BHP

Curtin University

The eDNA for Global Environment Studies (eDGES) Program

Annual Report 2023-2024

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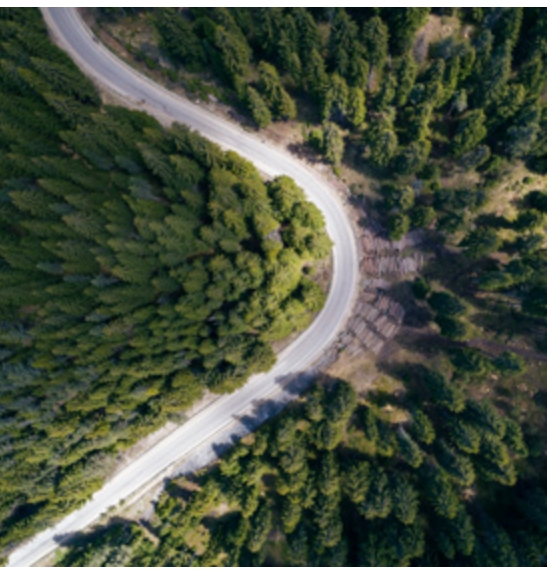

Executive Summary

This is the fourth and final annual progress report of the eDGES (eDNA for Global Environmental Studies) research program since its commencement in July 2020.

eDGES has grown to consist of six projects that are being carried out at the Trace and Environmental DNA (TrEnD) Laboratory at Curtin University (Perth, Australia) with an overriding aim of making significant contributions to biomonitoring in terrestrial, subterranean, wetland and marine environments. The portfolio includes four terrestrial and inland aquatic-based projects that pushes environmental DNA (eDNA) research well into new territory from both an analytical perspective (merging eDNA and genomics) and an environmental perspective (subterranean ecosystem in Australia and hypersaline lakes in Chile). There are also two marine projects, one developing new metabarcoding assays for detecting invasive marine species, and another project applying these assays to describe the marine benthic assemblages associated with infrastructure assets and natural locations.

eDGES has been a highly successful research program with significant contributions across all the projects within both applied and fundamental science. Moreover, the research group has established solid national and international networks through collaborations as a direct outcome of this program. All the details of the output and advances of each project are found in the following pages, but a few of the many eDGES highlights involve:

- A new high precision qPCR assay to monitor the elusive Pilbara olive python and demonstrating that water from rock pools is an excellent source for python eDNA detection – both results represent concrete advances that will significantly improve biomonitoring capacity of this endangered species.

- Sequencing and assembling more than 150 complete or near-complete mitochondrial genomes from invertebrate stygofauna of the Great Artesian Basin (SA) and Ethel Gorge (WA). This was done in collaboration with ARC Linkage Project 190100555 (Michelle Guzik) and has provided a tremendous step forward, both in terms of establishing rigorous methods for accurate, cost effective and reproducible monitoring of these systems, and by establishing a crucial reference data library for studying the diversity and evolution in this hidden world.
- The successful application of the first eDNA multi-assay approach to investigate hypersaline biodiversity in the Chilean precordillera – a design that allowed to detect eDNA from flamingos and other endangered species (e.g., *Heleobia atacamensis*) from water samples. A similar approach also enabled us to unveil bacterial and macroinvertebrate diversity at five different hypersaline lakes at the A-class reserve Rottnest Island in Western Australia.
- Improved detection of invasive marine species by the development of a new multiplex assay to detect the phyla Arthropoda and Mollusca, and the enhancement of the 16S public DNA reference database. These are important outcomes for surveying for the introduction of invasive marine species.
- Discovery of a previously undescribed species of oyster from remote areas in the Pilbara.
- The largest port eDNA survey ever conducted, with over 1100 samples collected in the Port Hedland area. The high throughput eDNA sampling methods used there will set a benchmark for surveys of native and invasive marine species in the future. The eDNA sequence analyses for this work were the first to be done on new BHP-funded NextSeq2000 sequencing platform (named “the Dekker”) shared by eDNA Frontiers and TrEnD lab.

As reflected in the subsequent pages these results are just the tip of the iceberg. To date a total of 22 peer reviewed eDGES research papers have been published including papers in prestigious high impact journals such as *Biological Reviews* and *Science of the Total Environment*. A similar number of papers are currently in the making.

Moreover, the eDGES team has been extensively represented at conferences, seminars and workshops worldwide, counting at least 12 eDGES-based research talks and posters over the years. Among many highlights was a key-note presentation at the widely attended “1st Australian and New Zealand conference on environmental DNA” in Hobart 2023.

Our annual eDGES seminars have been a tremendous success with increasing interest from stakeholders over the now three events held, with nearly 150 people attending directly in person and online, and a number more viewing the recordings. In this way we have transferred our broad learnings to the community regularly, and events such as these have helped foster strong relationships with our collaborators and help build new network partners.

The worldwide COVID-19 pandemic caused significant delays in sampling, travel, recruitment and sourcing necessary material for the planned activities, but thanks to agile project management the overall scientific and societal outcomes of this program have not been compromised. In fact, with eDGES round 2 now confirmed, we feel like the work is only about to start, and we expect the coming years to be even more successful, productive and exciting!

