

# HOW GENETICS CAN HELP SAVE FRESHWATER FISHES

Artwork: Theresa L Cole

Conservation geneticists **Luciano Beheregaray**, **Catherine Attard**, **Chris Brauer** and **Michael Hammer** report on innovative new ways to conserve and restore endangered fish populations.

**G**lobally, most freshwater habitats are moderately or highly threatened. They are often more vulnerable than terrestrial ecosystems of comparable size because they are linear, making it difficult for aquatic animals to recolonise regions after fragmentation and local extinctions. Freshwater fishes are of particular concern, for they are highly vulnerable to decline following disturbance. They have the highest extinction rate of all vertebrate groups.

The extinction crisis faced by freshwater fishes globally is an Australian reality. In the Murray–Darling Basin more than half of the 50 or so native fish species are considered threatened, and overall native fish populations have probably diminished by about 90% since European colonisation. Overall allocation of water for irrigation, altered flow regimes and record droughts have caused dramatic water shortages. Other threats include reclamation of wetlands, damage to stream banks and vegetation, increased nutrients, and predation and competition by introduced species such as carp, redfin, trout and mosquitofish. There is a lack of community and government awareness about the plight of native fishes. Efforts to protect and restore populations have mostly been directed at the few large species valued for fishing. Many of the smaller species need urgent attention.

In 2006 the water crisis in the Murray–Darling Basin reached a tipping point when a long drought, possibly

heralding a changed climate, peaked with record low inflows. By 2008 much of the lower Murray system was dry. Several native fishes were on their way to local extinction when a team of governmental and nongovernmental organisations mounted a rescue operation. South Australia's drought action plan was implemented to save five threatened species: the southern purple spotted gudgeon, Murray hardyhead, river blackfish, Yarra pygmy perch and southern pygmy perch. They were monitored in many sites, and rescues were conducted as their situation became perilous. Some sites were provided with water and some fish were moved to areas with water. Others were collected for captive breeding, with the goal to restore populations once the drought had broken.

Our group at the Molecular Ecology Lab at Flinders University led a program to captive breed four of the species using new genetic methods to assess and improve their survival prospects under changing conditions.

## The role of genetics in conservation

The Murray–Darling Basin is highly fragmented, with a multitude of dams and weirs and habitat losses that limit migration between fish populations. One of the problems faced by rare species in fragmented habitats is low genetic diversity due to inbreeding (when close relatives breed with each other) and genetic drift (the loss of genetic variants due



In 2008 and 2009 much of the lower Murray system dried up, necessitating the rescue of several threatened fishes about to go locally extinct. This old paddle steamer (used for accommodation) was left high and dry as the Lower Lakes near Goolwa, South Australia, receded. Photo: Michael Buddle

to chance during reproduction). Low genetic diversity is likely to reduce the reproductive fitness of rare fishes and limit their ability to adapt to environmental and climatic changes. Small, isolated populations are also highly vulnerable to chance events, such as disease or extended drought.

We are working in the emerging field of restoration genetics to address these genetic problems while recovering threatened fish populations. Genetic approaches are likely to play a major role in conservation in the future, given the extent of habitat fragmentation and the rate of environmental change.

The aim of 'genetic-based captive breeding' is to preserve as much as possible the genetic diversity found in wild populations. By working out family relationships (or pedigrees) we can design crosses in the laboratory between unrelated individuals to prevent inbreeding. Another way of conserving genetic diversity that is slowly becoming more popular is 'genetic rescue' in the wild. Individuals are moved between recently isolated populations to restore connectivity or to supplement local levels of genetic diversity. Genetic analysis can reveal a great deal of information about a species including life-history, ecological, and behavioural characteristics critical for their long-term persistence, allowing us to take action to optimise their potential to adapt to future environmental changes.

### Genetically informed captive breeding

About 250 adults of four of the five threatened species were rescued in the lower Murray system prior to their local extinction. We undertook breeding of the Yarra and southern pygmy perch and the Murray hardyheads, while the southern purple spotted gudgeons went to a partner organisation. Unfortunately, there were too few river blackfish for captive breeding.

