously hunted, poisoned and trapped in the area (Vidal, 2014). The reintroduction project not only aims to return the Southern pudu, but also initiate an *ex situ / in situ* project for one of the most charismatic Chilean temperate forest mammal. This will hopefully eventually increase population numbers via the “corridor” in the temperate forest of Lake Villarrica ecosystem.

Implementation: In 2000, after presenting the project and initiative to the Chilean wildlife authorities, Servicio Agricola y Ganadero (SAG), obtained the legal permit to start a breeding center for Southern pudu and other species such as the Guiñas cat (*Leopardus guigna*). Aiming not to capture free roaming animals, the center got focused in individuals that were orphans or had been attacked by dogs. Under the supervision of SAG wildlife officers (Villarrica bureau), the center started receiving wounded animals, for rehabilitation or as a breeders in case they could not be returned to the wild. The first three years were the hardest, since all the previous management experiences with exotic deer, such as Fallow deer (*Dama dama*) and Red stag (*Cervus elaphus*), were useless! The team had to understand the importance and effect of “nutrition” and “stress level handling”, plus social structure and behavior for this small deer.

After five years of operation, the project was able to rehabilitate about 70% of individuals attacked by Domestic dogs (*Canis lupus familiaris*), and released back

Author radio-collaring Pudu for release

to the wild by SAG officers into national parks. The center also had a continuous breeding success, forming several breeding units and groups in separated pens. After about a decade, the project team got a private piece of land donation to start a private reserve. A complex restoration effort started by stopping logging and livestock activities. After removing cows, horses and sheep, the team also initiated a reforestation plan with native species, mostly *Nothofagus* species. A restoration process controlling exotic species, including Domestic dogs, was also initiated. The team captured (chemical immobilization and darting) pudu and transported all the animals into the reserve, to start the breeding process in one big pen. As a legal framework the project and reserve were registered on the Chilean Environmental Ministry “Ministerio del Medio Ambiente” as a “conservation” and “preservation” private reserve unit.

During the reserve operation, the team detected that the site was being visited by pumas often, thus a capture/radio-collaring and monitoring process began with the permit of SAG to approach and understanding the ecology and behavior of pumas in the proposed reintroduction site. About five years after starting the conversion process of the farm into a reserve, the reintroduction process began by soft releasing “rehabilitated” and “captive born animals”. Sanitary considerations at the project include blood sampling, preventing 1-Bovine leukosis virus, 2-Paratuberculosis, 3-Brucellosis plus anthelmintic treatment during the preparation and evaluation to the soft-release process.

Post-release monitoring: Since the puma monitoring project was initiated in advance, the reserve was already under camera-trap surveillance. Even though at the beginning animals did not have radio-collars, monitoring was possible since individuals did not travel long distances or leave the reserve. Once funds were obtained, radio-telemetry was also added to the monitoring process, thus animals were monitored by two methods at the same time. After the first year of living in the wild, fawns were detected by camera-traps, and later also juveniles. Even though released individuals interacted with natural predator such as Pumas (*Puma concolor*) & Culpeo fox (*Lycalopex culpaeus*), the first predation event was not detected until 17 months, when a male (three years old) was predated by a puma.

After being released, two behaviors were detected on radio-collared animals: 1)

Exploring a distance of 300 m and returning to the release point during the first 15 days and then establishing a fixed territory; 2) Staying at the release site and gradually establishing a territory. The home ranges, (based on Minimum Convex Polygon) registered were 4 - 10 ha, with the same "estimation" made by camera-trap monitoring. Even though pudu are considered crepuscular or nocturnal, daylight activity is more common at the reserve, with very little "motion" indication coming out of collars at night, or camera trap monitoring. This could be due to the management policy at the reserve which has an "exclusion area" where only government wildlife officers and the project team can enter. Presently, the breeding center has about 50 pudus, and the reintroduction area has ~15 mature individuals that breed every year, living among the pumas and Culpeo fox, both natural predators.

Major dificultes faced

- Once the project started being successful, some other organizations involved in research & conservation started making observations and comments to the Chilean wildlife authority (Servicio Agricola y Ganadero), thus making every action and decision slower and more difficult.
- A decade has passed since the project began and the land value has increased dramatically. Thus the person who donated the land has begun making efforts to recover the land for his family. This has resulted in the loss of the buffer area.
- After years of respecting the "preservation area" at the core of the reintroduction site, the land donor has begun building trails for visitors.
- Attempts have been made to open and have visitors visit the breeding unit by the land donor thus putting at risk the long-term efforts made on this project.

Major lessons learned

- Even though captive-bred and rehabilitated pudu had failed on other projects when released to the wild, the success of this project probably reflects on the methodology that has made a difference.
- Private conservation projects/efforts are a real

Pudu in camera trap in the reserve

necessity in Chile, but a deep analysis on the legal framework must be adjusted, since today it just depends on ethical and moral intention of the project initiators.

- Handling and management of Chilean native deer is quiet demanding and needs proper care and is different from other deer species where any mishandling would lead to the project failing.
- SAG approval and cooperation is a vital component of the Project.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Under the actual weak legal framework in private conservation efforts in Chile, the future of the project, reserve and animals is uncertain. Even thought the technical execution has been successful.
- SAG, Villarrica Bureau, commitment with the Project has been crucial.
- Commitment and ethical attitude of the technical team of work toward the Project and animals.
- The experience of the working team to control exotic species.
- Universidad Santo Tomás partnership and volunteers.

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Reintroduction of the red-rumped agouti at Tijuca National Park, Rio de Janeiro, Brazil

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Introduction

Red-rumped agoutis (*Dasyprocta leporina*) are scatter-hoarding rodents from the family Dasyproctidae, weighing from 2 - 6 kgs. Their seed stealing-relocation behavior enhance distance and efficiency dispersal of large seeds. They are currently listed on IUCN and Brazilian Red Lists as Least Concern because of their wide range and large populations (Emmons & Reid, 2016). Agoutis indeed thrive in large forested areas, especially in the Amazon, but in small degraded fragments of Atlantic Forest they are absent or scarce (Galetti *et al.,* 2017). Agoutis are not listed on CITES

Agouti leaving release pen © Peterson de Almeida

Researchers tracking Agouti © Vitor Marigo

Appendices and its global population trend is considered stable, but the species is extensively consumed as bushmeat. The species is distributed from Paraná to Bahia states in the Atlantic Forest, a severely fragmented and defaunated biodiversity hotspot (Galetti *et al.*, 2017). Tijuca National Park (TNP) is an Atlantic Forest remnant within Rio de Janeiro. The history of habitat loss and dense urban surroundings explain its impoverished fauna and impossibility of unassisted recolonization. In the past, agoutis were locally extinct due to illegal hunting and fragmentation. The species had not been recorded in the Park for at least three decades, despite release efforts during the 1970s.

Goals

- Reestablishment of a self-sustaining population.
- Restoration of ecological interactions such as seed dispersal.
- Development of effective translocation techniques.

Success Indicators

- Establishment of home ranges by the reintroduced individuals.
- Breeding of released individuals.
- Unassisted growth of population.
- Evidence of restoration of ecological interactions.
- Population reaching regulation stage.

Project Summary

Feasibility: Agoutis are frugivore rodents with a plastic diet, consisting mainly of seeds and pulp. They are seed dispersers, and their seed scatter-hoarding and stealing-relocation behaviors enhance distance and efficiency of large seed dispersal. These generalist habits allow agoutis to persist even in small forest fragments, therefore the species is a good choice for an early stage refaunation programs (Galetti *et al.*, 2017). REFAUNA is a refaunation program (*i.e.* trophic rewilding, Svenning *et al.*, 2015; Fernandez *et al.*, 2017) that aims to reintroduce several species in the same area to restore lost ecological interactions due to defaunation. Part of TNP's forest had been cleared in the past and was restored

in the 19th century; today the vegetation is composed of typical Atlantic rain forest trees in different succession stages along with some exotic ones. The agoutis came from two semi-captive populations, most of them from Campo de Santana Municipal Park (with more than 500 agoutis). The animals were then transferred to the local Zoo (RioZoo) facilities for health evaluation. All examinations and quarantine were carried out at RioZoo. Animal reintroduction in an urban protected area in the middle of Brazil's second largest city provides an interesting opportunity for engaging a wide public on conservation biology.

Agouti acclimatization pen © Catharina Kreischer

Implementation: We started in 2009 the reintroduction process of *D. leporina* in TNP as the first stage of REFAUNA program. About 50 agoutis were live-trapped in the years of 2009 - 2010 and 2013 - 2014 by using Tomahawk® live-traps with fruits and vegetables as bait. The animals were then translocated to RioZoo facilities for quarantine, exams and preparation for release. During quarantine, animals were sexed, weighed, ear-tagged, and had their blood, feces and urine collected for clinical exams to ensure that released animals would not introduce pathogens to the release site. It is important to consider a trade-off between minimizing disease transmission risk in the reintroduction site and the level of stress the animals suffer during pre-release procedures, which may compromise post-release survival. Thirty-one healthy agoutis (17 females and 14 males) were fitted with Telenax® radio-collar transmitters and transferred to two acclimatization pens (10 x 10 m^2) with food supply located at TNP. They were released after 2 - 10 weeks using a delayed-release protocol.

Post-release monitoring: Animals with radio-tracking collars were followed using the "homing-in on the animal" observation method until the death of the individual or the equipment stopped working. In the first weeks after the release, each radio collared animal was monitored intensively, and after this period at least twice a week. This monitoring allowed to recording individual locations, breeding events, interactions with other co-specifics, species and environment and to access their survival rates. Released agoutis became independent from food supply, experienced high survival rates, and breeding was observed before 12 months after release.

Capture-mark-resight surveys were conducted at least twice a year from 2013 to 2017 to monitor population size. Captured animals were marked by freeze-branding and fur bleaching on both sides. Resight was performed by a set of

Agouti in camera-trap with a seed in the mouth
© Laboratório de Ecologia e Conservação de Populações

camera traps. Agouti population was estimated around 30 individuals within the monitored area fluctuated and overall growth of the population was positive, with all individuals being wild born since 2016. Thus, the population was clearly able to grow without the aid of further releases. Agoutis were seen consuming fruits and seeds from at least 23 species of plants and hoarding three of them. Currently agoutis are the only frugivores able to disperse seeds larger than 30 mm in diameter in TNP.

Major difficulties faced

- Neck wounds caused by the radio collars.
- High mortality during quarantine/acclimatization.
- Low capture rate of wild-born animals (high effort with few individuals caught) in order to estimate population numbers by capture-mark-resight method.
- Feral dog predation after release.

Major lessons learned

- Need to reduce quarantine and acclimatization time.
- Need to maintain males as separate as possible during prerelease phase.
- Agoutis easily adapted to wild environment even if maintained in captivity for generations.
- Bait for camera trap can influence cohesion.
- TNP can be used to test best management protocols.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- A large source of animals available and easily accessible for the

reintroduction.

- Agoutis do not have specific food requirements.
- Rapid breeding after release.
- High survival of released animals.
- Partnerships with ICMBio, RioZoo, FIOCRUZ, UNESA, UERJ and UFRRJ reduced significantly the costs of the project.

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Reintroduction of the brown howler monkey to Tijuca National Park, Rio de Janeiro, Brazil

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Introduction

Brown howler monkeys (*Alouatta guariba*) are currently listed on IUCN Red List as Least Concern and on App. II on CITES, whereas the subspecies *A. guariba clamitans* is nationally listed as Vulnerable in Brazil. However, IUCN and national assessments were conducted in 2008 and 2012, respectively, and these assessments must be updated due to high mortality of individuals in a yellow fever outbreak in southeastern Brazil which started in late 2016.

The species is distributed throughout the Atlantic Forest, a biodiversity hotspot which have lost over 80% of its former distribution, severely fragmented and defaunated (Galetti *et al.*, 2017). Tijuca National Park (TNP) is a defaunated Atlantic Forest remnant within Rio de Janeiro city that has been deforested and then restored. Its urban surroundings explain its impoverished fauna and impossibility of unassisted re-colonization. Defaunated forests such as TNP might not be viable in the long-term due to the lack of interactions between animals and plants. After the extinction of Brown howler monkeys, more than 100 years ago, no other large primate was left in TNP.

Brown howler monkeys © Luísa Genes

Goals

- To re-establish a self-sustaining population.
- To restore ecological interactions.

- To develop effective translocation techniques for howler monkeys.

Transporting howlers to release pen © Luísa Genes

Success Indicators

- A 50% survival rate in the first two years.
- Occurrence of births in the first two years.
- Maintenance of group cohesion.
- Evidence of restoration of ecological interactions.
- Population reaching regulation stage.

Project Summary

Feasibility: Brown howler monkeys form social groups with varying size. Although groups present hierarchies with one central male, the mating activities of other resident males are tolerated. They have flexible folivorous-frugivorous feeding behavior. Such habits allow the species to persist in small and degraded forest fragments, thus they are considered as potential candidates for early-stage reintroductions in a refaunation program.

REFAUNA is a refaunation program (*i.e.* trophic rewilding, Svenning *et al*., 2015; Fernandez *et al.*, 2017) that has started with the reintroduction of the Red-rumped agouti in 2010, followed by the Brown howler monkey in 2015. Both reintroductions were conducted at Tijuca National Park (TNP), with the main goal to restore ecological processes lost due to defaunation. TNP vegetation is composed of typical Atlantic rain forest species along with some exotic species. The habitat is considered suitable for howler monkeys, and presumed causes of extinction (deforestation and hunting) are no longer present. TNP is the most visited protected area in Brazil, with more than 2.6 million visitors in 2018. On the one hand, reintroductions in this urban protected area provide an interesting opportunity for engaging a wide public on conservation biology. On the other hand, this large number of visitors could be challenging when reintroducing social and charismatic species.

Implementation: Six individuals were released in TNP from 2015 to mid-2019. The first group consisted on four individuals (sex ratio 1:1), being two captive-born and two wild-born obtained from seizure. Prior to translocation, all individuals were held at Centro de Primatologia do Rio de Janeiro (CPRJ) for health exams, dietary re-education and establishment of group cohesion. We only observed one

Male with radio-telemetry anklet
© Luisa Genes

agonistic behavior during quarantine, from the central male to a juvenile (not included in the group). We translocated the four individuals to a 4 x 2 x 2 m soft release pen at TNP, where they stayed for three weeks prior to release. Every individual was monitored to check for possible wounds or abnormal behavior. After release, supplementary feeding was offered through a fruit box first placed on top of the pen, and then hanged 10 m high at the canopy. We continued supplementing the animals for two months after release.

Two months after the first release, the captive-bred male had to be retrieved back to CPRJ due to interaction with visitors and abnormal behavior. The other male started presenting the same behavior and we decided testing another approach: releasing it in a site with lower touristic visitation. One year after the first release, the captive-bred female also showed interaction issues with visitors. In this occasion we were able to bring another male from CPRJ to TNP, the fifth individual. They stayed together in the pen further away from most visited areas for two weeks in late 2016 and were then released.

The sixth individual was a juvenile that stayed for five months in the release pen, due to wounds caused by the radio transmitter and due bond trial with another female. The female was removed before releasing due to illness. After its released, this animal also presented abnormal behavior and, therefore, was also removed from TNP.

Post-release monitoring: We followed translocated individuals intensively for the first two weeks after release. Although intensive, monitoring avoided interactions with howlers. Most radio transmitters failed one month after release. Consequently, individuals were located through active search. Thereafter, monitoring consisted of weekly excursions. Animals were followed from 08:00 - 17:00 hours whenever possible. Their locations were recorded every 30 minutes and their diet was observed. Plant species consumed were recorded and further identified to assess the restoration of plant-animal interactions. Moreover, the interaction between released howlers and the dung beetle community was assessed through sampling the dung beetle community and through ecological function experiments (Genes *et al.*, 2019).

After the group remained together for about one month, one of the females left

the group and one male started to present abnormal behavior. Six months after release, one of the females gave birth to offspring, which had died after three months. The missing female was located visiting one of the few houses within TNP, where she was fed with fruits and egg by residents. She was captured and then released together with the fifth individual on the third release event. Few weeks later they separated, and this female was found dead (unknown cause) after a couple of months. This male was found with the remnant female from the first group two months after the couple disruption. From 2016 to 2019 two other infants from this new couple were born and are currently alive, forming one single group of four animals.

Howler forest habitat © Marcelo Rheingantz

Major difficulties faced

- Scarcity of source individuals.
- Impossibility of releasing new individuals due to yellow fever outbreak.
- Interaction of captive-born individuals with visitors.
- Radio telemetry equipment failure.

Major lessons learned

- Group cohesion is easily disrupted when the group is released.
- Radio telemetry equipment must be extensively tested in captivity to guarantee animal welfare.
- Release sites must be as far as possible from touristic hotspots.
- Captive-born animals must either be trained before release or be released together with wild-born individuals.
- Tijuca National Park is suitable to howler monkey populations.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Low number of individuals released.
- Occurrence of birth with high survival of juveniles (one birth per year, two of three survived until present).
- High survival rate in the wild, but low permanence rate of captive-born individuals (individuals had to be removed).
- Restoration of ecological interactions with predicted plant species and with dung beetles.
- Population reinforcement was not allowed due to a yellow fever outbreak.

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Translocation of Stephen's kangaroo rat preserving familiar neighbor groups in California, USA

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Introduction

Stephen's kangaroo rat (*Dipodomys stephensi*, SKR) is a nocturnal granivorous heteromyid rodent endemic to southern California. Kangaroo rats are ecosystem engineers and maintain grassland communities through their burrowing and seed caching behavior. SKR occur in open, flat areas with native grass or sparse coastal sage scrub, but much of the habitat within their historic range has been destroyed due to agriculture, housing, and industrial development. Development in southern California is occurring at a rapid pace, and mitigation translocations of SKR and other kangaroo rat species are frequently required, although rarely successful. Improving translocation methods is critical for the persistence of this species. SKR are listed as endangered under the United States Endangered Species Act, threatened under the California Endangered Species Act, and Vulnerable by the IUCN.

Goals

- Experimentally manipulate releases to determine whether familiarity with neighbors improves translocation success.
- Modify habitat at release-site prior to translocation to increase suitability for SKR.
- Establish a self-sustaining breeding population of SKR.
- Improve translocation methodology for SKR and other heteromyids.
- Mitigate for development at source sites and increase the number of populations on protected land.

Stephens' kangaroo rat fitted with radio-transmitter
© Debra Shier

Success Indicators

- Capture and translocate 50 individuals per year to maximize chances of success.
- Determine whether SKR translocated with familiar neighbors are more likely to settle, survive and reproduce compared to those translocated with unfamiliar SKR.
- Develop techniques to improve post-release fitness and establishment of SKR following translocation.
- Establish a population of SKR that persists for at least five years post-release.

Project Summary

Feasibility: Previous kangaroo rat translocations failed to establish viable populations with long-term persistence. High mortality, low site fidelity, and lack of post-release monitoring contributed to a history of unsuccessful translocations. In 1992, 599 SKR were translocated with no animals surviving at the site 11 months following release (O'Farrell, 1994), and a 2002 translocation resulted in 40% survival four months post-release with no surviving animals after one year (Spencer, 2003). Past translocations did not consider the social relationships within SKR populations. Although SKR are solitary and territorial, the dear enemy effect suggests that they may spend less time and energy defending their multi-purpose breeding territories from familiar neighbors compared to unfamiliar individuals (Temeles, 1994). Translocating animals with familiar conspecifics could increase the likelihood of translocation success by reducing aggressive interactions, increasing site fidelity and survival, and ultimately by improving reproductive output (Shier & Swaisgood, 2012). One mechanism by which social familiarity can improve translocation success is by helping to anchor released individuals. Reducing post-release survival has been identified as one of the 50 most important questions in all of conservation behavior (Greggor *et al.*, 2016).

Implementation: A total of 99 SKR were translocated; 54 in September 2008 and 49 in July 2009. Prior to translocation, we determined territory locations and neighbor relationships by trapping the source sites and tagging individuals with unique color combinations of reflective ear tags. An animal was assigned to a territory if it was trapped at the same burrow ≥3 times and was observed entering or chasing another animal away from the burrow (Shier & Swaisgood, 2012). After neighbor associations were mapped, SKR were trapped for removal from the source sites, fitted with VHF radio transmitter backpacks, and transferred to the release site. A protocol for soft release was created, which included acclimation cages for each individual. Acclimation cages consisted of an underground wire nest box (15.2 x 15.2 x 7.6 cm) set 30.5 - 38.1 cm underground, two corrugated plastic tubes (5 cm diameter) that connected the nest box to the surface, and an above ground wire retention cage (30 x 61 x 30 cm) filled with approximately 5 cm of dirt from the site. During the acclimation period, one tube was plugged and the other tube opened into the above-ground cage, allowing SKR access to explore and forage above-ground while preventing them from escaping. In response to

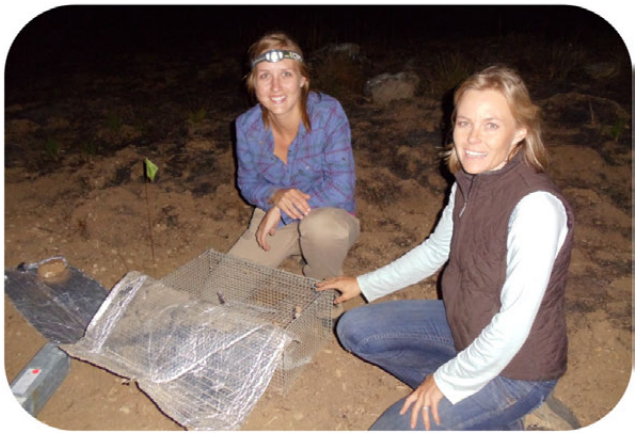
Harvester ants (*Pogonomyrmex rugosus*) invading cages a ring of plastic landscape divider was installed around each acclimation cage with insect adhesive (e.g. Tanglefoot) along the seam to prevent ants from getting into the acclimation cage. We held SKR in acclimation cages for one week and fed them a mix of seeds and lettuce daily. At the end of the acclimation period, the top cages were removed and animals were able to move freely on the site. We placed a battery-powered electric-tape fence around the site to deter coyotes and other mammalian predators.

Release site habitat with native grasses
© Debra Shier

Each year, half of the SKR were translocated with members of their neighbor group and half were translocated with unfamiliar individuals. Groups were matched for age and sex ratios, and released on the same night to ensure release timing did not confound the results (Shier & Swaisgood, 2012). Because non-native grasses restrict movement of SKR, in 2008, we reduced non-native grass cover on the 3.24 ha release site using a string trimmer. In 2009, an adjacent 4.45 ha release site was burned to reduce ground cover prior to SKR translocation. Both release sites were located on the Southwestern Riverside County Multispecies Reserve in southern California.

Post-release monitoring: For two days following both releases in 2008 and 2009 behavioral observations were conducted on a random subset of kangaroo rats. These observations revealed that SKR translocated with familiar neighbors initiated fewer fights and spent more time vigilant and foraging than SKR translocated with unfamiliar individuals. We used radio telemetry to track the burrow location of each animal every day for two months post-release, and once a week during the third month. SKR translocated with familiar neighbors traveled shorter distances and established burrows more quickly after release. Radio transmitters were removed during the three month post-release trapping surveys.

We conducted post-release trapping surveys monthly in year 1, at 1, 3, 6 and 12 months in year 2 and then annually for five years at each of the reintroduction sites. In both 2008 and 2009, we found higher rates of survival for SKR that were translocated with familiar neighbors compared to those translocated with unfamiliar individuals. Annual trapping revealed that more offspring were born to females translocated with familiar neighbors. We counted active burrows to compare SKR activity between plots where different habitat restoration techniques were used. There were more burrows, indicating greater SKR activity,



Transferring SKR to acclimation cage

in areas where non-native grasses were burned, followed by areas that were mowed, and the fewest burrows in untreated areas.

Major difficulties faced

- Gathering social data on the source population was time and labor intensive; however similar results could be accomplished through extensively trapping within small target areas and moving all neighbors simultaneously.
- Collecting a sufficient number of individuals to translocate in paired experimental and treatment groups required translocations to be conducted in two different years and from three source sites.
- Following the 2008 release, only 3 of 20 (15%) female kangaroo rats translocated with unfamiliar individuals survived to six months post-release.
- Intensive, annual management was necessary to reduce non-native grass cover on both release sites prior to and following translocations.
- Harvester ants invaded acclimation cages in 2008 and high levels of predation by Barn owls and meso-carnivores followed the 2009 release.

Major lessons learned

- Increasing social familiarity among members of a release cohort can be important to dampen dispersal, facilitate settlement and promote exhibition of important behaviors associated with fitness, and enhance survival and reproduction in SKR, and should be tested more broadly with other species.
- In cases where restoration of a release site is required prior to release, the method of site preparation has the potential to influence establishment and post-release success and requires more research.
- Implementation of soft-release protocols, including the use of acclimation cages and food supplementation, likely contributed to high post-release survival, but have not been directly compared to hard release.
- To manage non-targets at the release site, plastic landscape dividers are an effective means of excluding ground-dwelling ants from acclimation cages, but electric-tape fencing is not effective at reducing predation pressure as fencing posts provided perches for raptors and fencing did not deter mammalian predators.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- A self-sustaining population continued to persist nine years following release, with 148 individual SKR (19.25 SKR/ha) trapped on the original release sites in 2017, with many (n = 85) in reproductive condition.
- Social structure is important even in a solitary species and behavioral observations and fitness measures both show SKR translocated with familiar neighbors were more likely to settle at the release site and had higher survival and reproduction than SKR translocated with unfamiliar kangaroo rats.
- The translocation methods with acclimation cages, ant deterrence, and intact neighbor groups improved techniques for SKR soft release.
- Release site selection based on suitable soils, slope, aspect, and conservation status of the property, with vegetation management prior to and post release to maintain open ground and reduce non-native grass cover, facilitated long-term success.
- Collaboration with land managers and federal, state and local agencies was critical for the success of the program.

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Pre-release care, translocation, and radio tracking of a rescued Andean cat in Bolivia

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Introduction

The Andean cat (*Leopardus jacobita*) inhabits rocky habitats in the central Andes of Argentina, Bolivia, Chile and Perú, usually above 3,600 m (Villalba *et al.*, 2016), and at lower elevations in southern Argentina (Novaro *et al.*, 2010; Martinez *et al.*, 2008). It is one of the five most threatened felid species of the world and it has been categorized as Critically Endangered in Bolivia (Villalba *et al.*, 2009). It is also listed in CITES Appendix I. There is no record of any Andean cat in captivity. In March 2016, a sub-adult Andean cat was captured by local people in the town of Patacamaya (about 100 km from La Paz city) and delivered by the Bolivian Forestry and Environmental Police (POFOMA) to the Vesty Pakos Municipal Zoo (La Paz). There was no certainty of the specimen's origin and exact date of capture, therefore, it was decided to keep it in captivity until an appropriate solution could be decided. Behavioral studies showed that the individual should be released. We carried out pathogens analyses that came back negative (Napolitano *et al.*, 2019)

Released Andean cat © Juan Reppucci - AGA

and identified two potential sites with optimal habitat to release the individual in Sajama National Park.

Goals

- To return an individual to its natural habitat, contributing to the strengthening of an Andean cat population, and ensure its welfare while in captivity.
- Obtain biological information about the species, including health parameters (blood count, biochemical profile), morphometry and behavior.
- Release the Andean cat, ensuring the conditions of animal welfare and conservation of the individual and the wild population in the release area, minimizing the risks to its health and survival.
- To know the general behavior and the movement patterns of the released Andean cat and estimate its home range if it established territorial boundaries within Sajama National Park.
- To increase awareness about the species' conservation, mainly through the dissemination of the release operation, its safeguards and scope, and documenting the experience so the process can be repeated if needed.

Success Indicators

- The Andean cat is safely released following international guidelines, local regulations and specially generated local protocols.
- Behavioral studies allow adjustments in the management of the captive Andean cat, to safeguard its welfare and help in maintain natural behavior.
- Results of health studies to avoid any possibility that the animal could be harmful to wild populations.
- The individual is successfully monitored until its establishment in the release area or proximities.
- Elaboration of video and press release to increase general public knowledge regarding Andean cat existence and its conservation threats.

Project Summary

Feasibility: An inter-institutional *ad hoc* committee was established to define actions concerning the rescued Andean cat; it included representatives of the Bolivian environmental authorities (national, departmental and municipal levels), POFOMA, the Vesty Pakos Municipal Zoo, Andean Cat Alliance (known by its Spanish acronym, AGA), Wildlife Conservation Society - Bolivia and the Institute of Ecology of the San Andres Major University. The release site was selected considering the location where the individual was found, the existence of previous records of the species and the predisposition of Protected Area´s authorities and local communities to receive the Andean cat and collaborate once the individual has been released. During four days in July 2016, five potential areas were evaluated in Sajama National Park (SNP), to identify the most appropriate site for releasing the Andean cat, considering habitat features (rocky areas, highland

wetlands, vegetation cover), prey availability, human activity and accessibility. Technical representatives of the Committee participated with the collaboration of SNP park rangers.

Implementation: To proceed with the release of the Andean cat the Committee followed IUCN guidelines and Bolivian regulations. The Andean cat remained quarantined for 165 days in a special enclosure, adapted by the Zoo staff. During this period the cat was constantly remotely-monitored, its behavior was checked through camera traps and its health conditions were assessed through the implementation of five carefully-planned physical examinations. The first examination, after three days of receiving rehydration and feeding treatment at the Zoo, reported good physical condition, and a weight of 4.1 kg. In all assessments physical condition, hematological and blood chemical parameters were normal compared to that of domestic cats and wild felids of the genus *Leopardus* (data for the Andean cat did not exist) (Beltrán-Saavedra *et al.*, In review).

The Andean cat's blood was checked through PCR and serological tests for 17 pathogens relevant to felids, to prevent the introduction of those into the release area. All tests were negative (Napolitano *et al.*, 2019). A GPS-VHF collar (ATS™), provided by an Argentine team of AGA was fitted on the Andean cat to be able to monitor its behavior after release. The collar was programed to obtain one point every five hours daily, and one point every hour every seven days. According to this schedule the battery should last up to eight months. Three days before the release event, some elements used by the Andean cat in the Zoo´s enclosure, like stones, branches, sand and straw, were scattered around the chosen site, in order to reduce post-release stress. At 06:00 hours on 27th August 2016 the Andean cat was sedated at the Zoo and placed in a kennel for transport. During the trip to the SNP, physiological parameters were monitored and after complete recovery the cat was transported on foot to the exact location to be released. The Andean cat was released at 15:35 hours and monitored until 17:00 hours, during this time it showed normal behavior and movements.

Post-release monitoring: For 20 days after the release, the cat was monitored daily and intensively estimating general location trough the VHF signal making sure to not disturb it. No exact locations were pursued because this information was already being stored in the GPS collar and we prioritize to obtain information on presence and movements of the animal in the area. The Andean cat was detected almost daily over the first 12 days; in four non-consecutive occasions the signal came from the release area, and in other four from adjacent valleys, within a range of about 3 - 4 km (NE, NW and SW). No signal was obtained from either those areas or further away (up to 17 km) for the following seven days until 14th September. On 15th September, around 15:00 hours for the last time and for few minutes the signal was obtained, coming from the upper part of the valleys located over 3 km from the release site. Several searches were carried out between October and December simultaneously with two or three teams, covering suitable areas for Andean cat within the whole Sajama National Park. The release site was close to the Chilean border and a Chilean AGA team carried

out a search in the Chilean side, between 27th November and 2nd December, but no positive results were obtained. Finally, on 28th April 2017 an aerial search was carried out covering about 350 km², but no signal was obtained either.

Researchers doing site assessment in Sajama National Park © M. Lilian Villalba - AGA

It is relevant to highlight that the collar was also fitted with a mortality signal, that should trigger in case the animal was not moving for a long period of time. Throughout all these searches the mortality signal was not detected; therefore, it is more likely that either the cat dispersed far away from the release site, or that the collar failed.

Major difficulties faced

- There are no records of any Andean cats in captivity, therefore the Zoo team adapted and adjusted all procedures. This was also an unprecedented release experience in Bolivia.
- The pathogen analyses of blood samples were made as a facility with AGA´s trained experts in Chile, so CITES permits were needed to move the samples between countries. The complications for obtaining those permits delayed the release date.
- To obtain an appropriate tracking collar was also highly difficult and affected the release date.
- The lack of a permanent vehicle for field monitoring hampered the search of the Andean cat in regular intervals.
- Although local people from the protected area was informed and consulted about this operation, the fact of introducing a carnivore (even a small one like the Andean cat) made some concern; so, during the monitoring process, meetings with some communities was carried out to explain the project.

Major lessons learned

- It is fundamental to ensure that natural behavior of small wild cats temporally kept in captivity is preserved, as well as their health and welfare. Isolation from humans and permanent environmental enrichments of the enclosure with natural elements, are crucial to avoid the emergence of stereotyped behaviors or human habituation.
- Camera trap videos were a valuable tool to establish the Andean cat

ethogram, as a basis to monitor its behavior and assess its welfare; this also enabled timely and sound decision-making to modify and improve various aspects for maintenance in captivity and isolation.

- Environmental authorities, based on experiences like this and with the support of specialized committees, can establish general protocols to reduce bureaucratic response times, fulfilling with all the biological and veterinary criteria of IUCN. It is equally important that professionals and people related to wildlife care, know and follow IUCN reintroduction guidelines, local regulations and coordinate with respective authorities before carrying out the release of wild animals.
- It is important to anticipate the occurrence of similar cases and to be prepared with the necessary equipment. It is also essential to reinforce local laboratories to be prepared for genetic, parasitological and of pathogen analyses.
- There is a need to raise awareness and to generate a closer participation of people who live near release sites, so that they are part of these conservation processes.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- The maintenance of the Andean cat in quarantine was successful and an important achievement in the conservation history of the Andean cat. It was the first Andean cat maintained officially in captivity and the first being released after captivity.
- The conformation of an interinstitutional team, the knowledge, responsibility and willingness of the members was fundamental during all the process.
- The transportation and release process were successful, the Andean cat was sedated and adequately manipulated for this purpose.
- After the release, the Andean cat was daily monitored during 20 days, of which it was detected for nine non-consecutive days.
- The signal was lost after 20 days which precluded us from knowing the final outcome of the released individual. The loss of the signal could be due to a failure of the radio-collar as happened to other researchers studying the species in the high Andes (Reppucci, pers. comm., Villalba, unpublished), or it is also likely that the cat moved much further. A study made in the area of the release in 2018, which involved 36 camera traps and six months of permanent sampling did not detected the presence of this individual (from 18 photos with Andean cats evaluated; Huaranca, *et al.*, In prep.).

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Translocation of the Los Angeles pocket mouse to a protected reserve in California, USA

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Introduction

The Los Angeles pocket mouse (*Perognathus longimembris brevinasus*; LAPM) is a nocturnal, granivorous, heteromyid rodent endemic to open Riversidean alluvial fan sage scrub habitat (Williams *et al.*, 1993). The LAPM is one of eight sub-species of the little pocket mouse (*P. longimembris*) (Hall, 1981), and is listed as a Californian species of special concern as a result of serious population decline and range retraction from agricultural and urban development (Brylski, 1998). Additional threats to LAPM habitat include habitat fragmentation and invasive plants, especially annual grasses that eliminate bare ground (Western Riverside County BMP, 2011). As a conservation measure, we translocated LAPM from a water recharge project area in the San Jacinto river, California, USA. Our release site was approximately 8 km up-stream in a protected reserve. The translocation aided LAPM by reducing mortalities associated with the project. LAPM is not currently listed federally as an endangered species, however they are closely related to the critically endangered Pacific pocket mouse (*Perognathus longimembris pacificus*; PPM) (Williams *et al.*, 1993). Little pocket mice are smaller than other heteromyid rodents and a secondary goal of this translocation was to gain experience with the equipment and methodologies needed for relocation of heteromyids of this size.

