Project Plan SP 2020-068

Evaluating the application of eDNA and metabarcoding as biodiversity and monitoring tools

Ecosystem Science

Project Core Team

Supervising Scientist	Adrian Pinder
Data Custodian	Adrian Pinder

Project status as of Sept. 21, 2021, 3:21 p.m.

	Update requested

Document endorsements and approvals as of Sept. 21, 2021, 3:21 p.m.

Project Team	granted
Program Leader	granted
Directorate	granted
Biometrician	granted
Herbarium Curator	not required
Animal Ethics Committee	granted

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Biodiversity and Conservation Science Program

Ecosystem Science

Departmental Service

Service 6: Conserving Habitats, Species and Communities

Project Staff

Role	Person	Time allocation (FTE)
Supervising Scientist	Josephine Hyde	1.0
Technical Officer	Allan Wills	0.3

Related Science Projects

Proposed period of the project

Sept. 8, 2020 – July 1, 2023

Relevance and Outcomes

Background

One of the significant issues of the 21st century is the decline in biodiversity occurring globally, with consequences for ecosystem functioning and services [3,4]. Knowledge of the diversity, distribution, population and community trends of animals and plants is vital to making informed biodiversity conservation decisions. DBCA undertakes considerable ecological and environmental monitoring in support of conservation initiatives.

Traditional monitoring methods or assemblages have developed over many decades and have produced results that have significantly improved conservation management. However, methods for ecological monitoring is continually evolving, and in recent years techniques such as camera traps, audio recorders and satellite tracking have resulted in improved effectiveness of monitoring programs. Environmental DNA (eDNA) is another emerging technology that is starting to be operationalised as part of the monitoring toolkit. No one technology can provide all of the tools needed for ecological monitoring, but eDNA has some advantages over other methods in some situations.

Some existing methods are not ideal for detecting elusive or poorly known taxa[1,2,5] and can be laborious in time and effort. Significant taxonomic expertise is often required to identify or at least distinguish taxa, especially invertebrates, but such expertise is decreasingly available[6-8]. The similarity of some closely related species at the juvenile life stage and the presence of cryptic species can also hinder identification. Increasingly, genetic methods of biodiversity survey and monitoring, such as metabarcoding and specifically environmental DNA (eDNA), are being proposed as alternative methods to overcome some of these problems[2,9].

eDNA refers to the genetic material found in environmental samples, released from living or dead organisms, including extracellular DNA, whole cells and even whole organisms[2,10]. The DNA can originate from individuals that are occupying a location or deposited as they pass through. For example, if we sample a water body, DNA may have originated from animals that lived in the water or that visited the water body to drink. Studies increasingly advocate that eDNA studies are all that is needed, but, as with other monitoring tools, eDNA has limitations that need to be considered.

The amount of eDNA released into the environment is highly variable and is influenced by factors such as the species, life stage and biomass at the location sampled. Some environments, such as water, allows the eDNA to diffuse from its point of origin so that finding the exact location of the target species can be difficult. Many environmental variables can degrade DNA, including temperature, pH, UV, salinity, and microbial activity [11,12]. Therefore, the length of time that eDNA is available after shedding depends on its environment. While eDNA can

detect rare and cryptic species, it cannot determine population biology information, such as sex ratios and body condition.

This project will examine how eDNA can be effectively used as a monitoring tool, complementing existing monitoring methods and projects at DBCA and how eDNA can be incorporated into new biodiversity and conservation initiatives.

Aims

This project aims to apply eDNA and metabarcoding methods to a range of survey and monitoring projects to test whether they can effectively replace or supplement traditional ecological sampling.

Individual projects will include:

- A pilot project to develop eDNA protocols for detecting two species of conservation concern: the rakali (Hydromys chrysogaster) and Carter's freshwater mussel (Westralunio carteri) using eDNA.
- Testing utility of eDNA and metabarcoding for assessing responses of forest invertebrate communities to fire and silvicultural practices using FORESTCHECK sites and protocols.
- Using eDNA to investigate spatial and/or temporal patterns in wetland biodiversity (e.g. surveying for threatened fish in the Muir-Byenup Ramsar site).

Other potential applications include:

- eDNA from flowers to examine plant/pollinator interactions.
- Using meta-barcoding to examine the impacts of invasive redclaw crayfish on Pilbara river pool zooplankton communities (invasive animal grant application submitted to DAWE).

Expected outcome

This project will provide informed recommendations as to which kinds of research and monitoring questions these emerging genetic methods can be successfully applied to and develop protocols for collection and analysis of samples. Where these methods are successfully applied the individual projects will produce new knowledge to inform conservation decision-making. Projects will also contribute to sequence libraries of species present in the environments investigated, as well as a better understanding of temporal, spatial and environmental patterns of species occurrence.

Knowledge transfer

Development and testing of methods during this project will inform the design of similar survey and monitoring projects within DBCA, particularly within Biodiversity and Conservation Science, Conservation and Ecosystem Management and Regional and Fire Management Services. Results of projects will also contribute directly to current and future survey and monitoring initiatives, such as those carried out for threatened species management and forest management.

We will publish research findings in multiple formats, including technical reports, presentations, internal and external media, and peer-reviewed research publications. Research results will also be communicated in ways that allow their inclusion in DBCA processes such as species and area planning and the work of recovery teams. Raw and processed data and associated interpretations will be securely stored in appropriate corporate and external databases and repositories. These outputs and research agreements will also allow knowledge transfer to external researchers such as those within government agencies and universities.