Contents

Program Overview

The eDGES program focuses on developing and applying environmental DNA (eDNA) techniques to address challenges related to biodiversity loss and sustainability in our changing world.

Through our scientific achievements we seek to understand ecological and evolutionary changes in natural and artificial land and seascapes to develop new-generation tools for identifying and managing species, and foster biodiversity conservation through whole community studies afforded to us by eDNA.

Beyond the research, our goal is to translate this knowledge into outcomes for society that will ultimately enhance our collective wellbeing.

Key points

- Beyond conducting innovative research, the program sought multiple outcomes such as local and international PhD training, peer reviewed papers, associated grant augmentations (eg ARC Linkages), community education, and input to policy concerning the environment.
- We sought to engage the public with awareness events; seminars, annual progress meetings, and an end-of-program symposium.
- eDGES contributes to the strategic BHP-Curtin Alliance (signed July 2020), whose goal is innovation, education and research. The eDGES program is funded by BHP's Social Investment Framework, under the 'Environment' stream by contributing to "biodiversity conservation, water stewardship and climate change mitigation and adaptation."
- eDGES was formally commenced in July 2020 and this round concludes in June 2024.

Project Summaries

The six projects described here focus on improving, validating, or applying eDNA techniques to address biodiversity related questions of benefit the general public.

These projects represent new or developing applications of eDNA and extensions of the technology to help translate an emerging research technique into a functional biomonitoring tool for use by industry and governments to obtain new information on biodiversity which enables and facilitates sustainable management. The eDGES program will also generate fundamental new scientific knowledge on the evolution, distribution and threats to biodiversity in both the Australian region and Chile.

Project 1

eDNA monitoring and conservation genomics of the Pilbara Olive Python

Aims to merge eDNA monitoring with whole genomic analyses to study the Pilbara Olive Python (*Liasis olivaceus barroni*), resulting in deep population genomic insights and evolutionary ecology information to guide conservation priorities. A key outcome in this study will be the generation of the first Olive Python reference genome.

Project 2

Subterranean fauna detection and conservation

Develops new high-resolution eDNA tools for monitoring genetic diversity in subterranean species across different sites, in order to effectively manage their conservation and obtain new ground-breaking information into the evolutionary processes in this hidden world.

Project 3

Functional ecology of Chilean precordillera lakes: towards a Wetland Health Index for birds

By uniquely combining eDNA technology with isotope analysis this project aims to not only investigate the wetland biodiversity but also characterize ecosystem web dynamics from sediment microbes to the migratory Chilean flamingos. This will allow us to understand the food chain and identify keystone species that are indicators of wetland health.

Project 4

Improve the Sensitivity and Specificity of eDNA metabarcoding tools for Invasive Marine Species detection

This project aims to develop crucial reference validations for invasive marine species (IMS) and native congeners from the North West Shelf of Western Australia. This will greatly enhance the ability of eDNA tools to detect IMS in environmental samples with a high level of certainty.

Project 5

Benthic communities associated with port and harbour infrastructure in Port Hedland: Targeting invasive marine species

Environmental DNA samples will be collected to describe spatial patterns in the distribution of benthic organisms associated with artificial structures and natural habitats within and either side of Port Hedland. This sampling will also test for invasive marine species.

Project 6

Terrestrial ecosystem biomonitoring with eDNA across the tree of life: the Olympic Dam case study

The objective of this project is to validate cutting edge molecular tools to assess terrestrial biodiversity (soil microbial communities, plants, invertebrates, and vertebrates) and commence development of an eDNA based metric for evaluating the condition of terrestrial ecosystems. This approach to ecosystem monitoring will advance the study of individual species to understanding entire ecosystems and how species interconnect.

Project Outputs

Research from the program has generated new knowledge and is released in open publications, under the goal of contributing to public learnings and supporting biodiversity outcomes.

Publications

2024

Guzik, M.T., Stringer, D.N., Thornhill, J.C., Coates, P.J., Humphreys, W.F., van der Heyde, M., White, N. E., Sacco, M., Besaley-Hall, P., Hillyer, M.J., Wilson, N.J., Hosie, A.M., Kirkendale, L., Alexander, J., Huey, J.A., King, R.A, Cooper, S.J.B., Austin, A.D. (Submitted) Best practices for curating eDNA custom barcode reference libraries: an Australian case study. *Limnology and Oceanography*.

Guzik, M.T., Thornhill, J.C., van der Heyde, M., Stringer, D.N., White, N. E., Sacco, M., Beasley-Hall, P., King, R.A, Cooper, S.J.B., Nevill, P., Austin, A.D. (Submitted) Towards a global barcode reference library for subterranean fauna. *Science of the Total Environment*.

Perina, G., Camacho, A.I., White, N.E., Callan, S.K, Abello, J., Morgan, L. Guzik, M.T. (Submitted) An integrated study of *Atopobathynella* (Parabathynellidae, Bathynellacea) species reveals restricted distributions in a complex hydrogeological setting: two new species from the Pilbara (Australia). *Contributions to Zoology*.