Los Angeles pocket mouse

Goals

- Modify habitat at the release-site prior to translocation to increase suitability for LAPM.
- Develop an efficient and reliable translocation strategy for LAPM and other small heteromyids.
- Establish a self-sustaining breeding population of LAPM.
- Mitigate for development at source sites and increase the number of populations on protected land.

Success Indicators

- Capture and translocate at least 50 individuals to maximize probability of release success.
- Settlement and survival of LAPM at the release site.
- Evidence of successful reproduction of the translocated LAPM, measured by offspring at the site or reproductive males and females during post release monitoring.
- Establish a population of LAPM that persists for at least five years following release.

Project Summary

Feasibility: LAPM are endemic to open Riversidean alluvial fan sage scrub habitat in areas with suitable sandy soil (Williams *et al.*, 1993). This restricted range in southern California has a dense human population and LAPM are in direct conflict with the rapidly growing Los Angeles, Riverside and San Bernardino urban centers. In addition to direct loss of habitat, LAPM habitat is compromised by fragmentation and a variety of human impacts such as, artificial light pollution, illegal off-road vehicles, garbage dumping, human encampments and House cats. Our translocation project was a conservation mitigation measure for a water recharge project in occupied habitat within the dry riverbed of the San Jacinto river, California, USA. Our group has developed an effective translocation protocol for Stephens' kangaroo rat with a post-release monitoring program (Shier & Swaisgood, 2012). We modified this protocol to fit the smaller heteromyid rodent, LAPM (8 -11 g versus approximately 67 g). A release site was chosen by the United States Fish and Wildlife Service approximately 8 km up-stream from the source site in a conservation area managed by the Western Riverside County Regional Conservation Authority. This release site is within the same drainage with similar vegetation and predation pressure beyond the distance that LAPM could travel to return to the source site. We trapped the release site in advance and determined that the resident LAPM numbers at the release site were low enough to conduct a translocation and add new animals to the existing population via translocation.

Implementation: In May 2012, we trapped 122 LAPM (52 adult females and 70 adult males) from the source site over 21 consecutive nights (10,710 trap nights) immediately prior to the start of construction. Unfortunately, this time of year is not ideal for a translocation as it was in the middle of the breeding season, however we were tied to the construction schedule. We placed Sherman traps in grid lines with a trap every 10 m. LAPM were housed individually in a temperature-controlled facility, with sand, bedding material and a nesting chamber. We provided the mice with wild finch seed, romaine lettuce and small meal worms daily. At the holding facility seven females gave birth to a total of 23 pups. We supplemented the diet of pregnant and lactating females with whole powdered milk.

In preparation for the translocation, we had invasive grasses mowed and small

Release site habitat

Sycamore trees (*Platanus racemosa*) within 100 m of the release site removed as they could be used as perches by avian predators. To deter meso -predators, we applied Mountain lion urine (Mt.LionPee, Original Predator Pee) along the perimeter of the site once weekly for four weeks prior to the release. At the release site each LAPM was placed in an individual acclimation cage. Acclimation cages consisted of an underground nest chamber (15.2 x 15.2 x 7.6 cm) set 60 cm underground, two black plastic corrugated tubes (2.54 cm diameter) which connected the nest chamber to the surface, and an above-ground wire retention cage (20 x 20 x 20 cm). The acclimation cages were spaced 5 m apart in neighbor group clusters of 32 per group following Shier and Swaisgood (2012).

We installed plastic landscape dividers around acclimation cages to prevent invasion by Harvester ants (*Pogonomyrmex spp*). The placement of individuals in acclimation cages was based on neighbor relationships from geographical locations of trapped individuals from the source site. In addition, family groups of mothers and newly weaned offspring were placed in adjacent acclimation cages.

We fed mice a seed mix and lettuce daily while in acclimation. After one week in the acclimation cages, the above-ground portion of the acclimation-cages was removed. On 10th July 2012 we released 144 marked LAPM (52 adult females, 69 adult males, 12 juvenile females, 11 juvenile males) into the wild from their acclimation cages. We continued to supplement the mice three times a week with the seed mix until rains produced sufficient food resources (three months).

Post-release monitoring: We re-trapped the site for five consecutive nights at one and three months following release. During these two trapping bouts we captured 31% of the translocated LAPM. To assess long-term success, we continued to trap for five consecutive nights each year for five years post-release. At one year post-release we trapped 24 LAPM. 62% (N = 15) of captured LAPM were founders and N = 9 were unmarked LAPM. Two years post-release there were 31 LAPM and one was a founder. At five years post release in 2017 the population was strong with 67 LAPM trapped. We observed signs of reproduction during all years of post-release monitoring. LAPM have short life histories and it is thought that the unmarked LAPM at the site are the result of successful reproduction. However, because we have not conducted genetic analysis there is a possibility that LAPM were recruited from the surrounding area.

Major difficulties faced

- Initial trapping took place under a super moon and during the breeding season; this may have decreased the number of LAPM we were able to remove from the source site.
- The release site was in a protected natural area with restricted human access; however, people trespassed, rode off-road vehicles, dumped trash, and vandalized and stole equipment.
- Seven LAPM died in acclimation cages. They were found dead above ground after digging out all the soil from the top cage. They may have died from exposure or stress from trying to escape.
- In the absence of radio-telemetry, we were unable to distinguish between dispersal from the release site and mortality.

Digging acclimatization burrows
© Thea Wang

Major lessons learned

- When the release site is near residential neighborhoods the safety of the neighborhood should be taken into consideration. This particular neighborhood had high levels of vandalism and confrontations. People were not opposed to our conservation efforts, but our staff were vulnerable because we were in the field at night. We implemented a new protocol to include security into projects that are located in areas with moderate to high levels of crime and/or drug use.
- We were able to scale down the equipment for the smaller sized heteromyid. The pocket mice are more motivated than kangaroo rats to try and escape from the acclimation cage. To prevent the mice from spending too much energy trying to escape we shortened the burrow tube in the above ground cage and raised the floor of the top cage above the surrounding substrate.
- The new design of smaller acclimation cages can be used with an endangered subspecies of little pocket mouse.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- We trapped a large number (122) of LAPM out of the source site; which allowed us to release a large number into the receiver site.
- We used established techniques to translocate heteromyids, such as acclimation cages, predator deterrence, intact social groups and competitor exclusion.
- A self-sustaining population was successfully established; in 2017, five years post-release, 67 individual LAPM (35/ha) were trapped at the release and many (76.1%, n = 51) were in reproductive condition.

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Conservation translocation of banded and Shark Bay rufous hare-wallaby to Dirk Hartog Island, Western Australia

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Introduction

The Banded hare-wallaby (*Lagostrophus fasciatus fasciatus*) and Shark Bay rufous hare-wallaby (*Lagorchestes hirsutus bernieri*) are two small macropodid marsupials, listed as Vulnerable by the IUCN and under Australian Commonwealth and Western Australian state legislation. Although similar in appearance and behavior, these species are not closely related, with banded hare-wallabies the sole-living representative of the subfamily Lagostrophinae. Both taxa disappeared from the Australian mainland after European occupation, at least partially as a result of predation by non-native Red foxes (*Vulpes vulpes*) and Feral cats (*Felis catus*) (Woinarski *et al*., 2012), and the only remaining natural populations were found on Bernier and Dorre Islands in Shark Bay, Western Australia.

Subsequently, Banded hare-wallabies have been successfully translocated to Faure Island (also in Shark Bay) and Australian Wildlife Conservancy's Mt. Gibson Sanctuary, but the Shark Bay subspecies of Rufous hare-wallaby has never been translocated. Dirk Hartog Island is a large island (63,300 ha) in Shark Bay and while there are anecdotal reports that both taxa previously occurred there, no physical evidence (historical or subfossil specimens) were ever obtained. The successful eradication of Sheep (*Ovis aries*), Goats (*Capra hircus*) and Feral cats from Dirk Hartog Island by 2017 represented an opportunity to establish new populations of both taxa.

Banded hare-wallaby on Bernier Island © DBCA

Goals

- Establish new populations of both Banded and Rufous hare-wallabies.
- Maximize genetic

Hare-wallabies arriving by helicopter © DBCA

diversity of founder population of both taxa.

- Create a large, viable and genetically diverse populations of both taxa leading to delisting as threatened fauna.
- Re-establish ecosystem engineering processes mediated by the two taxa (e.g. browsing, grazing, modification of vegetation structure).
- Create a sufficiently robust and genetically diverse population which will provide a preferable source site for future reintroductions to other locations.

Success Indicators

- *Short-term:* Greater than 50% survival of translocated hare-wallabies in first four months.
- *Short to medium-term:* Body weight and condition maintained over nine months.
- *Short to medium-term:* There is evidence of recruitment in first nine to 36 months.
- *Long-term:* The area of occupancy increases.
- *Long-term:* Greater than 90% genetic diversity maintained after 10 years.

Project Summary

Feasibility: Dirk Hartog Island is geographically proximal to the only natural populations of both these taxa, with just 30 km separating the island from Dorre Island. Bernier and Dorre Islands have ostensibly similar vegetation to Dirk Hartog Island and all three islands fall within the same Interim Biogeographic Regionalization for Australia (IBRA) bioregion (Yalgoo). Since the destocking of sheep and goats commenced in 2005, vegetation has begun to recover with 38% of the island showing significant recovery (van Dongen *et al*., 2019). The subsequent removal of cats further increased the likelihood that the island could support hare-wallabies. While no specimens or subfossil remains have ever been obtained of either taxon from Dirk Hartog Island (Baynes, 1990), there is strong anecdotal evidence that both did once occur on the island (Shortridge, 1909).

Although formerly a pastoral property, Dirk Hartog Island became a National Park in 2009 and is managed by the Western Australian Department of Biodiversity, Conservation and Attractions (DBCA). However, the former pastoral lessees retain a small freehold property on the island and operate a vehicle barge which

permits public access to the island. Visitor numbers to the island are generally low with a maximum of 20 vehicles permitted in the National Park at any time.

Implementation: A trial translocation of 12 individuals of each taxon took place on 29th and 30th August 2017, in a 2:1 female to male sex ratio. The focus of the trial was to better understand the challenges that a translocation of these taxa would entail, before commencing a full-scale release. Animals were transferred by charter vessel (approximately five hours journey) and Rufous hare-wallabies were treated with a sedative (diazepam) to assist with maintaining animals in a calm state. Both species received selenium and vitamin E, which are thought to play a role in minimizing the risk of capture myopathy. All 24 individuals were fitted with radio-telemetry collars and were monitored daily by ground radio-tracking for 12 weeks post-release. After this period, collared individuals were recaptured for assessments of condition and collar-fit. Monitoring was reduced to once every 4 - 6 weeks by fixed-wing aircraft to assess ongoing survival and animals were recaptured in May 2018 for collar removal and health-checks.

After the success of the 2017 trial, a full-scale translocation was commenced in September 2018 with 90 Banded hare-wallabies and 50 Rufous hare-wallabies released, this time in even sex ratios. Again, 12 individuals of each taxon were fitted with collars and monitored in the same fashion. However, the translocation of animals from Bernier and Dorre Islands employed the use of a helicopter, reducing journey time to approximately 30 minutes, while also minimizing other negative sensory stimuli.

Post-release monitoring: During the trial translocation, Rufous hare-wallabies underwent significant weight-loss in the first 12 hours after capture, with an average of 13% reduction (up to 18%). It was assumed this related to stress-related urination and hypersalivation. By comparison, Banded hare-wallabies did not show significant weight loss. This was followed by the first mortality four days post-release, with a male Rufous hare-wallaby found dead. A subsequent necropsy indicated capture myopathy as the cause of death. However, no further mortalities were recorded until collars were removed in May 2018. Recaptures of collared hare-wallabies found most were in average or above-average condition and six Banded hare-wallabies and 10 Rufous hare-wallabies were recorded as having pouch young.

Rufous hare-wallabies slowly regained weight but only three exceeded

Hare-wallaby release habitat © DBCA

their original capture weight (although some animals would have lost weight after weaning joeys).

During the full-scale translocation, initial weight-loss in Rufous hare-wallabies was minimal (average 3% in first 12 hours) and this was largely attributed to the shorter time and reduced stressors in transit by helicopter. No mortalities of collared hare-wallabies were recorded in the full-scale translocation in 2018, but a road-kill rufous hare-wallaby was discovered by rangers in May 2019. Signage has been erected to encourage visitors to the island to slow down outside of daylight hours but the risk of vehicle collisions with hare-wallabies and other wildlife may need to be better articulated to the public.

Major difficulties faced

- Management of translocation stress, particularly in Rufous hare-wallabies, was a major consideration. This species is vulnerable to mortality through capture or stress myopathy (Cole *et al*., 1993), which is initiated by exertional stress.
- Capture of hare-wallabies on Bernier and Dorre Islands involved hand-netting at night using a spotlight to locate animals. This, and the need to access the islands by sea at night was a major challenge, especially when hampered by inclement weather.
- Intensive post-release monitoring was made more challenging by large over-night movements, particularly by Rufous hare-wallabies.

Major lessons learned

- Whether originally native to Dirk Hartog Island or not, both Banded hare-wallabies and Rufous hare-wallabies adapted quickly to their new environment and thrived.
- Reducing time in transit and employing a transport medium with less negative sensory stimuli (noise, motion, scent) helps minimize stress, particularly for vulnerable species like Rufous hare-wallabies.
- Hare-wallabies can be fitted with telemetry-collars without causing mortality from entanglement, assuming due care is used to achieve optimal collar-fit.
- Both Banded hare-wallabies and Rufous hare-wallabies undertake large movements during the initial post-release period but often return to their initial release location.
- Vehicles represent a potential source of mortality for hare-wallabies on Dirk Hartog Island, which is something that will need to be managed.

Success of project

Highly Successful*	Successful	Partially Successful	Failure

*** - ranking for both Banded hare-wallaby and Shark Bay rufous hare-wallaby**

Reason(s) for success:

- Successful removal of sheep, goats and cats from Dirk Hartog Island.
- Highly suitable habitat at release sites.
- Trial translocation undertaken initially to help inform and improve strategies for full-scale translocation.
- Expertise of field-team with collaring and handling of hare-wallabies and administering drugs.
- Large translocation numbers in a rapid, intense translocation session.

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Reintroduction of the eastern quoll to Mulligans Flat Woodland Sanctuary, Australia using trials, tactics and adaptive management

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Introduction

The Eastern quoll (*Dasyurus viverrinus*) is a small-to-medium marsupial that previously inhabited south-eastern Australia. Last seen on the mainland in 1967, its extinction has been attributed to predation by introduced predators, habitat loss and disease. It is now restricted to Tasmania and listed as Endangered by the IUCN Red List and the EPBC Act 1999. It is a nocturnal predator and scavenger with a diet of invertebrates, birds, small mammals, reptiles, fruit, and carrion. The species is sexually dimorphic with a mean adult body mass of 1,250 g for males and 850 g for females. Males have larger home ranges (mean 44 ha) than females (mean 35 ha). Females can carry a single litter of up to six young per year. Annual mortality in the wild is high, with 20 - 58% of juveniles surviving to their first breeding season with a life expectancy of three to four years (Godsell, 1983). The reintroduction site is Mulligans Flat Woodland Sanctuary (MFWS), a 485 ha area containing critically endangered Box-gum grassy woodland situated

Eastern quoll © Adam McGrath & Woodlands and Wetlands Trust

in north-east Canberra, Australian Capital Territory (ACT), Australia. MFWS is enclosed by predator-proof fencing to exclude introduced Red foxes, cats, European rabbits and hares, all of which were eradicated within the exclosure prior to reintroductions.

Goals

- To establish a population of Eastern quolls at MFWS.
- To demonstrate how the use of trials, tactics and adaptive management can improve reintroduction success.

Success indicators

- *Survival:* At least ≥67% of founders surviving after 42 days post-release for each trial reintroduction. This will indicate that the environment is suitable, and founders have settled into the landscape (*achieved in Trials 2 and 3*).
  - ⇒ Note that Eastern quolls that escaped the MFWS fence but were retrieved alive, or were transferred to another facility, were considered survivors, so we report here on 'true' survival (henceforth survival).
- *Condition:* At least ≥67% of founders maintaining body condition weight within 10% of their release weight after 42 days post-release (*achieved in Trials 2 and 3*).
- *Reproduction:* At least ≥67% of females successfully produce young by 42 days post-release for each trial reintroduction. This will indicate that the environment offers sufficient denning sites and can support lactating mothers (*achieved in Trials 2 and 3*).
- Additional success criteria include population growth, population persistence, genetic diversity and behavior, which will be monitored over the long-term.

Project Summary

Feasibility: The Eastern quoll reintroduction is a partnership project between The Australian National University (ANU), ACT Government, Mt. Rothwell Biodiversity Interpretation Centre, Woodlands and Wetlands Trust and James Cook University, and is part of the long-term Mulligans Flat-Goorooyarroo Woodland Experiment (www.mfgowoodlandexperiment.org.au). This major collaborative partnership combined funding for conservation on public lands, drew on the strengths of each organization, and built on the success of previous reintroductions such as for the Eastern bettong (*Bettongia gaimardi*).

A translocation proposal was prepared including the success criteria outlined above as well as a risk assessment (Manning, 2015). The predator-proof fence surrounding MFWS removed the threat posed by introduced Red foxes and cats, which are a major barrier to mainland reintroductions. Based on these, the reintroduction was approved by the Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE), Victorian Department of Environment, Land, Water and Planning, ACT Territory and Municipal Services and the ANU

Mulligans Flat release site © Mark Jekabsons

Animal Experimentation Ethics Committee.

Implementation: The reintroduction was undertaken as a series of three trials over three consecutive years (Trial 1 in 2016, Trial 2 in 2017, and Trial 3 in 2018). An additional translocation was conducted in 2019 to maximize the genetic diversity of the founding population (not reported here). We used the Translocation Tactics Classification System (TTCS, Batson *et al.*, 2015) as a framework to adapt our tactics between trials to achieve our strategies of maximizing survival and minimizing post-release dispersal. We also adopted an adaptive management approach, using monitoring to facilitate rapid learning and to implement interventions to improve reintroduction success (Wilson *et al.*, 2020).

In Trial 1, we translocated fourteen Eastern quolls (6 females & 8 males) to MFWS in austral autumn. No females were carrying pouch young because the mating period was yet to occur. Founders were selected from both captive (6) and wild (8) populations. Captive founders were sourced from Mt. Rothwell, and wild founders from free-ranging populations across 14 geographic regions in Tasmania which were separated by at least 15 km or a significant geographical barrier to eastern quoll dispersal (informed by a study of the genetics of these wild populations in Tasmania (Cardoso, 2014)). We took no more than two animals from each region to minimize impacts on the source population and maximize genetic diversity. Releases were carried out as soon as possible (i.e. animals were transported to the ACT, underwent health assessments, and released on the same day) and at night to minimize stress and to provide maximum time to explore MFWS and find a den before first light. No supplementary food was provided.

Despite significant modifications to our predator-proof fence prior to the first release, within days of the Trial 1 release, seven founders escaped over the MFWS fence. Of these, two were found deceased (assumed to be preyed upon by the red fox), three died from injuries whilst under observation in a care facility, and two were retrieved alive and released back into MFWS. Of the remaining seven founders, two were found deceased within MFWS, one was transferred to Mt. Rothwell due to its poor condition, and four (3 females & 1 male) survived without issue. In Trial 1, 43.8% of founders survived the establishment period, 37.5% maintained body condition, and 62.5% of females produced young.

In Trial 2, we translocated thirteen female-only founders, preferring those that were carrying pouch young that were fused to the teat (>60 days of age, ~25mm crown rump length, Bryant, 1988). Releases in Trials 2 (and also in Trial 3) were conducted from one of four central locations (separated by 50 m). This tactic aimed to maximize the distance over which a founder would travel before encountering the fence, while also allowing them to encounter food resources, den sites, conspecifics, or other features of interest before the fence. In Trial 2, 92.3% of founders survived the establishment period, 92.3% maintained body condition, and 84.6% produced young.

In Trial 3, we translocated 8 female-only founders, also preferring those carrying fused pouch young. No significant differences in survival or dispersal were found between captive and wild founders in Trials 1 and 2, so only the more genetically-diverse wild founders were selected in Trial 3. No significant weight loss was observed, so no supplementary feeding was provided. In Trial 3, 87.5% of founders survived the establishment period, 100% maintained body condition, and 87.5% produced young.

Post-release monitoring: Daily survival and den location were monitored using VHF or GPS collars for 42 days post-release because survival plateaued after this period in Trial 1. We conducted post-release health checks measuring weight, condition, and pouch occupancy every two weeks, though timing and frequency varied due to reproductive stage, weight fluctuations (influencing collar fit), logistical constraints, and ability to re-trap the targeted animal.

Major difficulties faced

- *Escapes:* Within days of the Trial 1 release, several founders escaped over the MFWS fence into the surrounding landscape and were preyed upon by Red foxes. This translocation was also complicated by issues with collar fit due to expanding neck sizes induced by breeding hormones (especially in males).
- *Weight loss:* By 14 days post-release in Trial 2, four captive-bred founders had lost >10% of their initial release weight. As an adaptive management intervention, supplementary food was deposited into their dens, in declining amounts as weights stabilized.

Major lessons learned

- *Males were poor founders:* In Trial 1 many founders, especially males, escaped over the MFWS fence within the first few nights of release.
- *Releases should occur in winter:* We hypothesized that the greater number of male escapes was exacerbated by the timing of release. Eastern quolls experience elevated breeding hormones in autumn, and this stimulates mobility and aggression in males, aiding them to acquire den sites and food (Godsell, 1983). We suspect that females may have also struggled to settle because they were being pursued by males and were likely to have elevated hormones. We adapted our tactics in Trials 2 and 3 by conducting

Research team © Swimming Wombat Photographics & Woodlands and Wetlands Trust

releases in winter, after the mating period. This had the added benefit of reducing stress and collar fit issues associated with breeding hormones.

- *Females should be translocated with pouch young:* Conducting releases in winter allowed us to translocate females with fused pouch young. This tactic meant we could translocate new male and female juveniles sired by either captive or wild males, and avoided the elevated male mortality and dispersal observed in Trial 1. We hypothesized that females with pouch young would invest in finding and maintaining a natal den, thereby reducing dispersal and potential escape from MFWS.
- *Dispersal affects the survival:* Founders that moved between dens on consecutive nights were more likely to escape and less likely to survive, and this den movement was lower for females and when den sharing with another founder.
- *Stress needs to be managed:* Founders in Trial 1 were released from bags by researchers, which may have increased stress. We adapted our tactics in Trials 2 and 3 and placed founders *in situ* in a den box (wooden box with a sliding door) with the door closed for one to two hours (delayed release). After last light, the door was opened from behind the den box and the founder could leave of its own accord. We hypothesized this would minimize stress and provide maximum time for founders to explore MFWS and find a den before first light.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- We released healthy eastern quolls into a fenced predator-free environment.
- We closely monitored the population post-release and adjusted our reintroduction tactics to reduce factors driving dispersal over the fence and mortality. Once we identified a way to establish founders inside MFWS we were able to reduce our effort and focus on translocating more genetically-diverse founders.
- We used the TTCS as a framework to improve our ability to identify, select

and design tactics to achieve our strategies of maximizing survival and minimizing post-release dispersal.

- We used trials in an adaptive management framework to make informed choices that improved reintroduction success. This approach is particularly important for threatened species reintroductions, where rapid decisions are often required despite the absence of complete knowledge.
- The passion, dedication, innovative thinking and collaborative effort of the partners and volunteers which made the planning, operations and research conducted in this project possible.

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Training and monitoring of orphan leopard cats by soft release in Miaoli County, north-western Taiwan

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Introduction

The Leopard cat (*Prionailurus bengalensis*) is a common wild felid distributed throughout Asia. It is listed as Least Concern by IUCN Red List. However the subspecies *P. bengalensis rabori* occurring on the Philippine islands is listed as Vulnerable, and the subspecies *P. bengalensis iriomotensis* occurring on Iriomote Island in Japan is considered Critically Endangered. Although the leopard cats adapt to diverse habitats, a combination of factors including human encroachment, habitat loss and fragmentation, and commercial exploitation have threatened leopard cats in many of their historic ranges. Road kills, the competition and spread of disease caused by invasive carnivores (e.g. dogs and cats), as well as killed in retribution for poultry damage, were all considered the threats to leopard cats across most of its Asian range. The populations of Bangladesh, India and Thailand are included in CITES Appendix I, while all other populations are included in Appendix II. In Taiwan, it is listed as Endangered in the Wildlife Conservation Act since 2009 (Council of Agriculture, 2009). This research was conducted in Miaoli County (24°17'N-24°44'N, 120°43'E-121°15'E) where is one of three counties (Miaoli, Taichung and Nantou) that where still have viable population of Leopard cats in northwestern Taiwan. Seven orphans were rescued and were later released at or near the site where they were found from 2012 to 2015, and the soft-release techniques were used. Past and recent human settlement and activities have contributed to a high level disturbance in this research area.

Leopard cat © Mei-Ting Chen

Goals

- To assist the individuals returning to the wild, adapting and surviving in the natural environment.
- To return the genes to the native populations for reducing losses caused by human

Leopard cat habitat with researcher © Wei-Jie Lee

interference.

- To investigate whether and how the individuals could survive and breed successfully.
- To establish soft-release techniques for rescued orphan Leopard cats.
- To develop the reintroduction protocol of the leopard cat for future reintroduction in the historic distribution areas.

Success Indicators

- Leopard cat survives in the wild.
- Settlement and establishment of the Leopard cats territory.
- Breeding success of the species in the wild.

Project Summary

Feasibility: When the orphaned Leopard cat was rescued, the individual was sent to the rescue center for health examination and therapy and the experts meeting evaluated the feasibility of the training-release project for the orphan Leopard cat. Diverse issues had been evaluated for understanding the tradeoff among the benefits, costs and risks and these include: 1) the conditions of habitat, such as local density of Leopard cat, food resource and competitors; 2) conservation status of population, which is Endangered in Taiwan; 3) conditions of the individuals which include the source, physical and mental conditions, risk assessment of contagious disease; 4) success assessment of release according to the technique of training and release, the interaction between individual and trainer, and the attitudes of locals towards leopard cats; 5) feasibility of monitoring, including radio-tracking and monitoring the body condition of the released individual by visiting it irregularly; and 6) the budget. The expense was the minor concern, because it had been evaluated that population size of Leopard cat in Taiwan could be 468 - 669 at present, and the government was committed to restoration the population. Successful orphan release cases are valuable for the reintroduction of gene source back to the population or re-establishment of

Leopard cat populations.

Implementation: When the individual was evaluated for release, implementation of training-release procedures were as follows: 1) determination of location for training and release, and communication with local people, 2) designing and building the wild enclosure, 3) training in wild enclosure for hunting skills and interaction with trainer, 4) training with the trainer in the wild for hunting skills and habitat adaption, such as environment, food resource, intraspecies- and interspecies-competitors, sympatric animals, 5) short-term release done progressively by increasing the independent time, and by decreasing the frequency of food provision, 6) anesthetization for final health examination and radio-collaring, and 7) release of the Leopard cat.

It is very important to communicate with local people for agreement of the Leopard cat release, because there are retributions for poultry predation issues. Besides, a single trainer can train the individual to avoid human activities and consume poultry. The trainer could collect the ecological information of the trained individual adapted to the habitat and be familiar with the sympatric wildlife by the observation of the trained individual's behavior. As well as detailed radio-tracking could help to identify the route of the trained individual in the wild over several hours to several days.

Post-release monitoring: After the release of trained individual, the first work was radio-tracking of the individual to know whether it could establish its territory on site or in nearby areas. The home range and core area could be calculated by radio-tracking, which showed how the released individual using the habitat.

The second work was to monitor the body condition of the released individual by direct observation by irregular visits, as well as to supply the food and medicine when the individual needed such intervention. This could evaluate the adaption of the released individual and could increase the survival rate of the individual. Moreover, long-term monitoring could provide more information such as breeding of the released individual This is scarce and valuable ecological information for Leopard cat at the same time for the other rare and elusive small wild cat species.

Overview of release site © Mei-Ting Chen

Major difficulties faced

- Determination of location for training and release.
- The attitudes of locals towards Leopard cats.
- Single trainer - in order to avoid too many

human contacts that may result in losing trainee's natural fear of humans.

- The trainer must have sufficient knowledge and experience in the wild, as well as familiarity with cat behavior.
- Limitation of the individual's starting training age, which being under three months old could be more easier and better interaction with the trainer rather than if it was older than three months old which would make it more difficult for wild training.

Major lessons learned

- Consideration of the social environment and atmosphere and the agreement by local people.
- Minimizing the individuals contact with people and artificial interference, such as the same single trainer, avoidance of artificial activity and avoidance of domesticated animals such as poultry that are kept by humans.
- Sufficient pre-release training in the wild, including training-release procedures 4) and 5).
- Intensive post-release monitoring, including the radio-tracking, body condition monitoring by direct observation of visiting irregularly, and supporting of food and medicine in urgent cases.
- Location selection for training and release in larger and continuous habitat with less human activity and in where the radio-tracking and monitoring is able to operate.

Success of project

Highly Successful	Successful	Partially Successful	Failure
1*	4*	1*	1*

*** - Out of 7 releases 1 was Highly Successful, 4 were Successful, 1 was Partially Successful and 1 Failure**

Individual soft-release projects:

- The first case was considered failed since the individual was trained in the wild for only one and a half months and then the radio-tracking signal was lost. The period of wild training was too short for the released individual and it failed to establish its territory on site and the wild enclosure was not built in the training site which could be the reason of this failure.
- In the second case, the researcher failed to monitor the released individual in the post-release monitoring period, because of the terrain.
- For the 3rd to 6th cases, those released individuals could survive independently and establish their territories on-site or in nearby areas which were monitored for five months, two months, seven months and five months respectively. However, the third and fourth one were captured (failed to find the body) or poisoned by humans on purpose in the post-release monitoring period, which was one of the threats to Leopard cats in

Taiwan.

- The seventh released individual was the most successful case in this research, which was monitored for three years and bred four times during the monitoring period.

Reasons for success:

- Determination of training and release site with the consideration of the attitude of local people (friendly to Leopard cats or not) and the feasibility of training, radio-tracking and monitoring.
- Sufficient pre-release training period in the wild, this could be achieved with a built enclosure in the wild.
- Adopted the soft-release techniques and detailed procedures (diverse prey provided and poultry avoided, single trainer, short-term release by progressively increasing the independent time, and by decreasing the provision of food gradually, etc.).
- Trainers with sufficient knowledge in leopard cat ecology, feline behavior, and experience in the wild.
- Sufficient post-release monitoring carried out.

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Reintroduction and supplementation of European ground squirrel populations in the Czech Republic

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Introduction

The European ground squirrel (*Spermophilus citellus*) is a rodent species classified as Endangered according to the IUCN Red list. Moreover, the species is classified as Critically Endangered in the Red list of vertebrates of the Czech Republic (CR) and belongs to the highest category of specially protected species in Czech legislation. Its conservation is hence of high priority and a national Action Plan for the European Ground Squirrel (EGS) has been implemented since 2008. Both reintroductions and supplementations discussed here have been performed as part of this Action Plan. Reintroductions are currently restricted to the protected landscape area České středohoří, as the first experimental area that offers suitable habitat and good potential to create a metapopulation. This metapopulation should possibly include stable existing populations Hrádek and Raná. Supplementations are at the present focused on small populations with abundance of up to 15 individuals, but with good potential regarding habitat quality and size and also management. In the future it is intended to use supplementations even for larger populations to prevent them from inbreeding. There are no geographical limitations for the supplementations within the CR, all populations are considered to be from the same genetic lineage.

European ground squirrel
© J. Matoušová

Goals

- To create a functional metapopulation of EGS in western part of the protected landscape area České

středohoří with a minimum abundance of 2,500 individuals (reintroduction goal).
- To ensure the long-term existence of maximum EGS populations in the CR (supplementation goal).

Success Indicators

- Confirmed successful breeding of released individuals (for both reintroductions and supplementations).
- Long-term persistence of new populations, ideally also with increasing abundance and expansion to surrounding suitable habitats (for reintroductions).
- Doubling the abundance of a particular population at that time within five years of last release, independent existence of the population for at least 10 years after last release (for supplementations).

Project Summary

Feasibility: The EGS is a socially living species that requires open habitats with permanently low vegetation cover. In the CR, these conditions are usually met at grassy airfields, natural or cultural grasslands - steppes, meadows, pastures, camps or sports fields, vineyards, gardens and orchards. All of these habitat types require at least some kind of human management, typically mowing or pasture, in order to constitute a suitable environment for EGS. Ensuring regular management at most EGS localities is an essential part of the Action Plan.

Considering reintroductions, the first step was to select suitable localities for this measure. The best localities were searched among known historical places with relatively recent occurrence of EGS in the area of interest and final ranking was

Table1: Summary of all reintroductions and supplementations of EGS carried out within the Czech Action Plan, including post-release monitoring results

Locality	Initial abundance	No. of released individuals / Monitoring results observed reproduction (R) / estimated abundance				
		2016	2017	2018	2019	2020
Písečný vrch	0	-	57/-/57*	57/R/80*	0/R/50	40/R/80*
Hliniště	0	-	-	-	85 /-/85*	0 /-/0
Karlovy Vary	8	22/-/30*	22/R/37*	0/R/50	0/R/100	0/R/100
Hodkovice	1	-	20/-/21*	20/R/35*	0/R/60	0/R/60
Roudnice	10	-	-	-	20/-/30*	20/R/55*
Břeclav	4	-	-	-	-	20/-/24*

* Population estimate include also number of released individuals

prepared based on criteria such as habitat suitability, overall area, isolation of each locality and its future prospects for needed management, property rights, etc. In advance before any release is actually performed, the selected locality is visited again to make sure it is well prepared (in some cases more demanding intensive management such as removal of shrubs can be applied). Financial aspects regarding future management of the newly created localities is of course of great importance.

Release enclosures for squirrels © J. Matoušová

Planning supplementations is carried out in a similar way. Suitable localities are selected from those with estimated abundance of 15 or fewer individuals based on monitoring results from the previous year. Final decision then takes into account size of each locality, its history and prospects regarding management, property rights and positive or negative view of respective landowners, overlaps with protected areas, etc. Of course, a necessary condition for reintroductions and supplementations is a sufficient and reliable source of individuals of suitable genetic origin. Due to the conservation status of EGS in the CR and limited options to provide spare animals from existing natural populations, this has been solved by establishing four breeding facilities (most of them started with animals brought from Slovakia) and by cooperation with zoos that keep EGS of acceptable genetic origin. During 2017 all of these sources were able to provide around 100 individuals every year for the purposes of reintroductions and supplementations.

Implementation: Carrying out EGS reintroductions and supplementations is highly demanding on coordination and cooperation. There is, for biological reasons, a relatively short time period (early spring or late summer), during which any releases of the species should be performed (we have so far always released in late summer). Therefore, all required administrative matters need to be taken care of in advance. This includes for example official agreements of relevant conservation offices or permissions from respective landowners. At the same time, up to date information about current state at selected localities, situation in breeding facilities or offers from zoos needs to be continuously collected. Consequently, a plan for given season can be prepared (where to carry out a reintroduction/supplementation, how many individuals to release at each locality, from which sources to bring animals in each case, etc.) and dates of releases can be set. The season plan has to be effectively communicated with all involved

Overview of release site © J. Matoušová

parties, so that everything is then prepared in time (needed equipment, trapping of EGS in breeding facilities, transports, veterinary checks, etc.). Very important is of course also keeping records of all released individuals. Sex, weight and age (juvenile or adult) are taken, as well as fecal and DNA samples, and the animals are individually marked with microchips.

