Emma Stevens preparing to collect a water sample in the Canning River as part of her masters research. Photo – University of Western Australia.

New emerging biodiversity monitoring techniques – exploring eDNA

By Nia Murray

What is eDNA?

Environmental research within Australia is reaching new heights, with the use of exciting and emerging non-invasive molecular techniques harnessing eDNA (environmental DNA). Organisms leave traces of DNA throughout their environment and this DNA or 'eDNA' is helping scientists discover the organisms that inhabit an environment with one single sample of water, soil, and other matter. The growing use of eDNA is assisting in discoveries of invasive species, endangered species survival and overall biodiversity monitoring, possibly creating an inexpensive complementary technique to many time-consuming traditional study procedures. A DNA sample from an ecosystem can also show scientists the presence of pathogens and invasive species aiding the management and restoration of these ecosystems.

For conservation, depending on the objective, eDNA metabarcoding or single species surveys can be used. Metabarcoding eDNA can identify multiple species from a single environmental sample, allowing for the identification of detectable biodiversity within an area. This can be used as a general survey tool to find species from different taxonomic groups which otherwise would require multiple different survey techniques, knowledge, and skills. Single species surveys include identifying and extracting only the DNA of one single species, assisting in the detection of specific threatened or invasive species. This can also assist in tracking populations for post release survival of a translocated/reintroduced species.

Although eDNA can provide valuable information on biodiversity, it is not able to provide information about an organism's age, condition, or breeding status, which may clarify the population biology of a target species. Knowledge of these still requires the use of traditional methods. Ultraviolet radiation, temperature, and weathering can degrade an eDNA sample, possibly causing limitations to collecting samples.

In the lab

It is most important to avoid contamination when collecting eDNA samples, otherwise the DNA found will not reflect the eDNA in the surveyed area. This requires, at the very least, gloves, pre-sterilised equipment, and completing any traditional testing methods after eDNA sampling.

There are several laboratories in Australia that conduct eDNA testing, including the DBCA Sid James Conservation Genetics Laboratory, the TrEnD Lab at Curtin University, the commercial lab eDNA Frontiers, enviroDNA in Melbourne, ecoDNA in Canberra and the Australian Genome Research Facility. These labs can receive a DNA sample from any environment, the DNA is extracted through a series of steps to remove organic material and retain the target DNA. For single species detection these labs analyse eDNA samples by running it through a qPCR machine to give a presence/absence result, similar to how advanced and sensitive Covid-19 tests are completed. When metabarcoding, the PCR machine replicates the DNA material extracted from the sample, creating more copies for a DNA sequencing machine to read. To then identify the organisms, DNA readings are searched in databases known as reference sequencing databases which include records of past collected and sequenced DNA. Unfortunately, the organism can only be identified if their sequence has been entered into a database. These databases are still being added to and include GenBank and BOLD. Many labs have their own databases dedicated to their study areas.

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What research has taken place and what has been discovered?

Canning River invasive fish detection

Western Australian freshwater ecosystems are commonly under threat by invasive fish species, disturbing their rich biodiversity. Invasive fish have been introduced throughout the Canning River by various processes, one being the public releasing pet aquarium fish. To combat this issue, barriers were placed throughout the river over the years in the hope of stopping the spread of the invasive fish.

A study involving DBCA and The University of Western Australia has been designed to investigate how effective these barrier modifications were in managing invasive fish movement three years after installation, and to obtain a general biodiversity survey of the river.

*The pearl cichlid (*Geophagus brasiliensis*) is an invasive species of the Canning River that is aggressive to native fish populations and degrade our waterways due to their sediment sifting feeding behaviour. It is an attractive aquarium species but Don't dump that fish. Photo – Paige Wilson.*

This study has looked at both traditional fyke netting (a traditional type of cylindrical fish trap that is easy for a fish to enter but difficult for them to leave) that is left overnight, and the new method of sampling eDNA. Samples were collected over a two-week period from the Canning River using two different eDNA methods. One where the water is collected and the DNA is actively filtered out using a pump, and a second method where the filters were placed in the river and DNA was allowed to accumulate onto the filter.

After the eDNA samples are collected, the traditional fyke nets can be used to compare the different methods, whilst providing fish population density, which eDNA cannot do. It is hoped that this data and future studies will assist in giving insights into what fish are currently utilising the river, how they move within and between the barriers, and which invasive species are present.

This highlights the importance of the Don't Dump That Fish campaign of public education about the negative effects of releasing unwanted pets into drains and waterbodies with their potential as invasive species.

