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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
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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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