Known experience with releases of EGS suggests that it is essential to force the animals to remain at the release site as long as possible and prevent them from panic escape reaction. This is usually ensured by releasing them individually into pre-drilled holes that are then temporarily closed by a heavy bottle or wooden stick and an extra protection for the first few days is provided by simple enclosures. Food and shelter in form of pallets with hay are also provided at the beginning to help the animals settle at the locality. In case of supplementations the new animals are usually released directly into existing burrow entrances of the local individuals, with the entrances again temporarily closed.

Post-release monitoring: After release, each locality is visited at least two to three times in the course of the next week, when the enclosures and bottles are removed. More frequent visits follow until first hibernation, during this time occurrence of new burrow entrances is observed. Afterwards, the localities are regularly checked as part of monitoring within the Action Plan. That includes one obligatory visit per year (but typically three) and provides results in form of population estimates and maps of inhabited area. Additionally, active burrow entrances are localized by GPS to get a more detailed picture of the inhabited area and at least one trapping session is usually performed to check the health and demography of the population.

Major difficulties faced

- Starting a sufficient number of breeding programs.
- Getting permissions from landowners.
- Ensuring finances for proper management and monitoring at new localities in the future.
- Finding the most effective release method for each situation.
- Coordination and time management during a short season.

Major lessons learned

- It is extremely important to release EGS to a suitable habitat and to ensure it will stay suitable in the future (that means mostly ensuring needed management, mowing or pasture, in the long run).
- The method of release has to force animals to remain at the release site and prevent them from panic reaction (closing animals temporarily in pre-drilled holes, offering hiding places and extra food at the beginning, etc.).
- For both reintroductions and supplementations, releasing in two consecutive years seems to be effective so far (we used 2 x ~60 individuals for a reintroduction and 2 x ~20 individuals for supplementations, other versions will likely be tested as well in the future).
- Needed permissions and other administrative matters must be dealt with well ahead of time.
- Proper coordination and communication with all involved partners is necessary.

Success of project

Highly Successful	Successful	Partially Successful*	Failure

*** - goals are set in a long-term perspective, therefore only some success indicators have been reached so far and the project continues**

Reason(s) for success:

- Proper theoretical preparation and planning, use of experience collected from literature or colleagues.
- Yearly availability of animals thanks to successful breeding programs.
- Emphasis on suitability of habitat in selected areas.

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The first community initiative in a rewilding project of orphaned African elephant calves from Reteti elephant sanctuary to Sera wildlife conservancy in Northern Kenya

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Introduction

Kenya's elephants occur in both savannahs and forests, although all are considered to be the savannah subspecies *Loxodonta africana africana*. The largest range areas for the savannah populations are the Tsavo ecosystem and its environs, and the Laikipia - Samburu ecosystem and contiguous areas to the north. The forest-dwelling populations occur mainly in the Aberdares and Mt. Kenya, coastal forests and Mt. Elgon. Between 1973 and 1990, elephant numbers in Kenya catastrophically dropped from some 167,000 to a minimum of around 20,000 (Douglas-Hamilton, 1989). From 1990, after the formation of a more effective management authority, the Kenya Wildlife Service (KWS) and the end of legal international ivory trade (through elevation of African elephants to Appendix I of CITES), the national elephant population gradually increased to about 35,000 in 2010 (KWS). IUCN data indicates that the estimated elephant range in Kenya is 110,972 km² translating to 19% of the total land area in Kenya. This project was undertaken in Samburu, Northern Kenya at Reteti, a community owned elephant sanctuary which was officially opened in 2016 with the aim of rescuing elephants in crisis, rehabilitating them and eventually releasing them to the wild. The elephant calves were released into a

Elephant in a mud-bath

neighboring Sera Wildlife Conservancy.

Goals

- Rescued elephant calves rehabilitated at Reteti and later successfully released to the wild.
- Minimizing contact between elephant's claves and humans at release site to avoid habituation.
- Minimizing risk to people and elephant.
- Promote interactions of the released orphaned elephants with wild elephant population at Sera.

Post-release monitoring of elephants

Success Indicators

- Released elephant calves to adapt to the new environment and with <5% mortality rate.
- Released calves interact with wild elephants at the release site.
- Released calves to maintain a good body condition score (3 - 3.5 out of 5) during and after release.
- Zero conflict with humans' post release.

Project Summary

Feasibility: Approval was sought from the Kenya Wildlife Service to initiate the project.

Weaning, training and handling: This begun nine months before the actual move. The calves were isolated from the group and kept in a separate stable. Bottle feeding was initially eight times a day every 24 hours and was dropped sequentially and by the time of translocation only two bottles feeding in a 24 hour period was done. The milk formula concentration dropped from 260 g to 130 g per feeding by the time of translocation as the calves browsed more on natural vegetation. Crate training started two months prior to the actual move. The three elephant calves were fitted with satellite collars to aid in post-release monitoring.

Disease risk assessment: The three male calves were screened for endo-parasites every month for three months before the translocation. Information was gathered on any elephant disease at Sera and there was no record on elephant disease outbreak at Sera for the last seven years. Body condition scoring and general health was assessed and ascertained to be good before the move.

Assessment of Sera as a suitable release site: Habitat assessment at release site confirmed enough forage, good security, minimal predator pressure (only three

Warges, Sosian, Lingwezi & Keeper

Spotted hyenas at release area) and hence low risk of predation. Water analysis was performed and levels were within acceptable limit as per World Health Organization (WHO) guidelines and in comparison with livestock guidelines (There is NO available elephant specific water requirement guidelines) except for high nitrate levels in one of the water sources within Sera. Surface water distribution is adequate

Socio-economics impact: Several meetings and public gatherings were held to sensitize the two communities about the move. The communities endorsed the move and jointly drafted a Memorandum of Understanding (MOU) between Sera and Namunyak (Reteti) for the management of translocated elephant calves.

Implementation:

Capture and loading: Two months prior to the move, a transport container was mounted on a loading ramp and crate training began. The three elephants were fed in the transport container to get accustomed to it. During the movement day the exercise went without hitches save for some delays due to the excitement created by observers. The animals looked calm and so decision was made not to use any tranquilizers during transport. The transport container was filled with browse cuttings and animals were checked every 30 minutes during transport.

Release of the elephants at Sera holding boma: The three calves were released onto a temporary holding boma at Sera Community wildlife conservancy. The boma had the basic requirements to provide shade, water and browse. There was also a mud bath for elephants to wallow and cool themselves off.

Post-release monitoring at Sera: At the time of release, the elephants continued with their normal routine of two bottles a day (06:00 and 18:00 hours). They fed on natural vegetation while being taken out of the boma to familiarize themselves with watering points. By day three, the elephants did not return to the boma and the keepers observed them from a distance. Interestingly they did not have the need for bottles and continued feeding on natural vegetation. At some point they met with wild elephants and followed them from a distance while maintaining their group. No aggression was observed between the newly released elephants and the wild elephant families/ individuals. Post release monitoring will continue and will include regular body condition assessments.

Major difficulties faced:

- *Seasonal changes:* Dry season can be extraordinarily dry leading to little available forage. Rainy seasons lead to accessibility challenges due to vehicles getting stuck during transport.
- *Uncertainty:* This being the first rewilding project there were lots of delays as no one was sure what would happen
- Lack of elephant specific drinking water guidelines.

Major lessons learned:

- The need to consult and involve communities as well as other stakeholders during planning and implementation phase.
- Preparation for movement need to start early ideally nine months before the movement. This will give time to the group to become cohesive and learn from each other while at the same time have enough time to forage on natural browse more than being bottle fed.
- The timing of the movement should be at the tail end of dry season or early wet season for the elephants to browse on more lush vegetation as soon as its sprouts. This will ensure that they are not nutritionally stressed and are able to improve or maintain good body condition.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Multi-stakeholder consultations during planning and implementation phase.
- Habitat and water analysis survey prior to the move.
- Experience and commitment from Reteti staff and by all persons involved.

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Reintroduction of European bison to the Vanatori Neamt Nature Park, Romania

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Introduction

The European bison (*Bison bonasus*) went extinct in the wild in 1927. A small population of captive individuals survived at a few European zoos, totaling 54 individuals in 1924 (Olech, 2008). Long-term collaborative breeding has resulted in the species recovering to more than 7,000 individuals today, and it is currently listed as Vulnerable by the IUCN. Modelling of the habitat required for sustaining large bison populations identified the Carpathian mountain range, which spans 260,000 km² across seven countries of Eastern Europe, as one of the only remaining places where European bison could potentially survive independently, and particularly the area from south-east Poland through Ukraine and into northern Romania (Kuemmerle *et al.*, 2011). The Carpathians supports populations of brown bears, wolves, lynx and wildcats but the largest herbivores (bison, aurochs and wild horses) were all driven to extinction around 200 to 400 years ago. European bison have been reintroduced to the northern Carpathians since the 1960s, with six separate populations established in Poland, Slovakia and Ukraine. The Vanatori Neamt Nature Park (VNNP), located in northern Romania towards the border with Ukraine, was therefore the ideal site for the first reintroduction of European bison in the country (Kuemmerle *et al.*, 2010).

Released European bison in VNNP © Felicia Aron

Goals

- To establish a viable, free-ranging population of European bison in the Vanatori Neamt Nature Park.
- To provide a model project that demonstrates the advantages of large mammal conservation and sustainable use of natural resources.
- To provide long-term economic benefits to the

local, national and regional economy.

- To provide a flagship species that serves the role of promoting public awareness and educational campaigns for the promotion of the conservation of biodiversity and sustainable development, locally, nationally and internationally.

Vanatori Neamt Nature Park © Felicia Aron

- To promote regional integration in the Carpathian Mountains chain.

Success Indicators

- Successful translocation of European bison from several source populations.
- High post-release survival and successful reproduction of released European bison.
- Appropriate range expansion and habitat use by the reintroduced European bison population.
- No poaching of released European bison.
- Replication of project model to other sites.

Project Summary

Feasibility: The Vanatori Neamt Nature Park (47°03'10" to 47°18'20" North, 26° 4'20'' to 26°22'32" East) is located in the Neamt County of Northeast Romania. It is part of the Eastern Carpathians and Moldavian Sub-Carpathians, at elevations of 365 to 1,231 m. The park covers an area of 30,818 ha, including 26,322 ha (85%) of natural mixed forests, 2,300 ha of pastureland, 900 ha of hayfields and 700 ha of urban areas (Catanoiu, 2007). Over half of the forest stands are aged over 100 years, peaking at 180 - 200 years. Average annual rainfall is 652 mm, and temperatures range from -3.7°C in January to +19.5°C in July. The park is a Natura 2000 site for species and habitats of community interest. Vanatori translates as “hunters”, and the area has been known as a hunting reserve since 1475, when hunting was the preserve of the Moldovian prince and his court (Catanoiu, 2007). The VNNP had the highest probability of effective law enforcement of 24 Romanian protected areas assessed by Iojă *et al.* (2010). The Cracău valley was identified as the first release site within the VNNP. It is an isolated forested valley of around 5,000 ha, with deciduous and mixed forests (altitude between 800 - 1,000 m), with low human impact. There is potential to

Released European bison in VNNP
© VNNP Administration

extend the European bison range northwards to allow connectivity with a herd already established in Ukraine.

Implementation: A fenced 180 ha acclimatization area was constructed near the VNNP visitor center to maintain a semi-wild European bison population, which has facilities for feeding, managing, and observing. European bison were sourced from within Romania, and also from Switzerland (in 2005), Germany (in 2005 and 2006), Sweden (in 2009), Ireland (in 2014) and the United Kingdom (2014). A 1 ha pre-release fenced area was constructed within the Cracău valley close to Chitele chalet, approximately 50 km south-west of the acclimatization enclosure.

The first five European bison were released in the spring of 2012, and a second group of five in the spring of 2013. Both groups were transferred from the semi-wild population. In April 2014, a group of six females was transferred directly to the pre-release enclosure from Port Lympne in the UK, having been sourced in the preceding months from Fota (2 females), Kingussie (1 female), Howletts (1 female) and Port Lympne (2 females), and was released two months later in June 2014.

Fourteen more European bison were released from 2015 to 2019, sourced from the semi-wild enclosure, making a total of 30 European bison released between 2012 and 2019 (Table 1), comprising 14 males and 16 females aged between 0.6 and 18.8 years at release (mean 6.2+/-4.7 years). All European bison were of the Lowland-Caucasian line and were subject to health-testing prior to transfer to the pre-release sites. Supplementary feeding was provided close to the pre-release enclosure during particularly cold spells.

Post-release monitoring: Six of the first 10 European bison released were fitted with VHF radio-collars prior to release. This allowed monitoring of factors including habitat preferences, refuges, group dynamics, and health status. Battery life for these transmitters was about two years. The VNNP team used this information to help advise on habitat suitability for subsequent European bison reintroduction projects in the Țarcu, Poiana Ruscăi and Făgăraș areas of the Southern Carpathians in Romania.

Two GPS collars were provided by The Aspinall Foundation, one was fitted to one of the six females released in June 2014, the other to a male that had been

Table 1. Summary of the growth of the reintroduced population of European bison in the Vanatori Neamt Nature Park, Romania: 2012 - 2019

Year	Releases	Deaths	Births	Population Size	Notes
2012	5	1	2	6	Release on 22nd March 2012
2013	5	0	0	11	Release on 28th March 2013
2014	6	1	2	18	Release on 24th June 2014
2015	2	0	1	21	Release on 13th March 2015
2016	3	0	1	25	Release on 15th March 2016
2017	4	0	6	35	Release on 14th March 2017
2018	1	0	3	39	Release on 26th March 2018
2019	4	3	7	47	Release on 10th May 2019
Total	30	5	22	47	19 males, 21 females, 7 calves

released in 2012. Despite some problems these collars allowed more efficient monitoring than had been possible with the radio-collars. The group of six females remained together post-release, generally feeding in the hay meadows during the summer months and moving into the forest areas in the winter months. They were joined by a male during the first winter post-release, and again during the second and third winters post-release. Five of the six females gave birth to a single calf each in the summer of 2017, three years post-release. The sixth female gave birth the following year, and three further births were reported in 2019. By the end of 2019, 25 of the 30 released European bison in the VNNP were still alive, 22 births had been reported, and the total population was 47 (Table 1). Of the five post-release deaths, four were males and one was a female, one occurred less than one year post-release, another two years post-release, and three at five or six years post-release. Survival to one year post-release is 96.2%, overall annual survival is 96.0%, and female annual reproduction is 30.3%.

With the help of the GPS collars, it was calculated in 2017 that the reintroduced European bison population was ranging across an area of 60,000 ha, incorporating a diverse range of habitats. Much of this ranging was by the males, the two female herds remained in ranges of approximately 10,000 ha each.

Major difficulties faced

- *Inappropriate legal status and management of European bison at a national level:* There is no national coordinated official policy regarding reintroductions and the reintroduced European bison populations, leading to incoherency in the relevant national legislation. As a result the management of the reintroduced populations is implemented in respect of environmental legislation but in disrespect of game legislation.
- *Lack of a national strategy for European bison:* This strategy, approved by the involved ministries, will provide actions to diminish the national-level

VNNP team tracking released bison
© VNNP Administration

threats for the European bison.

- *Issues with monitoring collars:* The first two GPS collars worked well except that they were both broken, at the point of the self-release mechanism, after 14 months for one on a female European bison and after five months for one on a male, apparently due to scratching behavior. The collars were recuperated, fixed and eventually refitted when appropriate opportunities arose.
- European *bison-human conflicts:* With the increasing European bison population some of the European bison travelled outside the boundaries of the VNNP, or approached villages within the VNNP. Some damage has been caused by the European bison, especially in winter time. At times the VNNP team has led European bison back towards the primary release area. Extensive pre- and post-release work with local communities has ensured that these occurrences have not led to negative impacts on the future of the project.
- *Competition with domestic animals:* EU subsidies provided to farmers have led to a recent revival of the local domestic livestock industry and therefore increased risks of competition between domestic livestock and reintroduced European bison.

Major lessons released

- Long-term approach is crucial to the success of such a project.
- The “soft” release strategy for the European bison reintroduction has been very successful, with the establishment in the same general area of captive, semi-free and reintroduced herds.
- Educational and public awareness activities dedicated to European bison have been essential.
- Establishment of a mechanism to provide benefits to the local economy through the European bison reintroduction has also been critical.
- A holistic approach proved very successful, which allowed not only to reestablish a keystone species in the ecological sense but also in a spiritual and cultural sense.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- A growing population of European bison has been re-established.
- Post-release survival and reproduction rates are high (births have outnumbered deaths by more than four to one).
- Ranging and habitat use is appropriate for the species and the site.
- There have been no cases of poaching of the European bison to date.
- The project model has been replicated in three other sites nationally (Țarcu, Poiana Ruscăi and Făgăraș Mountains). It may also be replicated in Suceava and Maramures, in order to connect the reintroduced population of VNNP with the Ukrainian populations.

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Reintroduction of plains bison to the American Prairie Reserve in Montana, USA

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Introduction

The Plains bison (*Bison bison bison*) is a subspecies of the American bison native to North America. It is listed as Near Threatened by the IUCN and as a Species of Concern by the state of Montana. Once numbering in the millions, bison were nearly extirpated in the 1800s due to widespread and intensive hunting (Gates *et al.*, 2010). Conservation efforts have brought the species back from near extinction, with an estimated 11,248 - 13,123 mature individuals according to the IUCN Redlist. However, there are concerns over long-term genetic diversity due to small herd size and cattle gene introgression (Gates *et al.*, 2010).

The Montana Glaciated Plains has been identified as a global priority for conserving grassland biodiversity due to the relative intactness of native habitats and diversity of flora and fauna (APR, 2018). This region comprises of sagebrush steppe and mixed grass prairie with intermittent streams that flow into the Missouri River. Here, the nonprofit American Prairie Reserve (APR) is attempting to establish a 1,416,399 ha reserve for large-scale conservation. APR has reintroduced bison since 2005 and to date have 866 bison and 169,818 ha.

Plains bison in the American Prairie Reserve

Goals

- Establish a population of at least 10,000 bison that is genetically diverse and ecologically functional.
- Restore the natural and variable herbivory pattern of year-round bison grazing.
- Establish a large reserve and network of reserve-friendly lands for large-scale biodiversity conservation and habitat contiguity.

- Use bison restoration to improve mitigation of and adaptation to climate change.
- Create conditions whereby bison conservation provides a diversity of benefits for the public.

Success Indicators

- A healthy, genetically diverse, and growing population of bison.
- Transition from rotational cattle grazing to year-round continuous bison grazing on managed lands.
- Build a land base of at least 404,685 ha by 2027 with wildlife-friendly fences on the reserve boundaries and removal of interior reserve fences.
- Restore the ecological role of bison (grazing patterns and other behaviors) to complement native vegetation restoration and ecosystem adaptation to climate change.
- Create a program of bison harvest with diverse cultural benefits; provide facilities for students, researchers, and artists to study bison; develop interpretive materials for bison viewing; and build public support for bison restoration.

Project Summary

Feasibility: The Montana Glaciated Plains has been identified as a global priority for grassland conservation due to the relative intactness of native habitats and existing biodiversity (APR, 2018). This region comprises of sagebrush steppe and mixed grass prairie with intermittent streams flowing into the Missouri River. Bison evolved in this landscape as the primary grazer and historically had a large impact on the ecosystem via grazing and other interactions (Gates *et al.*, 2010). APR aims to restore bison in order to restore grassland biodiversity on the landscape scale. However, cattle have been grazed here since at least 1915 (McMillan *et al.*, 2018) and currently exist on private and public lands. Therefore, the public is concerned that a transition from cattle to bison will negatively impact the region's ranching culture and industry. Other concerns include damage to personal property, public safety issues, disease transmission to other livestock, and impacts to vegetation and wildlife.

Implementation: In 2005, APR reintroduced 16 bison from Wind Cave National Park, South Dakota (USA) onto their private lands. Subsequent reintroductions from 2006 - 2014 include a total of 42 bison from Wind Cave National Park, 10 from Broken Kettle Grasslands Preserve in Iowa (USA), and 238 from Elk Island National Park in Alberta (Canada). These three source herds represent two genetic ancestries, contain no cattle genes, and have been free of brucellosis and tuberculosis for at least 30 years. To further increase genetic diversity, APR and the Fort Belknap Indian Reservation, which has bison of Yellowstone National Park lineage, have agreed to swap 12 bull bison. All reintroductions are conducted through soft release with the bison being corralled for one month and disease tested before release onto the land. Bison cow fertility has been 80 - 90% and population growth has been ~20% annually, similar to the rates found during

Plains bison herd in the American Prairie Reserve

the early years of bison restoration on the National Bison Range in Montana and in Yellowstone National Park (APR, 2018).

In total, APR owns 42,323 ha of private land and holds state and federal leases on 127,494 ha of public land, totaling 169,818 ha of the 1,416,399 ha goal. Bison currently occupy 15,129 ha. Leased land is managed by the Bureau of Land Management (BLM, a federal agency) and the Montana Department of Natural Resources and Conservation (DNRC), which have approved year-round bison grazing on two allotments totaling 7,284 ha. Four additional allotments, totaling 13,106 ha, are currently rotationally grazed by cattle and are proposed to transition to bison grazing, pending approval. Recently acquired private and public lands do not yet have reintroduced bison. APR has also restored native vegetation on 1,692 ha of previously cultivated lands.

Post-release monitoring: APR conducts biannual counts of the bison population, which is 866 as of fall 2019. Herd movement is also monitored using GPS radio-tracking collars. APR annually screens for disease by immobilizing or capturing a sample of the herd and drawing blood for testing. All animals that are sampled receive an ear-tag and RFID tag. Disease management follows the policies and regulations of the Montana Department of Livestock and the U.S. Department of Agriculture because bison in Montana are currently classified as livestock and are required to be managed as such. Additionally, APR follows the IUCN guidelines for managing bison health. APR bison have not tested positive for brucellosis or tuberculosis. APR minimizes manipulation of the population to allow it to develop a natural sex ratio and age structure. Hunting and translocating bison to other conservation herds have been used to reduce herd sizes that have increased to the carrying capacity of the lands. Mortality from competition among bulls, from native predators, and from other natural causes is permitted. Electrified boundary fences are actively monitored.

The response of vegetation and wildlife to bison reintroduction have been monitored since the first reintroduction. Kohl *et al.* (2013) found that bison had larger foraging patches, moved at a faster rate, and spent less time grazing and at water than cattle. McMillan *et al.* (2018) have found that APR land with year-round bison grazing had significantly higher plant species richness, higher compositional heterogeneity, and less invasive for abundance than land rotationally grazed by cattle on a nearby BLM allotment. Studies are currently

underway to monitor responses of riparian vegetation and bird and mammal communities.

Beginning in 2017, APR has hosted an annual community day to discuss bison management with local residents and students from afar. APR has also hosted volunteer groups, artists, Montana elected officials, Indian tribal leaders, Audubon organizations, native plant societies, and guests from the National Geographic Society. APR has donated two bison to the Smithsonian's National Zoo in 2014 as educational animals. APR's bison management has contributed a major share of the more than US$ 1.9 million in average annual expenditures by the organization in this region, exclusive of land acquisitions.

Major difficulties faced

- Gaining approval for year-round bison grazing and interior fence removal on the recently acquired public land allotments.
- Management/moving of bison on isolated properties due to inability to release them into the adjacent leased public lands year-round.
- Accessibility and activities are limited for visitors due to road conditions and the remote setting.
- Changing the local ranching community's negative perception of bison restoration.

Major lessons learned

- It will take many years, possibly decades, to reach the ambitious goal of 10,000 bison and 1,416,399 ha due to local and legislative opposition to bison restoration in Montana.
- Funding must remain secured for long-term management (property acquisition and fencing), scientific research, and monitoring.
- Large-scale restoration of this species requires partnerships with indigenous tribes and local, regional, national, and international conservation groups, researchers, donors, and supporters.
- To generate public and political support, the team needs communications specialists, marketing staff, and philanthropy managers who can harness the iconic status of the species and its story of restoration.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Bison have been reintroduced and the population is disease free, genetically diverse, and has grown to 866 individuals representing one of the largest conservation herds in existence.

- Year-round bison grazing has been implemented on portions of the reserve and McMillan *et al.* (2018) has shown that it has some ecologically beneficial effects on plant communities.
- The reserve has grown to 169.818 ha since 2005, but the reserve lands are currently patchily distributed.
- APR's bison management has contributed over US$ 1.9 million annual expenditures in the region, and APR continues to partner with willing landowners, tribes, researchers, and visitors for a diversity of financial and cultural benefits (APR, 2018).
- It is difficult to change the local community's and legislature's views of bison restoration. This is due to the local and statewide livelihood in livestock since the past century, agriculture as one of the largest industries in the state, and public perceptions of bison restoration regarding disease, private property impacts, reductions in cattle ranch numbers, and associated economic declines in rural communities.

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Welfare release of a zoo-born brown hyena in the Limpopo Province, South Africa

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Introduction

The Brown hyaena (*Parahyaena brunnea*) is endemic to southern Africa and is listed as Near Threatened on the IUCN Red List (Wiesel, 2015). Its total population size is believed to be under 10,000 mature individuals, and it suffers from persecution across most of its range, despite being primarily a scavenger with little evidence of predation on livestock (Mills, 2013; Wiesel, 2015). The Brown hyaena is rare in captivity, with just 12 living individuals reported in ZIMS in November 2019, of a total of 271 reported to have been held in captivity historically (177 are reported as dead, and 61 as undetermined or pending confirmation). Four of the reported living captive Brown hyaenas are in South Africa, four in Europe, three in North America and one in Asia. There is currently no apparent conservation justification for a global captive-breeding program for Brown hyaenas, and the existing captive population is too small to be useful as an assurance population or as a primary source for reintroduction projects. This case -study concerns a single male Brown hyaena born in captivity in the United Kingdom and released in a fenced reserve in the Limpopo Province of South Africa, primarily for welfare rather than conservation reasons.

Goals

- Transfer of a captive-born Brown hyaena to its natural habitat within the native range of the species.
- Release of the hyaena within a protected area.
- Post-release monitoring of the hyaena.
- Assessment of the success of the release.

Male Brown hyaena in South Africa © SanWild

Success Indicators

- Survival of released hyaena to at least one year post-release.
- Demonstration of appropriate feeding behavior, such as scavenging the kills of large predators such as leopards.
- Demonstration of appropriate social behavior.
- Demonstration of appropriate predator-avoidance behavior.
- Successful reproduction.

Project Summary

Feasibility: The male Brown hyaena was born on 19th June 2009 at Port Lympne Reserve in Kent, UK, the only male in a litter of three thought to be the first captive births of this species in the UK and the first in Europe for a over decade. The two females were subsequently transferred to other European zoos to join two lone males, but there was no other female in captivity in Europe to be paired with the young male. Therefore an alternative solution outside of Europe was looked for, primarily motivated by welfare rather than conservation concerns. In 2012, discussions commenced with the SanWild Wildlife Sanctuary in Limpopo province in the north of South Africa, located approximately 40 km west of Kruger National Park, who were willing to try to rehabilitate and release the hyaena into their private fenced reserve.

The SanWild Wildlife Sanctuary is just over 2,600 ha in size. It comprises a mixture of habitats and varied terrain, including flat grassland, thick bush, rocky hills, and some water holes. The reserve was founded by the late Louise Joubert primarily for the rehabilitation of rescued wildlife. A small number of rescued Brown hyaenas had been released to the reserve prior to this release, as had various other species. A pack of African wild dogs (*Lycaon pictus*) was held in one large enclosure, with sub-groups allowed out into the main reserve four at a time to be able to hunt; these hunting parties would always return to the rest of the pack in the enclosure due to their tight social bonds.

All necessary infrastructure and staffing for the proposed rehabilitation and release of the male hyaena were therefore in place at SanWild. The long-term commitment of the Joubert family to the site meant that post-release support and monitoring would be undertaken, which was one of the pre-requisites for implementing the project from The Aspinall Foundation's perspective. Post-release support and monitoring have been important components of other translocation projects implemented or supported by The Aspinall Foundation (e.g. Catanoiu *et al.*, 2021; King *et al.*, 2012).

Implementation: Once all necessary permitting and health-testing was completed, the male Brown hyaena was sedated and crated at Port Lympne on 12th August 2013, arriving the following day at Johannesburg airport in South Africa. Following a short delay at customs it was a simple 5.5 hour drive to SanWild. It was already dark when the transport crate was unloaded into the pre-release enclosure. The crate was opened the same evening. The team returned early the next morning and found the hyaena sitting calmly in the bushes in the

center of the enclosure. There was no evidence of any digging along the fence-line or any other sign of attempts to escape from the enclosure. Over the following weeks the hyaena settled well into the pre-release enclosure, including showing good survival instincts such as storing excess food in an old aardvark burrow. He also made contact with some other hyaenas through the enclosure fence.

SanWild wildlife sanctuary © Richard Barnes

Post-release monitoring: The Brown hyaena was released into the fenced reserve at SanWild in 2014, approximately six months after his arrival in South Africa. Post-release monitoring was undertaken by the reserve staff, primarily during routine anti-poaching patrols. Direct observations were regularly made during night patrols. Post-release supplementary feeding was provided close to his pre-release enclosure, so direct observations were often made there. Informal body condition observations showed that he remained in good health following release. In March 2015, approximately one year post-release, he was observed in good physical condition at 02:00 hours about 11 km from his pre-release enclosure, illustrating that he was ranging extensively throughout the reserve. Around the same time he was observed feeding on a hare, although it is unclear if he hunted it or scavenged it. In July 2015, he was observed in the company of another Brown hyaena, of undetermined sex. In August 2016, three years after arriving in South Africa and approximately 2.5 years post-release, camera-trap images showed him scavenging at 21:30 hours on a carcass killed earlier that night by a leopard. At this stage the SanWild team considered him to be “fully rehabilitated”. By June 2019, over five years post-release, the SanWild counter-poaching team still reported observing him occasionally during their night patrols, and regularly see his spoor. The team report that he remains in good physical condition.

Major difficulties faced

- Lack of vaccinations suitable for hyaenas that could have been utilized prior to transfer or release.
- Some paperwork issues were encountered upon arrival in South Africa.
- Release of zoo-born animals is often questioned as a justifiable strategy.

Major lessons learned

- A zoo-born carnivore can be successfully rehabilitated and released.
- A soft release process was successful in this case.
- Post-release supplementary feeding was probably an important factor in the successful release.
- Despite the lack of vaccinations there were no apparent disease issues encountered in this case.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Post-release survival of the brown hyaena for at least five years.
- Demonstration of appropriate feeding behavior including scavenging the kill of a leopard.
- Demonstration of appropriate social behavior with conspecifics.
- Insufficient evidence to determine reproductive success.

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Reinforcement of an isolated Javan silvery gibbon population on Mt. Tilu, West Java, Indonesia

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Introduction

The Javan gibbon (*Hylobates moloch*), also known as Silvery gibbon or Moloch gibbon, is endemic to Java and is categorized in the IUCN Red List as Endangered (Andayani *et al.*, 2008). The species is listed under CITES Appendix I, and has been protected by Indonesian law since 1924. The use of land for economic activities has resulted in disturbance to forest security in the form of encroachment, illegal logging, forest and land fire, and illegal trade in plants and wildlife (MoEF, The State of Indonesia's Forests 2018). Habitat fragmentation has led to the isolation of remaining populations (Smith *et al.*, 2017), many of which are small or have already been locally extirpated.

The Javan gibbon is considered a high conservation priority in Indonesia and is the subject of rehabilitation and release programs. There is an ongoing population reinforcement project in the forests of Mt. Tilu Nature Reserve near Bandung in West Java, with captive-born and rescued wild-born gibbons sourced for release from the Javan Primate Rehabilitation Centre located in the protected forest area of Mt. Tikukur. The project is managed within the Javan Primates Conservation Programme (JPCP), run by the Directorate General for Conservation of Natural Resources and Ecosystem, Ministry of Environment and Forestry (KLHK), in partnership with The

Javan gibbon on Mt. Tilu © The Aspinall Foundation / KLHK

Aspinall Foundation (TAF) Indonesia Program.

Goals

- To return confiscated and captive-born Javan primates to their natural habitat.
- To re-establish a viable population of the Javan gibbon in and around the Mt. Tilu Nature Reserve, West Java, Indonesia.
- To raise public awareness, especially amongst the younger generation, about the importance of wildlife and habitat conservation.
- To provide opportunities for national students to participate in field-based conservation of primates.

Success Indicators

- High post-release survival of released Javan gibbons.
- Reproduction by released Javan gibbons.
- Increased population of Javan gibbons in the Mt. Tilu Nature Reserve.
- Increased viability of the Javan gibbon population in the Mt. Tilu Nature Reserve.
- Increased public awareness not to keep or trade Javan gibbons.

Project Summary

Feasibility: From December 2007 to April 2009 a field survey was conducted of 22 potential gibbon sites outside of National Parks in West and Central Java, comprising 106 forest blocks (Wedana *et al.*, 2010). New Javan gibbon populations were discovered in the protected forest reserve of Mt. Tambakruyung, Ciwidey, and in the western part of Mt. Tilu Nature Reserve, southern West Java. Fifteen groups of Javan gibbons were found at Mt. Tilu, comprising 43 individuals, occupying around 2,000 ha (20 km²) in the southern and western parts of the reserve. Gibbons were absent from all other areas of Mt. Tilu, which contains 8,000 ha (80 km²) of mountain forest habitat. Based on these extensive survey results, the Mt. Tilu Nature Reserve was identified as a suitable site for Javan gibbon conservation through

Post-release monitoring team © The Aspinall Foundation / KLHK

population reinforcement by releasing captive-held gibbons.

Mt. Tilu Nature Reserve and private tea plantation
© Tony King / TAF / KLHK

Mt. Tilu (7.156°S 107.530E; 1,030 to 2,140 m elevation) has a mountainous topography with an average rainfall of 2,534 mm/year. Ninety tree species have been identified (30 families), 50 of which form part of the diet of Javan gibbons. Release site management is undertaken as part of the Mt. Tilu NR collaboration conservation management with BBKSDA, a 650 ha privately-owned tea plantation (PT. Chakra) located inside the Nature Reserve, and representatives of local community groups. The education and awareness strategy focusses on local villages, involving all levels such as elementary and high school environmental programs, plantation workers, and local people/village leaders.

Implementation: Pre-release is undertaken at the Javan Primate Rehabilitation Centre (JPRC), 15 km from Mt. Tilu. This involves disease screening, behavioral training, and diet modification to reflect food availability at the release site. All gibbons underwent extensive pre-release testing and preparation that followed a detailed disease risk analysis. Several captive gibbons tested positive for the Hepatitis B virus (HBV). We organized a field survey to test for the existence and prevalence of HBV in wild Javan gibbon populations, and in collaboration with Public Health England this resulted in the identification in wild and captive populations of a HBV strain specific to Javan gibbons (jgHBV). The virus does not appear to cause disease in gibbons, and therefore the research demonstrated that captive gibbons shown to have the specific Javan gibbon strain of HBV can be considered for release.

Transport to the release site is in individual crates, by vehicle to Mt. Tilu then carried to the specific release sites. The gibbons spend a few days or weeks in simple net cages at the release site prior to final release. As recommended by Campbell *et al.* (2015), gibbons are released in bonded pairs if we have successful pairings during the rehabilitation period. However, a few gibbons are not easy to pair. In such cases we release them alone in an area where we have identified a single existing wild gibbon present. Using this method we have successfully released single individuals who have gone on to pair in the wild.

Forty Javan gibbons have been released in and around Mt. Tilu from 2014 to 2019 (Table 1), including 19 males and 21 females, aged 10 months to 18 years

Soft-release cage on Mt. Tilu
© Tony King / TAF / KLHK

at release. Seven were gibbons transferred from the captive population held at Port Lympne and Howletts in the UK, two were captive-born at the JPRC rescue center, and the other 31 were gibbons rescued by KSDAE from the illegal wildlife trade in Indonesia. Most (33) have been released within the Mt. Tilu Nature Reserve, four in the nearby small and isolated Patengan Nature Reserve (in 2018 and 2019), and three in the Kanaan protected forest (in 2019).