Tan K.S., Tan S.K., Lukehurst S.S., Wells F.E. (2024) Assessing the threat to the Australian marine environment of the oyster genus *Magallana* in Singapore. *Raffles Bulletin of Zoology*. (accepted)

Tan S.H.M., Wells F.E., Lukehurst S.S., Strong E., Sanpanich K., Duangdee T., Ambarwati R. and Tan K.S. (2024). Unravelling the *Brachidontes variabilis* species complex (Bivalvia: Mytilidae) of the Indo-Pacific region. *Journal of Molluscan Studies*. (accepted)

Wells F.E., Lukehurst S.S., Fullwood L.A.F., Harvey E.S. (2024). Distribution of intertidal rock oysters in the Pilbara, Western Australia. *Management of Biological Invasions* 15(1): 131–143. https://doi.org/10.3391/mbi.2024.15.1.08

Wells F.E., Duangdee T., Sanpanich K., Lukehurst S.S. (2024). Status of the invasive charru mussel *Mytella strigata* (Hanley, 1843) in the upper Gulf of Thailand five years after it was first surveyed. *BioInvasions Records* 13(1): 69–82. https://doi.org/10.3391/bir.2024.13.1.07

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Beasley-Hall, P.G., Murphy, N.P., King, R.A., White, N.E., Hedges, B.A., Cooper, S.J.B., Austin, A.D., Guzik, M.T (2023) Time capsules of biodiversity: Future research directions for groundwater-dependent ecosystems of the Great Artesian Basin. *Frontiers in Environmental Science*. https://doi.org/10.3389/fenvs.2022.1021987

Campbell, M. A., Laini, A., White, N. E., Allentoft, M. E., & Saccò, M. (2023). When nets meet environmental DNA metabarcoding: Integrative approach to unveil invertebrate community patterns of hypersaline lakes. *Journal of Oceanology and Limnology*, 41(4), 1331–1340. https://doi.org/10.1007/s00343-022-2151-9

Laini, A., Stubbington, R., Beermann, A.J., Burgazzi, G., Datry, T., Viaroli, P., Wilkes, M., Zizka, V.M.A., Saccò, M. and Leese, F., 2023. Dissecting biodiversity: assessing the taxonomic, functional and phylogenetic structure of an insect metacommunity in a river network using morphological and metabarcoding data. *The European Zoological Journal*, 90(1), pp.320-332.

Mousavi-Derazmahalleh M., Ellis R.J., D'Rozario B.L., Berry T.E., Peverley G., Dawkins K.L., Campbell M., White N.E. and Allentoft M.E. (2023). Rock pools as a source of environmental DNA for the detection of the threatened Pilbara olive python (*Liasis olivaceus barroni*). *Front. Environ. Sci*. 11:1187545. doi: 10.3389/fenvs.2023.1187545

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Saccò, M., Mammola, S., Altermatt, F., Alther, R., Bolpagni, R., Brancelj, A., Brankovits, D., Fišer, C., Gerovasileiou, V., Griebler, C., Guareschi, S., Hose, G. C., Korbel, K., Lictevout, E., Malard, F., Martínez, A., Niemiller, M. L., Robertson, A., Tanalgo, K. C., Guzik, M.T., ... Reinecke, R. (2023). Groundwater is a hidden global keystone ecosystem. *Global Change Biology*, 30, e17066. https://doi.org/10.1111/gcb.17066

Simpson T.J., Wellington C.M., Lukehurst S.S., Huerlimann R., Veilleux H., Snow M., Dias J., McDonald J.I. (2023). Development of a real-time PCR (qPCR) method for the identification of the invasive paddle crab Charybdis japonica (Crustacea, Portunidae). *PeerJ* 11: e15522. https://peerj.com/articles/15522/

Takahashi, M., Saccò, M., Kestel, J. H., Nester, G., Campbell, M. A., Van Der Heyde, M., ... & Allentoft, M. E. (2023). Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. Science of The Total Environment, 162322. https://doi.org/10.1016/j.scitotenv.2023.162322

van der Heyde, M., White, N.E., Nevill, P., Austin, A.D., Stevens, N., Jones, M. and Guzik, M.T., (2023). Taking eDNA underground: factors affecting eDNA detection of subterranean fauna in groundwater. *Molecular Ecology Resources*. 0: 1-18. https://doi.org/10.1111/1755-0998.13792

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Saccò, M., Blyth, A. J., Douglas, G., Humphreys, W. F., Hose, G. C., Davis, J., ... & Halse, S. A. (2022). Stygofaunal diversity and ecological sustainability of coastal groundwater ecosystems in a changing climate: The Australian paradigm. *Freshwater Biology*, 67, 2007 – 2023. https://doi.org/10.1111/fwb.13987

Saccò, M., Guzik, M. T., van der Heyde, M., Nevill, P., Cooper, S. J., Austin, A. D., ... & White, N. E. (2022). eDNA in subterranean ecosystems: Applications, technical aspects, and future prospects. *Science of The Total Environment*, 153223. https://doi.org/10.1016/j.scitotenv.2022.153223

Saccò, M., Campbell, M. A., Nevill, P., Humphreys, W. F., Blyth, A. J., Grierson, P. F., & White, N. E. (2022). Getting to the root of organic inputs in groundwaters: stygofaunal plant consumption in a calcrete aquifer. *Frontiers in Ecology and Evolution*, 202. https://doi.org/10.3389/fevo.2022.854591