First, we analysed their DNA (from fin clips) to estimate the relatedness between each fish. We then played matchmaker and designed crosses between unrelated individuals. These were grouped in tanks to mimic the natural breeding system of each species. For each species we created 7 to 11 breeding groups, each housed in a 1000 or 2000 litre tank. We were delighted to welcome into the world 1100 to 2000 offspring for each species

over two breeding seasons in 2011 and 2012. Even more pleasing was that there was minimal or no loss of genetic diversity in the first generation born in the lab compared to the diversity found in the rescued, wild caught populations.

Finally, in late 2011 after the drought had ended, with jubilation, extensive media coverage and the participation of South Australia's premier and water minister, the first 1000 offspring were released into the wild. There were further releases over the next two years into the lower Murray.

### Genetic monitoring

We then used a genetic approach to monitor the fate of the offspring in the wild. Between 2011 and 2014 our partner organisations (SARDI Aquatic Sciences, Aquasave-Nature Glenelg Trust, and South Australian Department of Environment,

Water and Natural Resources) captured some of each species, removed fin tissue for analysis and returned them to the wild. We compared genetic information from the fin clips with information from tissues we had collected before the captive-born fish were released.

So far we have done this for the two pygmy perch species. The results for the southern pygmy perch were very encouraging. Of the 71 captured during initial monitoring, 19 were fish that had been released and 52 were wild-born offspring. This showed that the fish are both surviving and breeding. The prognosis for the reintroduced Yarra pygmy perch is not so certain. They have naturally very low levels of genetic variation, one of the lowest ever reported for a freshwater fish, which makes it difficult to genetically distinguish between them. Of the 13 captured during early monitoring we classified eight as possible offspring of the captive-bred individuals. It will be some time before we know if the reintroduced populations of either species are self-sustaining.

If they aren't self-sustaining, we could continue the genetic-based captive breeding to regularly supplement the wild population. But there is a high risk that the captive population will lose genetic diversity and adapt to captivity, reducing the survival, reproduction and growth of those released, and impairing their ability to adapt to change. ▶

Genetic approaches are likely to play a major role in conservation in the future.



Southern pygmy perch. Photo: Michael Hammer



Yarra pygmy perch. Photo: Michael Hammer



Southern purple spotted gudgeon. Photo: Michael Hammer



Murray hardyhead. Photo: Gunther Schmida

## MURRAY–DARLING FISHES ON THE BRINK

### **Southern pygmy perch** (*Nannoperca australis*)

This fish, which grows to about 8.5 centimetres, lives in small groups on the vegetated margins of still or gently flowing streams, billabongs and other waterways. It occurs across southeastern Australia, but recent genetic work suggests it could consist of two species, one in the east and the other more inland.

The southern pygmy perch was once widely distributed in the Murray–Darling Basin, probably continuously from the upper to the lower reaches of the Murray. With recent large-scale extinctions from floodplain wetlands in the middle Murray, it survives now in small population fragments mainly in tributaries of the upper Murray and pockets of the Mount Lofty Ranges and Lake Alexandrina in the lower basin. Our genetic tests show that its populations are isolated with no gene flow and low genetic diversity. Many appear to have been affected by inbreeding.

### **Yarra pygmy perch** (*Nannoperca obscura*)

This fish, which grows to about 7.5 centimetres, lives in small groups (sometimes with southern pygmy perch) in slow-flowing or still waters with lots of vegetation. It occurs patchily in Victoria and South Australia. The fragmentation of its habitat and its poor dispersal ability make it highly vulnerable to local extinctions. We have found that it has very low genetic diversity. It is listed nationally as vulnerable.

### **Southern purple spotted gudgeon** (*Mogurnda adspersa*)

This fish, which grows to about 12 centimetres, lives among plants, rocks or snags in wetlands and creeks with slow-moving or still waters. It occurs in tropical to temperate coastal drainages in eastern Australia, and in the Murray–Darling Basin. Genetic evidence suggests it consists of more than one species. It is considered common on the coast but endangered in the Murray–Darling Basin. Scattered populations survive in northern Darling River tributaries, but the species was considered extinct across a large area of the southern Murray and Murrumbidgee Rivers until the recent discovery of a population in a small wetland in the lower Murray.