Post-release monitoring: Although not all could be followed intensively, most of the released gibbons were observed on a daily basis by the monitoring team for at least a few months post-release. Subcutaneous VHF-transmitters were used on six gibbons but were not very effective due to the density of the forest and the hilly terrain. Therefore monitoring is undertaken using traditional tracking techniques, by local people employed by the project in collaboration with West Java BKSDA forest rangers and the tea estate. Local students from various local Universities also help to conduct post-release research, such as collecting data on diet.

Of the first 33 Javan gibbons released, to the end of 2018, one is still monitored over five years post-release, another was monitored for over three years post-release, and 11 more for over one year post-release. Only three are confirmed dead (one male during the soft-release process), one infant female at five months post-release, and one male killed by a Javan leopard at eight months post-release). Survival to one year post-release is therefore between 39% and 91%. The large range of uncertainty is to due to the difficulties of monitoring many of the released gibbons for more than a few months post-release. Survival to two months post-release is between 65% and 98%. Four births have been reported within the program so far (Table 1). Two of the births, in 2016 and 2019, were to a wild female who paired with a male released in 2014. The pair and the two infants were still regularly observed to the end of 2019. The other two reported births were to a released female in 2018 and to a wild female paired with a released male in 2019.

Table 1. Summary of Javan gibbons released in and around the Mt. Tilu Nature Reserve till the end of 2019

Year	Released	Confirmed deaths	Post-release births	Notes
2014	3	1	0	2 others released but recaptured and re-released subsequently
2015	4	0	0	-
2016	2	1	1	Birth to a wild female paired with released male
2017	14	0	0	-
2018	10	1	1	Includes two released to Kanaan Protected Forest. Birth to a released female
2019	7	0	2	Includes one released to Kanaan PF and four to Patengan NR
Total	40	3	4	-

Major difficulties faced

- Limitations of tracking equipment for post-release monitoring.
- Identifying additional forest locations for releases.
- Difficulties in rehabilitating some individuals who have spent considerable time in unnatural or unsuitable captive situations.
- The presence of predators in the release site (especially Javan leopards).

Major lessons learned

- The pre-release socialization stage must be as natural as possible, considering the natural behavior of the species, including in the selection of foods and of enrichment. The pre-release phase is very important in determining the ability of individual gibbons to survive in the wild.
- Gibbons are very sensitive and selective species, especially pairs, so the rehabilitation process requires a long time.
- Health testing is critical for good selection of individuals for release.
- In the absence of effective tracking devices post-release monitoring must be conducted intensively on a daily basis for the initial 1 - 2 months after release. Just one or two days without monitoring makes it very difficult to locate the gibbon again.
- The success of a rehabilitation and release program lies in the management of post-release monitoring.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Post-release survival of the Javan gibbons appears to be high, although data is difficult to quantify due to several individuals being lost to monitoring within just a few months.
- Post-release reproduction has been observed, by a released female gibbon and also by a wild female paired with a released male. However many of the released gibbons are not monitored long enough to confirm reproduction or evaluate reproductive rates.
- The project is helping conserve the habitat of the release site, and the Javan gibbon population.
- The project is raising public awareness on the curbing of the illegal wildlife trade and on wildlife conservation, at local and national levels, including through participation in some releases and through national media.

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Reinforcement of isolated populations of Javan grizzled langur and western Javan ebony langur at Mt. Tilu, West Java, Indonesia

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Introduction

The Javan grizzled langur (*Presbytis comata*), also known as Javan surili, and the western subspecies of the Javan ebony langur (*Trachypithecus auratus mauritius*), also known as Javan lutung, are both endemic to western Java, and are considered Endangered and Vulnerable respectively on the IUCN Red List, with populations of both species in decline (Nijman & Richardson, 2008; Nijman & Supriatna, 2008). The use of land for economic activities has resulted in disturbance to forest security in the form of encroachment, illegal logging, forest and land fire, and illegal trade in plants and wildlife (MoEF, The State of Indonesia's Forests 2018), including the live capture of wild primates for the illegal wildlife trade.

This case study refers to reinforcement projects for the two species in the isolated forests of Mt. Tilu Nature Reserve near Bandung in West Java, with captive-born and rescued wild-born langurs sourced for release from the Javan Primate Rehabilitation Centre located at the foot of Mt. Patuha in the protected forest area of Mt. Tikukur. The project is managed within the Javan Primates Conservation Programme (JPCP), run by the Directorate General for Conservation of Natural Resources and Ecosystem, Ministry of Environment and Forestry (KLHK), in partnership with The Aspinall Foundation (TAF) Indonesia Program.

Javan grizzled langur © Tony King / TAF / KLHK

Student undertaking post-release monitoring
© The Aspinall Foundation / KLHK

Goals

- To return confiscated and captive-born Javan primates to their natural habitat.
- To increase the viability of isolated populations of Javan grizzled langur and western Javan ebony langur in and around the Mt. Tilu Nature Reserve, West Java, Indonesia.
- To raise public awareness, especially amongst the younger generation, about the importance of wildlife and habitat conservation.
- To provide opportunities for national students to participate in field-based conservation of primates.

Success Indicators

- High post-release survival of released primates.
- Reproduction by released primates.
- Increased viability of the Javan grizzled langur and western Javan ebony langur populations in the Mt. Tilu Nature Reserve.
- Increased public awareness not to keep or trade Javan primates.

Project Summary

Feasibility: Mt. Tilu Nature Reserve (7.156°S 107.530E; 1,030 - 2,140 m elevation) was selected as a suitable site for the reinforcement of primate populations, primarily for Javan gibbons (Wedana *et al.*, 2021) but also for Javan grizzled langurs and Javan ebony langurs, following extensive surveys from 2007 to 2009 of 22 sites outside of National Parks in West and Central Java (Wedana *et al*., 2010). During 14 transects at Mt. Tilu, Javan grizzled langurs were recorded 25 times, in groups of one to 10 individuals, and Javan ebony langurs 12 times (Wedana *et al.* 2010). Mt. Tilu is isolated from other forests by deforested lowlands. The cause of declines of langur populations in and around Mt. Tilu probably include hunting and habitat destruction and fragmentation.

Mt. Tilu has a mountainous topography containing 8,000 ha (80 km²) of mountain forest habitat with an average rainfall of 2,534 mm/year. Ninety tree species have been identified, comprising 30 families and mainly dominated by Fagaceae, Moraceae, Lauraceae, Myrtaceae, Rubiaceae, Euphorbiaceae, Theaceae, Magnoliaceae, Meliacaeae and Podocarpaceae (Wedana *et al.*, 2010). Release site management is undertaken as part of the Mt. Tilu NR collaboration

conservation management with BBKSDA, a 650 ha privately-owned tea plantation (PT. Chakra) located inside the Nature Reserve, and representatives of local community groups. The education and awareness strategy focusses on local villages, involving all levels such as elementary and high school environmental programs, plantation workers, and local people/village leaders.

Semi-structured interviews of local people in the tea plantation in 2012 and 2013 illustrated that they respected the local rules about not hunting or cutting the forest; however both activities continued to be carried out in the forests of Mt. Tilu, primarily by “outsiders” (Meg Selby, unpublished PhD research report, 2013). Local people also noted that forest quality in the tea plantation was improving under the current management but that wildlife was not recovering at the same rate, and were positive about the project to help wildlife recover.

Implementation: Pre-release is undertaken at the Javan Primate Rehabilitation Centre (JPRC), 15 km from Mt. Tilu. This involves disease screening, behavioral training, and diet modification to reflect food availability at the release site. All langurs undergo extensive pre-release testing and preparation that follows a detailed disease risk analysis. The langurs spend a few days or weeks in simple net cages at the release site prior to final release.

Thirteen Javan grizzled langurs have been released between 2015 and 2019 (Table 1), including seven males and six females. Eight of these were transferred from the captive population in Europe, and five were rescued by KSDAE from the illegal wildlife trade in Indonesia. Ten were released on Mt. Tilu, the other three in the nearby sites of Patengan Nature Reserve (one pair in 2016), and Mt. Tikukur Protected Forest (one solitary male in 2019). Thirteen western Javan ebony langurs have been released between 2016 and 2019 (Table 2), including two males and 11 females, as three groups of four released in Mt Tilu (2016 to 2018), and then a single female released in the nearby Kanaan Protected Forest (in 2019). All were individuals rescued by KSDAE from the illegal wildlife trade in Indonesia.

Post-release monitoring: Released langurs are monitored post-release whenever possible, by The Aspinall Foundation in collaboration with West Java BKSDA forest rangers, local trackers and university students. Four of the 13 Javan grizzled langurs released are known to have died within the first-year post-release, one due to predation by a Javan leopard seven months post-release, one due to consumption of a poisonous plant four months post-release, and the other two within two weeks post-release for reasons thought to be related to digestive issues. Four others have been monitored for over three years post-release (three of them for over four years), the other five survived well for a few months post-release but could not be monitored any longer due to difficulties in locating them. Survival to one year post-release is therefore between 31% and 69%, and thought to be at the higher end of this range. Survival to one-year post-release for rescued individuals is between 60% and 80%, for zoo-born individuals it is no higher than 63%. Of the four that have been successfully monitored, three are females that have all joined wild groups and each has given birth post-release,

and one is a male that also joined a wild group, one month-post release. One of the females to give birth was captive-born in the UK.

There have been no reported deaths amongst the 13 western Javan ebony langurs released, but only four have been successfully monitored for over one year post-release. Survival to one year post-release is therefore 100% for the langurs that have been successfully monitored, but sample size is low. The four females successfully monitored joined wild langurs soon after release and two have subsequently given birth to surviving offspring.

Major difficulties faced

- The gut of the Javan grizzled langur is very complex, both anatomically and physiologically. They are predisposed to dysbiosis which can be rapidly fatal, causing lower post-release survival rates than in other Javan primates.
- Limitations of tracking equipment for post-release monitoring.
- Identifying additional forest locations for releases.

Table 1. Summary of Javan grizzled langurs released in and around the Mt. Tilu Nature Reserve till the end of 2019

Year	Males released	Females released	Confirmed deaths	Reported births	Notes
2015	2	3	1	0	-
2016	2	3	2	2	Includes one pair released in Patengan NR
2017	2	0	1	0	-
2018	0	0	0	0	-
2019	1	0	0	1	Includes one male released in Mt. Tikukur PF
Total	7	6	4	3	-

Table 2. Summary of western Javan ebony langurs released in and around the Mt. Tilu Nature Reserve till the end of 2019

Year	Males released	Females released	Confirmed deaths	Reported births	Notes
2016	1	3	0	0	-
2017	1	3	0	0	-
2018	0	4	0	0	-
2019	0	1	0	2	Includes one female released in Kanaan PF
Total	2	11	0	2	-

- Difficulties in rehabilitating some individuals who have spent considerable time in unnatural or unsuitable captive situations.
- The presence of predators in the release site (especially Javan leopards, Black eagles and Serpent eagles).

Local participation and public awareness at Mt. Tilu
© The Aspinall Foundation / KLHK

Major lessons learned

- The pre-release socialization stage must be as natural as possible, considering the natural behavior of the species, including in the selection of foods and of enrichment. The pre-release phase is very important in determining the ability of individual langurs to survive in the wild.
- Javan grizzled langurs and Javan ebony langurs live in groups with a simple hierarchical structure, namely one-male multi-female. If there are other males in a group, there are many potential problems. Therefore the determination of group composition prior to release should adjust to the characteristics of wild langur groups, both in terms of the number of individuals and the sex ratio.
- Health testing is critical for good selection of individuals for release.
- In the absence of effective tracking devices post-release monitoring must be conducted intensively on a daily basis for the initial 1 - 2 months after release. Just one or two days without monitoring makes it very difficult to locate the langur again.
- The success of a rehabilitation and release program lies in the management of post-release monitoring.

Success of project

Highly Successful	Successful*	Partially Successful	Failure

*** - ranking for both Javan grizzled langur and Javan ebony langur**

Reason(s) for success:

- Post-release survival of the Javan ebony langurs appears to be high, although data is difficult to quantify due to several individuals being lost to monitoring within just a few months.

- Post-release survival of the Javan grizzled langurs appears to be lower, although some have survived and integrated into the wild population.
- Post-release reproduction has been observed in both species, including in a zoo-born female Javan grizzled langur. However many of the released langurs are not monitored long enough to confirm reproduction or evaluate reproductive rates.
- The project is helping conserve the habitat of the release site, and the langur populations within it.
- The project is raising public awareness on the curbing of the illegal wildlife trade and on wildlife conservation, at local and national levels, including through participation in some releases and through national media.

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Reinforcement of isolated Javan ebony langur populations in East Java, Indonesia

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Introduction

The Javan ebony langur (*Trachypithecus auratus*), also known as Javan lutung, is endemic to Indonesia and is listed as Vulnerable on the IUCN Red List. Two subspecies are currently recognized; *T. auratus mauritius* has a restricted distribution in west Java, and *T. a. auratus* occurs in eastern Java and on the smaller islands of Bali, Lombok, Pulau Sempu and Nusa Barung. The use of land for economic activities has resulted in disturbance to forest security in the form of encroachment, illegal logging, forest and land fire, and illegal trade in plants and wildlife (MoEF, The State of Indonesia's Forests 2018), including live capture of Javan ebony langurs for the illegal wildlife trade (Nijman & Supriatna, 2008). This case study refers to two reinforcement projects of eastern Javan ebony langurs, in the highland Coban Talun and the lowland Kondang Merak Protected Forests. Langurs for release are sourced from the Javan Langur Rehabilitation Centre located on the edge of the Coban Talun forest. Most released langurs are rescued from the illegal pet trade in Indonesia, others are from the European captive population. The project is managed within the Javan Primates Conservation Programme (JPCP), run by the Directorate General for Conservation of Natural Resources and Ecosystem, Ministry of Environment and Forestry (KLHK), in partnership with The Aspinall Foundation (TAF) Indonesia Program.

Goals

- To return confiscated and captive-born Javan primates to their natural habitat.
- To reestablish viable populations of the Javan ebony langur in the Coban Talun and Kondang Merak Protected

Released Javan ebony langur at Coban Talun
© Made Wedana / TAF / KLHK

Kondang Merak lowland forest © Tony King / TAF / KLHK

Forests, East Java, Indonesia.

- To raise public awareness, especially amongst the younger generation, about the importance of wildlife and habitat conservation.
- To provide opportunities for national students to participate in field-based conservation of primates.

Success Indicators

- High post-release survival of released Javan ebony langurs.
- Reproduction by released Javan ebony langurs.
- Increased population of Javan ebony langurs in the release sites.
- Increased viability of the Javan ebony langur populations in the release sites, and in the long-term across the natural range of the species.
- Increased public awareness not to keep or trade Javan ebony langurs.

Project Summary

Feasibility: The release sites were selected following population and habitat surveys at numerous sites across East Java in 2010 and 2011 (Wedana & Kurniawan, 2011; see Wedana *et al.*, 2013). Following these surveys, the choice of the first release site was the Coban Talun Protected Forest, located within the Raden Soerjo Forest Park. Another survey was undertaken in March 2012 to conduct detailed studies of biodiversity, threat potentials and vegetation, including wild food abundance (Kurniawan, 2012). Coban Talun PF comprises approximately 250 ha of forest from Mount Pusungrawung to the eastern slope of Mt. Biru and is a form of highland rainforest ecosystem with evergreen trees. Elevation ranges from 1,300 - 2,200 m a.s.l.. Coban Talun is managed by a state-owned forestry enterprise Perum Perhutani. According to information from local residents, the number of langurs previously in the 1980s and 1990s could reach hundreds of individuals. The number has decreased dramatically as a result of uncontrolled poaching, and only six groups were found in 2010 - 2012 with an average of 15 individuals per group. The carrying capacity of Coban Talun was estimated at approximately 15 groups.

The second release site selected was the Kondang Merak Protected Forest, a lowland rainforest located by the coast south of Malang covering ~1,500 ha but with only 28 langurs in four groups inventoried in 2011 (Wedana & Kurniawan, 2011). All coastal rainforests in this region are severely damaged and

fragmented, and langur populations are isolated and highly threatened (Wedana & Kurniawan, 2011). The Kondang Merak PF is also managed by the state-owned forestry enterprise Perum Perhutani.

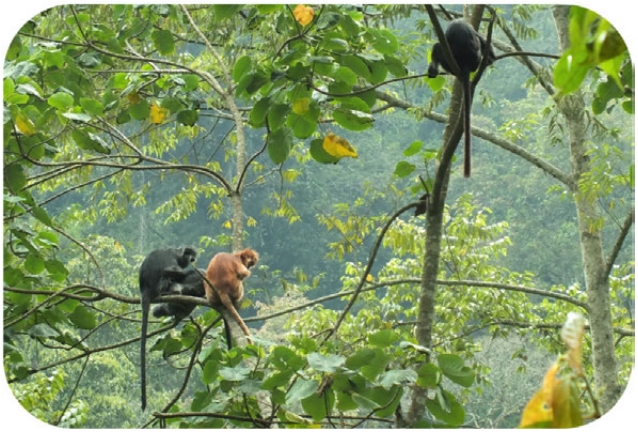
Implementation: The pre-release phase is undertaken at the Javan Langur Rehabilitation Centre (JLRC) in East Java, located only 4 km from Coban Talun, and approximately 50 km from Kondang Merak. The average rehabilitation period for the langurs prior to release is 10 months, including three months of quarantine. All necessary pre-release procedures, such as final veterinary exams and behavioral observations, are undertaken during the pre-release phase. All langurs undergo extensive pre-release testing and preparation that follows a detailed disease risk analysis. Transport to the release site is in individual cages, either carried as a backpack for the 1 - 2 hour journey to Coban Talun, or driven to Kondang Merak and then carried to the release site. Simple cages are constructed at the release sites to allow a soft-release process. The first releases used wooden cages built high in the trees (Wedana *et al.*, 2013), more recent releases have used net cages constructed from ground level to the mid-storey. During this habituation phase the langurs are introduced to natural leaves which will become their food in the forest. After about five days the langurs are released.

A total of 128 East Javan ebony langurs have been rehabilitated at the JLRC from 2011 to 2019. Of these a total of 93 langurs have been released, 32 at Coban Talun and 61 at Kondang Merak (Table 1). Sixteen originated from Howletts and Port Lympne in the UK, two from Beauval Zoo in France, seven were captive-born at JLRC, and the remaining 68 were rescued by KSDAE from the illegal wildlife trade in Indonesia.

Post-release monitoring: Intensive monitoring of the released langurs is conducted by The Aspinall Foundation in collaboration with East Java BKSDA forest rangers. Eighteen university students have also conducted field research at the sites. The monitoring team records movements and types of food consumed. Subcutaneous VHF-transmitters were used on a few langurs but were not very

Table 1. Summary of Javan ebony langurs released at Coban Talun and Kondang Merak between 2012 - 2019

Year	Langurs released (Coban Talun)	Langurs released (Kondang Merak)	Confirmed deaths	Reported births
2012	13	0	0	0
2013	0	0	0	2
2014	10	0	0	3
2015	0	14	2	2
2016	0	15	2	8
2017	0	18	0	10
2018	0	8	1	3
2019	9	6	0	2
Total	32	61	5	30



Released Javan ebony langurs at Coban Talun
© Iwan Kurniawan / TAF / KLHK

effective due to the density of the forest and the hilly terrain. Twenty-six langurs have been monitored for over two years, including eight for over five years and another for over seven years. Many of the released groups associated with wild langurs. Some wild individuals joined released groups, and some released individuals joined wild groups. Home-range sizes for the released groups are estimated at between 6 - 21 ha, depending on the condition of the vegetation cover and the availability of natural foods. At least 121 species of plants have been observed to be consumed by released langurs in Kondang Merak, and at least 35 species at Coban Talun. Vegetation consumed includes trees, vines, epiphytes and shrubs.

Of the first 70 langurs released, to the end of 2017, only four are confirmed dead, 40 were monitored for at least one year post-release, and 12 could not be monitored after only a short time post-release, giving a first-year survival rate between 69% and 93% (excluding the 12 langurs that were not monitored), and considered to be at the higher end of this range as all disappearances were due to difficulties of maintaining monitoring and recognizing individual langurs rather than any evidence of possible mortality.

A total of 30 births have been reported to 28 released female langurs to the end of 2019 (60% of females released to the end of 2017), with one female having three reported births, including five births to female langurs captive-born in the UK. One of the offspring of a UK-born female has also been reported to have successfully given birth. The estimated population size at Coban Talun has increased from 90 in 2010 to 135 in 2019.

Major difficulties faced

- Limitations of tracking equipment for post-release monitoring.
- Identifying additional forest locations for releases.
- Difficulties in rehabilitating some individuals who have spent considerable time in unnatural or unsuitable captive situations.

Major lessons learned

- The pre-release socialization stage must be as natural as possible, considering the natural behavior of the species, including in the selection of

foods and of enrichment. The pre-release phase is very important in determining the ability of individual langurs to survive in the wild.

- Javan ebony langurs live in groups with a simple hierarchical structure, namely one-male multi-female. If there are other males in a group, there are many potential problems. Therefore the determination of group composition prior to release should adjust to the characteristics of wild langur groups, both in terms of the number of individuals and the sex ratio.
- Health testing is critical for good selection of individuals for release.
- In the absence of effective tracking devices post-release monitoring must be conducted intensively on a daily basis for the initial 1 - 2 months after release. Just one or two days without monitoring makes it very difficult to locate the langur again.
- The success of a rehabilitation and release program lies in the management of post-release monitoring.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Post-release survival of the Javan ebony langurs appears to be very high.
- Post-release reproduction has been observed frequently, and reproduction of the F1 generation has also been reported.
- Population estimates at the release sites have increased since the start of the reinforcement projects.
- The project is helping conserve the habitat of the release sites.
- The project is raising public awareness on curbing the illegal wildlife trade and on wildlife conservation, at local and national levels, including through participation in some releases and through national media.

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Reintroduction of collared peccary in the Iberá wetland, northeastern Argentina

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Introduction

Collared peccaries (*Pecari tajacu*) are listed as of Least Concern by the IUCN and included in CITES Appendix II, except for populations inhabiting Mexico and the United States. The species is classified as Vulnerable in Argentina, where specialists speculate that habitat loss and transformation, and increasing levels of poaching and hunting caused a 30% population decline within the last 15 years. Indeed, Collared peccaries were partially eradicated from at least three and driven to extinction in two provinces (Camino *et al.*, 2019). Located in northeastern Argentina, Corrientes is one of the provinces where Collared peccaries disappeared around the mid-20th century. Corrientes is home to the Iberá wetland, a 13,000 km^2 complex of federal (i.e., Iberá National Park) and provincial reserves that protects an interconnected system of marshes, large ponds and streams associated with subtropical grasslands and remnants of native forests. Collaborative efforts implemented by governmental agencies and non-governmental organizations led to a steady recovery of wildlife and natural habitats in the Iberá wetland; thus, creating the conditions to revert extinction processes. Indeed, since 2015, Foundation Rewilding Argentina has been reintroducing collared peccaries in Iberá National Park (28.65491 S, 57.43166 W). Here, we summarize results obtained during the first five years of work.

Collared peccary with young © Rafa Abuin

Goals

- To restore Collared peccaries to the Iberá wetland.

Success Indicators

- Number of groups formed.
- Percentage of groups that are no longer supplemented with food.
- Percentage of groups that reproduced.
- Percentage of

reproductive events that resulted in piglets surviving ≥6 months.
- Number of self-sustainable populations established.

Project Summary

As a result of improved conservation and legal status and habitat restoration the Iberá wetlands became suitable for the reintroduction of Collared peccaries. Threats like hunting and poaching were minimized and, in some locations, even halted by law enforcement and patrolling. Furthermore, Collared peccaries were successfully reintroduced in Texas, USA (Litondo, 1993), lending support to reintroductions as tool to restore this species. Provincial and federal wildlife agencies were reluctant to allow translocations of wild peccaries (*see major Difficulties Faced*). Thus, we resorted to zoos and wildlife shelters to obtain the needed individuals (*see Reasons for Success*). We obtained permits to transport and release peccaries from provincial and federal Wildlife Agencies, according to existing regulations. Overall, the public supported the reintroduction of this species, but governmental officers were concerned regarding large and small agricultural producers, who could see peccaries as having similar impacts than Feral pigs (*Sus scrofa domestica*), being the latter invasive and harmful to agriculture and animal husbandry. To overcome this misconception, we held several meetings with these officers and explain them the differences between peccaries and Feral pigs. As a result, we could reintroduce a few groups and once officers realized that peccaries would not impact agricultural activities, we obtained a full approval for the translocation project.

We obtained 205 collared peccaries from eight zoos and wildlife shelters. We transported peccaries from their home institutions to our quarantine facilities using 70(l) × 40(w) × 50(h) cm crates made of iron and wood. Peccaries were placed individually in each crate to avoid fights. Distances between home institutions and quarantine facilities varied between 700 - 1,500 km and trips lasted from 12 - 36 hours. If temperatures were high, we stopped frequently to refresh the animals with a hose and cold water. Only four animals died during transportation.

Once at the quarantine facilities all animals underwent a sanitary screening. We used results from the screening to classify animals as either potentially fit or unfit for reintroduction. The latter included animals diagnosed with foot and mouth disease, classical and African swine fever, bluetongue disease, brucellosis, vesicular stomatitis, pseudorabies (Aujeszkys disease), and porcine reproductive and respiratory syndrome. The presence of one or more of these diseases would result in animals removed from the project. However, sanitary screening proved that all animals were fit for reintroduction. We observed some mortality during the quarantine because of fight-related injuries.

The quarantine period lasted on average 83 (range = 45 - 137) days. Length of quarantine depended on animal conditions at arrival and time elapsed until all health check-ups and analyses were finished. Before release, we treated animals for internal and external parasites using 300 µg/Kg (Dectomax®, Zoetis AR Laboratory), ricobendazole 7.5 mg/Kg (Axilur®, MSD Animal Health), praziquantel

5.5 mg/Kg (Cestodan®, Köning Laboratory), and friponil 1% pour on formulation 1mL/10 Kg (Ectoline®, Boehringer Ingelheim).

Peccaries fit for release were moved to pre-release pens, where we fed them daily with a mix of non-native and natural foods. Time spent in pre-release pens varied from 7 - 30 days. If small groups were consolidated at arrival, animals would spend a short period in pre-release pens. Conversely, if small groups had yet to be formed, animals would spend more time in pre-release pens. In some cases, we collared all individuals that we freed, but as the number of entanglement events increased, we began collaring only a few (*see major Difficulties Faced*). We implemented an intensive post-release monitoring scheme based on radio-telemetry because peccaries struggled identifying native sources of food; thus, periodic food supplementation was need. Food supplementation lasted ~30 days to a year, but time decreased as we gained experience regarding the native foods that peccaries preferred. Food supplementation was terminated when we observed that animals would not lose weight despite a steady reduction in the amount of food we provided. However, if after some time animals lost weight, food supplementation was reinstated. Interestingly, we observed that individuals that were thriving after release would teach recently freed animals how to find food resources in the wild. Thus, we began establishing mobile pre-release pens with naïve individuals in the territories of stablished ones. Established solitary males taught naïve females, and groups of females taught naïve males.

Out of 167 individuals released in four different locations, 32% died from collar and agonistic-related injuries, starvation, drowning and unknown causes. At least 21 groups became established, 16 (76%) of which are completely independent (i.e., food supplementation was terminated) and 19 (90%) are formed at least by one female and one male. Of the later, at least 11 (58%) reproduced ≥1 times. Out of 19 recorded reproductive events (i.e., piglets were observed), 11 (58%) resulted in at least one piglet surviving ≥6 months. Currently, two self-sustaining populations thrive in two locations, whereas two populations, still under management, are being established in other two locations. More releases are planned for 2020.

Major difficulties faced

- Federal and provincial wildlife agencies would not issue permits to translocate wild individuals. They perceived translocations as a hazard to source populations rather than a conservation tool. As a result, we were limited to reintroduce captive animals provided by zoos and wildlife shelters.
- Misconceptions about the potential for peccaries to harm agricultural activities.
- Captive animals required lengthy quarantines, and intensive veterinary care and management before and after release extending project times, costs and increasing risks.
- Although critical for post-release monitoring, collaring was problematic.

Individuals tended to lose weight after release, which translated in decreasing levels of fat around the neck. Thus, collars became loose increasing the frequency of entanglements, where animals would get entrapped with a front leg through their collars (i.e., between the collar and the neck). This often resulted in severe injuries and even deaths.

Researcher at release site © Rafa Abuin

- Often, we lacked information about group membership of the animals that we incorporated in to the project. Thus, large groups were kept in the same pen and then released. Large group size increased the frequency of intraspecific strife, which resulted in injuries, deaths, and triggered undesirable early dispersal. Early dispersal of uncollared individuals hindered food supplementation during the critical first weeks after reintroduction.

Major lessons learned

- Favor large (e.g., 40 × 40 m) over small (e.g., 20 × 20 m) pens during quarantine when social relationships between individuals are unknown. Large corrals allowed large groups to split into ≥2 small consolidated ones and individuals being able to avoid aggressive encounters. This strategy decreased the frequency of agonistic encounters that were resulting in severe injuries and sometimes deaths.
- Favor small mobile over large static pre-release pens at release locations. Mobile pens allowed releasing groups at different sites within the reintroduction area; thus, decreasing risky agonistic encounters with previously released groups that could have stablished their territories nearby.
- Radio-collaring is key as VHF radio-collars were critical to implement intensive post-release monitoring and managing (*see Major Difficulties Faced*). Locating animals periodically allowed for food supplementation (see below) and treatment of injuries and infections.
- If captive animals are reintroduced, then implement a food supplementation scheme. Hundred percent of the animals we reintroduced originated from captive facilities and took them time to develop skills to find native sources of food. Without food supplementation, most of the animals would have died soon after release. We suggest including native foods gradually, so they completely replace non-native food at the time of release.
- Group size matters at the time of release. Releasing large groups (≥15

individuals) led to fission processes that resulted in some groups lacking radio-collared individuals hindering monitoring and management (e.g., food supplementation). Small groups also facilitated the use of small mobile pre-release pens. We suggest releasing consolidated small groups (≤5), identified during the quarantine or in prerelease pens, each of them with two radiomarked adults, preferable the dominant male and the dominant female.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Intense consultation with practitioners before the implementation of the project.
- Availability of institutions that had Collared peccaries and were willing to support the project.
- Experienced personnel able to write proposals, secure funds, establish positive relationships with source institutions and authorities, implement and build safe transportation strategies and equipment (e.g. crates), manage animals at quarantine facilities, evaluate sanitary issues, monitor released animals, and communicate results to different stakeholders and the general public.
- High capacity to respond rapidly to unexpected situations (e.g. animals having their forelimbs entangled with the collar).
- Adequate funding to support long term work and face unpredicted situations.

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Supplementation of the white saxaul in its native habitat in Abu Dhabi, United Arab Emirates

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Introduction

White saxaul (*Haloxylon persicum* Bunge) is a perennial, small tree found in the deserts of Abu Dhabi, United Arab Emirates (UAE) (Jongbloed, 2003). It grows in shallow depressions along the slopes of dunes and is tolerant to extremes in temperature, light and water availability (Al-Khalifah, 2007). They occupy a geographically distinct and well-delineated area in the south of Abu Dhabi City, where they form a characteristic species-poor plant assemblage on low dunes (Al Dhaheri *et al*., 2017). The shrub, belongs to the family Amaranthaceae, grows up to 4.5 m high. The wood is heavy and coarse and the bark is spongy and water-soaked. The leaves are so small that it appears leafless, giving it a dull grey appearance. *H. persicum* woodland in Abu Dhabi is described as ‘dew forest' because of the way in which the leaves trap the condensation of fog moisture. The condensed water droplets then drip onto the ground around the bush and serve as an auto-watering mechanism. This process prompted the coining of the term 'dew forest' (Aspinall & Hellyer, 2004).

H. persicum is linked to the culture and traditions of UAE, has medicinal and economic importance. These woodlands are microhabitats for birds and small mammals and supports high biodiversity. *H. persicum* has yet to be assessed by IUCN, but it is regionally assessed as Endangered on the IUCN Red List.

H. persicum is one of the endangered species in Abu Dhabi Emirate with a declining population. Therefore the Environment Agency-Abu Dhabi (EAD) has been constantly monitoring the species as part of its conservation activities. As chances of regeneration of the species are extremely low due to lack of rainfall

White saxaul © EAD

and adverse climatic conditions, EAD decided to supplement the existing population by planting more individuals. This project started by an initial seed collection whereby seeds are collected and germinated under a controlled environment. They are then transplanted and monitored to ensure a successful growth.

Goals

- Enhancement of the only existing native population.
- To develop a reservoir for the seeds to promote natural regeneration.
- To develop a reservoir for the seeds for future *ex situ* conservation.
- Develop a seed propagation protocol for *H. persicum*.
- Improve the biodiversity of the habitat associated with the species.

Success Indicators

- To enhance species population through propagation by seeds.
- Successful germination of the seeds in the nursery.
- Identify suitable planting site for the seedlings.
- Successful results of the planting.
- Plant Micro-Reserve established and protected.
- Restriction of livestock activities in the area.

Project Summary

Feasibility: *H. persicum* has a restricted distribution in Abu Dhabi and is of outstanding conservation value because this is the only natural occurrence in the UAE and in eastern Arabia in general (Al Dhaheri *et al.*, 2017). The species has been in severe decline and the existing stands are at threat due to loss of their natural habitat and overgrazing. No natural regeneration of the species was recorded in the wild due to environmental extremes and lack of rainfall. The natural habitat for *H. persicum* in Abu Dhabi is locally known as Al Ghadha Protected Area (N 23.921305°, E 54.266781°), with a total area of 1,088 km^2, which represents the eastern distribution limit of the species in the Arabian Peninsula, which is officially

Habitat overview of White saxaul plants © EAD

Aerial view of White sexual strip planting © EAD

protected under UAE Federal Laws.

A detailed distribution map of the species was created and provided us with an estimate of the population size. In order to supplement the native population, we decided to have a new patch of the species in the same habitat with in the protected area.

Implementation: For supplementation of natural population (*in situ* conservation) actions, seeds were collected from the existing wild population. Seeds were collected from their natural habitat from the end of December to the beginning of January during the years 2016 - 2017. Seeds were sown in potting bags soon after collection, without any treatment, in a substrate that consists of soil and peat, and sown at a depth of 3 mm and moisturized.

After about two weeks, 23% of the seeds germinated and the seedlings remained in the potting bags for up to one year, until they attained a height of about 50 cm. The seedlings, were then transplanted to the nine planting sites which each have a distance of 3 km between patches and each patch contained ~300 plants/patch. The patches were irrigated for the first five months and then irrigation was stopped for the next seven months and the success rate without watering was found to be 70%. After seven months the patches were irrigated once every two weeks. All planting locations were recorded with a GPS, and mapped in order to be easily identified for long-term monitoring.

Post-planting monitoring: After transplantation, the monitoring was carried out on a monthly basis and individual plants were counted and assessed for their growth and survival and monitored for flowering and fruiting.

Major difficulties faced

- The seeds are highly viable for about two weeks after which the viability drastically reduces.

- There is a low rate of germination.
- The seeds are sensitive to soil depth for germination.
- As post-plantation monitoring is on-going the plants are still in a vegetative state as flowering and fruiting has not been recorded.