Tasks and Milestones

Initial Project Planning (FORESTCHECK/RAKALI): Jul - Sep 2020
Primer Design: July - Dec 2020
River sampling (Rakali): Sep + Dec 2020
Soil sampling (FORESTCHECK): Nov 2020
DNA extractions Tests: Sep – Nov 2020
River DNA extractions: Jan 2021
River DNA PCRs: Jan-Feb 2021
Masters Student (Canning River Project): Feb - Dec 2021

Masters Student (Rakali Mapping Project): Feb 2021-July 2022
TERN Sampling: Autumn 2021

References

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Study design

Methodology

Unlike traditional survey methods, eDNA does not involve capturing or visualising a live organism but rather DNA of an uncertain origin. Before sampling begins for an eDNA project, there are several essential considerations regarding sample design. Observing a target species relies not only on the species of interest being present at the sampling location but also on presence of their eDNA in the field sample, in the DNA extraction and that the PCR binds and captures the target DNA sequence. False negatives can happen at any stage along this pipeline, and therefore design of a robust eDNA sampling protocol must take this into account. Many projects either use extensive pilot studies or mathematical models to understand how many samples and replicates are needed to obtain the required overall sensitivity. The final experimental design should be matched to the observation process, and the question being asked, but some commonalities are valid for all eDNA projects.

The first thing that should be determined for each new eDNA project is the question that needs to be answered and the budget for the project. eDNA requires rigorous sampling and observing a species eDNA can generally be broken down into three different factors; the sample size, the molecular assay design, and field implementation. The goal and the project's budget will influence how much emphasis is placed on these factors. There has been a suggestion that when targeting rare species that there should be more sites sampled but less replicates at each site, whereas when more common species are targeted there should be fewer sites sampled but more intense sampling at those sites. However, many species have limited inferences about site occupancy or field time can be expensive or limited, for example, difficult to reach sites. In these cases, taking fewer samples in the field can be compensated for by increasing the number of replicates in the lab. The number of molecular replicates (qPCRs/PCRs) needed is linked to the probability of detecting eDNA in the samples. The higher the probability there is that there is eDNA in the sample present, the lower the number of molecular replicates, with a minimum of 4 suggested to detect eDNA at a site using qPCR.

Environmental DNA studies are not static, and it is not unusual for sampling protocols to change over time as methods improve and understanding of how different site characteristics influence occurrence, capture and detection of eDNA. The main ways that studies are designed is to start with a pilot project, mathematical models and lab experiments. Understanding the limits of qPCR assays for each new primer combination and capturing the extraction methods should be the first step when designing a new protocol. Using existing species knowledge, environmental data and mathematical models, combined with the project question, helps to design a robust sampling protocol that can be refined over time with or without pilot projects.

Two initial sub-projects are being designed to get this research underway. These are 1) a meta-barcoding project to survey for ground-dwelling invertebrates in a selection of FORESTCHECK northern jarrah forest sites that examine responses to fire history and 2) a pilot project to use eDNA to survey for two species of conservation concern. These are the rakali (native water rat) and one of its primary prey (the threatened *Westralunio carteri* mussel) in rivers around Perth and the south-west.

The FORESTCHECK project sites were selected using historical sampling data to include past metadata and previous studies. Five sites per plot were selected as the target is a broad presences/absences of invertebrate genotypes rather than a very fine in-depth species index project. Leaf litter and soil samples from the same location were collected to increase the range of invertebrates detected. Enough material from each site was collected to do multiple extractions to increase the likelihood of rare species detection and qPCR was selected over PCR for the amplification step as it has a higher detection rate for low DNA samples.

For the rakali/mussel project we are collaborating with both Rivers and Estuaries Science in DBCA and Department of Water and Environmental Regulation (DWER). DWER's Healthy Rivers program has been used to determine sampling locations. Sampling methods in the field were based heavily on the public protocol released by the United States Geological Survey. As this project is very targeted, using both primers and probes that are species specific, and the project is currently in the pilot stage, fewer samples were taken in the field and more molecular replicates at the qPCR stage are being employed. These methods may change as the protocol is developed.

DNA from positive replicates will be pooled together to increase the DNA amplified from individual samples, and then sent for NGS at AGRF for sequence verification. Bioinformatics analysis of the resulting sequence data will be performed on the Pawsey high-performance computers, mostly following a modified PEMA pipeline. As much as possible, the sequences will be blasted against custom libraries created specifically for the WA region.

Biometrician's Endorsement

granted

Data management

No. specimens

None

Herbarium Curator's Endorsement

not required

Animal Ethics Committee's Endorsement

granted

Data management

Genetic data will be published on a public database, most likely GenBank, in line with best practice guidelines. In the interim genetic data will be stored on an external hard drive and the Pawsey Supercomputuer while data analysis is being undertaken. Other data, such as spatial information and species occurrence information arising from the genetic work, will be stored in appropriate corporate databases such as Data Catalogue.

Budget

Consolidated Funds

Source	Year 1	Year 2	Year 3
FTE Scientist	1.0	1.0	1.0
FTE Technical	0.3	0.3	0.3
Equipment			
Vehicle			
Travel			
Other			
Total	40000 (including allocation from Forestcheck)	40000 (including allocation from Forestcheck)	40000 (including allocation from Forestcheck)

External Funds

Source	Year 1	Year 2	Year 3
Salaries, Wages, Overtime			
Overheads			
Equipment			
Vehicle			
Travel			
Other		13000	
Total		13000	