Research Note | September 2025

Trialling eDNA to detect giant burrowing frog

This note summarises the results of a pilot project that investigated if environmental DNA (eDNA) could be used to detect giant burrowing frog (*Heleioporus australiacus*) in the Eden region. This work was carried out by scientists at EnviroDNA and ecologists from Forestry Corporation of NSW as part of the Coastal Integrated Forestry Operations Approvals (IFOA) Monitoring Program. An independent expert peer reviewed the results. This project is one way the monitoring program is considering new techniques and testing how they can be used and applied.

The *giant burrowing frog is listed as vulnerable in NSW under the* Biodiversity Conservation Act 2016. *Recent monitoring of three known populations in the Eden region using traditional survey methods have not detected the species in two locations. The eDNA pilot investigated if DNA present in water samples could be used to improve detection rates or identify new populations. eDNA is a relatively new and powerful technique, which could provide cost-effective means to enhance existing monitoring. The pilot found that while eDNA successfully identified giant burrowing frog in pools where tadpoles were present, results were inconsistent in flowing water or at nearby downstream locations. Further investigation of detection distances and stream flow conditions are needed to fully understand the usefulness of eDNA to detect giant burrowing frog.*

Since 2008, the Forestry Corporation of NSW (FCNSW) have monitored the giant burrowing frog on state forests under a Species Management Plan (SMP) required under the Coastal Integrated Forestry Operations Approval (IFOA). The SMP identifies three population management zones within the Eden region: Broadwater, Yambulla, and Yurammie. The SMP aims to identify breeding sites, record site characteristics to predict potential habitat, investigate adjacent breeding sites, monitor population changes over time, and determine efficient survey techniques.

The SMP requires FCNSW ecologists to conduct surveys to monitor adult frogs and tadpoles in suitable habitats. However, the traditional survey methods have only found significant frog numbers in Broadwater, with no recent sightings in Yambulla and Yurammie. As such, low detection is hindering effective population monitoring.

The Natural Resources Commission (the Commission) worked with a cross-agency technical working group, scientists, ecologists, and an independent frog expert to investigate if environmental DNA (eDNA) could provide an alternative survey method to detect giant burrowing frog. Studies have found eDNA a highly effective and cost-efficient technique to rapidly determine the presence of rare aquatic species.[i]

Adult giant burrowing frog (*Heleioporus australiacus*)
Image credit: Alarmy

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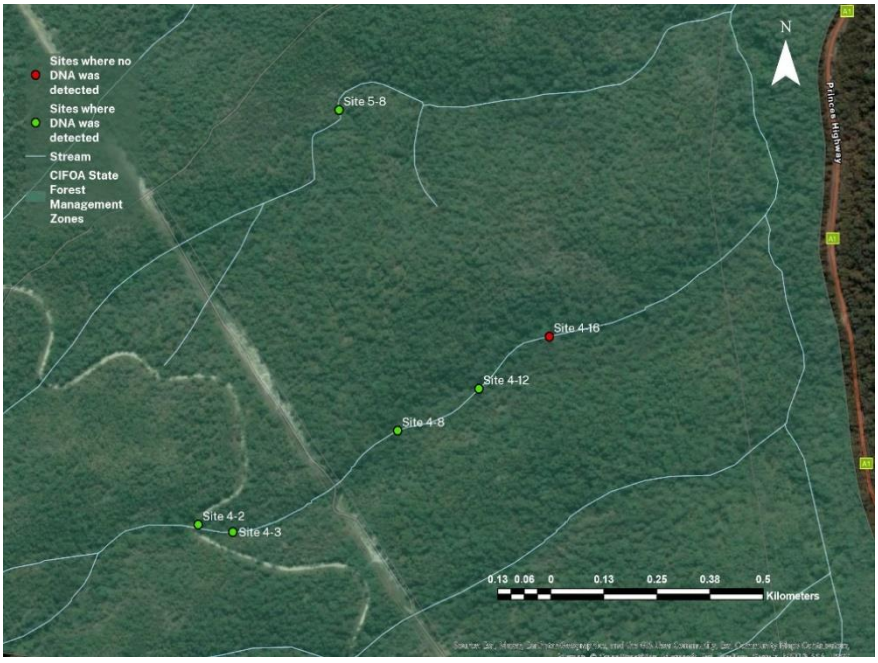
What is eDNA? How can it help monitoring?

eDNA refers to DNA extracted from samples from environments such as water or soil. Species that live in or near the water or soil leave behind traces of their DNA in faeces, mucus or in shed skin or hair. For aquatic species, water samples are filtered to capture DNA, which is then analysed in a laboratory using an assay to find what species the DNA belongs to. By comparing the collected DNA sequences to a database of known species, the presence or absence of the species can be determined. Before this analysis can happen, the assay for the target species must be developed and tested, first in the laboratory and then using field samples. This helps researchers understand how well the assay works for different population sizes, conditions and distances from where the species is living. Once established, the cost savings from using the eDNA approach include less time in the field to determine if the species is present.

Pilot design and sampling

The Commission engaged scientists from EnviroDNA to test and validate an eDNA assay for the giant burrowing frog, recommend the sampling approach, and analyse the DNA samples collected. The scientists recommended sampling five different sites in different conditions (wet/dry) and collecting five samples at each site, along with recording physical observations of the presence or absence of giant burrowing frog tadpoles. Each collected sample would then be tested three times in the laboratory for the presence of giant burrowing frog DNA.