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Saccò, M., White, N. E., Harrod, C., Salazar, G., Aguilar, P., Cubillos, C. F., ... & Allentoft, M. E. (2021). Salt to conserve: A review on the ecology and preservation of hypersaline ecosystems. *Biological Reviews*, 96(6), 2828-2850. https://doi.org/10.1111/brv.12780

Saccò, M., White, N. E., Campbell, M., Allard, S., Humphreys, W. F., Pringle, P., ... & Allentoft, M. E. (2021). Metabarcoding under brine: microbial ecology of five hypersaline lakes at Rottnest Island (WA, Australia). *Water*, 13(14), 1899. https://doi.org/10.3390/w13141899

2020

Burgazzi, G., Laini, A., Ovaskainen, O., Saccò, M., Stubbington, R. and Viaroli, P., 2020. Communities in high definition: Spatial and environmental factors shape the micro-distribution of aquatic invertebrates. *Freshwater biology*, 65(12), pp.2053-2065

Conference/Seminar/Workshop Presentations

1st Australian & New Zealand eDNA Conference in Hobart, 14-17th February 2023:
Poster: "Development of a multi-assay approach for detecting invasive marine species (IMS) for use in biosecurity applications." Presented by Sherralee Lukehurst.
Presentation: "Dark taxa in dark environments: eDNA in subterranean systems". Presented by Nicole White.
Presentation: "An overview of eDGES program 4 and 5" delivered by Prof Morten Allentoft.
Presentation: "Looking under the mat: benchmarking computational methods for analyzing microbial datasets" delivered by Dr Matthew Campbell.
Key-note presentation: "eDNA through an aDNA lense: lessons from a related discipline" delivered by Prof Morten Allentoft.

eDGES project 2 was presented in Adelaide at an ARC Linkage grant writing workshop in March 2023 by Nicole White.

eDGES project 2 was presented at the final ARC Linkage meeting LP109100555 "Taking eDNA underground: transforming assessment of subterranean ecosystems" in Perth, 27th February 2024 by Nicole White.

eDGES project 2 was presented by Dr Nicole White and Dr Mieke van der Heyde at the WABSI workshop "Integrating eDNA into bioassessment and monitoring of subterranean fauna in WA", 28th February 2024.

14th International Conference of inland salt lakes and salinas (ISSRL (International Society for Salt Lake Research)) – Dr Mattia Saccò

A short presentation on the eDGES project has been delivered by Dr Mattia Saccò and Dr Nicole White in quality of guest speakers at the 18th Annual WA Wetlands Conference 2022 in Cockburn, Perth.

Southern eDNA Society airDNA workshop, Canberra June 2024 "Comparative analysis of spiderweb-derived and air borne eDNA for terrestrial biodiversity monitoring" delivered by Assoc Prof Paul Nevill

DCCEEW Workshop, Darwin June 2024 "Is eDNA metabarcoding an effective approach for the monitoring of mine site restoration? Tips and tricks from 6+ years of research" presented by Dr Mieke van der Heyde and Assoc Prof Paul Nevill

Terrestrial Conservation

Project 1

eDNA monitoring and conservation genomics of the Pilbara Olive Python

Summary

This is an ambitious project aiming to merge environmental DNA (eDNA) monitoring with genomic analyses to study the Pilbara Olive Python (*Liasis olivaceus barroni*) – an endangered Australian terrestrial top predator. We will develop new eDNA markers which can detect the Pilbara Olive Python in soil and water samples.

We will also sequence and bioinformatically assemble a complete reference genome of the Pilbara Olive Python, creating the foundation for cutting-edge research on population genomics and evolutionary ecology. By establishing this ambitious, novel and holistic molecular framework, this project will create new and important tools which will facilitate the monitoring and evaluation of conservation priorities, practices and policy for the Pilbara Olive Python.

Figure 1. Pilbara Olive Python (L.o.barroni) captured in Afghan Pool, Newman, WA by R.J.Ellis

Implementation

The project started on time however due to Prof Allentoft not being able to travel to Australia, it was decided to postpone the PhD-component of project until 2023. This change implies that this project has been extended and is not expected to end until the PhD is handed in (in 2026).

Staffing and management

- **Prof Morten Allentoft** – Principal investigator, head of TrEnD lab and chief scientist in eDNA Frontiers at Curtin University. Overall project lead and primary supervisor of students.
- **Dr Nicole White** – Co-principal investigator and Research Fellow in TrEnD lab at Curtin University. Co-supervision of students and oversees the laboratory work.
- **Dr Mahsa Mousaviderazmahalleh** – Postdoctoral fellow working on snake genomics and bioinformatics (funded by Morten's research "start-up package"). Mahsa will co-supervise the PhD student on bioinformatic analyses.
- **Mr Ben Heyward –** PhD student (2023-2026) studying the application of shotgun sequencing for python eDNA detection.

Key collaborators

- Vertebrate Genome Project (VGP, https://vertebrategenomes project.org/). World renowned consortium that facilitates the genome sequencing and assembly.

- Prof Stephen van Leeuwen, Curtin University. Extensive knowledge on python ecology and assists with strategic decisions and funding opportunities.
- Biologic Environmental Survey Pty Ltd. Consultancy working on python eDNA together with eDNA frontiers and TrEnD laboratory.
- Associate Prof Shyam Gopalakrishnan, Globe Institute, University of Copenhagen. A leading computational biologist with expertise in genome analyses.
- Associate Prof Bill Bateman, Curtin University. Expertise in reptile ecology and on the PhD supervisory team (along with MA, NW, MM).