Monitoring translocated wallabies

Monitoring marsupials using traditional capture methods can prove a challenge. Trapping is invasive and difficult as many marsupials don't enter traps readily, while photo-monitoring methods aren't always effective as most marsupials have no identifiable features. New work by DBCA analysing scats (faecal matter) for DNA from bilbies, and banded hare-wallabies (amongst others) has provided a breakthrough in monitoring these elusive, trap shy species. This is allowing researchers to monitor the effectiveness of management including the translocation of threatened species.

Analysing eDNA from the scats of the threatened banded-hare wallaby has provided a non-invasive monitoring technique which has been used to study translocated animals. Scientists can generate a unique genetic fingerprint for individual animals and identify their home range, gender, and genetic diversity. Early results from the translocation to Dirk Hartog Island are very promising and have shown the population increasing and interbreeding within two to three years. Photo– Richard Manning.

The banded hare-wallaby has become extinct in the wild on Australia's mainland and efforts are underway to recover the species by undertaking conservation translocations to islands and fenced areas where they are safe from feral predators such as cats and foxes. As a pilot release in August/September 2017, 12 rufus and 12 banded hare-wallabies were translocated from Bernier and Dorre islands to Dirk Hartog Island, followed by a full-scale translocation of 90 banded and 50 rufus hare-wallabies in October 2018. Banded hare-wallaby translocations also occurred in 2017–2018 from Faure Island to Mt Gibson Wildlife Sanctuary. DBCA staff on Dirk Hartog Island are undertaking a rigorous monitoring program following the release of the translocated species to ensure they are settling in and establishing new territories.

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As banded hare-wallabies are not easy to capture, a new method using genetic analysis of hare-wallaby scats is helping DBCA staff to identify and count individuals in their new environment. Originally achieved using genetic markers known as microsatellites, DBCA are now using a new marker type known as SNPs, which has enabled a higher throughput method of genetic analysis. SNP – or single nucleotide polymorphism – refers to a change in a single nucleotide in the genome sequence of an individual. Sampling multiple SNPs in an animal's genome enables researchers to generate a unique genetic fingerprint for each individual, which then serves as a method of non-invasive ID tagging.

From the DNA obtained from each scat, scientists are then able to identify and count the number of individuals in the sampling area and each animal's home range based on the distribution of their scats. Analyses indicated an increase in the number of banded hare-wallabies in the survey area between 2019 and 2020, indicating the hare-wallabies are establishing well. Not only can they tell individuals from their scats, they can also determine the animal's gender and estimate the genetic diversity in the population.

Genetic diversity is important, as it helps a population to better adapt in their new environment. Results from genetic diversity tests found that individuals from separate source populations were interbreeding, which is a good sign for population growth and genetic adaptation. All this information was gathered, non-invasively, using scat eDNA.

eDNA in WA's pollen

Animal and insect pollinating species are on a decline from environmental stressors and visual surveys to monitor pollinating activity are time consuming giving limited data for scientists to work with. The use of eDNA in partnership with visual surveys can detect these important pollinating events in greater detail and provide insight into understudied flora and fauna species interactions. Joshua Newton and colleagues at Curtin University conducted a study comparing eDNA metabarcoding with visual surveys in the Helena and Aurora Range of WA. They were able to detect interactions between flowers from seven species with diverse floral morphologies with birds, mammals and insects, and discover the changing ecology of pollinating species in WA.

The differences between techniques were striking, eDNA identified 59 pollinating taxa, where visual surveys identified only 16. Interestingly, the visual and eDNA survey results did not significantly correlate. The eDNA results left many bee visits undetected, such as the introduced European honeybee which were visually detected on 13 plants, yet honeybee DNA was detected on only four. Visual surveys also detected native bee species, which were left undetected during DNA surveys. It is possible that environmental factors have caused DNA degradation. The use of eDNA metabarcoding did assist with detection of nocturnal species. During visual surveys, no nocturnal taxa were recorded visiting the flowers, however the eDNA surveys detected species of moth, and nocturnal mammal species including the western pygmy possum.

The difference in visual and eDNA survey results perhaps implies that the use of both survey methods is most effective for accurate results on pollinator species and flower interactions. Using eDNA can provide otherwise undetectable information on pollination ecology and assist in future conservation efforts.

Visual and eDNA survey results did not significantly correlate. Survey data from the eDNA identified 59 pollinating taxa, where visual surveys identified only 16 with few overlapping results. This suggests both methods are complimentary. Graph – Joshua Newton et al.