### **Murray hardyhead** (*Craterocephalus fluviatilis*)

This fish, which grows to 7.5 centimetres, lives on the margins of lakes, wetlands, backwaters and billabongs in the lowland floodplains of the Murray and Murrumbidgee river systems. It was once widespread in the Murray–Darling Basin, but is now extinct in NSW and listed nationally as endangered.

### **River blackfish** (*Gadopsis marmoratus*)

This medium-sized fish, which grows to about 45 centimetres, lives in freshwater streams in the southeast, typically in habitat with woody debris (snags). It has suffered substantial declines, thought to be mainly due to siltation of streams and changes to river flows. It is considered endangered in South Australia.



This is a release site near Hindmarsh Island for fish born in the genetic captive breeding program. Photo: Chris Brauer

Our findings will enable intelligent translocations to boost genetic connectivity and help populations survive.

We tested the genetic risks of continued captive breeding in southern pygmy perch left over from the restoration program. There was minimal loss of genetic diversity in the first generation, but the second generation were considerably less diverse than the original rescued population. This was due to using individuals from only a proportion of the original breeding groups, as well as a skewed reproductive contribution of breeders (some fish produce more offspring than others). This multigenerational captive breeding program should not be used as the sole reservoir of genetic diversity for supplementing reintroduced wild populations.

### Genetic rescue

Rare Murray–Darling Basin fishes may struggle to persist in many sites given the range of threats, small populations and low levels of genetic diversity. Several species which probably once inhabited much of the river system are now represented by a few small populations, particularly in the highly fragmented upper reaches of the system.

One way to boost their prospects is to supplement declining populations with fish from genetically healthier populations. To identify populations that need genetic rescue and those that could be a source, we need to analyse the distribution of genetic diversity and migration patterns across the range of a species. Ideally, we would also assess how regional differences in climate, topography etcetera have affected genetic diversity and the persistence of local populations. That can be achieved with new powerful ‘genomic’ techniques that generate data from thousands or millions of DNA regions, as opposed to conventional ‘genetic’ studies of a dozen or so DNA regions.

In recent years our team has amassed extensive DNA data for over 3000 fish of several species across their ranges. For some species we have also characterised environmental conditions at each sampling site. We can learn a great deal from their DNA, which enables us to distinguish natural patterns from those resulting from recent human actions.

We found that the small, isolated populations are genetically distinctive. The DNA data suggest their isolation is sometimes very recent, post-dating European colonisation. They also have signatures in their DNA of recent declines, indicating they had higher levels of genetic diversity in the past.

Although the basin-wide picture is gloomy, our findings will enable intelligent translocations to boost genetic connectivity and help populations survive. New genetic material in some populations can build their resilience to climate change. We

discovered that southern pygmy perch populations from the lower Murray–

Darling Basin are adapted to hotter and drier conditions than those elsewhere in the basin. Translocating them to populations in upper catchments could help those populations adapt to the increasing aridity and climate variability predicted for the basin. This is the opposite to the typical ‘local is best’ policy of using local sources for restoration. ■

**READING:** Attard CRM, Möller LM, Sasaki M, et al. 2016. A novel holistic framework for genetic-based captive breeding and reintroduction programs. *Conservation Biology* 30:1060–69 ■ Brauer C, Hammer MP, Beheregaray LB. 2016. Riverscape genomics of a threatened fish across a hydroclimatically heterogeneous river basin. *Molecular Ecology* DOI: 10.1111/mec.13830 ■ Cole TL, Hammer MP, Unmack PJ, et al. 2016. Range-wide fragmentation in a threatened fish associated with post-European settlement modification in the Murray–Darling Basin, Australia. *Conservation Genetics* DOI: 10.1007/s10592-016-0868-8 ■ Hammer MP, Bice CM, Hall A, et al. 2013. Freshwater fish conservation in the face of critical water shortages in the southern Murray–Darling Basin, Australia. *Marine and Freshwater Research* 64:807–21.

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