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
Successful PhD graduate	The PhD-component was postponed due to COVID followed by several other delays. Budget and timeline were then revised to accommodate for a PhD scholarship.	Ben Heyward started in May 2023
Assemble two complete genomes	The Pilbara Olive python genome has been fully assembled (at VGP) and the Kimberley olive python genome is now in the final stages.	Almost done
Develop olive python qPCR assay	This assay has been successfully developed and is now in the final testing stage.	Final testing
Identify best eDNA substrates	We have published a paper on using eDNA from rock pools for python monitoring, and we are now systematically testing other substrates from enclosures.	One paper published, one to go.
Estimate eDNA decay rates	Preliminary DNA decay work was done in Ben's Honours project, and this is now laying the foundation for this PhD chapter.	Tank experiments scheduled for late 2024
Test eDNA shotgun sequencing	Preliminary data from honours project exist. New samples have been collected and this work is ready to take off.	We have a reference genome, we have the samples, and we have a NextSeq, so we are ready!
Population genomics	Still in planning	Scheduled for last year of PhD

Overview of outputs and outcomes

The overriding aim of the project was to improve biomonitoring of olive pythons and halfway through this PhD project, we have already contributed significantly by:

- Showing that water in rock pools is an excellent substrate for olive python eDNA monitoring (published as Mousavi-Derazmahalleh *et al*. (2023) Rock pools as a source of environmental DNA for the detection of the threatened Pilbara olive python *Liasis olivaceus barroni*, Frontiers in Environmental Research – Figures 2 & 3 below).
- Developing a high precision qPCR assay for olive pythons that is now being written up for publication (first data chapter in Ben's PhD).
- Sequencing and assembling the first complete genome of the Pilbara olive python in collaboration with the Vertebrate Genome Project, (VGP). The quality of the genome is exceptional with assemblies into full chromosomes and an N50 value of 203,7 Mb (see table below). The assembly of the Kimberley Olive python (*Liasis olivaceus olivaceus*) genome is in final stages.

Table 1. Assembly statistics of Pilbara Olive python

Assembly statistics	
Genome size	1.5 Gb
No. of chromosomes	17
GC %	40
Scaffold N50	203.7 Mb
BUSCO %	95.38

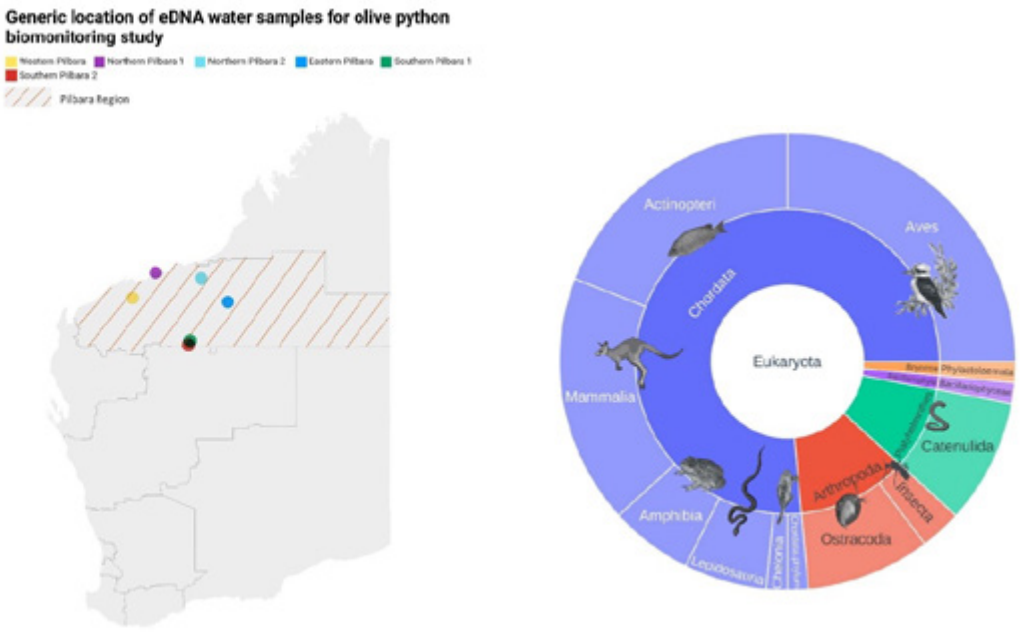


Figure 2. (left) Map of Western Australia showing regions where eDNA water samples were collected. (right) Pie chart displaying overall diversity found in the samples. The size of each piece is proportional to the number of taxonomic assignments after data curation.