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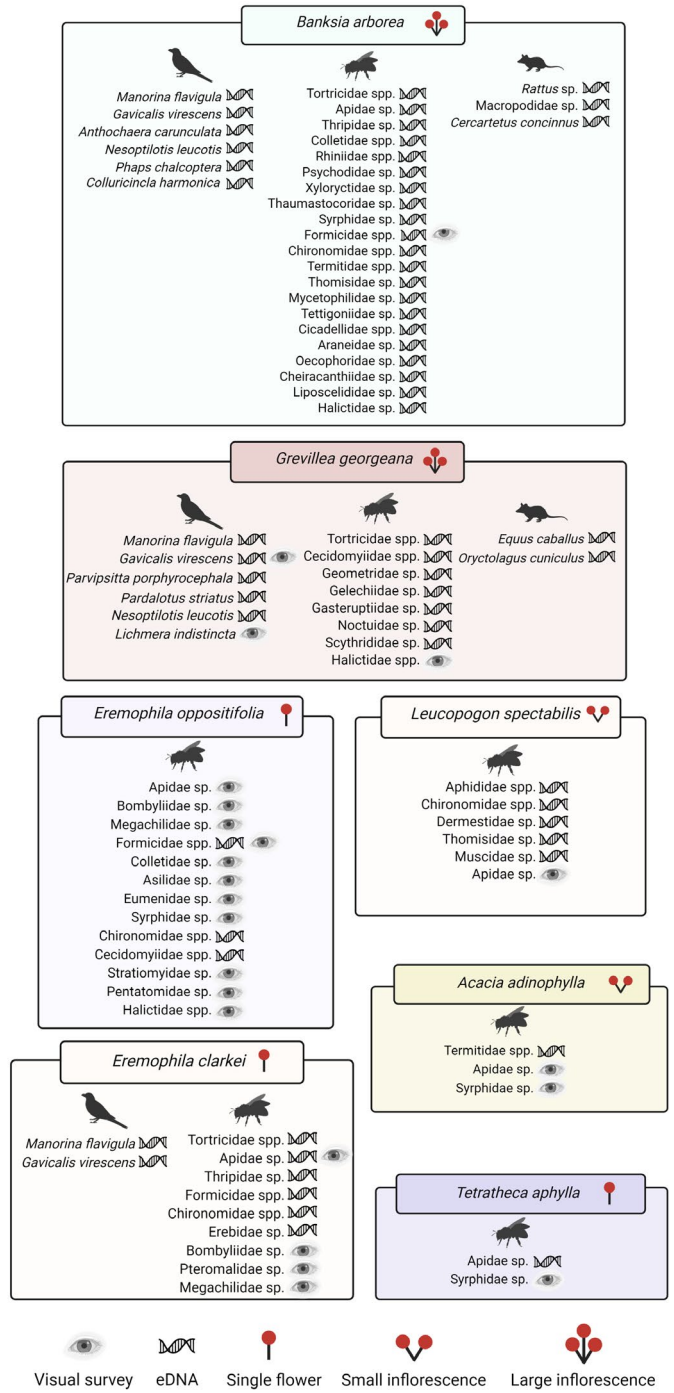