Major lessons learned

- The viability of seeds is found to be low.
- Seeds germinated only at a burial depth of about 3 mm.
- Propagation by seeds has been found successful.
- Protection against grazing from Camels (*Camelus dromedarius*) and off-road driving.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Seedlings were grown to a mature size before transplantation.
- Seedlings were transplanted in the same habitat.
- Protection of the transplanted area from livestock grazing and off-road driving.
- Regular irrigation pattern.

Acknowledgments

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Experimental population augmentation of critically endangered Julian's hibbertia, Sydney, Australia

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Introduction

Julian's hibbertia (*Hibbertia spanantha* Toelken & A.F. Rob.) is a decumbent to sprawling subshrub ~30 cm high. It is a member of the *Dilleniaceae*, a family of mainly shrubs, with 11 genera. It occurs in dry sclerophyll forests, with light clay shale sandstone transition soils, in the highly urbanized Sydney Basin Bioregion (OEH, 2018). *Hibbertia spanantha* was formerly described in 2015 (Toelken & Robinson, 2015) and is restricted to four disjunct populations, which cumulatively contain around 100 stems. The number of genetically distinct individuals is unknown as this species may produce ramets (clones). Under the IUCN Red List categories; restricted geographic distribution, very low numbers of mature individuals and projected or continuing decline, *Hibbertia spanantha* has been listed as Critically Endangered under both Australian Commonwealth (EPBC Act, 1999) and New South Wales state (BC Act 2016) legislation.

An experimental augmentation translocation commenced in 2017 to buffer one population, of eight plants (some probably clonal), from stochastic threats and anthropogenic impacts of nearby urban development. This translocation successfully established 19 additional individuals (of an original 25). Learnings will be applied to large-scale translocations, when the genetic health of parent populations can be confirmed.

Hibbertia spanantha flower © C. Doyle

Goals

- Identify best practice propagation and plant maintenance requirements through an experimental pilot translocation.
- Immediately buffer the wild population, consisting of approximately eight stems, from stochastic threats through introducing additional plants.
- Engage community and local governments in conservation activities.
- Use population genetics to inform future augmentations and establish a larger bolstered self-sustaining population.

Success Indicators

- Successful propagation, >90% strike rate and identification of best propagation medium (varied fertilizer level or home soil use) to facilitate plant establishment, survival and reproduction.
- Survival of >80% transplanted individuals in 12 months post-planting including evidence of reproduction, flowering and fruit.
- Local council and community volunteers involved in plant propagation, maintenance and monitoring. Showcased project in Ku-ring-gai Council. Envirotube episodes https://www.youtube.com/user/greenstylenorth
- Population relatedness and genetic health identified and, best combination of genetic material identified for large scale augmentation (underway).

Project Summary

Feasibility: *Hibbertia spanantha* is known from only four populations, the largest containing ~89 stems (some suspected ramets) and the smallest restricted to one plant. Each population is highly fragmented, with an area of occupancy (AOO) no greater than 100 m^2. It's restricted range, small population size, low area of occupancy and low observed reproduction, compounded by threats from urbanization in the Sydney Basin, have resulted in it being a recommended population augmentation candidate. Undertaking these translocations required collaboration and support from one private ecological consultancy, three local government authorities and licensing from the State Government authorities. Due to budgetary constraints, in-kind contributions from local government staff and community volunteers were essential when planning the propagation, planting and subsequent maintenance and

Chantelle Doyle counting flowers © Simon Dunne

monitoring of translocated plants.

Ross Rapmund monitoring plant growth
© C. Doyle

Implementation: The production of viable seeds in *Hibbertia spanantha* is sporadic. Therefore, plants were propagated from cuttings collected from the wild population. Cuttings were taken from semi-hard wood and transferred to potting medium two months later. Striking and propagation success was >90%. A total of 25 plants were grown in 125 ml forestry tubes for the experiment. Twenty of these were potted using a customized nursery, premium potting medium of organic (composted pine bark and coir peat) and inorganic matter (fine sand), identified by the nursery as maximizing growth in Australian native species. Nutrient loading was achieved using two varied applications (high [~7.5 g additional fertilizer] and low [~3.5 g additional fertilizer]). Home soil collected from parent population site was mixed at 50:50 ratio with nursery standard mix and divided between the remaining five 125 ml forestry tubes. The fertilizer rate in home soil was equivalent to 0.5x low, or 7.5g/625ml to ensure plants were not nutrient depleted. Nutrient levels were reapplied across all treatments at the same ratios in January 2018, during repotting.

Fertilized individuals were expected to perform better in the nursery environment, where water and light were not limited, but anticipated to experience transplant shock when translocated. Conversely, plants grown with exposure to 'home soil' were expected to perform worse in the nursery environment, where nutrients were limited by the growing medium, but exhibit higher rates of growth and reproduction in the first 12 months post-planting. Plant growth (width and height) and health was monitored monthly for seven months in the nursery and identified a significant impact of high nutrient treatment on plant growth only, although chloritic growth was observed in all treatments except home soil. Propagated plants were installed in a randomized block design, with five individuals planted in each of five blocks. Each block included 1 - 2 plants from each nutrient/home soil treatment. Plants were watered in with 5 L/plant, by hand using watering cans. Follow up watering was undertaken at a rate of 4 L/plant per plant every two days for the first week, then 4 L/plant every three days for the second week. Plants were then hand watered weekly for the next month, at a rate of 3 L/plant per plant.

Post-planting monitoring: Plant growth and reproduction post-planting was monitored monthly for 12 months (358 days). Growth measurements were taken

Hibbertia spanantha fruit © C. Doyle

as orthogonal widths and converted to a growth index. Reproduction measures were time of first flowering, peak flower number (count) and time to 50% flowering production (T50). Reproduction monitoring increased to between weekly and fortnightly during peak flowering (November - January). Water stress affected most individuals during the experiment post-planting, resulting in leaf drop and spot flowering. Monitoring identified home soil treatment and higher soil nutrients significantly impacted peak flower production. There was also evidence that home soil and high and medium nutrient treatments buffered plants against stress, although due to limited replication further investigation is required.

A larger augmentation will occur based on the results and recommendations from the assessment of population relatedness and genetic health.

Major difficulties faced

- Limited seed material, therefore plants propagated from cuttings giving limited genetic diversity.
- Staffing requirements for post planting maintenance; hand watering required over 115 L/plant in addition to 1,220 mm of natural rainfall.
- Budget constraints meant monitoring relied on invaluable assistance from community volunteers/in-kind contributions.
- Limited number of transplants; 25 plants translocated to augment the existing eight, meant that interpreting significant results of treatments was limited.

Major lessons learned

- Plants had much higher water requirements than planned and are sensitive to water stress.
- Nutrient loading (with slow release fertilizer) pre-planting and growth in home soil (despite lower nutrients) may improve establishment through buffering against stress, but a larger trial is required to confirm.
- Vegetative propagation expedites translocation where immediate buffering is required but can present longer term problems where relatedness/ clonality of the parent plants is unclear.
- Larger numbers of translocated plants are required for subsequent

plantings.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Conservation augmentation had >80% survival after 12 months (19 of 25 plants) but required intensive hand watering.
- Plants showed evidence of reproduction, prolific flowering but fruiting was negligible. This may be due to low genetic diversity and may require subsequent plantings of plants from other populations. Pollinators are also unknown.
- Limited number of plants in the pilot translocation meant reduce ability to analyze significant results (and inform subsequent planting).

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Translocation of a threatened rainforest herb to mitigate wildfire risk and extend the species range in Victoria, Australia

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Introduction

Tall astelia (*Astelia australiana*) grows in the understory of cool temperate rainforest in the Central Highlands and Otway Ranges of south-east Australia. Its current known range consists of 15 sites and its overall abundance is estimated at 10,000 (Cutler & Murphy, 2010). Both the Tall astelia and its cool temperate rainforest community were once more widespread and are listed as Threatened (Vulnerable) at a State level (FFG Act, 1988). Tall astelia is also listed as threatened (Vulnerable) at a National level (EPBC ACT, 1999). Tall astelia populations have declined by 68% over the 25 years since it's monitoring program began in 1993. Threats to the species include plant pathogens (*Phytophthora* and *Pythium* species), browsing by introduced Sambar deer (*Rusa unicolor*), and wildfire. Low light availability in the rainforest understory in the absence of canopy disturbance limits the species recruitment and can contribute to mortality (Parker, 2018). The Tall astelia provides habitat and food for many native bird and mammal species including Eastern pygmy possum (*Cercartetus nanus*), Swamp wallaby (*Wallabia bicolor*), Lewin's honeyeater (*Meliphaga lewinii*) and Silvereye (*Zosterops lateralis*) (Parker, 2018).

Female Tall astelia fruiting

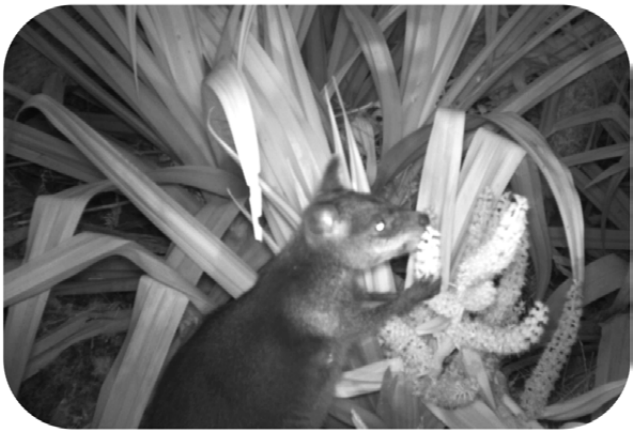
Goals

- To examine if translocation is a viable management option for this species.
- To minimize the risk of a large wildfire impacting multiple sites/ populations.
- To extend the species range.
- To increase genetic diversity within translocated populations.
- Restore a population at a heavily

reduced site.

Success Indicators

- At least 80% survival of translocated individuals after two years.
- Reproduction in translocated populations.
- Population growth over time at sites.



Pollination by Swamp wallaby captured in a camera trap picture

Project Summary

Feasibility: The Tall astelia rainforest habitat is characterized by an overstory of Myrtle beech (*Nothofagus cunninghamii*), Southern sassafras (*Atherosperma moschatum*), and Blackwood (*Acacia melanoxylon*) with the occasional emergent Mountain ash (*Eucalyptus regnans*). The middle stratum is typically comprised of the Soft tree fern (*Dicksonia antarctica*) and Rough tree fern (*Cyathea australis*). Fern species including Hard water-fern (*Blechnum wattsii*) and King fern (*Todea barbara*) dominate the understory and rich array of fern species that grow as epiphytes on the trees including Kangaroo fern (*Microsorum pustulatum*) and Common finger-fern (*Grammitis billardierei*) and Filmy fern species (*Hymenophyllum sp*.). The sites range in elevation from 400 - 800 m a.s.l. and are characterized by sloping topography that form a gully with a watercourse flowing through them. This topography and wet vegetation mean that these sites are less likely to be impacted by fire than the surrounding upslope sclerophyll vegetation. The Otway Ranges site is located in a National Park and one site in the Central Highlands is in a Regional Park managed by Parks Victoria. The other 13 sites are located in Special Protection Zones within State Forests which are managed by the State Government Department of Environment, Land, Water and Planning.

Implementation: We conducted a translocation trial to determine if this was a viable management option for the species. This trial involved translocating 54 individuals from one source site into three sites recipient sites: control site (within the source site); in a locally absent site (on the same creek line as the source site in an area where the species is absent); and, in an rainforest site 25 km away from the source site where the species does not occur. The trial was a success with between 83 - 88% of individuals surviving two years post translocation at each of the three trial sites. We then applied for and conducted a translocation of 200 individuals from six source sites, including the Otway Ranges site, to create five new sites and to increase the number of Tall astelia in one of initial trial sites (absent site) and re-stock a heavily browsed site post-fencing.

Post-planting monitoring: We continue to visit translocation sites on an annual

Tall astelia in a translocation site

basis to monitor survival and reproduction. Of the 254 translocated plants 22% (56) have died. This includes 13 of the original 54 trial translocation plants four years post translocation and 43 plants from the 200 translocation plants two years post translocation. Nineteen of the 43 plants that have died in the translocation were in the heavily browsed site that was re-stocked. Mortality across all sites is mostly due to herbivory and disease in the translocation sites. Some sites have performed better than others and this highlights the importance of finding translocation sites that are free of disease and herbivores or are fenced. Some individuals may not have survived the translocation due to them having damaged or few roots when they were taken from the source site. Microsite selection within sites also had an effect on the survival of translocated individuals with wet and muddy areas closest to the watercourses not suitable for having higher mortality.

We have just completed a genetic analysis of the species across its range to understand its gene flow dynamics and help to inform future translocations. This understanding will assist us to determine which source sites are best to use to increase genetic diversity of translocated populations. It will also provide guidance on the required proximity of translocations to encourage gene flow between populations.

Major difficulties faced

- Finding translocation sites that are suitable and located in reserves and within special protection zones.
- Changing recipient sites (permit amendments) if they are identified as unsuitable.
- The remote location of some source sites makes it difficult to select individuals from them for translocation.

Major lessons learned

- For translocation sites to be suitable they need to be free of disease and deer.
- Visit potentially suitable translocation sites to check suitability (see above) before applying for a permit to translocate.
- Use care to get the roots and rhizome when moving individuals from the

source site.

- Small to medium sized individuals translocate the best.
- Avoid planting individuals in the wet-muddy areas adjacent to watercourses and in areas that have low light in the understory due to full canopy.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Reproduction has occurred at two translocation sites to date.
- Reproduction is dependent on enough light being available in the understory due to canopy disturbance so need to plant beneath canopy gaps to encourage more reproduction.
- Survival at two translocation sites was reduced due to disease and or browsing by herbivores.
- Survival in one site was reduced as the microsite some were planted into was not suitable.

Acknowledgments

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Experimental translocation of the threatened banded ironstone wedding bush in Western Australia

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Introduction

Ricinocarpos brevis R.J.F.Hend. & Mollemans (Euphorbiaceae) is declared as 'Rare Flora' under the Biodiversity Conservation Act 2016 (WA) in 2005, and listed as Endangered under the Environment Protection and Biodiversity Conservation Act 1999 (Commonwealth). It is currently ranked as Critically Endangered (CR) under World Conservation Union (IUCN, 2001) Red List criteria B1ab and 2ab (based on severely fragmented populations and continued decline in, extent of occurrence and area of occupancy). Project location is in the Eastern Goldfields region, north of the Southern Cross town-site, in Western Australia. The species is a short range (~100 km) endemic recorded from just three locations. It occurs on the mid to upper debris slopes of rocky ironstone and weathered basalt ridges. These ridges are rich in minerals and the main threats to the species are mining and exploration activities (DEC, 2016).

The species is a non-lignotuberous, monoecious shrub growing up to 1.8 m in height, with evidence of flowers being insect-pollinated and seeds being ant-dispersed (DEC, 2016). Recruitment is likely to be episodic pulse-events, with soil moisture availability the principal driver of seedling emergence, in this semi-arid environment with ~300 mm annual rainfall (Elliott, 2019; Turner, 2018).

Ricinocarpos brevis male flower © Carole Elliott

Goals

- To improve the understanding of methods to translocate *Ricinocarpos brevis*.
- Assist in establishing *R. brevis* populations on waste rock landforms and/or other areas disturbed by mining.

Ricinocarpos brevis habitat © Carole Elliott

Success Indicators

- Success of the translocation is defined where 10% or more of the translocated individuals remain alive two years following translocation.
- Translocations are expected to provide information of value in the context of determining appropriate methodologies for the establishment of *R. brevis*.

Project Summary

Feasibility:

Habitat: Waste rock landforms were prepared to a mining standard that requires landform stability (i.e. erosion control) and a restoration standard that includes ripping to alleviate compaction, application of topsoil and surface sown seeds of local native species. Soil profiles are incomplete, often with a base profile of mixed large, small and fine scale waste rock fractions, which provide durable substrates for achieving landform stability; and a surface cover of topsoil that is ideally applied to a depth of <10 cm, whilst maintaining favourable soil-moisture properties for plant establishment.

Species: "*Ricinocarpos brevis* is an upright shrub 1 - 1.8 m in height and 1 - 1.5 m in width. The leaves are obovate, 10 to 25 mm long and 4 to 6 mm wide with strongly recurved margins and stellate hairs on both surfaces. Flowering generally occurs during the cooler months but may flower following large out of season rainfall events. Fragrant white flowers have been recorded from March to July, with the species having separate male and female flowers on the one plant (monoecious). Fruits contain up to three seeds (one ovule per ovary segment) and develop during spring, expand to around 12 mm in length and dehisce explosively in October/November." (DEC, 2016).

Socio-political and economic issues: "Mineral exploration leases cover the areas where *Ricinocarpos brevis* is known to occur. The occurrences of *R. brevis* has

Experimental translocation site © Carole Elliott

caused, and has the potential to cause, negative economic impacts through compliance with the environmental impact assessment and approvals process, and the potential restriction of future access to underlying iron ore deposits. There is also potential for translocation sites to complicate future access to mineral resources." (DEC, 2016).

Note: Text in quotation ("…") is quoted directly from the species Interim Recovery Plan, issued by the Department of Environment and Conservation (DEC, 2016).

Implementation:

Translocation:

Recipient sites (disturbed, waste rock landform) for experimental translocations were selected based on:

- Being close to natural populations (i.e. landscape connectivity).
- Land tenure and risk of future mining activity.
- Safe and ongoing accessibility to sites located within mining activities.
- Landscape aspect (e.g. preference for southern facing aspect).
- Rehabilitation stage of waste rock landform (i.e. capacity to install translocation).
- Physical and chemical assessment of soils (i.e. similar to natural population).
- Capacity to install infrastructure (e.g. irrigation).

Experimental translocations were installed yearly in April - June in 2013 - 2017, and designed to compare the success of tubestock planting and direct seeding. Tubestock experiments used 24 plants per treatment and tested up to five treatments in each translocation year. Treatments investigated to determine tubestock survival included shading, fertiliser, irrigation, propagation source (seed or cuttings), plant age, water holding crystals and biodegradable pots. Direct seeding experiments, used eight replicates of 25 seed for each treatment and tested up to six treatments each year. Treatments that were investigated included aspect (north or south-facing), shade, irrigation, seed burial, seed enhancement (priming or pelleting) and water holding crystals. Each replicate (tubestock or seed) was individually fenced to protect against herbivory.

Cultural/tribal: “A search of the Department of Indigenous Affairs Aboriginal Heritage Sites Register has identified four sites of Aboriginal significance within the vicinity of the populations of *Ricinocarpos brevis* on the Windarling Range (DIA, 2010). Liaison has been included as a recovery action to ensure there will be Indigenous engagement in relation to the recovery actions posed in this plan.” (DEC, 2016).

Phytosanitary concerns: Translocated tubestock were propagated and grown in an accredited nursery (Kings Park and Botanic Garden Nursery) prior to planting in translocation sites, thereby posing no inherent phytosanitary risks.

Post-planting monitoring:

Monitoring: Intensive monitoring of tubestock and direct seeding was conducted regularly after installation: at 1 - 2 months (late autumn); 4 - 5 months (spring); 7 - 8 months (early summer); 9 - 10 months (late summer) and 12 months (early autumn) for every year of the translocation program. Monitoring involved quantification of seedling emergence, survival, growth, health, and reproduction.

Results: Evaluation showed that shading, irrigation and older tubestock increased seedling emergence, survival, plant health and growth. However, the magnitude of these increases depended on seasonal rainfall, as these approaches were more effective in average or above average rainfall years. For example, overall seedling emergence (autumn to spring) for 2015 was lower (1.7%) than 2016 (4.5%), despite experimental irrigation, and was most likely affected by reduced rainfall over this period (2015 was 54% below average and 2016 was 13% below average).

Major difficulties faced

- Poor knowledge of plant establishment into waste rock landforms.
- Challenge of installation of irrigation systems on engineered waste rock landforms.
- Increasing frequency/severity of drier winters (climate change).
- Unknown horticultural capacity of species and how to transfer them to natural environments..

Major lessons learned

- Knowledge of population genetic structure is important for making informed decisions on sourcing material.
- Establishment on waste rock landforms is possible, with greater survival of tubestock derived from seed rather than cuttings.
- Older tubestock (8 - 18 months) establish better than younger tubestock (<6 months) due to reduced impact on root systems during planting.
- Shading and irrigation improved establishment, growth and survival of tubestock and seedlings.
- Soil moisture is the primary driver of seedling emergence, establishment

and plant survival.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Three of the five translocations resulted in >10% survival after two years.
- Information gained from these experimental translocations guided the development of appropriate methods for translocation of the species (Elliott, 2018).
- Investment in research provided critical information for successful plant establishment and an experimental framework that identified and refined the best approach for future translocations.

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Strengthening and increasing the restoration process of several rare and threatened tree species within the Araucaria Forest region in southern Brazil

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Introduction

The Araucaria Forest of Southern Brazil is of one of the world's most endangered ecosystems. Many plant species are highly threatened mainly due to the reduction and fragmentation of habitat and the spread of invasive species. Several programs and projects have been developed for the conservation and restoration of this region. However, forest restoration rarely includes endangered species, mainly due to the difficulty of obtaining seeds and seedlings from these plants. To address these constraints Chauá Society, a local NGO, focusses on researching and disseminating best restoration practices for 46 rare and threatened tree species.

This case study will focus on activities carried out between 2014 - 2019 on three species listed as Vulnerable by the IUCN: 1) *Butia eriospatha* - an ornamental grassland palm with edible fruits; 2) *Cedrela fissilis* - a tree with high economic and ecological importance; 3) *Ocotea porosa* - a tree readily exploited for timber and woodwork. The three species listed

Araucaria forest © Raphael Santos

on Brazil's Red List: 1) *Trithrinax acanthocoma* (Vulnerable), 2) an ornamental Palm tree (*Ocotea odorifera*) (endangered) - a tree that suffered high exploitation for timber and aromatic oils, and 3) *Quillaja brasiliensis* (endangered) - a tree used for soap making and for wood.

Goals

- To locate and map 60 new individuals of each species (12 mother trees with a minimum distance of 50 m between trees) across five populations for seed collection and for ecological studies within an adequate genetic variability.
- To collect seeds from all mapped individuals for ecological research and seedling production in the Chauá Nursery.
- Establish at least four new populations of each six tree species by reintroducing seedlings into protected areas and also monitor their survival and growth rates for at least three years.

Success Indicators

- Number of new populations and individuals found of each species in each remnant.
- A similar amount of seeds collected from for each mapped mother tree of each species.
- Number of seedlings planted and results of monitoring activities.

Butia eriospatha © Sociedade Chaua

Project Summary

Feasibility: Southern Brazil's Araucaria Forest is part of the Atlantic rainforest eco-region which historically dominated the landscape, yet present coverage is estimated at less than 1% of remnant of old secondary damaged forest (Castella & Britez, 2004). Currently the landscape is an agro-mosaic with fragments of generally secondary forest less than 50 ha (Ribeiro *et al.*, 2009; Tabarelli *et al.*, 2010), high-yield agriculture (Fonseca *et al.*, 2009), and alien species monoculture plantations for timber and paper. This severe fragmentation improves the risk of extinction of several species. Threatened populations are

Chaua Nursery © Sociedade Chaua

expected to continue decreasing even if no further degradation occurs (Metzger *et al.*, 2009), due to genetic erosion (Sork & Smouse, 2006). Forest restoration interventions that include a broad range of species are a challenge, mainly due to the availability of native species seedlings in nurseries. Despite the increasing demand for restoration (by law, buffer zones of river and springs shall be preserved or restored), landowners and local institutions are not aware of the importance of diversity in restoration, due to the loss of cultural and economic ties to the forest. Public and private nurseries typically produce a small selection of threatened species. Sites left to regenerate naturally also do not hold endangered species, usually because of the lack of them in neighboring plots. Chauá Society collaborates to promote change in this arena searching for new populations, mapping trees, producing seedlings, reintroduction and monitoring planting, and producing technical and scientific information about rare and threatened species. This case study demonstrates efforts to reintroduce and monitor individuals of six endangered species from the Araucaria Forest.

Implementation: Over the past five years, we looked for and monitored the phenology of mother trees aiming to identify and collect mature fruits and seeds. We recorded 473 mother trees from *B. eriospatha* in seven protected areas. Seeds were collected from 24 of them, which resulted in 2,112 seedlings. From these, 221 were planted and monitored. For *C. fissilis,* we found 114 mother trees in 16 protected areas, and seeds were collected from 15 of them, resulting in 894 seedlings, 293 of them were planted and monitored. The species *O. odorifera* was represented by 58 mother trees in 14 protected areas, and seeds were collected from eight of them. 617 seedlings were produced and 431 of them were set in the field. For *O. porosa,* 136 mother trees were distributed in 20 protected areas, and 30 of them have produced seeds that resulted in 2,111 seedlings - 682 planted. *Q. brasiliensis* is extremely rare, just three mother trees in two protected areas were identified, and seeds from one tree resulted in 267 seedlings. A total of 256 saplings were under evaluation in the field. *T. acanthocoma* occurred in just two protected areas, and from 169 mother trees, nine produced 1,154 seedlings that resulted in 750 individuals in the field.

Post-planting monitoring: Seedlings were planted in sites located along the original distribution area of the species, and the maintenance was performed by partners quarterly. All sites were monitored until three years after planting, and we

measured the survival rates, diameter at the ground and total height; maintenance failures, when present, were registered. *B. eriospatha* had an average survival of 85% at good maintenance routine against 47% under failures on maintenance. Average increase of height was 0.3 cm/month and of diameter was 0.7 mm/month. *C. fissilis* presented a large variation in survival rates among sites - from 83% to 6%. In general, it is very sensitive to frost, diseases and to the absence of maintenance. The average of height increase was 0.5 cm/month and diameter was 0.1 mm/month. *O. odorifera* presented a high survival rate - 96%; the height increase was 0.8 cm/month and diameter 0.1 mm/month. For *O. porosa*, the same parameters were 90%, 0.4 cm and less than 0.1 mm. Light conditions have a high influence in survival and growth of *Q. brasiliensis* - seedlings planted in open areas were 25% more successful in survival and they grew four times faster than those in shadow areas. The average survival was 85%, height increase was 2.4 cm/month, and diameter was 0.2 mm/month. Average parameters for *T. acanthocoma* were 81% for survival, 0.4 cm/month for height and 0.5 mm/month for diameter increase. Despite the whole difficulty to find seedlings of threatened and rare species in nurseries and the lack of information on initial growth for them, these results indicate that these species can be successful on growth and survival parameters in the field, and with good maintenance, can be a source of biodiversity recovery for the Araucaria Forest.

Major difficulties faced

- Find viable number of mother trees.
- Establish seed collection protocols.
- Control the maintenance of planting sites.
- Maintain regular monitoring for three years.
- Data management for subsequent analysis.

Major lessons learned

- The commitment to basic research surrounding seed storage, *in vitro* seed germination, saplings development monitoring and species ecology are essential to conduct effective restoration.
- The survival of endangered Araucaria Forest species in restoration is generally similar to that of common species.
- Increasing the diversity of native species in the nurseries depends greatly on the awareness and understanding of the local population and experts about the relevance and problems associated with endangered species.
- Site selection is very important for future success of new population establishment.
- Regular maintenance for at least the first two years is a key element to the survival of seedlings once they are planted.

Success of project

Highly Successful	Successful*	Partially Successful	Failure

*** - this was the success of the project for all the six species *Butia eriospatha*, *Cedrela fissilis*, *Ocotea porosa*, *Trithrinax acanthocoma*, *Ocotea odorifera* & *Quillaja brasiliensis*.**

Reason(s) for success:

- The partnership and funding for the initiative from the Global Trees Campaign, through Fauna & Flora International.
- The partnership with other local and regional NGOs and nurseries.
- The help of voluntary work and interns.
- A highly skilled technical team focused on knowledge creation and transfer and on capacity building.

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The reintroduction of the water soldier in Italy: a failed experience

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Introduction

The Water soldier (*Stratiotes aloides* L.; Hydrocharitaceae) is a floating aquatic macrophyte native of central-northern Europe and Siberia. *Stratiotes* stands represent protected nesting or reproduction sites for invertebrates, birds and fishes. Despite it is considered “Least Concern” globally, the species is declining in Europe and it is currently Extinct in the Wild (EW) in Italy at the southern edge of its range (Rossi *et al.*, 2013). Threats to the species were identified in the eutrophication of water due to the intensive use of fertilizers in agriculture (e.g. Italy) and to the percolation of organic nitrogen in areas of intense livestock breeding (Abeli *et al.*, 2014).

Stratiotes typically floats in summer and sinks in autumn/winter. The Water soldier is a dioecious species which further complicates its reintroduction because of the need to identify proper source material for both sexes. In the specific case of the reintroduction of *Stratiotes* in Italy, we could identify the original Italian female genotype preserved *ex situ* in a private nursery, but no local male plants were found. So, as explained below suitable male plants have been identified in other European populations with similar genetic characteristic of the original Italian female population.

Wild population of the Water soldier in the Danube Delta © M. Costa

Goals

- Find genetically suitable male plants as source material for reintroduction.
- Identify a suitable release site in the area where *Stratiotes* occurred in the past.
- Create a self-sustainable population of *Stratiotes aloides* in Italy after its extinction.

Success Indicators

- Female population released and refloating after the first winter sinking.
- Male population released and refloating after the first winter sinking.
- Sexual reproduction achieved three years after reintroduction.

Project Summary

Feasibility: *S. aloides* was declared extinct in the wild in Italy about 20 years ago when female plants were last seen near the Mantua Lakes (Lombardy, Northern Italy). The original Italian genotype was thought to be lost when few female plants were found in a private nursery. This gave a new boost to the reintroduction of the species. Threats for the species where only supposed on the basis of the available literature (Smolders *et al.*, 2003; Zantout *et al.*, 2011). A specific research to clarify the role of eutrophication in the local extinction of *Stratiotes* was started with the additional aim to identify suitable sites for reintroduction. Water quality was evaluated in sites where the species occurred in the past and in potential reintroductions sites and compared with current *Stratiotes* stands across Europe. The study revealed that most of the potential reintroduction sites were compromised but a partially suitable site could be identified in a large ditch few kilometers from the original *Stratiotes* site near Mantua, within the River Mincio Regional Park (Lombardy).

Population genetic analyses were performed to identify suitable source populations for male plants among several European and Siberian populations of *Stratiotes*. Genetic characteristics of the sampled populations were compared with those of the Italian female plants. The study revealed a complex genetic pattern across Europe, likely due to long-distance dispersal mediated by waterfowl (Orsenigo *et al.*, 2017). However, some genetic similarities were found with male plants from the Netherlands and from the Danube Delta (Romania; see Orsenigo *et al.*, 2017).

Implementation: A total of 530 *Stratiotes* individuals were released in a large ditch near La Piuda (Soave di Porto Mantovano), Mincio Regional Park, in two phases. Thirty plants were released in in October 2011 and 500 plants were released in April 2012.

Post-planting monitoring: The post release monitoring revealed that none of the released individuals survived. The 30 individuals reintroduced in autumn 2011 were not found in spring 2012 and one of the hypothesis was that these few plants dispersed in the large ditch. However, of the 500 plants released in spring 2012 only 5% were found in July 2012 and eight individuals were last seen in August 2012. Causes for this quick decline were not completely understood and may be related to the water quality or to the negative effects of long-term *ex situ* cultivation. We also hypothesized that the ditch was too deep to allow enough light to reach the bottom and stimulate plant flotation after winter. Considering the negative outcome of the reintroduction of the female population and the lack of alternative sites, the project was stopped, and the male plants were not reintroduced.

Measuring water quality variables © T. Abeli

Major difficulties faced

- Massive production of female plants from few individuals cultivated *ex situ*.
- Identify the real causes of failure.
- Identify alternative reintroduction sites. Most of the historical Italian range of the species is totally compromised in terms of habitat quality.

Major lessons learned

- Water depth is an important ecological factor to be considered for the reintroduction of *S. aloides* and other partially floating macrophytes.
- Populations close to a release site may not be the most (genetically) suitable as source material for reintroduction, due to complex dispersal patterns.
- For some species, habitat conditions may be compromised to a point that prevents any viable reintroduction attempt.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for failure:

- The ditch where the species was reintroduced was likely too deep.
- Values of some variables related to water quality (e.g. nitrates) were not evaluated as potential threats, incorrectly.
- Several ecological factors at the release site were only superficially considered (e.g. water temperature, seasonal changes in water parameters).
- Lack of frequent monitoring after the release.

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Conservation and reinforcement of the sand stock in the coastal dunes of Central Italy

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Introduction

Malcolmia littorea (L.) R. Br. (recently renamed *Marcus-kochia littorea* (L.) Al-Shehbaz) is a suffruticous chamaephyte belonging to the Brassicaceae, typical of the West Mediterranean coastal dune habitats. It is characterized by a disjointed distribution across Portugal, Spain, France, Morocco, Algeria and Italy (Greuter *et al.*, 1986). Due to human-derived threats, such as habitat fragmentation, urbanization, and tourism, it is considered under extinction risk in several regions, including the Spanish autonomous community of Asturias (Fernandez Prieto *et al.*, 2014). It is classified as Vulnerable in the Spanish province of Alicante (autonomous community of Valencia; Serra Laliga, 2007). Along the French coasts, a 30% decline has been recorded in the last 30 years, although it is not yet included in the French Red List (source: http://flore.silene.eu). In Italy, the species is classified as Critically Endangered (CR) according to the IUCN category and criteria (Rossi *et al.*, 2013) and over the last seven years (2012 - 2019), the total number of reproductive individuals decreased from ~600 to ~260. Here, one of the four sub-populations have been completely removed and none of the remaining ones grows within protected or restricted areas. The conservation status of this species has never been assessed at global or European levels.

Sand stock flower © Sara Magrini

Goals

- To conserve the Italian population through its *ex*

situ conservation in seed banks.

- To develop a reintroduction protocol through field trials and experimental reintroductions.
- To monitor the number of individuals of the reintroduced sub-populations.
- To establish at least two new sub-populations in the historical distribution range in order to reinforce the native Italian population.

Sand dune habitat at TAS © Sara Magrini

Success Indicators

- To conserve seeds from all the Italian sub-populations of the study species in at least three different seed banks.
- To investigate the best way to reintroduce the species, including a thorough understanding of the species' seed biology.
- To increase the number of reproductive individuals in the Italian distribution range and decrease the extinction risk in Italy.

Project Summary

Feasibility: Three sites were selected for the experimental reintroductions, based on the ecology, logistics and historical distribution:

1) Torre Olevola (TOL): This site has public access and is affected by high human impact (tourism and urbanization) and the coastal vegetation is highly fragmented (high disturbance); an area of 1,800 m^2 of the sand dune system, facing SE, was selected.

2) Site of Community Importance Litorale di Torre Astura (TAS): This site is included in the Nettuno Artillery Training Area; the public access is limited to the summer season (low disturbance); an area of 2,000 m^2 of the well-developed sand dune system, facing SW, was selected.

3) Rio Martino (RIO): This site has public access but is included in the Circeo National Park and managed by the Italian National Forest Service (medium disturbance); an area of 1,300 m^2 of the well-developed and lightly fragmented sand dune system, facing SW, was selected.

Implementation: For all three sites, the field trials were carried out only after obtaining permissions from the authorities managing the respective lands. Every

Researcher planting *M. littorea* at TAS
© Sara Magrini

year between 2009 and 2013, seeds were harvested from at least 10 plants per native sub-population, dried, cleaned and then stored at +5°C until they were used for propagation or direct sowing.

TOL: In 2010, about 4,800 seeds randomly selected were sown, in groups of 100, in 48 plots. In particular, 24 pairs of plots were set, with one plot of the pair treated by removing the occurring vegetation (gap creation) and the other one acting as control (vegetation not removed).