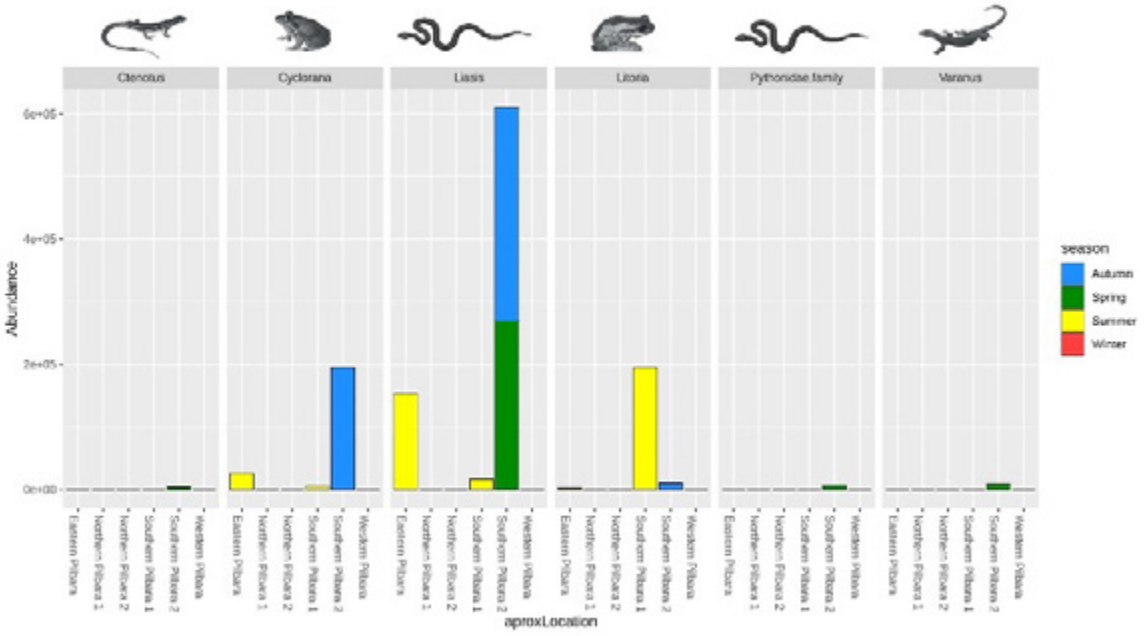


Figure 3. Abundance of reads assigned to reptiles and amphibians genera across the study locations and seasons.

Figure 4. Blood sampling of a Pilbara Olive python at Perth Zoo. A high-quality reference genome of Pilbara Olive python was built from sequencing and bioinformatic assembly of this blood sample.

Further important outcomes include:

- Ben Heyward successfully completed his Hns project on python eDNA decay, and this will now lay the foundation for chapter 3 in his PhD thesis (Figures 5 & 6).
- Preliminary results from the DNA degradation study (Hns project of Ben Heyward) were presented as a keynote presentation (M. Allentoft) on the 1st Australian and New Zealand eDNA conference in Hobart, Feb, 2023.

Overview of any unexpected impacts on the Project and/or beneficiary group

- The main challenge has been the physical absence from 2020 to 2022 of Prof Allentoft in Western Australia to personally interact with the team and collaborators.
- Two unsuccessful rounds of RTP scholarship applications have also resulted in delay but we have now recruited Ben Heyward for a PhD project and he has just started (April 2023).

Leveraged funding and value-add activities

- Dr Mahsa Mousaviderazmahalleh has accepted a continuing position (as lecturer) at Curtin University ensuring her physical presence and dedication throughout the rest of the project. This will greatly benefit data interpretation, PhD supervision on bioinformatics, and maximise utility of the python genome.
- Prof Morten Allentoft, Dr Mahsa Mousaviderazmahalleh, Dr Nicole White (and others) were successful in obtaining an ARC Discovery grant to commence in 2024. Also, Allentoft is still awaiting decision on a pending ARC Future Fellowship application. However, in both cases reviewers highlighted the eDGES program as a tremendous success, an excellent example of industry engagement, and a very strong foundation for leveraging ARC funding.

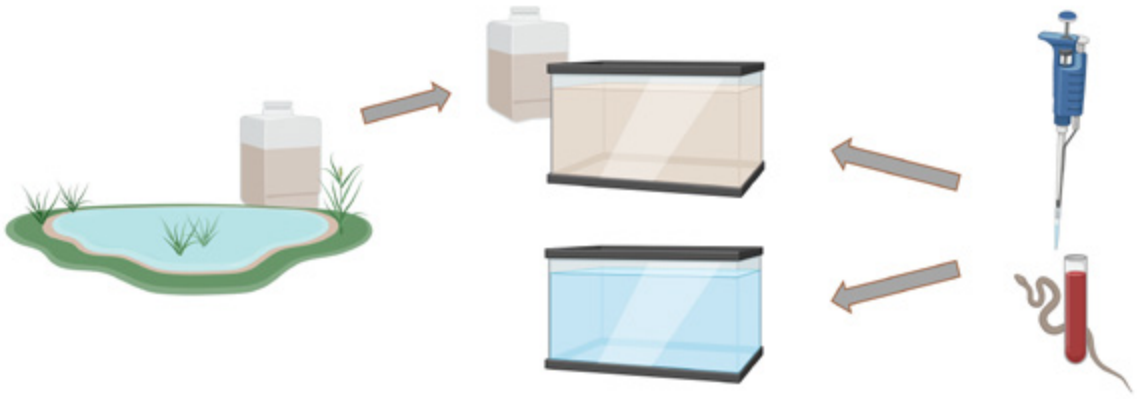


Figure 5. Two twenty litres milli-Q ultrapure and lake water tanks were spiked with 200 µL of Olive python blood. Samples including three replicates were collected at time 0, 1, 3, 6, 9 and 12 hrs, followed by daily sampling for a week, and thereafter twice a week for three weeks. Control samples were collected prior to the blood spike.