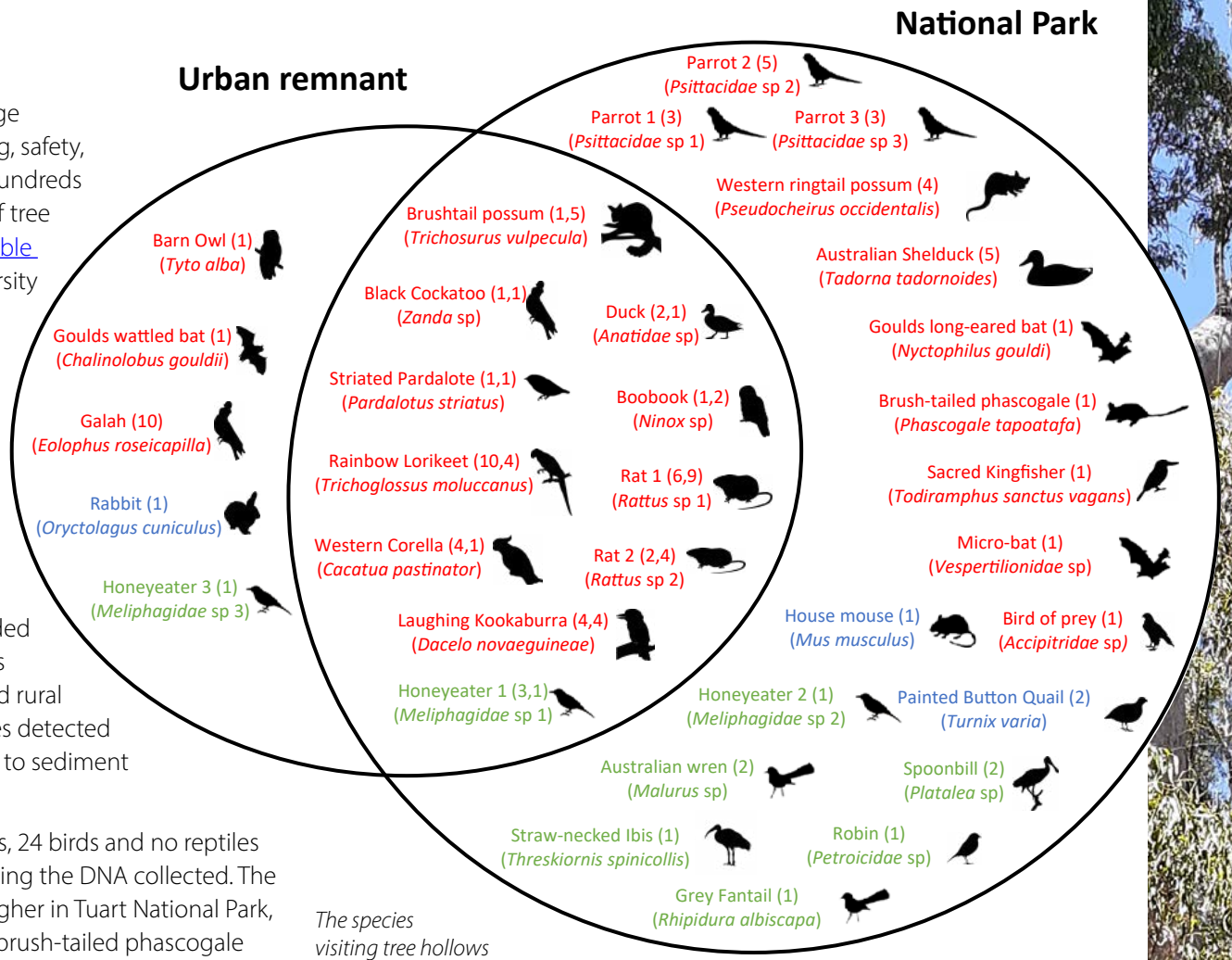
Who is in our tree hollows?

Tree hollows provide vital habitat for a range of species, acting as a resource for breeding, safety, and sleeping. With tree hollows needing hundreds of years to form in old trees, and the rate of tree clearing, the number of tree hollows available are in decline. Scientists from Curtin University used Kings Park, Bold Park, and the Tuart National Park as study sites to assess species interactions with tree hollows, while also comparing the efficacy of two sampling methods, extracting DNA from collected sediment samples versus using roller swab samples.

Using the non-invasive method of collecting eDNA, 138 samples were taken from 93 tree hollows. These samples included 93 roller samples and 45 sediment samples around urban Kings Park and Bold Park, and rural Tuart National Park. The roller swab samples detected a greater species richness of 19, compared to sediment samples which detected 13 species.

Thirty-four vertebrate species, 10 mammals, 24 birds and no reptiles or amphibians were detected after calibrating the DNA collected. The number of species detected overall was higher in Tuart National Park, including detection of the cryptic species brush-tailed phascogale (*Phascogale tapoatafa*). The two sites shared 11 common species with the invasive rainbow lorikeet (*Trichoglossus moluccanus*) being the most common hollow user. The graph shows the species found and provides a comparison between the urban Kings Park and Bold Park and the rural Tuart National Park sites.

The use of eDNA provides accurate insight into the species visiting tree hollows and valuable information on the impacts of invasive species such as the rainbow lorikeet to assist bushland managers.



The species visiting tree hollows in urban remnants and a national park, shows a higher number of species found in Tuart National Park compared to the urban remnant bushland at Kings Park and Bold Park. Red indicates a known hollow user, green is a non-hollow using bird and blue is a possible prey species. The number of detections is recorded in brackets with the invasive rainbow lorikeet being the most common hollow user. Graph– Joshua P. Newton et al.

A non-invasive sampling method involved tree climbing to collect roller swabs and sediment samples. Here Simon Cherriman from iNSIGHT Ornithology is high up in a tuart tree collecting eDNA. Photo – Joshua Newton.

Contact

Nia Murray
DBCA
email nia.mry@outlook.com