TAS: In 2013, 200 non-hardened adult (13 months old) and 200 juvenile (six months old) plants were transplanted in plots of 10, keeping the two ages separate. Half of them were planted on the slope facing the sea and the other half on the slope facing inland. Also, 500 seeds were sown, in plots of 100, in two areas characterized by high density of alien plants, specifically *Carpobrotus* spp. and *Agave americana* L., respectively.

RIO: Between 2014 and 2015, 50 hardened adult (12 month old) and 50 juvenile (seven month old) plants were planted in plots of five in the interdune (portion of sand dune system between mobile and fixed dunes), keeping the two ages separate.

Post-planting monitoring: In all cases, seedling emergence and plant survival was recorded monthly during the first year of the trial. After the first year, visits to reintroduced sub-populations were conducted on an annual basis during the reproductive season, to record the number of reproductive individuals. In TOL, the results of the experiment demonstrated that gap creation facilitated seed germination and seedling emergence. Thus, a significantly higher number of germinated seeds were found in the treated plots compared with the control plots; although only 5% of seeds introduced in the plots germinated during the first year. Since the monitoring in 2012, when 101 adult individuals were counted, this reintroduced sub-population continued to grow up to an estimated 5,000 adults in 2019.

In TAS, a very low number of juveniles survived after the first year of reintroduction, compared to adult plants. Even if, initially, more adults survived in the slope facing inland than on the slope facing the sea, the plants planted on the sea-facing slope were able to establish and reproduce better, reaching ~3,000

adult plants in 2019 against 30 on the slope facing inland. Regarding the sowing trial, no seedlings were ever recorded in the area with a high density of *Carpobrotus*, while 10 seedlings were recorded after one year in the area with a high density of *Agave*. After another year, a total of 62 seedlings were recorded and in 2019 a total of 66 adult plants were recorded.

In RIO, as in TAS, a very low portion (8%) of juveniles survived compared to adult plants (95%) and in 2019 a total of 112 adult plants were recorded.

As a result of these interventions the number of Italian sub-populations doubled (from three to six) with the total number of reproductive individuals increasing from ~260 to more than 8,400.

As highlighted in De Vitis *et al.* (2018), the small Italian sub-populations showed a decreased germination response, compared to other European large sub-populations, as a likely result of the reduced population size. This reinforcement intervention may be beneficial to restore good seed fitness levels, and so a good reproductive ability, in the native Italian sub-populations.

Major difficulties faced

- Dealing with landowners and constructors who were concerned by the presence of an endangered species, with the possibility of restrictions on land-use.
- Absence of fences may have led to increased mortality by humans (e.g. trampling) rather than being natural.
- Marking individuals would have been beneficial to the experiments; however, in order to make the plant less visible to the public (due to public access of the reintroduction sites) and due to specific plant growth (e.g. during summer many stems dry up and break away from the plant) it was taken the decision not to mark the individuals.
- When reintroducing both by planting and sowing, the difficulty exists of separating out the results from natural seed dispersal (e.g. wind, ants, herbivores).

Major lessons learned

- Reintroduction by seeds may lead to very low establishment results in the first year, but may lead to the formation of a soil seed bank, according to the species' ecology, and to the establishment of a persistent population.
- When reintroducing by seeds, gap creation facilitates seed germination and seedling emergence.
- The study species is able to germinate and establish in sites with high human-derived disturbance and high density of *Agave americana*, but does not in sites with high density of *Carpobrotus* spp.
- Reintroduction by seeds is more cost-effective than reintroduction by plants as it requires fewer procedural steps and resources.
- Reintroduction with hardened adult plants (≥12-month old) is more successful than with juveniles.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Italian genotypes successfully stored in three seed banks (Germplasm Bank of Rome Botanical Garden, Italy; Tuscia Germplasm Bank, Italy; Millennium Seed Bank, Royal Botanic Gardens, Kew, UK).
- The baseline data collected on seed germination and during the reintroduction trials have been critical to the development of a reintroduction protocol.
- The reintroduced populations act as a reinforcement for the native Italian sub-populations, with a 32 fold increase in the number of reproductive individuals and a decrease of the extinction risk of the species in Italy.

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Translocation of gennari milkvetch on Mt. Albo, Lula, Central-Eastern Sardinia, Italy

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Introduction

Astragalus gennarii Bacch. *et* Brullo (Fabaceae) is a dwarf shrub forming a dense, compact, spiny cushion, 20 - 80 cm tall. It is characterized by woody stems, densely branched, with persistent stipules and rachis in the old parts of the branches. Imparipinnate leaves, raceme 2 - 4 flowered and corolla white to yellowish. Seeds are reniform, brown-olivaceous, often blotched, smooth and laterally compressed. The flowering season of *A. gennarii* occurs from May to June, and fruits mature from June to July (Bacchetta & Brullo, 2006). *Astragalus gennarii* is an extremely narrow endemic plant to CE-Sardinia, with a single population, consisting of approximately 40 - 45 mature individuals, that grows on Mesozoic limestones in a restricted area of Monte Albo (Punta Turuddò, Lula; Bacchetta & Brullo, 2006; Cogoni *et al.*, 2014).

Currently this plant species is not listed in any international, national or local regulations (Cogoni *et al.*, 2014), however, due to the low number of reproductive individuals and for its narrow distribution, *A. gennarii* was assessed as Critically Endangered (CR) at global and regional level (Cogoni *et al.*, 2014; Orsenigo *et al.*, 2018).

Goals

- To introduce plants in sites less affected by landslide phenomena and human disturbance.
- To increase the population size, and specifically, to boost the number of mature individuals.
- To increase the

Astragalus gennarii in typical habitat
© Gianluigi Bacchetta

probability of population survival and the reproductive rate.

- To determine the conditions for a successful reintroduction of this species.
- To make the results of this project available for future plant reintroduction trials in the Sardinian and Mediterranean contexts.

Success Indicators

- Long-term survival rate of transplanted individuals.
- Plant growth and plant development patterns.
- Flowering and fruiting rates of the established plants (from the third year).
- Number of established seedlings.
- Number of recruited seedlings becoming reproductive (from the third year).

Project Summary

Feasibility: *Astragalus gennarii* is a chamaephytic spiny cushion, whose pulviniform habit is an adaptation to the ecological characteristics of the dry and windy habitats, typical of the Mediterranean mountains where the species occurs at ~700 - 1,055 m a.s.l. (Bacchetta & Brullo, 2006; Cogoni *et al.*, 2014). From a bioclimatic point of view, the species falls in the Mediterranean pluviseasonal oceanic bioclimate, between the upper meso-Mediterranean and lower supra-Mediterranean thermotype and upper sub-humid ombrotype (Bacchetta & Brullo, 2006). *Astragalus gennarii* is an orophilous plant species that grows in dwarf shrub communities belonging to the *Carici caryophylleae-Genistetalia lobelii* Klein 1972 (Bacchetta & Brullo, 2006; Cogoni *et al.*, 2014), associated with other endemic species such as *Cerastium supramontanum* Arrigoni, *Cephalaria mediterranea* (Viv.) Szabó, *Sesleria insularis* subsp. *barbaricina* Arrigoni, *Santolina corsica* Jord. *et* Fourr., *Brassica insularis* Moris, *Dianthus sardous* Bacch., Brullo, Casti *et* Giusso, *Clinopodium sardoum* (Asch. *et* Levier) Peruzzi *et* F.Conti and *Stachys corsica* Pers. (Bacchetta & Brullo, 2006). This plant community can be related to the habitat of community interest “Endemic oro-Mediterranean heaths with gorse” (Code 4090), subtype 31.75 - Cyrno-Sardinian hedgehog-heaths. Secondly, some individuals grow at the edge of the *Quercus ilex* L. forest or in garrigues dominated by *Cistus* spp.

Astragalus gennarii is an extremely narrow endemic plant to central-eastern Sardinia and, according to the regional responsibility criterion, deserves particular interest in conservation. The main threats to the unique small population of *A. gennarii* are principally represented by natural factors (landslides processes), negative effects of unregulated grazing, and by tourist and recreational activities, in particular hikers. These threats could cause a reduction of the population and modifications in the habitat quality (Cogoni *et al.*, 2014). In order to increase the number of mature plants, the translocation was planned as a reinforcement of the existing population (Fenu *et al.*, 2019).

Implementation: The translocation of *A. gennarii* has been carried out in an ecologically suitable site near the natural population, where the main threats,

related to landslide phenomena, unregulated grazing and human disturbance, were less important. All preparatory activities of *A. gennarii* translocation (e.g. site management actions and fence erection in order to prevent grazing) were started in April 2017, but due to the adverse weather conditions the translocation was postponed for the following year. In the first phase, seeds of *A. gennarii* were directly collected in 2016 from the remnant plants and, at the same time, stored in the Sardinian seedbank (BG-SAR) for future recovery or restoration programs and to implement an “active collection”, which might be used in the future for producing further plants.

Translocation by Forestas staff © Giuseppe Fenu

The plants of *A. gennarii* were multiplied in a public nursery of Forestas Agency (Agenzia Forestale Regionale per lo Sviluppo del Territorio e l'Ambiente della Sardegna, Autonomous Region of Sardinia) located near the natural population, by both vegetative stems and seeds. In both cases, the substrate of the pots was taken from the natural population area and no horticultural treatments were adopted; all survived plants were cultivated in a nursery for two years before the transplantation. In February 2018, a suitable area was selected and fenced in order to reduce the unregulated grazing and the human disturbance; some preliminary management actions (e.g. natural vegetation removal, superficial plowing of the land, etc.) had been done to open suitable spaces to reintroduce the transplants (Fenu *et al.*, 2019). In early March 2018, overall 350 plants were transplanted in the selected site with the support of the staff of the public administration of Forestas. Each transplanted plant has been labelled for following monitoring activities (Fenu *et al.*, 2019).

Post-planting monitoring: The management actions, in particular the natural vegetation control and the restoration of the protective fence damaged by wild and domestic animals, were regularly continuing. Finally, a species-specific monitoring protocol was planned and implemented for this translocation, in order to ensure its sustainability. In particular, during the first two years, all transplanted plants were monthly monitored from March to December, recording the growth, the survival rate of transplanted plants and the effectiveness of fence protection in the selected site. Flowering and reproduction outputs of the established plants and number of new established seedlings are planned to be monitored after three years from the transplanting action. Starting from the second year after the release, the monitoring activities will be carried out six times per year (bi-

monthly). From the start of the flowering of the transplanted individuals, the phenological parameters will be also measured, in order to determine the long-term sustainability of the *in situ* actions. Preliminary results of the monitoring indicated a high survivorship rate of the transplanted individuals.

Major difficulties faced

- *Suitable site selection:* As often happens, the best site falls into private land and the owners are not in favor of carrying out conservation actions like translocations.
- *Grazing and human limitation:* The suitable area is negatively affected by unregulated grazing and human disturbance that require specific protective measures to increase the survival probability of transplants.
- *Natural vegetation control:* The control of natural vegetation after the release often requires periodic management actions.
- *Summer drought:* The summer aridity represents a critical factor for plant persistence, in particular during the first years.
- *Management action post-planting and monitoring activities:* These activities require considerable economic resources, mainly due to personnel and travel costs.

Major lessons learned

- The selection of an appropriate area and microhabitat, often unique to the species, where the probability of plant survival and reproduction is greater, is the key to a successful reintroduction.
- The multiplication in nurseries for this plant species is easier and faster using vegetative stems rather than seeds.
- The plant multiplication in nurseries located near the selected areas reduces the probability of maladaptation of transplants.
- The management actions could be necessary even after the plant release and the related costs should be considered at the beginning of the translocation.
- The inclusion in the translocation program of public authorities, volunteers, and local stakeholders significantly improves the success of the program and determines an effective cost reduction.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- High number of survived transplants.
- Positive impact of the fence to protect transplanted plants from the negative effects of unregulated trampling and grazing.
- Presence of numerous new seedlings.
- Collaborative support of public administrations and local stakeholders.

Acknowledgements

This translocation was carried out in the frame of the Care-Mediflora project (80% financially supported by the MAVA Foundation). The authors wish to thank all Forestas staff who gave an essential contribution to the Care-Mediflora project (http://www.care-mediflora.eu/).

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Reintroduction and conservation introduction of the sea flax in Mallorca, Spain

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Introduction

The Sea flax (*Linum maritimum*) is a perennial herb of the *Linaceae* family with woody layer branched and yellow flowers in corymbiforme panicle. It grows in salt meadows from 0 to 10 m a.s.l. It is distributed in the Mediterranean from south Europe to North Africa, and it is present in Mallorca, Corsica, Sardinia and Cyprus. In Mallorca, it was located at the north-east of the Island, only in two populations. The last register about one of the populations was in 1950 (HJBS-Bonafe 2152, VIII-1950), where the collector says “next to be disappeared because of the effect of the surrounding crops - very unusual” and currently, this population is listed as disappeared. During November 2000, J.L. Gradaille collected seeds from the other population to store in the Soller Botanic Garden seed bank (accession number: 5046), and in February 2001, G. Alomar brought a plant from the same population for the living plant collection in the Garden (accession number: 010030). In 2006, some plants were found in the wild population, but it was never seen again. It is protected under Habitat Directive 92/43/EEC (Mediterranean salt meadows), and in the Balearic Catalogue (BOIB 131, 2017). It is catalogued as CR in the Red Book of Balearic Islands Vascular Flora (Saez, 2017).

Sea flax

Goals

- Establish at least two populations of *Linum maritimum* in Mallorca.
- Use the seeds collected in 2000 to restore the same population and to verify its viability.
- Restore the meadow salty habitat where the wild population was more than 10 years ago.
- Find another place to introduce a new population.
- *Ex situ* conservation as a guarantee for the wild populations once it has been re-introduced.

Success Indicators

- Achieve a normal life cycle in the re-introduced population after two years of monitoring.
- Number of plants obtained for reintroduction tasks (viability of seeds stored).
- Number of fruits production in the new populations after the first year of sowing.
- Number of recruitments in the new stabilized populations.
- Number of accessions collected from the new populations to conserve in the seed bank.

Project Summary

Feasibility: The main threats facing this species are the pollution of water resources, the drying of the soil and the increasing human pressure on the habitat. The first step to determine the feasibility of the Reintroduction Plan is to restore the habitat which the plants need to survive. The loss of the habitats is the main reason for which a species can disappear. In our case, the Mediterranean salt meadows are a Habitat Directive protected, but specifically in Mallorca they are not a common habitat. We were lucky that the place of one of the last view populations of *L. maritimum* in Mallorca is now a natural park, so this means at less "protection" when our project ends. In the other hand, the other cited population in Mallorca, in Capdepera was not possible to restore because the habitat had changed a lot. It was near a stream close to farm fields, and now there is only fields and the watercourse has been cemented. Besides this, we did not get any seeds from this population at Capdepera. We therefore decided to reintroduce the population in the Natural Park of S'Albufera and to look for another place in the Natural Park with a similar habitat.

The other important thing to consider is to have good plant material for the reintroductions. In our case, we used the accession conserved in the seed bank from 2000 expecting a good germination. The seeds are dehydrated with silica gel and conserved in frozen conditions (-18ºC). To use them for restoration they need to be defrosted and hydrated again very slowly. The germination of *Linum maritimum* has no problems with fresh

Sea flax plant with close-up of flower

Transplanting Sea flax

seeds, and we proved that the conserved ones were also viable. We use as well seedlings of *L. maritimum* collected from the living plant collection in the Soller Botanic Garden.

Finally, is important to have a good team (staff, collaborators, volunteers, etc.) and as well a group of stakeholders to support the action plan. In addition to the people working in the CARE-MEDIFLORA project, and the team from S'Albufera Natural Park, we had the collaboration of the nearby company Grupotel Parc Natural who give us irrigation water during the first year of the project. Communication with the media has been important to involve and raise the awareness of the communities with this project.

Implementation: To implement the Reintroduction Plan, we contacted the Species Protection Services from the Balearic Government, who has the legal responsibility on protected species on the Island. After getting the necessary permission, the second step was to contact the stakeholders who could be affected by the recovery plan. In our case, the Director of Albufera Natural Park who was happy with the project and gave us all the help with his team and as well the collaboration of the company close to the place where the population had to be reintroduced (Grupotel Parc Natural). The land was cleaned of invasive species and competitive ones like some young *Pinus halepensis* and basically the species *Schoenus nigricans.*

At the same time, in the Botanic Garden we start the germination of conserved seeds 17 years ago and the plant production from the seedlings collected in the Garden. To avoid hybridized plants is important to maintain the collection isolated and to know very well the reproduction biology of the species.

Post-planting monitoring: In the original site, we reintroduced 53 individuals during 2017. In the beginning only the survival of each individual was monitored and, during the summer the number of flowers and fruits produced each week. The number of new stems was monitored as well at the beginning as an indicator of growth. From the next spring, we monitored the number of seedlings in three plots of 50 x 50 cm as an indicator of population dynamics. At the beginning, there was a total of 118 seedlings, and at the end of the project in June 2019 only 23 of them had survived.

On the other hand, for the new introduced population, the protocol was the same, but until the end of the project in June 2019 we did not record any new

recruitment. We planted 71 individuals in November 2017, and by June 2018, they were all alive and flowering but after the summer, only 38 plants survived. The habitat of the new introduced population was not as good as the habitat for the reintroduced one because of drought.

After the first sowing in 2017, we have arranged for irrigation once a week courtesy of Grupotel Parc Natural who give us the facilities and monitoring has been done once a week during the first year and then once a month until June 2019.

Major difficulties faced

- Restore the habitat and find a new good place with suitable humidity conditions.
- Control of competitive species who can occupy the habitat due to its more resistant capacity to drought.
- Survival of plants after the first year when the irrigation was stopped.

Major lessons learned

- The restoration of the appropriate habitat is one of the mains goals to restore a population.
- The second one is to have plants and/or seeds which are better from the original population for restoration.
- The techniques on how to grow the species *ex situ* is an important step to design the recovery plan (reproduction biology, habitat requirements, etc.).
- A wider number of stakeholders ensures clarity and transparency for the project.
- Communication networks are important tools to have in mind during the project to involve the people with the problem.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- The project can be rated as highly successful in more than six years minimum after the restoration, when the populations do not need to be monitored, and the plant spreads to other sites with a good *ex situ* collection of seeds from the newly restored populations.
- The population where the habitat is better (the reintroduced one) is more successful than the introduced one where the habitat has been not easy to locate and is not the best.
- Now, two years after the recovery plan, we can say that there are individuals alive, flowering and fruiting; and with an interesting recruitment

only in one of the populations. We still need to monitor both populations for some years more to be sure that the species is not endangered in Mallorca.

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Conservation introduction actions: difficulties encountered with *Anchusa crispa* Viv. subsp. *crispa* – a threatened subspecies in Corsica, France

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Introduction

Anchusa crispa Viv. is a rare species, which is protected at the national level, listed in Annex II and IV of the European Habitats Directive 92/43/EEC and considered as "Endangered" due to national and regional IUCN Red Lists (UICN France *et al.*, 2018; Delage & Hugot, 2015). In 2018, this taxa was split in two subspecies: *valincoana*, a strict Corsican endemic of the west coast and *crispa* on the east coast (Paradis *et al.*, 2018). If *crispa* subspecies is located in several localities in the northwest Sardinian coast, in Corsica only two little populations could be found. They are found strictly on sandy littoral habitat, and the species suffers numerous anthropogenic impacts due to beach activities on properties without a clear legal status. For many years, different tools were used such as *in situ* conservation and legal enforcement actions. Due to this we decided to create new populations on managed land in two locations around 25 km north of where natural populations are found. The sites were Gradugine (Serra di Fiumorbu district) and Del Sale (Aleria district), both belonging to the "Conservatoire du Littoral" (Cdl) (French coastal protection agency) and managed by guards of the "Collectivité de Corse" (CdC). The work on the subsp. *crispa* was coordinated by National Botanical Conservatory of Corsica (CBNC)'s staff.

Goals

- Creating safety populations on properties with clear legal status and presence of guards in charge of

Anchusa crispa plant, Favona © Piazza

land management.

- Stabilizing the number of specimens in natural populations.
- Research on population creation as well as specific techniques.

Success Indicators

- Long-term population persistence.
- Increasing number of individuals in Corsica.
- In this species restoration also consider all other species of different taxa present at the sites.
- Extension of the area of presence of the sub-species in Corsica.

Project Summary

Feasibility: *Anchusa crispa* is an endangered species, with national conservation measures defined in the National Action Plan (PNA) (Piazza, 2012). In this document, ordered and approved by the French Ministry of Ecology, new population's creation in protected areas is expected and the CBNC carried out the operation. This aims to improve the conservation status of threatened Mediterranean plant species in Mediterranean islands of CAREMEDIFLORA program, financed by MAVA Foundation and in Corsica by OEC.

First, the question was to find suitable land for introduction. Three major criteria have been implemented: 1) Environmental conditions (land size, humidity, substrate organic matter's percentage, plant competition), 2) threats (erosion or sandy storm cover, land uses and practices), 3) land legal status (public or private). For 13 potentially favorable sites each criterion were evaluated. Finally, the two best-rated sites have been selected with an appointed expert namely Gradugine and Del Sale (Paradis, 2014). Then legal permits for protected plant collection and introduction have been submitted to the French Environment Ministry office in Corsica (DREAL Corse), and the project has been approved by the Regional Scientific Council of Natural Heritage of Corsica (CSRPN), and the National Council for Nature Protection (CNPN) of the French Environment Ministry. From the program point of view, rules were defined in a partnership agreement between the local project leader (OEC), the land's owner (Cdl) and the land manager (CdC).

Overall view of the habitat, Canella © Paradis

As conservation is not only a biological point of view, legal and social rules must be always taken in account, even if it needs time, and usually some more in managed areas. But, being on protected and managed land is a security to ensure the sustainability of actions (Godefroid *et al.*, 2010).

Transplanting *Anchusa crispa*, Favona © Piazza

Implementation: The second and third steps were seed collection and germination tests from the two natural populations. They were carried out by CBNC, and plants were produced on agreement bases by a private nursery specialized in wild species cultivation. *In situ* introductions were initiated in November 2017 to take advantage of the first autumn rains and therefore increasing the chances of success. All the 172 plants, of six months old, were introduced, without mixing provenance. CdC, CBNC and Cdl carried out the action jointly. Each specimen has been described, mapped and photographed.

Post-planting monitoring: According to the protocol defined by the CBNC, monitoring by CdC started in February 2018. This protocol take in account survival rate, mapping, photographing and plant description (character, diameter, phenology, etc.) identifying and mapping seedlings, vegetation dynamics. Monitoring has to be done monthly during the first five years. Afterwards, only an annual monitoring in spring will be sufficient. In addition of this protocol, due to their regular management mission, guards give us extra observations as presence and impacts of wild animals, specific climatic events, activities impacts which is important information for highlighting results.

Presently, only 20 months of monitoring have been done, and no serious conclusion can be formalized. Nevertheless, we have first results to share:

- *Spring 2018:* The recovery rate reached 73% and 87% of them flowered and fruited.
- *Autumn 2018:* The recovery rate declined to 27% due to the Adrian storm (29th October 2019). Due to this climatic event, a large quantity of sand brought by the sea was covered the two plantation areas and only 4% of plants survived.
- *Spring 2019:* The recovery rate was the same as in November 2018, but stabilized at 4%, and 100% of those plants were flowering and fruiting. In addition, nearly 400 germinations appeared from the soil seed bank, established in 2018, but, because of the young age of the plants, with a poor flowering and fruiting rate (less than 2%).

Due to this the seed bank cannot be rebuilt this year due to storms as well as the summer drought but nevertheless, monitoring has to be maintained to draw all the lessons from this experiment.

To conclude, we would like to acknowledge all our partners, institutions and collective political will engaged to preserve the species including enough grants, and synergy in-between stakeholders. Nevertheless, this invaluable support is not sufficient to insure success of this type of action. Some essential factors are not manageable: 1) climatic and environmental conditions, 2) species ability to colonize new environments. This case reflects difficulties encountered with plant recovery actions and preserving existing natural localities is better than trying to recreate "nature". Translocation actions are expensive, require extensive knowledge on biology, ecology, geography and all with uncertain results. Translocation must remain as the last chance intervention.

Major difficulties faced

- Many uncontrollable factors in *in situ* such as climate (drought, increased numbers and intensity of storms); negative influence of wildlife (Wild boars, ants); problems related to the use of sites and competition from other plants.
- Time required to obtain regulatory and land licenses.

Major lessons learned

- Need to safeguard natural populations.
- Difficulty with some taxa to get successful results in translocation operations.
- Need to have a good knowledge of the taxa and their habitats.
- Need for long-term monitoring and regular presence on the site.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- No control on environmental conditions e.g. climatic conditions and natural habitat.

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Horstrissea dolinicola: a steno-endemic, threatened plant on Mt. Ida (Psiloritis), Crete, Greece

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Introduction

Horstrissea dolinicola Greuter, P. Gerstberger & B. Egli (Apiaceae) is a dwarf perennial, entirely glabrous and extremely geophytic, with a fusiform to cylindrical taproot 1 - 2 cm in diameter. Only about 10% of its total length usually emerges above ground level at flowering time. It is an endemic of Crete, Greece and only found on Mt. Ida (Psiloritis), the mountain range of central Crete, at about 1,500 m a.s.l. This species was first described in 1990 and it is the only species belonging to the genus *Horstrissea*, which is closely related to the genus *Scaligeria*. It co-occurs with many other plants endemic to the calcareous mountains of Crete such as *Arum idaeum*, *Asperula idaea, Centaurea idaea, Cirsium morinifolium, Draba cretica, Prunella cretensis*. *Horstrissea dolinicola* has been categorized as CR (Critically Endangered) according to IUCN Red List Criteria B1ab (ii, v)+2ab(ii, v); C2a(i); D ver 3.1.

It is also considered one of the Top 50 Mediterranean Island Plants (update 2017) by the IUCN/SSC Mediterranean Plant Specialist Group. As a threatened and endemic species to Crete, it is protected by the Greek Law 3937/2011. The unique population of the species is found within the Natura 2000 site GR4330005 - Oros Idi (Vorizia, Geranoi, Kali Madara).

Horstrissea dolinicola flower

Goals

- Develop tools for assessing the conservation status of the species in the long-term
- Develop a protocol for translocation to reinforce the population.
- Actively involve the local stakeholders in the conservation of the species.
- Improve the legal protection status of the species.
- Removal/Limitation of the pressures on the population.

Success Indicators

- Production of a detailed distribution map of the species.
- Establishment of permanent monitoring plots and elaboration of the monitoring methodology.
- Successful results of the protocol for translocation experienced.
- Plant Micro-Reserve established and legally secured.
- Legal measures for the restriction of livestock activities in the area.

Project Summary

Feasibility: *Horstrissea dolinicola* grows in a few limestone sinks (dolines) in a region where many goats and sheep graze during the summer season. Its unique population is confined to an area where livestock farming is promoted by policies that support livestock development in the region. The species (and most of its co-occurring plants) is well adapted to withstand extreme grazing pressure, however livestock installations such as small containers for animal feeding and watering establishments, present a threat to the species' survival. In addition, places where it is located are adjacent to the road network. These sites also face the greatest pressure as they are used as car parking areas and illegal deposition sites (stones, etc.); they are also threatened with a possible widening of the road network.

Concrete *in situ* and *ex situ* conservation actions have been planned and implemented in the framework of the project CARE-MEDIFLORA, funded by the MAVA Foundation (2016 - 2019). Initially, a detailed distribution map of the species population was created and the size of the population was more accurately estimated (approximately 300 individuals); a management plan was elaborated for *in situ* translocation actions in the area. At the same time, a close collaboration was developed with the local stakeholders, mainly managing authorities and farmers: despite initial hesitations, the Municipal Authority and the local farmers concluded that this unique element of the area's natural environment should be protected; it was agreed to move part of the livestock installations to adjacent areas and administrative measures to be adopted according to the national biodiversity legislation for protected species and areas by the Decentralized Administration of Crete - Forest Directorate of Rethymnon. More specifically the establishment of a Plant Micro-Reserve (PMR) for the species was planned taking the legal status of a “Protected natural formation” (Greek Law 1650/1986 as amended by Law 3937/2011). The official

Horstrissea dolinicola habitat

decision for the creation of the PMR of 3.8 ha was made by the Decentralized Administration of Crete (Government Gazette of Greece: FEK D/250 18-5-2020).

Following the project CARE-MEDIFLORA, a new project funded by the Mohamed bin Zayed Species Conservation Fund was initiated in May 2020 in order to continue conservation actions for this plant until 2022. An information sign has been placed at the established Micro -Reserve and monitoring and reinforcement actions will be continued.

Implementation: For *in situ* conservation actions, namely the reinforcement of the natural population, seeds of an accession of 2007 from the same area, stored in the Seed Bank of MAICh, were used. The ecophysiology of seed germination of this species is already well studied (Fournaraki & Thanos, 2009; Fournaraki, 2010): the seeds of this species have developed a rare germination strategy characterized by double morpho-physiological dormancy; during the first year, after an extended period of low temperatures (5 - 10°C), the release from morphological dormancy is observed, followed by elongation of the underdeveloped embryo, radicle emergence and development of a single cotyledon (pseudomonocotyly). However, the epicotyl remains dormant. By the end of spring, the above ground part (cotyledon) dries out while a small tuber has already developed. During the second year, again under low temperatures, the tuber sprouts (removal of epicotyl dormancy) and produces long petiolate, lobed leaves. According to all experimental evidence, seedling establishment takes place during the second spring after seed dispersal (occurring in autumn).

For the production of seedlings used in this project, 230 seeds were sown on agar gel and incubated in growth chambers at optimum germination conditions in October 2016. After about four months, 61% (142 seeds) of the seeds germinated while 20% were characterized as “dead” or “empty”. Germinated seeds were placed in cell trays with enriched substrate consisting of white sphagnum peat and remained in the nursery for about 2 - 3 months. In the beginning of May 2017, they were transplanted into 90 paper pots and transferred to the field, in three locations of one site; they were covered with a wire cage for protection from grazing and other activities. In addition, seeds were directly sown in small paper pots in November 2017 in three locations of another site. At each location, 60 seeds were sown (180 seeds in total) and they were also covered with a protective cage. All planting locations were recorded with a GPS, and mapped in order to be easily identified for long-term monitoring. Moreover, a small area of

330 m^2 of the natural population was fenced for the purposes of long-term monitoring of the effect on the population after removing grazing pressure.

Post-planting monitoring: For the year 2018 the conservation actions were monitored during four visits (in May, August, September and October). In every monitoring visit:

- The introduced individuals were counted i.e. the number of plants found.
- The status of the protective cages was also recorded.

The last monitoring of May 2019 showed 70% success in seedling survival, while only 8% of seeds germinated.

After the end of the CARE-MEDIFLORA project, monitoring of the actions is planned to be performed twice per year by MAICh and with the active involvement of the management authorities of the area (Forest Directorate of Rethymno and/or Management Body of Protected Areas of Central and East Crete). For long-term monitoring, permanent monitoring plots have also been established within the pre-existing population, in order to evaluate the impact from eventual future degradation of the natural environment.

Major difficulties faced

- Restore the habitat after removing pressures affecting it.
- Convince local stakeholders to take action for this species.
- Bureaucracy in legal procedures.
- The time needed for getting the results of the monitoring actions.

Major lessons learned

- Consensus and active involvement of local stakeholders and authorities is a pre-requisite for successful conservation actions.
- Good knowledge of plant biology and ecology significantly contributes to successful recovery actions.
- Time is needed in order to have a clear figure of the success of any *in situ* action.
- The knowledge and material gained from *ex situ* conservation of plant species contributes to the success of translocation (recovery) actions.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Development of good collaboration with local stakeholders after extensive consultation and mutual agreements.
- Knowledge of the species' biology and ecology after long-term studies.
- Available material from prior *ex situ* conservation of the species in the Seed Bank of MAICh.
- Exchange of knowledge and experiences among the partners of the international project CARE-MEDIFLORA and available resources by the project.
- Networking: steady exchanges over an extended period among the partners of the GENMEDA - Network of Mediterranean Plant Conservation Centers.

Acknowledgments

We thank the MAVA Foundation for supporting the CARE-MEDIFLORA project. We also thank all the people who helped us with the restoration of *Horstrisssea dolinicola* in Crete and particularly, the staff of the Forest Directorate of Rethymno and of the Municipality of Anogeia and the local farmers in the area. Finally, we thank the Mohamed bin Zayed Species Conservation Fund for funding the continuation of our conservation actions for this plant.

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Translocation of *Dianthus rupicola* Biv. subsp. *rupicola* in the Nature Reserve "Isola Lachea e Faraglioni dei Ciclopi", Sicily, Italy

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Introduction

Dianthus rupicola Biv. subsp. *rupicola* (fam. *Caryophyllaceae*) is an endemic species of southern Italy (Campania, Basilicata and Calabria) and Sicily. It is a chasmophyte that grows on steep rocky outcrops and coastal cliffs within the thermo- and meso-Mediterranean bioclimatic belts, from sea level up to about 800 m a.s.l. It forms small shrubs with erect stems and pink flowers that appear from late spring to autumn (Pignatti, 1982). Flowers are insect pollinated and seeds are dispersed by gravity, lacking clear long distance dispersal mechanisms. In Sicily, *D. rupicola* subsp *rupicola* is found mostly on carbonatic substrates, except for a couple of very few-numbered populations growing on volcanic rocks located in the north-eastern sector of the island. This taxon is included in the Annex II and IV of the Habitat Directive (92/43/EEC) and is listed in the category "Least Concern" in the Red List of the Italian Flora (Rossi *et al.*, 2013) while the species is evaluated as "Near Threatened" at European level (Bilz *et al.*, 2011). The main threat to *Dianthus rupicola* subsp. *rupicola* is represented by habitat modification, both in terms of rock quarries or urban sprawl (e.g. touristic infrastructures, etc.) and, likely most severe, by invasive alien species (IAS) as, for example, *Opuntia* sp. pl.

Goals

- Seed collection, development of germination protocols and plant production (nursery).
- Establishment of a new population within the Nature Reserve "*Isola Lachea e Faraglioni dei Ciclopi*" (eastern Sicily).
- Eradication of invasive alien species (mostly *Opuntia ficus*

Close-up of *Dianthus rupicola* flower

-indica L.) in an area of ~10,000 m^2 .

- Restoration of the natural vegetation within the reserve using native species (e.g. *Euphorbia dendroides* L.*, Matthiola incana* L.*, Olea europaea* L. var. *sylvestris, Pistacia lentiscus* L. and *Rhamnus alaternus* L.).
- Implementation of a long-term monitoring plan.

Success Indicators

- Achievement of a germination percentage >85% for the seeds collected and used in the project.
- Production of 300 plants from seeds (sampled on volcanic population) to be used for the introduction.
- Establishment of viable population of *Dianthus rupicola* subsp. *rupicola* in the Nature Reserve "*Isola Lachea e Faraglioni dei Ciclopi*".
- Establishment of viable populations of *Euphorbia dendroides*, *Matthiola incana, Olea europaea* var. *sylvestris, Pistacia lentiscus* and *Rhamnus alaternus.*
- Reduction (75%) of the area invaded by IAS.

Project Summary

Feasibility: In Sicily, there are just two populations of *Dianthus rupicola* subsp. *rupicola* growing on volcanic substrates. One is located within the SCI ITA 070004 "*Timpa di Acireale*" and another one is found close to the Vallone Calcarone (Militello Val di Catania). Both are threatened by soil erosion and by invasive alien species, particularly by *Opuntia ficus-indica*. The loss of habitat and the competition with invasive alien species are, globally, recognized among the major drivers of biodiversity loss. To address these points within the CARE-MEDIFLORA project and to preserve the unique adaptation of the "Timpa di Acireale" population, we decided to introduce this genotype to a nearby volcanic islet, namely Isola Lachea, falling within the Nature Reserve "Isola Lachea and Faraglioni dei Ciclopi". The Lachea islet has been managed as a private property for part of its history and there is still plenty of exotic vegetation on it. Therefore, the introduction of *Dianthus rupicola* subsp. *rupicola* has to be coupled with by the restoration of the native vegetation and the eradication of invasive alien species.