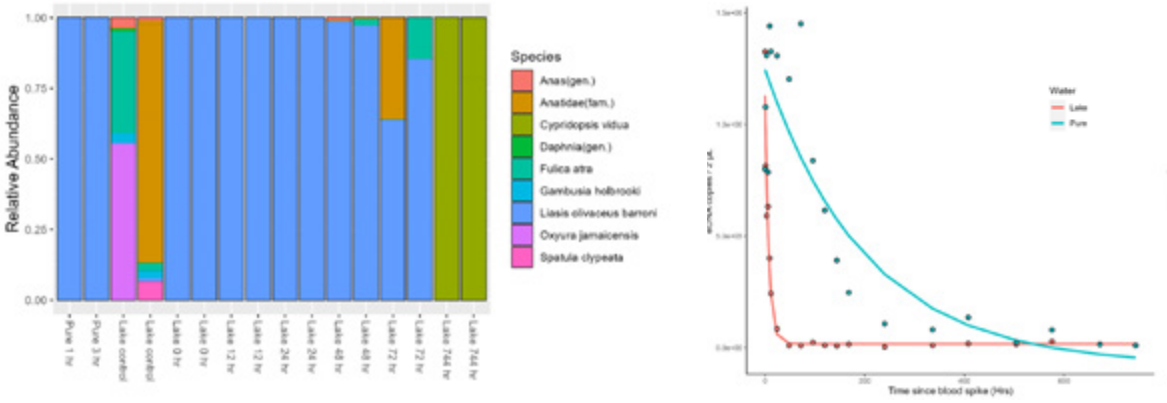


Figure 6. (left) Metabarcoding results of selected samples (2 pure tank samples, 2 lake tank control replicates, and 6 lake tank time points in duplicate). Relative abundance is displayed on the y-axis. Any taxa that could not be assigned to species level is shown as the lowest common ancestor. (right) eDNA copy number/2 µL (y-axis) against exponential decay models fit to both tanks (orange = lake; blue = pure) over 1 month (x-axis).

Figure 7. Substrate sampling at Perth Zoo conducted in February 2024: (left) Prof Morten Allentoft; (right) Ben Heyward (PhD student).

Challenges

- Because of the COVID pandemic Prof Allentoft only moved to Australia in February 2022. This has been a major obstruction for the project, but it has not compromised the research or the objectives. Only the timeline.
- Two unsuccessful rounds of RTP scholarship applications have also resulted in delays.
- The VGP has worked considerably slower than anticipated for the genome assembly. This implies that we have not been able to start this part of the work yet, as we are still waiting for the Kimberley Olive python (*Liasis olivaceus olivaceus*) genome for use in comparative analyses.

Key strategies

- We have re-budgeted the project, so it no longer requires external funding for the PhD scholarship. Hence, we have recruited Ben Heyward for the PhD and he started in May 2023. This PhD project will form the major research output from this point onwards.
- We have adjusted the timeline of the project to end in mid-2026, assuming a 3-year PhD project.
- The delay in genome assembles is no longer a major concern because the overall timeline has been adjusted to align with the PhD project.

Figure 8. (Clockwise from above) Pilbara Olive python (*L.O. barroni*) in water; Python Pool, Millstream NP; *L.o.barroni* basking in sun adjacent to pool. Photos by R.J.Ellis

Learnings

- The TrEnD lab has gained a lot of valuable experience with obtaining permissions (ethical, research, sampling, CITES, export etc) related to the sampling of living animals for genomic research – including the timeframes involved.
- We are currently establishing pipelines for eDNA shotgun sequencing and the results we have already obtained from Ben Heyward's hns project have provided invaluable insights ensuring that the remainder of the project rests on much firmer ground.

Communication

We have not yet substantively communicated this project to the public. This is primarily because it is a PhD project in the making.

- Minor communications include a news item that went to air in June 2021 on Channel 9 News following Perth Zoo's python bleed where team member Dr Nicole White was interviewed and footage was later released on Facebook.
- Reference is made on the eDGES website (https://www.edgesprogram.org).
- Once we have both genomes fully assembled and annotated, we expect to release public communications showing python blood sampling in Perth Zoo and explaining what our project is about.
- Short videos have been filmed to promote the project and describe its goals and outcome. They will be publicly available on the new eDGEs website once completed (https://www.edgesprogram.org).

Sustainability

Sourcing Pilbara and Kimberley pythons for the genome assembly work through agreements made with Perth Zoo and a private snake owner, has saved substantive logistics, unnecessary wildlife harassment, human time and associated emissions involved in field work.

Next Steps

The PhD project of Ben Heyward has been structured as follows: (i) review paper on shotgun sequencing approaches in eDNA (ii) Development of an olive python qPCR assay (iii) testing substrates with shotgun sequencing, qPCR and metabarcoding (iv) DNA decay experiments (v) Population genomics.

Figure 9. DNA collection of *L.o.barroni*.

