



How Can We Improve Frog Conservation in Fragmented Landscapes? Closing the Gap with a Novel Genetic Approach

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Habitat clearing, degradation, and fragmentation is occurring faster than ever before, causing species declines and extinctions. Many landscapes now exist as heterogeneous mosaics of land-use types, with patches of native vegetation between vast expanses of modified habitat. Within these landscapes, a significant concern is how species' movements will be impacted, and this is especially true for frogs as their dependency on aquatic and terrestrial environments makes them particularly sensitive to habitat modification.

At the landscape level, movement is vital for population persistence as it helps to maintain genetic connectivity. Populations that suddenly become isolated across the landscape are at an increased risk of becoming inbred and experiencing negative fitness consequences, such as reduced survival and development. Maintaining genetic diversity in fragmented populations is therefore crucial for preventing further species' declines.

However, the degree to which habitat fragmentation leads to population fragmentation depends on the landscape and the species. We know that mobility and gene flow is often restricted in fragmented landscapes in taxa that are highly mobile and have large area requirements (such as many

birds and mammals). The effects of habitat fragmentation on the genetic structure of less mobile taxa have received comparatively little attention. Information on the genetic structure of frogs under habitat fragmentation is lacking, and equally, detailed movement data, particularly of juveniles (whose dispersals are thought to be key to population connectivity), is unavailable for most frog species. Information paucity is one of the greatest barriers to effective frog conservation, and this is especially true for Australian frog species, of which more than one in six are threatened with extinction.

It is estimated that as many as seventy percent of Australia's frog species are intolerant of habitat modification. However, protected areas and land set aside for nature conservation make up less than a quarter of our nation's land area. Unless more effective land management practices are adopted, Australian frogs are likely to undergo further declines.

For species occurring in fragmented habitats, identifying the levels of genetic diversity and gene flow across the landscape can help to identify vulnerable populations, inform the most appropriate scales of land management, and prioritise areas to protect or restore. This information can vastly improve

conservation outcomes. Unfortunately, studies on gene flow and movements of frogs in modified landscapes have been mostly limited to European and North American species that undertake annual migrations to breeding ponds. There is little data from Australian frog species, which have vastly different life histories and ecological associations, including stream breeders that do not undergo distinct annual migrations.

In particular, we know surprisingly little about the effects of habitat modification at the species level. However, it is clear that different species, sometimes even those that are closely related and occur in the same habitats, can respond in contrasting ways to habitat modification, with some declining and others thriving. The project sought to understand why using the Endangered booroolong frog (*Litoria booroolongensis*) and the common stony creek frog (*Litoria wilcoxii*) as model species.

Booroolong frogs were once broadly distributed across New South Wales

Top: Gracie is a PhD candidate at the Centre for Ecosystem Science at the University of New South Wales and the Australian Museum Research Institute. Her research focuses on the effects of habitat modification on frogs. She also works as a FrogID validator at the Australian Museum, where she identifies frog species from audio recordings submitted to the national citizen science project, FrogID.



The Endangered booroolong frog (*Litoria booroolongensis*) in typical stream-side habitat. Image: Gracie Liu.



A male stony creek frog (*Litoria wilcoxii*) in breeding colour. Image: Gracie Liu.

and north-eastern Victoria, but their numbers have plummeted since the mid-1980s. Today, their declines continue to be strongly driven by habitat loss and degradation. They now only occur in a small part of their former range and are listed as Endangered under the Environment Protection and Biodiversity Conservation Act 1999 and Critically Endangered by the International Union for Conservation of Nature. They are rare in the Central Tablelands and were even presumed extinct from the Northern Tablelands for more than forty years (until they were rediscovered in 2017).

Yet, stony creek frogs appear to be thriving in these very landscapes. Despite being closely related and sharing many superficial similarities with booroolong frogs (similar life histories, physical appearance, broad habitat preferences), both species occupy riparian habitats and often the same stretches of stream. However, stony creek frogs are common and non-threatened.

Given the importance of genetic diversity and population connectivity for ensuring population resilience, could differences in gene flow and genetic variation be shaping the species' different responses under habitat modification and fragmentation? The project will use Diversity Array Technology sequencing (DARtseq), a next-generation sequencing approach to test this prediction. Specifically, the project aims to:

1. Assess and compare genetic diversity and gene flow in the booroolong (declining and endangered) and the stony creek frog (common and secure) within a highly fragmented landscape,
2. Determine whether the genetic structure of these two species is related to their threat status, and
3. Use the information to identify management units for species conservation.



Over the spring and summer of 2020-2021, the project collected over 120 genetic/tissue samples from booroolong and stony creek frogs across their shared geographic range in the New South Wales Central Tablelands. Under an approved scientific license and animal ethics protocol, a small tissue sample from one of the frog's toe pads (which can regrow) was collected; collecting a tissue sample guarantees that there will be enough suitable quality DNA to analyse. Booroolong frogs persist in several disjunct locations in the region, which features a range of land-use types, including pine plantations, agricultural and private land, and native vegetation. The project sampled several sites representing many of the major river systems in the region that are known to support booroolong and the more common stony creek frog.

These genetic samples will be sequenced and analysed using DARtseq. DARtseq is more time- and cost-effective than traditional molecular techniques and improves power to detect population differentiation at small scales relevant to management. It will allow the researchers to explore and directly compare the fine-scale genetic structure within and between booroolong and stony creek frog populations.

The data from the project will enhance the ability to assess the conservation value of various land management techniques for species living in highly fragmented landscapes. For example, it can help to determine whether increasing connectivity between populations (via landscape restoration or habitat corridors) will be a worthwhile strategy.

The information acquired from the project will also contribute directly to the conservation management of booroolong and stony creek frogs. Combined with the detailed movement and microhabitat use data that has been gathered from radiotracking these frogs, the project will help build a comprehensive picture of the species' movements and habitat requirements. With this information, we can identify genetically unique or vulnerable populations, prioritise important habitats and, ultimately, improve the conservation management of some of Australia's most vulnerable species.

FUNDS PROVIDED BY THE AUSTRALIAN WILDLIFE SOCIETY will be used to sequence and analyse the frogs genetic/tissue samples using Diversity Array Technology sequencing (DARtseq).



This Page: The New South Wales Central Tablelands consists of a variety of habitats, including pine plantations, agricultural land, and national parks. Images: Jodi Rowley and Gracie Liu.