Transplanted *Dianthus rupicola* plant

Our target species was absent from the islet, but the environmental conditions are suitable for it, and may have occurred here in the past. Currently, the introduction area is managed by CUTGANA, an institution devoted to the management and conservation of natural ecosystems. All the actions (eradication of IAS, plants introduction, natural vegetation restoration, and long term management plan) have been agreed and realized by us, in collaboration with CUTGANA.

Transplanting *Dianthus rupicola* plants in cleared sites

In order to maximize the survival rate of the introduced specimens, we planted both juvenile plants and seeds. The plants were produced from seeds collected from the population of “Timpa di Acireale”, previously tested at the Seed Bank of the University of Catania to assess their viability and germination requirements.

Finally, the location of the introduction site within a protected area and the involvement of the CUTGANA staff will ensure the protection of the new population and the monitoring of invasive species.

Implementation: The Botanic Garden of the University of Catania, in collaboration with a private company, provided the facilities and the expertise to produce *Dianthus rupicola* subsp. *rupicola* from seed. In addition, plants of *Euphorbia dendroides*, *Matthiola incana*, *Olea europaea* var. *sylvestris*, *Pistacia lentiscus* and *Rhamnus alaternus* have been produced for the habitat restoration.

Before reintroducing the plants, the manual eradication of *Opuntia ficus-indica* from about 10,000 m^2 of the nature reserve was carried out with the support of a specialized company, CUTGANA personnel and staff from the University of Catania. Eradication of *Opuntia ficus-indica* was not performed on the steepest cliffs, also for not causing slope instability, but the nature reserve is being thoroughly monitored for any establishment or resprouting of *O. ficus-indica* in the eradicated areas. On February 2018 and March 2019 the planting of *Dianthus rupicola* subsp. *rupicola*, as well as of *Euphorbia dendroides, Matthiola incana, Olea europaea var. sylvestris, Pistacia lentiscus* and *Rhamnus alaternus* have been carried out.

Eradicating *Opuntia* cactus (*left*) and cleared transplant site (*right*)

Post-planting monitoring:

- The survival rate of introduced individuals is our success indicator both for the introduction of *Dianthus rupicola* subsp. *rupicola* and for the habitat restoration.
- Number of surviving individuals will be periodically counted on sample areas.
- Since the end of the project, the site will be monitored twice a year by the staff of CUTGANA in collaboration with the University of Catania.
- The area where *Opuntia ficus-indica* was eradicated will be also monitored by the staff of the nature reserve and, if necessary, new eradication actions will be implemented.

Major difficulties faced

- Removal of *Opuntia ficus-indica* from the whole reserve.
- Irrigation of the newly planted specimens, due to scarcity of water available in the islet.
- Restoration of the steepest cliffs of the Nature Reserve.
- Excessive presence of rats and seagulls.
- Reachability of the islet in some periods of the year (i.e. autumn - winter).

Major lessons learned

- Before introducing a population to a new site, the habitat has to be restored to the appropriate conditions for the species to be introduced.
- The use of juvenile plants instead of seed is more effective.
- Development of germination and cultivation protocols for the species to be reintroduced is fundamental.
- Occurrence of potential damaging animals has to be monitored.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Lack or scarcity of water.
- Excessive summer drought may reduce survival rate of the plants.
- Control of potential damaging animals, e.g. rats.

Acknowledgments

We thank the MAVA Foundation for supporting the CARE-MEDIFLORA project. Many thanks are also due to all the people who gave their contribution to the *in situ* conservation of *Dianthus rupicola* subsp. *rupicola*, especially the CUTGANA staff.

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Population reinforcement of a critically endangered urban endemic hawkweed in Milan, Italy

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Introduction

The taxonomy of *Hieracium* L. (Asteraceae) is very intricate since this genus includes numerous taxa that reproduce by obligate or facultative apomixes. The high variability of the genus results in a high number of taxa (over 6,000). One of these taxa is endemic to the old town of Milan: *Hieracium australe* Fr. subsp. *australe* (Orsenigo *et al.*, 2019). This taxon was described in 1848 on samples collected from the walls of Milan (Fries, 1848) and after almost one century of oblivion from its last report (Fenaroli & Zahn, 1927), it was recently rediscovered, on the walls of the Sforza Castle (Galasso *et al.*, 2012). In 2016, only four individuals were counted and the species was considered "Critically Endangered" (CR) (Fenu *et al.*, 2016). So, a reinforcement of this population was necessary to avoid its extinction.

Mature individual of Hawkweed
© G. Larroux

Goals

- Protect the few surviving individuals in order to guarantee their reproduction.
- Study the reproductive biology of the species.
- Produce plants with seeds collected by surviving individuals.
- Reinforce the small surviving population of Milan's Hawkweed.
- Identify suitable release sites in the historical area of occurrence of the taxon.

Success Indicators

- Increase the number of plants.
- Clarify germination ecology of the taxon.
- Create a stock of *ex situ* individuals for future translocation actions.
- Reintroduce new populations in ancient walls of the city of Milan.

Project Summary

Transplanting Hawkweed on walls © G. Larroux

Feasibility: In 2016, seeds were collected from the four remaining individuals of *H. australe*. An amount of seeds was used to study the germination ecology, identifying the most favorable germination period. Seeds of *H. australe* subsp. *australe* show a light physiological dormancy, since a part of seeds germinate immediately after the dispersion (autumn), while the remaining ones germinate in spring, after the winter period. This will probably allow the species to avoid the risk that particularly cold winters, or particularly dry springs, will compromise the survival of all germinated plants.

Implementation: Four new plants were planted in May 2017, while at the beginning of December 2017, 39 small pots each containing 3 - 4 plantlets, were planted, divided into three small sites, close to each other. Moreover, 200 seeds were placed in the crevices between bricks of the walls. At the same time, the area where fenced to protect the few remaining adult plants.

Post-planting monitoring: Twenty-one pots (54%) survived 18 months after the operation and two of the new release sites showed high mortality rates, only one seedling originated from seeds. Causes of mortality were not completely understood, but could be probably identify with competition with other plant species including the alien invasive *Ailanthus altissima* (Mill.) Swingle, lack of soil and moss substrate due to a previous chemical weeding action and excessive summer drought. Traces of predation by Knurled snail (*Helix aspersa* Müller, 1774), have been found on winter leaves, although this seem to have only limited consequences on plant survival. In July 2019, 40 individuals were observed with an increment of the population of 900% in three years.

Major difficulties faced

- Plant the small pots in the cracks between bricks of the vertical walls.
- Avoid plant mortality during the summer drought period.
- Only one of the reintroduced plants flowered after 18 months.

Major lessons learned

- *H. australe* subsp. *australe* is sensitive to competition with other plant species (mostly alien).

- Summer drought stress seem to have a negative impact on young plants of *H. australe.*
- Seeds are not successful for translocation actions of *H. australe.*
- Fall is the better period for translocation of seedlings, to limit the negative effects of summer drought.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- The fence around the area of growth of the original population.
- Collaboration with the municipal administration that manages the growing area of Milan's Hawkweed.
- The detailed study of reproductive biology of *H. australe.*

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Translocation to prevent extinction in face of global change: the case of the sterile relict tree *Zelkova sicula*, Sicily, Italy

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Introduction

Zelkova sicula Di Pasquale, Garfì & Quézel is a narrow endemic tree listed in the Italian Red List of vascular plants and reported as 'CR' (Critically Endangered) according to IUCN criteria (Garfì *et al.*, 2017a). This species counts only two extant sub-populations located between 350 and 500 m a.s.l.. They colonize the bottom of little stream catchments of the outcropping base-rich volcanic rocks of the Hyblaean Plateau (SE Sicily), where they benefit from a seasonal water supply. Both sub-populations have been discovered recently (in 1991 and 2009, respectively) and are about 17 km distant from each other (Garfì *et al.*, 2011). There are approximately 260 and 1,540 individuals respectively, and both of them probably represent the result of century-lasting clonal spreading (root suckers) from two single triploid, non-reproductive, plants (Christe *et al.*, 2014). Hence, *Z. sicula* may be considered one of the rarest plants worldwide. Not capable anymore to

Flowering branchlet of *Zelkova sicula*
© G. Garfì

spread out from its refugial sites, this species is threatened with increasingly frequent wildfires and extreme climatic events (Garfì *et al.*, 2017a). Consequently, translocation probably represents the most effective strategy to ensure its survival in face of the rising intensity of stress and disturbance linked to ongoing global change.

Goals

- Increase the number of self-sustained sub-populations of *Z. sicula*.
- Use both known genotypes to form each new sub-population.
- Better understand the ecological requirements of *Z. sicula* by following its response under new climatic conditions assumed to be more suitable than the current ones.
- Reduce the risk of extinction due to stress and disturbance factors linked with global change, e.g. extreme drought and wildfires.
- Set up the most effective criteria to ensure further translocation of the species in other areas of Sicily.

Success Indicators

- Number of plants successfully established after planting.
- Growth performance in terms of total height and main shoot length.
- Survival rate after 10 years.

Zelkova sicula two years after transplanting © G. Garfì

Project Summary

Feasibility: Due to the sexual sterility of *Z. sicula*, the design of a dedicated vegetative propagation protocol was needed. Since *Z. sicula* revealed extremely recalcitrant to either *in vivo* or *in vitro* techniques, this task required many trials over a number of years and though finally effective, in the end it allowed producing a rather low number of new plants (Carra *et al.*, 2019). Multi-year field observations highlighted the low fitness of the target species within its native habitat. Therefore, basic knowledge about its ecological requirements had to be improved. This issue took into account the data on growth performance recorded on a plant cultivated in

less constraining conditions (at 820 m a.s.l.), the study of the biogeographical and palaeobotanical literature concerning the niche width of the entire genus, several field surveys of many populations of the closest biogeographical relatives, namely *Z. abelicea*, on Crete (Greece), and *Z. carpinifolia*, in Azerbaijan and Georgia.

Watering after transplantation © G. Garfì

In addition, to ensure long-term conservation and sustainability, protected areas and public properties were preferred for the final site selection (Garfì *et al.*, 2017b). Concerning this point, sometimes complex administrative procedures for permission were needed; in some cases, where authorization was denied despite the assumptions of ecological suitability, we were forced to select alternative neighboring locations.

Implementation: After exploring 19 potential sites located in the mountainous ranges and protected areas of Sicily, four sites were selected for plantations, situated respectively on Nebrodi Mts. (Bosco Tassita, 1,309 m a.s.l.), Madonie Mts. (Bosco Pomieri, 1,340 m a.s.l.), Sicani Mts. (Bosco Ficuzza, 1,015 m a.s.l.) and Hyblean Plateau (Bosco Pisano, 507 m a.s.l.). All the planting areas are managed by the Sicilian Regional Forest Agency (DRSRT) and lie within mixed forest stands including deciduous broadleaved trees (*Quercus*, *Acer*, *Fagus*, *Castanea*) and subordinated *Taxus baccata* trees, with the exception of Bosco Pisano, where *Quercus suber* is the dominant species. The selected 3,000 - 5,000 m^2 large plots were fenced in order to protect the new plantations from browsing damages due to domestic and wild herbivores.

Zelkova plants were obtained from both *in vivo* and *in vitro* propagation techniques, using plant material collected from the two native sub-populations. Before their transfer in the wild, plantlets were acclimatized for one growing season in a forest nursery located under meso-Mediterranean climatic conditions. Planting was carried out in several stages. It started in June 2016, with 15 plants per site, respectively at Bosco Ficuzza and Bosco Tassita. It continued in the following winter with 11 additional plantlets in these two sites, and the translocation of 25 plants at Bosco Pomieri and Bosco Pisano, adding up 102 plants in 2016. A second planting step was done between September and December 2017, adding 19 plants in each of the four sites, thus raising to 178 the total of translocated plants. Planting was carried out in the understorey of existing forest communities, following locally uneven spatial patterns, and preferring half-shade conditions and streamside when possible.

Heavy snowfall damage at Bosco Ficuzza © G. Garfì

Special procedures were adopted to maximize the survival rate, like the use of hydrogel (a polyacrylate which absorbs a high amount of water and releases it slowly) in the planting hole, in addition to mulching with biodegradable mats to reduce plant evaporation. Watering was provided during the first summer after planting to prevent potential drought stress. A micro-weather station was set up in each site in order to collect environmental data useful to address future translocations, namely rainfall, temperature, air humidity and soil water content at three different depths (top soil, 30 and 60 cm).

Post-planting monitoring: Immediately after the first plantations and until present, a monitoring activity has been carried out at least once a month during the growth season (April to September). For each plantlet basic data were recorded at planting, such as population of origin, propagation technique (*in vivo* or *in vitro*), micro-environmental conditions (light regime, micro-topography, potential competition). Periodical data collection included survival rate, length of the main shoot, phenology, visible pathogens (herbivore insects, fungi) and physical damages (climatic stress, accidental breaks). The amount of plants that have survived up to now is extremely high: in total 169 plants (95%) are alive, five died in 2017 (two at Bosco Pisano, one at Bosco Ficuzza, two at Bosco Tassita) and four in 2018 (one at Bosco Pisano, two at Bosco Ficuzza, one at Bosco Pomieri). The mean yearly growth rate was satisfactory, ranging from 11.1 - 21.1 cm depending on the site and the year.

At the three most elevated sites some plants suffered from shoot breaks due to heavy snowfall during winter 2017 - 2018, and in spring 2019 also due to low temperatures. Significant insect defoliation was observed during two years on the plantations at Bosco Tassita and Bosco Ficuzza, whereas a number of plants in all sites showed low vigor, probably due to excessive shade from the pre-existing tree canopy. Therefore, it is planned to carry out thinning trials during the next winter.

Major difficulties faced

- The setup of effective *in vivo* or *in vitro* propagation techniques has been extremely time-demanding and still needs refinements to improve the success rate.

- The low number of available plants for translocation limited the possibility to test a wider range of ecological conditions in order to assess the most suitable ones.
- Underestimated insect damages and breakage due to heavy snow significantly reduced/prevented the potential yearly height growth.
- Unexpected cold stress may be invoked as the responsible for the low vigor of some plantlets at the most elevated sites.
- The administrative procedures needed to obtain the authorization to select and perform the plantations have often been time-consuming and hampered by excessive bureaucracy.

Major lessons learned

- Site selection must be done after obtaining the full support and agreement of all local stakeholders.
- Since the release in public areas is believed to be an essential prerequisite, the time span necessary for granting permission should be adequately evaluated in the translocation planning process.
- Based on a cross-control of the growth rates and the few casualties that have occurred up to the present day, some of the selected sites seem to be too cold (at least during winter season). Hence, a longer time-series of local climatic data is needed to identify the real climatic niche of the target species in order to find out the most suitable sites where further nuclei could be released.
- The shade regime should be the object of accurate evaluation through time and the eventuality of specific silvicultural interventions (e.g. thinning the canopy of pre-existing tree cover) should be included in a post-planting conservation planning.
- Up to present the plants issued from *in vitro* propagation seem to respond better in terms of overall growth rate and vigor. This aspect needs to be better understood, hence monitoring must be continued for at least the next five years.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success/failure:

- Most of the plants responded very well to translocation, and the planting procedures as well as the post-planting watering have played an important role in ensuring their establishment at the very early stage.
- The planting and the very good establishment rate of the plants originating from both native subpopulations will ensure the survival and the effective conservation of the whole genetic diversity of the species.
- The effective cooperation of the staff (from field workers to officials) of the

Sicilian Regional Forest Agency that was involved in all the concrete planting steps was crucial for the successful results.

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Population re-establishment of the threatened Georgian almond in Eastern Georgia (Shida Kartli)

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Introduction

The Georgian almond (*Amygdalus georgica* Desf.)(Rosaceae), is a woody plant native to Georgia (Davlianidze *et al.*, 2018). Threatened by livestock grazing and habitat conversion for construction, the species is included as Endangered (EN B2a(i,ii)) in the Red List of Georgia (2014) and Vulnerable (VU) in the Red List of the endemic plants of the Caucasus: Armenia, Azerbaijan, Georgia, Iran, Russia, and Turkey (2014) (Nakhutsrishvili *et al.*, 2014). Field surveys (2014 - 2019) confirmed extant populations in three localities of central and eastern Georgia including in Shida Kartli (villages Sveneti and Tedotsminda) and Gare-Kakheti (Sagarejo, Kochora Mountain). The species was not relocated in several sites recorded in botanical sources such as Teleti (Tbilisi), Trialeti (Tsalka) and Igoeti (Kaspi).

To address the growing risks to remaining populations, an international partnership between the National Botanical Garden of Georgia and Botanic Gardens Conservation International was initiated in 2014 with funding from Fondation Franklinia. Carried out in the framework of the Global Trees Campaign, an initiative to safeguard the world's most threatened tree species, the project aimed at integrated *in* and *ex situ* conservation, including reintroduction in the environs of Igoeti, as well as the establishment of *ex situ* collections at the National Botanical Garden of Georgia, Tbilisi.

Amygdalus georgica wild population, Gori municipality Shida Kartli
© National Botanical Garden of Georgia

Goals

- Comprehensive distribution knowledge of the populations.
- Establishment of *ex situ* conservation and display collections, including seed banking and living plant holdings.
- *In situ* conservation, including establishment of a new population where the species formerly occurred.

Success Indicators

- A species distribution map.
- Best practice propagation protocol for this species.
- *Ex situ* conservation collections with >250 seeds respectively at the National Botanical Garden of Georgia and Millennium Seed Bank in the United Kingdom, as well as >100 saplings at the National Botanical Garden of Georgia.
- One *ex situ* display collection at the National Botanical Garden of Georgia, Tbilisi.
- *In situ* reintroduction with at least 100 saplings near village of Igoeti, Kaspi municipality, Shida Kartli region.

Project Summary

Feasibility: To promote technical capacity as well as influence national policy as regards integrated *in* and *ex situ* conservation of threatened plants native to Georgia, the Georgian almond was identified as a priority, exemplar species, based on features such as threat status, accessibility of propagules in the wild, as well as socio-economic and educational values. Growing in woodlands on slopes as well as in ravines with alkaline soils, the plant occurs under meso-xerophilous and xero-mesophilous conditions in the foothill and lower montane zone of the central and eastern parts of the country. Valuable properties of the species include its high content in amygdalin used for instance in medicine, and its ornamental amenity potential. These values also facilitate the use of this plant in public outreach programs to raise awareness of the country's biodiversity conservation needs.

Implementation: From 2014 over a period of three years, a series of complementary and integrated conservation activities were undertaken including:

- Field surveys to establish the species' distributional range and ecology in Georgia.
- Collection of propagules and soil samples to study and develop best-practice propagation techniques for the establishment of *ex situ* collections (seeds and living plants) and stocks of saplings for *in situ* recovery.
- In close collaboration with the National Forestry Agency and Kaspi municipality, demarcation of a plot of some 25 m^2 near the village of Igoeti in central Georgia to trial reintroduction.
- Creation of a display collection at the National Botanic Garden of Georgia in the capital Tbilisi, accompanied by interpretational public outreach

materials to promote the species as a botanical treasure of national pride.

Reintroduction plot of *Amygdalus georgica* near Igoeti, Kaspi municipality, Shida Kartli
© National Botanical Garden of Georgia

Post-planting monitoring: Evaluation and monitoring carried out twice a year since 2016, has highlighted the maintenance and further development of key project results including:

- *Comprehensive knowledge of extant populations: Amygdalus georgica* recorded in two municipalities of central and eastern Georgia including Gori (Shida Kartli) and Sagarejo (Kakheti); population records from the settlement Trialeti (Tsalka municipality, Kvemo Kartli) and Teleti ridge (Tbilisi) were not corroborated;
- *Amygdalus georgica* best-practice propagation protocol*:* established methods for seed germination and raising of seedlings facilitate the generation of plants for use in future recovery programs.
- Seed banking and living collections: 520 and 300 seeds respectively stored in the Seed Bank of the National Botanical Garden of Georgia and the Millennium Seed Bank of the Royal Botanic Gardens, Kew in the United Kingdom (Mikatadze-Pantsulaia *et al.*, 2017), and over 200 individuals in the living *ex situ* collection of plants of the Caucasus of the National Botanical Garden of Georgia.
- *Reintroduction:* 90% survival rate of 100 saplings planted, and 2.5% germination and survival rate of 200 sown seeds.
- One display collection of *Amygdalus georgica* in the National Botanical Garden of Georgia, accompanied by a panel exhibit with information on species including distribution, ecology, uses and conservation efforts.

These achievements continue to be monitored by the National Botanical Garden of Georgia. The viability of the *ex situ* seed collections held at the National Botanical Garden of Georgia and the Millennium Seed Bank in the United Kingdom is periodically evaluated as part of the general protocol for seed storage held at these institutions. *Amygdalus georgica* collections are being established also at other botanic gardens in Georgia such as Kutaisi Botanical Garden, providing a model example of national networking. The use of *Amygdalus georgica* in outreach events engaging local residents and schools contributes to raising the profile of the conservation needs of the native Georgian flora at large, both within the public and government. The high survival rate and growth development of the saplings in the reintroduction plot in Kaspi municipality with

Ex situ propagation at the National Botanical Garden of Georgia, Tbilisi
© National Botanical Garden of Georgia

individuals starting to bear fruit, is paving the way for natural regeneration of the species.

As this has been one of the very first plant conservation initiatives in Georgia with a focus on reintroduction, this project serves as an exemplar for the development of future species recovery programs in the country.

Major difficulties faced

- *Years with erratic seed set:* Propagule collection and targets for propagation could not be achieved in the first year due to limited seed set.
- *Development of a best-practice propagation protocol:* During the three-year project period, it was not possible to establish best-practice for vegetative propagation using cuttings, and hormonal treatment; use of analogues of auxin “Kornevin” (indole-3-butyric acid), heteroauxin and rooting gel “Clonex” had a very limited success rate (<2%).
- *Environmental conditions:* The climate in the location of the reintroduction site is challenging (very dry conditions during summer and autumn), in addition to grazing pressure by rodents and livestock; as a result, substantial project resources had to be spent on securely fencing the site whilst regular watering of the saplings during the extended summer period was essential to establish the saplings in last year of the project period.

Major lessons learned

- Although seed set in *Amygdalus georgica* is seemingly erratic in the wild, germination under *ex situ* conditions generally is vigorous and occurs on average within six months after sowing; this is particularly relevant for the success of short, three-year funded projects and should be considered when working on other species with more challenging and/or longer germination features.
- *Ex situ* conservation was particularly vital at the start of the project as details of the location of the *in situ* reintroduction needed extended consultations with the local authority and the National Forestry Agency; changes may occur at any time of the project and substitutes for sites of *in situ* recovery action should be kept as an alternative, for instance in the case of persisting climate hazards or political changes.

- The National Botanical Garden of Georgia continues regular monitoring of the reintroduction site following the BGCI project. The population re-establishment model developed during the project will be applied to other Georgian species of high conservation concern in Georgia.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- *Partnership*: An international, interdisciplinary collaboration that can capitalize on a wide range of expertise areas.
- *Planning:* A realistic goal and overall, achievable outputs within the agreed timeframe based on a comprehensive review of existing knowledge of the species during the project proposal preparation.
- *Proficiency:* An ability not only to draw on existing practical capacity but to learn from 'trial and error' such as in species propagation efforts, though different techniques (such as vegetative propagation) require refinement beyond the three-year project period to test a wider array of methods and materials.
- *Promotion:* An inclusive approach to informing and engaging all concerned project stakeholders from the start - from local communities (landowners, shepherds, etc.) to authorities and environmental NGOs, based on their needs, interests and use of popular communication means, including TV and social media.

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Experimental translocation of the endangered pearl-like *Androcalva* in south-west Western Australia

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Introduction

Androcalva perlaria (Malvaceae) is protected under the Biodiversity Conservation Act 2016 as the species was gazetted as Declared Rare Flora (DRF) in January 2008. It is officially ranked as Endangered (EN) based on The International Union for Conservation of Nature (IUCN, 2001) criterion C2a(i) due to less than 400 individuals remaining across six populations (Whiteley *et al.,* 2016). This species was first collected by botanists from the south coast of Western Australia in 1993. A second collection was made in September 2005 during mine survey work less than 40 km from the type location. *Androcalva perlaria* occurs over a very short range (~1,600 km^2), on sandy-clay soils in seasonally-waterlogged sites around the small wheatbelt town of Wellstead, approximately 100 km east of Albany, Western Australia. Over 80% of the vegetation around Wellstead has been cleared for agriculture and most populations are found in bushland fragments on farmland. However, one of the largest populations is found in a roadside reserve. The second largest population occurs within a proposed mine site. *Androcalva perlaria* is not currently listed under the Australian Government Commonwealth Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act).

Small *Androcalva perlaria* plant *in situ* © S. Turner

Goals

- Assess plant performance across two different translocation sites.
- Determine whether differences in plant performance are based on the use of different propagation material (seeds or cuttings).

- Understand if the use of an anti-stress agent (aspirin) and a slow release native fertilizer improves plant performance and survival.
- Gain a better understanding of the factors affecting translocation success and provide a more insightful site assessment process.

Success Indicators

- *Initial success of plantings (after one year):* Survival of at least 75% beyond their first summer when planted into sites currently supporting natural *A. perlaria* populations.
- *Medium term success of plantings (after two years):* Survival of at least 50% beyond their second summer when planted into sites currently supporting natural *A. perlaria* populations.
- Translocated plants behave physiologically in a similar way as naturally occurring plants when assessed in different seasons.

Project Summary

Feasibility:

Habitat: Androcalva perlaria occurs on sandy-clay in seasonally-waterlogged sites adjacent to *Eucalyptus occidentalis* wetlands, growing with *Anarthria laevis, Acacia cyclops*, *Actinodium calocephalum* and *Patersonia occidentalis*. Due to extensive clearing and other impacts, site selection was a significant challenge. After extensive review, two sites were deemed potentially suitable: 1) large wetland reserve (Mettler Lake Nature Reserve) that appeared similar to natural *A. perlaria* habitat, and 2) site where *A. perlaria* already naturally occurred. The second site was a proposed minesite to act as a control, as natural *A. perlaria* plants within this population were very healthy, and there were no signs of diseases, pests, or grazing.

Species: Androcalva perlaria is a quick growing seeder shrub that is readily propagated via the use of seeds, cuttings, and tissue culture (Nikabadi *et al.*, 2010; Whiteley *et al.,* 2016). It is low spreading and grows 0.5 m high by 1 m wide. Flowers are light cream and observed between September & December. The fruit is green-grey with a velvety hairy covering (Wilkins & Whitlock, 2011). Plants produce large numbers of small black physically dormant seeds, which form a persistent long-lived soil seed bank that is stimulated to germinate in response to fire (Turner *et al.,* 2013). Seedlings reach reproductive maturity within 12 months of germination.

Socio-political & economic: The implementation of the translocation was viewed as unlikely to cause significant adverse social and economic impacts as both of the sites selected for the translocation were either a Class A nature reserve with secure long-term conservation tenure or on a proposed mine site which had already been approved to be cleared thus the nature for undertaking the translocation was for research outcomes rather than purely for conservation purposes (Department of Parks and Wildlife, 2014).

Planting underway at Meter site © C. Elliott

Implementation:

Translocation: The site at Mettler Lake Nature Reserve (~400 ha) had been burnt several years prior and was also fenced to exclude herbivores. The translocation site (~50 x 50 m) was located on the western side of the wetland and situated on the side of a gently sloping ridge within seasonally waterlogged wetlands. The site selected on the proposed mine was a similar size but left unfenced (no herbivore activity). This site was also open with relatively sparse overstory and understory present. Tubestock (derived from cuttings and seed) were produced six months before field installation in July 2014. Within each site, 80 plants were randomly placed in lines 1 m apart, numbered then measured, with a subset also receiving fertilizer and/or an anti-stress agent (aspirin solution).

Cultural/tribal: Indigenous communities interested or involved in the region affected by this plan have not yet been identified. The Aboriginal Sites Register maintained by the Department of Indigenous Affairs does not list any significant sites in the vicinity of both translocation sites. However, not all significant sites are listed on the Register (Department of Parks and Wildlife, 2014).

Trans-border: Not applicable.

Veterinary/phytosanitary: Re-introduced plants were propagated using standard horticultural hygiene practices and grown in an accredited nursery (Kings Park and Botanic Garden Nursery) prior to field planting.

Post-planting monitoring:

Monitoring regime: Monitoring was undertaken after the first month and then every six months for the first year and then annually thereafter. Data collected included recording the number and health (1 to 5 rating system) of surviving plants, height, plant width, and reproductive state. Other metrics recorded included soil temperature and moisture, soil physical attributes, soil chemical attributes and details of the vegetation communities present. To understand a little better the physiological attributes of the translocated plants LiCOR (Photosynthesis, stomatal conductance and transpiration) and pressure bomb (predawn and midday water potential) measurements were undertaken and compared with measurements recorded for naturally occurring plants. These were collected during mid-summer (dry season) and mid-winter (wet season). These

data were used to provide a comprehensive snapshot of all the similarities and differences across both translocation sites in terms of plant performance and site attributes.

Proposed minesite natural habitat © S. Turner

Results: After two years of establishment plants at the proposed minesite had performed much better in terms of overall survival (91.3 ± 3.1%), plant health (4.5 ± 0.6) and growth (100 ± 39 cm wide) compared to the Mettler site where plants performed much more poorly i.e. lower survival (41.3 ± 11.8%), poorer plant health (2.8 ± 0.5) and smaller plants (33 ± 14 cm wide). Within both sites consistent and significant treatments effects were noted such as better performance of seed produced plants and fertilizer also modestly improving some plant attributes. Physiological measurements found that plants at the Mettler site were under significantly more water stress during summer and their photosynthetic capacity was also much lower. Translocated plants on the proposed minesite were overall physiologically similar to naturally occurring plants during winter as well as summer.

Major difficulties faced

- Locating suitable habitat that may support translocation of the species.
- Creating *in situ* planting spots among compacted soil with numerous existing plant roots.
- Logistics of obtaining physiological measures of plants (i.e. predawn water potential measurements) due to remoteness.
- Negotiations among stakeholders to agree to the translocation proposal and experimental approach within a suitable time frame.

Major lessons learned

- Holistic habitat assessment greatly improves translocation site selection.
- Good site selection is critical for high survival and growth.
- Plants produced from seeds performed better than those produced from cuttings.
- Results of aspirin treatment as an anti-stress agent were inconclusive with no significant benefit in terms of plant performance determined.
- The use of soil probes to monitor moisture and temperature and quantification of basic soil attributes provided standardized baselines for

comparisons between sites.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Higher survival was due to increased soil moisture at the natural *A. perlaria* population site, compared to the wetland reserve site which was generally much drier.
- The wetland reserve site did not exactly match the natural *A. perlaria* sites, with minor differences in vegetation structure and some soil attributes noted.
- Sustained adequate investment in research provided key information for obtaining high plant establishment through using an experimental framework that identified key factors that regulate translocation success.

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Restoration of marsh angelica population in the Czech Republic

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Introduction

Marsh angelica (*Angelica palustris*) is classified as data deficient taxon in the IUCN Red List. The species is listed in Annex II of the Habitats Directive (Council Directive 92/43/EEC) and is also classified as strictly protected plant species according to the Bern Convention. At national level it is classified as critically endangered taxon according to IUCN criteria in the Red List of vascular plants of the Czech Republic and it also belongs to critically endangered specially protected plant species. An Action Plan for Marsh Angelica has been implemented in the Czech Republic since 2000.

The distribution range of Marsh angelica is European-Westsiberian. The plant was in the past documented from eight localities in the Czech Republic, but it disappeared from most of them before 1950. Reintroduction has been implemented at two lowland central Moravian localities: Hrdibořické rybníky and Černovírské slatiniště. Hrdibořické rybníky was the last site where the species persisted until 1986. At the beginning of the reintroduction both sites had relatively good potential regarding habitat and future management.

Goals

- To maintain the species at its last recent locality in the Czech Republic (Hrdibořické rybníky).
- To establish viable population at one historical locality.
- To maintain the species in two *ex situ* rescue cultures with representative genetic variability.

Marsh angelica © Jan Vrbický

Success Indicators

- Existence of viable populations of at least 1,000 flowering plants for three to five consecutive years without any further reintroduction at the last recent locality and one historical locality.
- Existence of two *ex situ* rescue cultures with at least 30 flowering plants per year and a large seed bank.

Project Summary

Feasibility: Marsh angelica is a stout (75 - 145 cm tall) biennial, seldom shortly living perennial plant from the Carrot family (*Apiaceae*). It flowers once in its lifetime, usually from June to September. The occurrence of species is bound to fens. It requires mucky and boggy soils that are rich in calcium. It only grows on moist soils with permanently high level of groundwater. It does not tolerate stronger drying out of the rhizosphere, but is not adapted to longer-term flooding either. The decline of the species in the Czech Republic was caused by several impacts. Most historical localities were in the past strongly influenced by ploughing, draining (including pumping of drinking water) and input of nutrients from fertilization of surrounding fields. At present some of the biggest problems are unstable water regime, overgrowing of the habitat due to insufficient management and devastation of plants by non-native slugs.

The restoration of Marsh angelica population required relatively small funds. At the beginning of the Action Plan larger amount of money was needed for restoration of sites (restoration of water regime at the last site of Marsh angelica, cutting down self-seeded shrubs and trees, sowing of regional species mixture to low-diversity grasslands originally restored on arable land). After establishing the *ex situ* culture of Marsh angelica the cultivation of the species is easy and high amounts of seeds can be produced with relatively small funds. Most costs go to habitat management with special mowing carried out in places with populations of Marsh angelica forming only one third of this money.

Searching for seedlings in a site flooded by European beaver © Jitka Uhlíková

Implementation: The *ex situ* rescue culture was established at the end of the late 1980s from the remaining plants at Hrdibořické rybníky site. Attempts to restore a population of Marsh angelica have been going on since 1990 but regular activities started in 2000 when the Action Plan for Marsh angelica was approved. Activities aimed at reintroduction of Marsh angelica took

place at Hrdibořické rybníky and Černovírské slatiniště sites. Marsh angelica was reintroduced by planting of seedlings, but mostly by sowing of seeds to 1 x 1 m plots and later with enough seeds also by larger scale sowing (~3 plots of 10 x 10 m yearly). Activities focused on regular management were also part of conservation activities. Two studies focusing on genetic variability and relationships among populations of Marsh Angelica from the Czech Republic, Hungary, Poland and Germany were carried out. The study from 2010 showed that genetic variability of the Czech population is smaller than the variability of the Polish and Hungarian populations, but it is still high enough to keep relatively good likelihood of survival for the species. The study from 2016 used sequencing of selected chloroplast regions. The data showed that the Czech population is somehow unique compared to the neighboring countries.

Post-planting monitoring: Population size is assessed every year. Monitoring consists of counting flowering plants and non-flowering plants with leaves bigger than 15 cm. This size limit was chosen because it is nearly impossible to count plants with smaller leaves in tall vegetation. Other data such as infestation by insects or slugs are recorded and then the conservation status and trend of the population is evaluated. Ground water level is continuously measured by automatic data loggers. The Action Plan seemed to be successful at the Hrdibořické rybníky site until 2013. A spontaneous population was established at Hrdibořické rybníky in 2003, but seeds were added in the following years as well, ending in 2011. More than 2,364 flowering plants of Marsh angelica were recorded there in 2013, which was the highest number of flowering plants since the beginning of the conservation activities. In 2014, the site was flooded due to activity of European beaver and only five flowering and 30 non-flowering plants were present. The large seed bank present at the site after the intense flowering in 2013 was very likely destroyed by the still water that remained there for the whole summer of 2014. Further restoration of Marsh angelica population was negatively affected by Spanish slug consuming the seedlings. Only few dozens of plants were found in the last two years.