As adult giant burrowing frogs rarely enter the water, detecting adults using water samples would be highly unlikely, so sampling was restricted to seasons when tadpoles were present.



Survey sites in the Broadwater Population Management Zone

Ecologists from FCNSW collected samples in the Broadwater Population Management Zone, where recent surveys had found the frog was still present. The species must be present when testing the method. The ecologists also recorded the number of tadpoles in the pool at or upstream of each site where eDNA samples were collected.

They sampled five sites in dry conditions when in-stream pools were disconnected, and three sites in wet conditions when pools were connected by flowing water.

A long period of dry weather meant the wet condition survey took place a year after the dry condition survey. It was not possible to resurvey all sites that had been surveyed in dry conditions.

Results of eDNA pilot to detect giant burrowing frog

EnviroDNA analysed the collected samples to test field sensitivity of the eDNA assay and determine if it can reliably detect the presence and absence of the species. While giant burrowing frog was detected using eDNA methods, the results were inconsistent. The frog's DNA was positively detected at 4 out of 5 sites sampled during the first sampling event (pools disconnected) and 2 out of 3 sites sampled during the second sampling event (pools connected). However, results were mixed, indicating there are some conditions where the assay may have limited sensitivity. This could be due low abundance of tadpoles, because the species occupies the site infrequently, or due to flow conditions at the time of sampling.

While giant burrowing frog could be detected, further sampling is needed to fully understand if the eDNA approach can be used for this species over the range of conditions anticipated.

Results of giant burrowing frog DNA detection from each site surveyed

Site No.	Site description	Results for detection of giant burrowing frog DNA
Disconnected pools in dry conditions – five samples collected at each site		
4-16	Separated pools, some with minimal visibility. No tadpoles observed within 300 metres upstream.	▪ No DNA detected in any samples
4-2	Separated pools. Approximately 30 tadpoles observed.	▪ DNA detected in all samples
4-3	Sample 1 - 5 or more tadpoles in top shallow pool; samples 3 and 4 - 10+ tadpoles observed in pools; samples 2 and 5 - no visibility in pools.	▪ DNA detected in all samples
4-8	No tadpoles observed. Creek disappeared upstream but water was flowing throughout the site.	▪ DNA detected in two samples ▪ No DNA detected in three samples
5-8	Separated pools. Sample 1 – 1 tadpole observed in the pool sampled and 2 tadpoles observed in the pool above; samples 2 to 5 – no tadpoles observed.	▪ DNA detected in three samples ▪ No DNA detected in one sample ▪ One sample was inconclusive*
Connected pools with water flow after rain – five samples collected at each site		
4-8	Lightly flowing creek with numerous tadpoles observed in pool.	▪ DNA detected in all samples
4-12	Creek with water flowing lightly. 10 tadpoles observed approximately 100 metres upstream.	▪ DNA detected in three samples ▪ No DNA detected in one sample ▪ One sample was inconclusive*
4-16	Tadpoles observed 300 metres upstream.	▪ No DNA detected in any sample

* Inconclusive results indicate one of three DNA tests for a sample detected giant burrowing frog DNA.

EnviroDNA used the results to model the optimum number of samples needed to detect the giant burrowing frog and to recommend an appropriate sampling protocol. They found testing three filtered water samples collected at each site, with two repeat tests per sample, achieved a detection probability of 95 percent or higher.

More research will address limitations

An independent expert reviewed the pilot method and findings, confirming that eDNA can reliably detect the presence of giant burrowing frogs in areas where tadpoles are present. However, the method needs further investigation to understand its usefulness to detect the presence of giant burrowing frog under a range of flow conditions and distances from known tadpole locations. Increasing the number of sites surveyed could potentially improve the reliability of eDNA detections.

The review found while eDNA is a useful tool for detecting the presence of giant burrowing frogs in pools with visible tadpoles, it may not detect adult frogs or reliably detect the species downstream of where tadpoles were observed during higher stream flows.

For example, the pilot did not detect the species at a site located 300 metres downstream from where tadpoles were observed. This could be due to low tadpole numbers or stream flow conditions at the time of sampling. Slow-moving water may have limited eDNA dispersal, while high flows may have diluted the eDNA past detectable levels.

Image: courtesy of Rohan Bilney, FCNSW

Future investigations should sample at various distances downstream (e.g., at the source, 50 metres, 100 metres, and 200 metres), and record the tadpole density at each sampling point. This information will help determine the effectiveness of eDNA techniques for giant burrowing frog and would provide clearer insight into the relationship between distance, environmental conditions, and eDNA detectability. Further testing at sites in the Broadwater Population Management Zones are recommended prior to extending eDNA sampling in the Yambulla and Yurammie population management zones.

More information

This work is part of the species-specific fauna monitoring strategy within the Coastal IFOA monitoring program. The reports detailing the pilot results and the independent review can be found on the Commission's website.

[i] Villacorta-Rath C, Hoskin CJ, Strugnell JM and Burrows D (2021) 'Long distance (> 20 km) downstream detection of endangered stream frogs suggests an important role for eDNA in surveying for remnant amphibian populations.' *PeerJ*, *9*, p.e12013.