Attempts to reintroduce Marsh angelica to Černovírské slatiniště were not successful. The site has unstable water regime and therefore restoration of spontaneous population is more difficult. The only chance for restoring the species there seems to lie in the establishment of a large seed bank on larger areas of the site, so that the species can grow every year at least in parts of the site with optimal water conditions needed at the moment.

Major difficulties faced

- Unstable water regime caused either by former drainage or by activities of the European beaver.
- Destruction of plants by non-native slugs.
- Getting permissions from land owners to carry out proper management.

Major lessons learned

- It is extremely important to have localities with suitable habitat, water regime and management and to ensure their stability in the future.
- Finding out the optimal way of reintroduction is essential. Sowing of small amount of seeds to plots with soil cleared of vegetation was unsuccessful - less than 10 % of seeds germinated. Planting of seedlings had a much better result.
- A viable spontaneous population can collapse within one season if the water regime changes strongly.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for failure:

- A spontaneous population has not been established yet. Existence of the species in the Czech Republic is still dependent on reintroduction activities.
- Unstable water regime - when groundwater level depends on weather, it is very difficult to establish a population of Marsh Angelica because it is a biennial plant that flowers once in its lifetime and needs optimal water conditions during both years.
- Establishment of a large seed bank on larger areas of the sites has not yet been successful.

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Reintroduction of an endemic plant *Minuarti smejkalii* in Czech Republic

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Introduction

Minuartia smejkalii is inconspicuous, densely tufted perennial herb and belongs to the species of priority European interest according to Habitats directive 92/43/EEC, Annex II. It is also included in the Convention on the Conservation of European Wildlife and Natural Habitats and the IUCN international Red list. According to the Czech law, **M. smejkalii* is one of specially protected plant species included in the critically endangered species category. The species is an obligate serpentinophyte and thus it occurs only on serpentine. *M. smejkalii* prefers sunny habitats with sporadic vegetation such as rock platforms, shallow serpentine soils and sparse grasslands in open pine forests.

M. smejkalii is Czech endemic species, whose worldwide distribution is limited only to two nearby areas, both included in the NATURA 2000 network. The first area is in the Želivka Site of community importance (SCI) - in part of the territory protected as National natural monument (NNM) Hadce u Želivky (six populations). The second area is in the Hadce u Hrnčíř SCI (one population). *M. smejkalii* was reintroduced to one site in the Želivka SCI in 2018, where the species occurred in the past, but went extinct in 2012. Further, three current populations were reinforced.

Minuartia smejkalii in natural habitat

Goals

- To restore habitat condition at project sites.
- To maintain the species in *ex situ* populations to preserve its genetic diversity (in botanical gardens and in private gardens).
- To increase population size at three selected localities.
- To re-establish viable population at one historical locality.

Success Indicators

- Self-reproducing *ex situ* population containing at least 600 individuals.
- Creation of network of local gardeners cultivating the species including 10 gardeners for each site.
- Self-reproducing viable populations on three recent localities (at least 100 individuals depending on the size of the locality) and one historical locality (at least 600 individuals).

Project Summary

Feasibility: *M. smejkalii* prefers open serpentine rocks or open pine forests. The decline of the species was caused mainly by changes in the land used, e.g. absence of forest grazing or raking of litter. Sites were artificially afforested and serpentine bedrock covered by high layer of humus. Such conditions led to increased shading and competition (Pánková *et al.*, 2011). Suitable source of materials for species reintroduction was determined by genetic analysis and hybridization experiments. The results showed that plants from both areas should be kept separately, seeds from different populations within each site should be used for species reintroduction and only native seeds should be used for reinforcement of populations (Stojanova *et al.*, under review).

Implementation: Restoration of sites as well as species reintroduction was based on Management plans for both areas (Rešlová, 2011; Klaudys, 2012), but the extent of these actions was more intensive then described in these documents. We thus needed special permission from the state organs of nature protection. The action was implemented by the financial contribution of the EU, the Life program (project LIFE for Minuartia, LIFE15 NAT/CZ/000818, www.kuricka.cz) and the Ministry of Environment of the Czech Republic. The first step of the project was restoration of habitat conditions. Some trees and shrubs were cut to open the pine forests. High humus layer, together with ruderal species and mosses, was removed from the site manually and by a digger to uncover serpentine bedrock. Serpentine rocks covered by serpentine vegetation were created for *ex situ* conservation: large rocks (more than 30 m^2) were created in Botanical garden of CAS in Průhonice for Hrnčíře area and in Visitor Centre Vodní dům for Želivka area. Small rocks (~5 m^2) were built in the private gardens.

The major limitation was availability of seeds in the nature. Seeds were thus collected in several subsequent years and also population reinforcement and reintroduction were divided into two years. We used seeds collected in native

Overall view of the reintroduction site

population for population reinforcement and seed mixture from Želivka SCI for species reintroduction. While *ex situ* population in Visitor center Vodní dům was established from mixture of seeds from several populations in Želivka SCI, private gardens obtain seeds only from the largest one. *Ex situ* population in the Visitor center Vodní dům was established in 2017 by sowing 2,300 seeds and planting of 400 juvenile plants. Additional 1289 juvenile plants were transplanted in 2018. *Ex situ* population in the Botanical garden in Průhonice was established in 2018 by planting 150 individuals. The network of private gardens is continuously developing. Currently, we have 20 gardeners involved in the project, who care totally about 1,500 individuals.

Reinforcement of populations was done in 2017 by sowing of more than 9,000 seeds and by planting 472 juvenile plants. In 2018 additional 111 individuals were planted. Species reintroduction started in 2018 by planting 1,101 individuals. Additional ~500 individuals will be planted in autumn 2019.

Post-planting monitoring: Total population size, plant survival and size (e.g. plant size, number of flowering and non-flowering stems, seed viability) and number of new seedlings is evaluated every year in natural as well as in artificial populations. Size of all natural individuals is evaluated, while transplanted individuals are evaluated only in permanent plots. Seed viability is evaluated in standardized conditions in growth chambers in the Institute of Botany of the CAS. Habitat conditions are described every year by recording of phytocenological relevés of 1 x 1 m, evaluation of temperature and soil moisture, chemistry (prior and after management interventions) and canopy openness. Additional parameters -gardeners care and state of the rock are recorded in the private gardens. Monitoring showed that sowing is not successful method of species support since only 14 individuals established in the nature (zero in the gardens). Planting of young plants was more successful, although we had problems with low stability of the newly created rocks in the gardens leading to high mortality of plants in some gardens in the first year. Nevertheless, plant survival was high in the first year (72% in the botanical gardens and 74% in private gardens), but declined in the second year (22% in the botanical gardens and 28% in private gardens), but new seedlings were able to substitute the dead plants so the populations are increasing. There was, however, difference between gardens.

Similarly, plant survival was very high in the nature: 64 - 78% for reinforced

Transplanting plants at the reintroduction site

populations and 58.6% for the reintroduced population after the first year. More than 95% of the individuals was flowering and we observed very high number of new seedlings on all the sites. Evaluation of vegetation composition and habitat conditions in the nature showed that the restoration was successful, and the sites are slowly colonized by target species.

The monitoring will continue in the following years to ensure that the reintroduction as well as population reinforcement was successful and led to establishment of viable populations.

Major difficulties faced

- Low availably of seeds in nature, especially in small populations.
- Very low germination of sown seeds in nature.
- Low plant survival in the gardens in the first year caused by low stability of the newly created rocks.
- Establishment of balance between keeping the most natural conditions in the gardens (no watering, maintenance of competition) and plant survival (sometimes plants have to be watered to support their survival in extreme drought periods).
- Discussion with scientific community about involvement of local people into *ex situ* protection and about reinforcement of current populations.

Major lessons learned

- Successful *ex situ* conservation should be based on the scientific results including assessment of genetic structure of the population and potential hybridization risks.
- For *ex situ* conservation it is crucial to create conditions as similar to conditions on natural sites as possible to maintain species adaptations. Nevertheless, it is necessary to establish a balance between natural conditions and plant survival.
- It is necessary to test different approaches for species reintroduction and select the best one, which is cost-effective, feasible and guaranties high

success.

- Including local people to *ex situ* conservation led to creation of very cost-effective network and increased the awareness of the species and the area among the local people.
- Since extreme drought could increase plant mortality, the reintroduction should be done on partly shaded sites and plants in *ex situ* cultures could be watered in extreme conditions.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Habitat conditions were restored and target species started to spontaneously spread at the sites.
- Majority of planted individuals of *Minuartia smejkalii* survived and flowered.
- New seedling of *M. smejkalii* appear at the target sites, some of them are already flowering.
- Local people are still interested to enjoy the *ex situ* conservation; they are interested in species as well as site protection.
- Since extreme drought could increase plant mortality, the reintroduction should be done on partly shaded sites.

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Reintroduction and supplementation of long-stalked pondweed populations in the Czech Republic

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Introduction

The Long-stalked pondweed (*Potamogeton praelongus*) is an aquatic plant species classified as Least Concern according to the IUCN Red List. Moreover, the species is included as Critically Endangered into the Red List of vascular plants of the Czech Republic (CR) and belongs to the highest category of specially protected species in Czech legislation. About 90% of its localities have disappeared due to eutrophication, water pollution, successional extinction and destruction of its natural habitats, and introductions of herbivorous fish. Therefore only one native population in the oxbow of the Orlice River near Hradec Králové (OR) in Eastern Bohemia exists today. Its conservation is hence of high priority and a national Action Plan for the species has been implemented since 2003. Both reintroductions and supplementations discussed here have been performed.

Long-stalked pondweed © R. Prausová

Reintroductions are currently restricted to the areas where this species was formerly found - Orlice, Ploučnice basins. Supplementations were performed several times into OR. The species was experimentally grown in several revitalized pools in the Protected Landscape Area Kokořínsko (LPA). Plants for reintroduction are provided from a rescue culture in the Institute of Botany of the Czech Academy of Science in Třeboň (IB CAS), that was established from individuals collected in OR.

Goals

- To create 3 - 6 new populations of LSP in areas where it was formerly found - the Orlice, Ploučnice basins (reintroduction goal).
- To ensure the long-term existence

of the last native population of LSP in OR (supplementation goal).
- To ensure the long term existence of maximum LSP populations in the CR (supplementation goal).

Success Indicators

- Confirmed successful growth of out-planted individuals (for both reintroductions and supplementations).
- Long-term persistence of new populations, ideally also with increasing cover (for reintroductions).
- Long-term persistence of the last native population, ideally also with increasing cover (for supplementations).
- Long-term conservation of localities with LSP populations, i.e. optimal water characteristics and plant communities composition (for both reintroductions and supplementations).

Project summary

Feasibility: The LSP is a perennial aquatic plant that grows especially in unpolluted, mesotrophic, 0.2 - 2.0 m deep water on humic or sandy soils, clayish, muddy or peaty beds. It occurs in lakes, river floodplains (river-oxbows, pools), moderately flowing water, ponds, and reservoirs. In most localities, LSP grows in neutral to slightly alkaline waters. The species is endangered by eutrophication, which results in high phytoplankton and filamentous algae biomass and low summer water transparency. Parts of shoots covered with algae necrotize and die (Prausová *et al.*, 2015). Eutrophication of the Czech localities of LSP resulted in its being replaced by natant broadleaved pondweeds, *Nuphar lutea,* in small and shallow pools with littoral species and aquatic moss *Calliergonella cuspidata.* The growth of LSP is also limited by the influence of animals, especially herbivore fish *Ctenopharyngodon idella* and Mallard duck (*Anas platyrhynchos)*. Czech populations reproduce only clonally even though germination tests confirmed a germination ability (after interruption of their dormancy) of achenes gathered in Czech localities (Prausová *et al.*, 2013, 2015). The success of seed germination and subsequent growth of its plantlets in natural sites in the CR is unlikely because of low water transparency, threat by herbivores and competitive organisms, and disturbances like floods. Clonal growth and a rapid extinction of most of the Czech populations caused a current very low genetic variability of LSP in the CR.

Activities implemented from the Action Plan include a selection of potential localities (both native and established). Localities for reintroduction of LSP, a regular site and LSP population monitoring, and mainly revitalization of the OR and an insurance of sustainability of sites with LSP populations. Considering reintroductions, the first step was to select suitable localities for this measure. The best localities were searched in areas where was LSP formerly found, i.e. in the Orlice (East Bohemia) and Ploučnice (North Bohemia) basins. Optimal parameters of water in selected localities were: summer water transparency more than 0.5 m, total alkalinity of at least 1.5 mekv.l^{-1}, (moderately hard or hard water),

A - Plants from *in vitro* culture transferred into the rescue culture B - shoots after one growing season in the outdoor rescue culture in IB CAS, Třeboň © A. Kučerová

pH 7 - 9 (neutral to mildly basic), shading of water surface 15 - 50% (protection against overheating), electric conductivity 110 - 410 $\mu S.m^{-1}$. Important factor also was a character of sediment on the bottom (only a thin layer, i.e. 2 - 5 cm, of mud on sandy or clayish sediment), and a plant community composition (absence of competitive broadleaved plants, aquatic moss and filamentous algae). The plants used for reintroduction and supplementation of LSP into selected localities and OR came from the rescue culture at IB CAS. The reintroductions have been performed since 2005 to 2018 into about 40 localities. Only two localities showed to be suitable for this species (Kašparovo Lake in the Orlice River basin, Heřmaničky - oxbow of the Ploučnice River in the North Bohemia).

Success of reintroduction was negatively influenced by unpredicted weather development, and sudden change of water level and floods that damage planted shoots with an undeveloped root system. Negative impact of herbivorous fish, ducks or chemicals - pesticides occasionally occuring in water of Orlice River, were observed for several times.

Implementation: At first, all required administrative matters had to be taken care of in advance. This included or example official agreements of relevant conservation offices or permissions from watercourse manager (i.e. Povodí Labe, state enterprise). The number of outplanted shoots depends on the number of successfully grown shoots in IB CAS in a particular year. Since 2011 were plants for reintroduction grown from apical shoot segments of a tissue *in vitro* culture. The tissue *in vitro* culture (30 clones) was established by Mr. Pásek (from achenes provided by IB CAS) in frame of a project supported by EEA/Norway funds in 2009 - 2010 (Prausová *et al.*, 2011). About 250 shoots with rhizomes for reintroduction of LSP have been grown in the rescue culture in IB CAS every year. Cultivated plants have been transported from IB CAS to Hradec Králové for being out-planted into the potential localities in July and August. Number of 3 - 150 shoots have been planted into one of 3 - 5 selected localities during a summer.

Monitoring, growing and outplanting of *P. praelongus* shoots were financially supported in frame of the Action Plan.

Post-planting monitoring: Planting success has been verified in the autumn and during the subsequent growing season when the water transparency was convenient for this. The number of bunches and particular shoots (all, fertile, sterile) have been recorded during monitoring every year. Microsites with LSP population were localized by GPS to get a more detailed picture of its occurrence. The failure of the out-planting could be caused by using a low number of planted shoots, plants of small size and their low adaptability to the new environment. It is really complicated to predict weather development and the time of sudden change of water level or flood. Shoots carried away by the stream have a very low possibility to take root in a proper site. Using of pesticides and fishing (mainly herbivorous species) show to be strong and unpredicted factors in success of LSP reintroduction.

Heřmaničky - oxbow of Ploučnice River with reintroduced population © R. Prausová

Major difficulties faced

- Unsuitability of most of historical localities for the LSP reintroduction (irreversible changes of their site conditions).
- Unpredictability of weather development, sudden changes of water level and floods.
- Insufficient protection of localities against occasional pollution by pesticides.
- Insufficient protection of localities against illegal introduction of non-native herbaceous fish, semi-wild ducks (hybrids of domestic and wild ducks).
- Frequent changes of site conditions correlating with climatic fluctuations and extremes.

Major lessons learned

- It is extremely important to outplant LSP to a suitable habitat and to ensure it will stay suitable in the future (that means mostly ensuring needed site conditions).
- The time of out-planting to adapt to the weather and consequences of sudden changes of site conditions.
- For both reintroductions and supplementations, to use more than 100 shoots with well developed rhizomes/locality.
- Needed cooperation with watercourse manager (i.e. Povodí Labe, state

enterprise), and Czech Fishing Union (mainly regional association).

- Proper coordination and communication with all involved partners and public influencing the landscape in a surrounding of LSP localities is necessary.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Only some success indicators have been reached in reintroduction and supplementation so far and the consequences of revitalization of the last native locality are expected. The number of localities with particularly successful reintroduction is less than what is stated in the original plan.

Reason(s) for success:

- Proper theoretical preparation and planning, use of experience collected from literature or own research.
- Yearly availability of shoots for planting thanks to the tissue *in vitro* culture.
- Unpredictable development in weather and sudden changes of water level and floods.
- Complications in communication with fishmen and public influencing the surrounding of localities (use of pesticides, etc.).

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Conservation in a changing climate: reinforcement of the critically endangered, endemic Yuanbaoshan fir on Mt. Yuanbao, Guangxi, China

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Introduction

The Yuanbaoshan fir (*Abies yuanbaoshanensis*) is listed as Critically Endangered by the IUCN and as a Grade I State Protected wild plant in China. Throughout China, there are 21 *Abies* species, typically distributed in the mountains of the northeast and southwest regions. Four of the 21 species are endemic and found in the mountains of southern China, namely *Abies banshanzuensis*, *A. ziyuanensis, A. yuanbaoshanensis* and *A. fanjingshanensis*. Living beyond the typical distribution range of *Abies* species, these four types of firs are considered as relict species of the Pleistocene glaciation (Xiang, 2001). Among them, the Yuanbaoshan fir is confined to Yuanbao Mountains, Rongshui County of North Guangxi. The Yuanbaoshan fir occurs from 1,750 to 2,050 m a.s.l. with a very restricted distribution of 18.6 ha on the top of mountain and a cool and wet climate (Li *et al.*, 2002).

Goals

- To initiate the reinforcement of the Yuanbaoshan fir enhancing its resilience in the face of climate change over the next 40 years.
- To understand the adaptive capacity of the Yuanbaoshan fir outside of its current range.
- To apply the experience and lessons learned from the Yuanbaoshan fir to the reintroduction of other tree species in the future.

Yuanbaoshan fir © Ding Tao

Success Indicators

- At least 400 - 500 Yuanbaoshan fir planted seedlings survive after the first phase of the project.
- No human disturbance found around reinforcement sites.
- The seedlings have been planted outside of their current range, in three habitat types with different altitudes.
- A mechanism for long-term monitoring and after care for seedlings is in place after the first phase of the project.
- The comprehensive plan and annual report of the reinforcement has been issued after the first phase of the project.

Project Summary

Feasibility: Yuanbaoshan Nature Reserve was gazetted in 1982 and the habitat of Yuanbaoshan fir has been well protected (Huang. 1998), but the total global population is found in this reserve - around 300 mature individuals and 600 immature trees. Sub-adult trees are rare, many of these trees are less than 15 years old and regeneration is limited. From 1999, the Guangxi Institute of Botany started their research on this species. This has including the ecological and biological characteristics, discovering maturity of this species to occur between 40 - 50 years of age and the species preference for cool, wet, mountainous habitat. According to research on the scattered and restricted distribution pattern of the four fir species in southern China (Xiang, 2001), it has been concluded that, with a warming climate, the species' range is shrinking and it is being confined to the tops of the mountains. With global warming continuing, they are most likely to become extinct as they have nowhere further to go (Xiang, 2001). From 2012, direct conservation action started including quadrat sampling monitoring, regular patrolling, sustainable seed collecting, breeding, community engagement, community participatory conservation, genetic diversity research. This has been possible through the support of FFI China/the Global Trees Campaign, Guangxi Biodiversity Research and Conservation Association, Yuanbaoshan National Nature Reserve Management Bureau, Guangxi Institute of Botany and Guangxi Forestry Department. By the end of 2015, direct conservation action supported by these partners led to significantly reduced anthropogenic threats including trampling by tourists and cattle, grazing and non-timber forest product collection.

Yuanbaoshan fir cones © Ding Tao

Furthermore, in 2015, 2,000 seedlings were propagated successfully and the reintroduction plan was approved by all partners.

Yuanbaoshan fir habitat © Ding Tao

Implementation: Prior to the first phase of this reintroduction project, running from 2016 - 2020, some specific preparation was conducted. The nursery and seedling propagation techniques were developed, genetic diversity analysis was conducted and release sites were selected. Site selection was conducted by all partners, based on distance to a water source (minimized), distance to human disturbance (maximized) and in the Experimental Zone of Yuanbaoshan Nature Reserve as the rule of nature reserve only permitted the experiment to occur in the experiment zone. The result was a group of five sites, at the altitudes of 1,500 m, 1,600 m and 1,700 m, all under the altitude of the current habitat. The site above the altitude of the current habitat will be selected in other nature reserves for the second phase. The nursery was built and managed by Yuanbaoshan Nature Reserve, supported by FFI China, Seedling propagation and genetic diversity research was managed by Guangxi Institute of Botany. The genetic diversity research on the fir using ALFP (Amplified fragment length Polymorphism) approach showed a very low level, and does not show any likely sub-population (Wang *et al.*, 2004), this indicated that seeds should be collected from mother trees.

In total, 2,300 seedlings were propagated from the 31 mother trees, fieldwork aiming to maximize genetic diversity of seedlings collected also factored in ease and safety of access as well as seeds availability. Between 2016 - 2018, 250 seedlings were planted over the wet season of June in five sites over the three different altitudes and more than 250 seedlings will be planted in 2020 and 2021. During the planting, we ran an experiment to test the effect of adding root soil from the species' current habitat which is hypothesized to contain beneficial mycorrhizal hyphae. Half of all planting holes, n = 125, were filled with mycorrhiza -containing root soil and half were filled in with soil from the present habitat.

Post-planting monitoring: Immediately after planting and every six months hence, seedling height and basal diameter were recorded. Seedlings were cared for during weekly patrols of the reserve by forest staff. Any weeds or branches within a 75 cm radius around seedlings were removed and, in the dry season, watering took place weekly over the first year following planting. Guangxi Forestry Department have prioritized and funded this reintroduction program for the day-to-day management of the Reserve. Guangxi Institute of Botany undertake the

Post-plantation monitoring - Luo Dingming
Yuanbaoshan Nature Reserve

technical and training support to implement the reintroduction and monitoring while sharing monitoring data and research findings. By the end of 2019, across the five sites, there was an average height increase of 3.1 cm with an average 0.4 cm basal diameter increasing. There has been a high survival rate of 93%, with all 18 seedling deaths attributed to drought or the impact of fallen branches from the canopy. By the end of 2019, analysis of the mycorrhiza-containing root soil data showed that seedlings survival and growth in the two soil types was not significantly different. However, we believe this experiment should be repeated and, going forward, we plan to obtain expert guidance.

Major difficulties faced

- The Yuanbaoshan fir reaches maturity after 40 - 50 years, so it is difficult to see an impact on the population even after a successful first phase of reinforcement. This impacts the degree to which all partners feel committed and motivated towards delivering the reinforcement plan over the long term.
- The Yuanbaoshan fir tree is located on steep mountainous terrain in dense forest and is between 15 - 25 m in height which makes canopy seed collection and the monitoring of health and phenology challenging.
- The cone-bearing of the Yuanbaoshan fir exhibits a cyclical pattern in terms of cone quantity, which significantly varies between years (Tang *et al.*, 2001). Over the whole Yuanbaoshan fir population between 2006 to 2019, cones appeared in large numbers (more than 200 cones on every tree) on average every fourth year. This irregular production of cones means collecting seeds for the nursery is very opportunistic leading to low numbers of seedlings.
- The research on mycorrhiza-containing root soil of the natural habitat needs to be refined and developed over time.

Major lessons learned

- Basic biological, ecological and genetic research have been extremely important to prepare appropriate seedlings and choose planting sites. Without this basic research, much time and resources would have been wasted.

- Achieving zero human disturbance in the nature reserve, especially in the core zone of the Nature Reserve, through enhancing patrols, awareness raising and increasing community participation through the piloting of ecotourism has been crucial to the project's success.
- Effective collaboration between the scientific team and the reserve staff and managers has enabled the success of the project and a joint stewardship of the data generated. This has been due, in part, to a stable workforce and regular communication between the partners involved.
- Actively ensuring low project costs through promoting in-kind funding has maximized the sustainability of the project, especially considering its long term nature.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- By the end of 2019, 250 seedlings have been prepared and planted, with a reintroduction plan supported by all partners. More 250 seedlings will be planted in 2020 and 2021, and there are 2,000 seedlings growing in the nursery.
- The scientific team have been cautious and within this first stage carefully considered the adaptive capacity of the fir in the plan of the reintroduction, and so the sites selection have been implemented carefully.
- Clearly defined roles within the team along with a sustainable monitoring and after-care regime in place, that have make sure the sustainability over a long time for the success of the reinforcement.
- The comprehensive plan and annual report of the reinforcement has been established which will lead to future monitoring, evaluation and adaptive management.

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Reintroduction planning and implementation of rare species Danyang aster, Yeoju, Republic of Korea

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Introduction

Aster altaicus var. *uchiyamae* Kitam is a biennial plant in the family of Compositae (Aster), collected in 1902 by T. Uchiyama in Suanbo area, South Korea and recorded in 1937 as a variant of *A. altaicus* by Kitamura. It is endemic to Korea, and is called 'Danyang aster' in Korean. Rosette type of above ground parts remain in winter and it blooms in the following year. Wild habitats are in the channel fill deposits and soil is composed of sand and gravels. It is known that the species is distributed in a wide range of the channel fill deposits of southern Hangang River. However, currently it is found in very limited areas. In 2005, the Ministry of Environment in Korea listed the species in the Second Class of Endangered Species and the Korea Forest Services listed it in 2002, as a Rare and Endemic Plant.

The population size of the species depends on the geographical conditions of the habitats, *i.e.* flooding (NIBR, 2010). It has rosette form to tolerate the harsh ecological conditions such as low minerals in soil, and high temperature and radiation in summer (Matsumoto *et al.*, 2000). Germination of seeds begins spring but it last longer period because of the moisture contents in the coarse sandy soil (Mitsuko *et al.* 2005).

Danyang aster in its natural habitat

Transplanting the species in a quadrat

The wild habitats of the species became narrower due to the construction of dams or barrages that block water flow, and dredging. These pressures changed the seasonal flooding pattern, introduced the competitive vegetation like Gramineae and became unfavorable environment for the species (Muranka *et al.*, 2012). We have been monitored, since 2002, the population of the species in a natural habitats on Dori Island, Yeoju, Korea. The number of individuals in the area and the size of the distribution kept decreasing dramatically. Some sub-groups of the species on the Island disappeared during monitoring. We aim to reintroduce propagated plants to prevent the extinction of the species and to increase the density/number of individuals.

Goals

- To plan both *in-* and *ex situ* conservation strategies of *A. altaicus* var. *uchiyamae*.
- To reintroduce and recover the number of individuals to the level in 2012 (minimum number, 23 plants/m^2).
- To connect with fragmented populations in Dori Island.
- To remove competitive and invasive vegetation and sediment preventing plant growth.
- To implement a regular monitoring to the translocated site over 10 years.

Success Indicators

- Natural increases of individuals from the reintroduced ones.
- Density balances of age classes.
- Connecting the neighboring meta-populations.
- Red List category of the species changes from endangered to vulnerable.

Project Summary

Feasibility: Most of natural habitats disappeared and the largest natural habitat was on Dori Island, Yeoju. However, the ecosystem of the area was disturbed since 2010 by invasion of other vegetation due to the construction of dams and dredging of rivers discovered during monitoring activities. The populations are

Overview of habitat and surrounding areas

fragmented and species numbers reduced rapidly. Fortunately, the species produces many seeds and seeds easily germinate in favorable environment. In addition, seeds disperse by wind that facilitate to expand the habitats naturally after the reintroduction. Reintroduction of either whole plant or seeds to increase the individual numbers. In order to increase the efficiency of the reintroduction, we collected seeds from the several populations to maintain the genetic diversity, propagate in green house and transplant in the target areas.

Implementation: The species grows on substrate near rivers and these soils are filled with gravel and rocks and lack nutrients. However, the natural habitats have deposited clay and sands covered with the gravel that has resulted in the invasion of competitive vegetation. There is a need to remove excessive soils, gravel and rocks and resulting competitive vegetation. After restoration of the soil environment for the species, 2,000 propagated plants were transplanted in a density of 5 plants/m^2. Continual removal of excess soils and competitive vegetation until the species is well established. Protective nets were installed around the small plants to prevent grazing by wildlife and survival and growth of the reintroduced plants was monitored until the end of the year.

Post-planting monitoring: Plants were reintroduced in July 2019 and observed the survival, flowering, and fruiting throughout the winter. Most of plants survived and with a survival up to 93%. Despite the protection net animals grazed some plants to death. The plants have a rosette type of growth, which is typical of this species. Some individuals, 25 of them, grew fast and produced flower stalks and seeds. Among them, 14 plants had aborted seeds and 11 had sound seeds that were expected to disperse the following spring.

Major difficulties faced

- A lack of research on the ecology of the species due to the rareness of the species and a narrow range of habitats.
- Continuous climate changes especially high temperature and drought during the spring in the habitats that hinder germination and growth of seedlings.
- Construction of dams in river changed geographical features of the habitats that is not suitable for this species. It needs regular artificial management to

restore its condition to previous favorable conditions.

- Increased damage by animals.

Major lessons learned

- The species is bi-annual but some have shortened life cycle that can complete within a year.
- Period of high temperature and drought kept increasing in the natural habitat while we carried out our monitoring.
- The precipitation patterns have changed and the period of flooding has increased that results in plant death. There is a need to consider the “ground level” for reintroduction to avoid flooding the plants for a long time.
- More genetically diverse sources provided to maintain the diversity.
- Long-term monitoring on the ecosystem including the species is needed.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Reintroduced plants established well and survived at the first trial restoration site.
- Survival rates was high and some individuals produced flowers and seeds within a year although there are very few.
- It is early to determine the success of the project because the period of monitoring is very short.
- Artificial management was conducted to increase survival rate; it will take more time to stabilize without human intervention.

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Conservation and re-introduction of red horntail orchid, a nationally extinct orchid species in Singapore

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Introduction

Bulbophyllum maxillare is a beautiful native species of Singapore. It has two common names, first is the Chinbone *Bulbophyllum* and the second is the Red horntail orchid. Recent taxonomic revision (Vermeulen, O'Byrne & Lamb, 2015) has put *Bulbophyllum blumei*, *Bulbophyllum masdevalliaceum* under *Bulbophyllum maxillare*. The warm growing epiphyte grows on trees from sea level up to 800 m. The ovoid pseudobulb is light green in color, up to 4 cm long by 1.5 cm in diameter, each bearing a single soft fleshy leaf, 8 - 15 cm long by 1.5 - 2.5 cm across. The flower measures 6 - 9 cm long by 2 - 2.5 cm across. The petals are reddish purple with light yellow edges. The lip is purple and yellow.

Close up of *Bulbophyllum maxillare*

The species is distributed in West Malaysia, Sumatra, Borneo, the Philippines, Papua New Guinea, the Solomon Islands and Australia. In Peninsula Malaysia and Singapore, the species are found growing on old mangrove and near rivers. In Singapore, it was first collected at Kranji in 1890 and was last collected by Henry Ridley, from Kranji in 1892. Since it is nationally extinct, we propagated the species from nursery materials and the species was successfully reintroduced to many areas in Singapore.

Goals

- To conserve the species by raising seedlings from seeds.
- To reintroduce the seedlings to its natural habitat, parks and roadside trees.
- To monitor the growth of reintroduced plants.

Success Indicators

- To propagate the species from seeds effectively.
- To ensure the species are reintroduced successfully into the natural habitats, parks and roadside trees.
- To ensure the reintroduced plants continue to survive after the reintroduction.
- To find out the best conditions for reintroduction.

Planting of orchids on rain trees

Project Summary

Feasibility: *Bulbophyllum maxillare* was first collected by J.S. Goodenough at Kranji in 1890 and was last collected by the director of the Botanic Gardens, Henry Ridley, from Kranji in 1892. Since then, the species has not been rediscovered and became nationally extinct. The species was propagated from seeds by using nursery materials from Peninsular Malaysia.

Implementation:

Seedlings culture: Propagation of seeds is the most effective way to conserve orchid species. A modified KC medium is used for the germination of seeds; once germinated, seedlings are transferred to a second medium (Arditti, 1977; Yam, 2013; Yam *et al.*, 2013). Seedlings are grown to about 4 cm tall, which takes about 12 months. They are then ready to be transferred to the nursery.

Propagation of seedlings in nursery: Seedlings should generally be planted in groups of about 30 into community pots, using small charcoal pieces and brick chips. When the seedlings are well established, they are divided and planted individually on slab of wood. Plants are ready for reintroduction when each seedling has three to four new shoots and a healthy root system.

Reintroduction: The seedlings were planted on trees during the rainy season, from the beginning of October to December. .

Time of planting: The best time for planting tropical orchid seedlings is before or during the rainy season. In Singapore, the rainy season starts around October and lasts until January. Most of our plantings have been carried out from late September through November. Seedlings planted during these periods have established themselves quickly, producing new shoots and roots. Once the roots of epiphytic species have attached themselves to the bark of the host tree, they can absorb water and nutrients directly from the environment.

Planted orchid growing vigorously at Zhenghua Nature Park (*left*) & flowering at Pasir Ris Park (*right*)

Host trees: Trees that support more epiphytes tend to be better hosts than those with fewer epiphytic plants, as their presence indicates that conditions are suitable for epiphytic species. Of all roadside trees that are suitable for epiphytes, the rain tree fosters the most luxuriant growth of epiphytic plants. The most common epiphyte found on rain trees is *Asplenium nidus* (Bird's nest fern) and *Dendrobium crumenatum* is the most common orchid.

Planting: The seedlings were planted under the proper environmental conditions, for this species, they were planted on mature host trees with other epiphytes and with 30 - 50% shade. Slabs of wood with established seedlings were secured on tree trunks and/or branches with horticultural wires and/or nails. Seedlings were reintroduced to Sungei Buloh, Singapore Botanic Gardens, Telok Blangah Hill Park, Dairy Farm Nature Park, MacRitchie Reservoir, Pulau Ubin, Holland Road and Napier Road, Pasir Ris Park and several neighborhood parks throughout Singapore.

Post-planting monitoring: Some of the reintroduced plants have grown for more than 10 years in various nature reserves, parks and on roadside trees. Some 80 - 90% of the plants planted under the optimal environmental conditions have survived, and most of them have flowered and fruited. Our hope is that these plants will be able to self-propagate by seed; in this way, our reintroductions can help to enrich the development of the ecosystems in which they have been planted. Their flowers may attract and support viable populations of pollinators, which may in turn allow the development of viable seeds. These seeds may be dispersed to nearby locations, and if mycorrhizal fungi are present, germination will be possible, allowing the recruitment of new native orchids into these habitats.

Major difficulties faced

- Seedlings planted at exposed areas did not survive.
- Some of the seedlings were removed by monkeys.

Major lessons learned

- The species grow more vigorously in areas with 50 - 70% shade with high relative humidity.
- Plants that are grown under 50% shade flower more often than those that are grown under 70% shade.
- Trees that support more epiphytes tend to be better hosts than those with fewer epiphytic plants.
- The best time for planting is during the rainy season.

Success of project

Highly Successful	Successful	Partially Successful	Failure

Reason(s) for success:

- Seedlings were grown to a mature size in the nursery before reintroduction
- Reintroduced plants were planted on the right host trees.
- Reintroduction were carried out just before or during the rainy reason to ensure the seedlings establish well after planting.
- More than 80% of the plants planted under the optimal environmental conditions have survived, and many of them have flowered and fruited.

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