Key activities planned for the remainder of the project

2024:

- We expect to have both olive python genomes assembled within 2024.
- The olive python qPCR assay (Chapter 2) will get published in a peer reviewed journal.
- All sequencing and qPCR done for the substrate testing (Chapter 3)

2025:

- Conduct DNA decay tank experiments (chapter 4)
- Start population genomic sequencing and analyses (chapter 5)
- Field trip to sample wild pythons for population genomics and possibly engage with relevant schools and traditional owners.

2026:

- Finish analyses, write up, publish the chapters and Ben will hand-in the PhD thesis.
- Publish a comparative study on the two python genomes (Dr Mousaviderazmahalleh as first author) and enable public access to those.

Figure 10. Substrate sampling at Perth Zoo (left to right: Prof Morten Allentoft, Dr Mahsa Mousavi-Derazmahalleh, Mr Ben Heyward).

Subterranean Conservation

Project 2

eDNA for Subterranean fauna detection and conservation

Summary

In this project, running in parallel with ARC Linkage Project LP190100555 – "Taking eDNA Underground: Transforming assessment of subterranean ecosystems" with Michelle Guzik and Mieke van der Heyde, we developed a novel eDNA methodological framework for subterranean biodiversity monitoring in groundwater ecosystems using new assays and rigorous sampling methods for accurate, cost effective and reproducible monitoring of these systems. In addition, sixty-five novel invertebrate mitochondrial genomes from the Great Artesian Basin (GAB; Figure 11) in SA and Ethel Gorge in WA were successfully sequenced (for examples see Figures 12 and 15). Establishment of this complete mitochondrial genome data set is a huge technological step forward in the available reference data for subterranean and groundwater fauna that will facilitate future innovations in eDNA detection (OTU identification and assay design). The mitochondrial genomes also offer several advantages for species barcoding with establishing species delimitation, as these genomes can help determine species boundaries and assess cryptic or closely related species. By comparing the genetic distances among individuals or populations, new species and distinct lineages can be identified for conservation and protection.

Figure 11. An artesian spring located in the Coward Spring complex within the Lake Eyre supergroup were sampled for eDNA analyses and invertebrate fauna for mitochondrial genome sequencing.

Further activities for this project included 180 eDNA water samples from the GAB to investigate and field validate the new assays developed (currently underway). Additionally, these 55 aquifer eDNA samples (Wellfields A&B) and 125 spring water eDNA samples were metabarcoded with two assays (18S and Bact16S) showing springs contain a higher diversity of organisms than aquifers (Wellfield A & B; Figure 16). One additional COI barcoding assay will be sequenced at the end of May 2024 to complete this dataset for analyses and publication.

Phylogeographic analyses of the mitochondrial genomes combined with eDNA metabarcoding can provide an assessment of species boundaries, intraspecies variation and examine the geographic distributions of these unique underground communities (Figure 14). Subterranean invertebrates in groundwater systems have been identified as proxy or surrogate species for conservation. In many cases these animals are endemic to specific groundwater systems and have adapted to the unique environmental conditions of these systems. Due to these species short-range endemism, they are often found in a limited geographic range and can serve as effective indicators of the overall health and condition of the groundwater system they inhabit. Their conservation can help protect and maintain the ecological integrity of these unique and complex systems.

'Dark taxa' refers to groups of organisms that are poorly known or understood, often due to a lack of comprehensive taxonomic study, insufficient sampling, or limited available data. These taxa are considered 'dark' because they exist in relative obscurity, with little information available about their biology, ecology, distribution, or evolutionary relationships. Mitochondrial genome sequencing has shown a linage of Isopoda Amphisopidae as 'dark' when compared to the others of this group that were sequenced (Figure 13). This lineage is known only from sequence data and cannot be linked to any physical specimen or resolved taxonomic name.

Implementation

This project commenced activity as planned in Q4 2020 with a modified focus commencing sampling in the GAB rather than the Pilbara. Three fieldtrips undertaken by BHP due to COVID restrictions resulted in the collection of 192 ground- and artesian-spring water samples for eDNA metabarcoding, in addition to 93 GAB spring invertebrate samples collected from four spring complexes (i.e. Coward, Hermit Hill, Lake Eyre South and Wangianna) for mitogenome sequencing. The second location of focus for this project, Ethel Gorge in the Pilbara Western Australia, resulted in 18 subterranean invertebrates for mitogenome sequencing. Furthermore, tissue samples obtained from the Western Australian Museum for two critically endangered species of blind cave gudgeon, *Milyeringa veritas* (Cape Range) and *Milyeringa justitia* (Barrow Island) were also included for mitogenome sequencing. The complete mitochondrial genomes resulting from this project greatly enhances the DNA reference database, or in other words, 'moving beyond the standard barcode' to facilitate eDNA sequencing and marker development and examine intra-species diversity across broad geographical gradients in both South and Western Australia for this project.

Staffing and management

- **Dr Nicole White** – Principal investigator and Project lead, Research Fellow, Trace and Environmental DNA lab and Subterranean Research and Groundwater Ecology Group at Curtin University. Nicole leads coordination of the overall project management, communications and scientific output.
- **Prof Morten Allentoft** – Principal investigator, head of TrEnD lab and chief scientist in eDNA Frontiers group at Curtin University.

Key collaborators

- **Dr Michelle Guzik** – ARC Research Associate, Australian Centre for Evolutionary Biology and Biodiversity at the University of Adelaide. Michelle is Project Lead of ARC Linkage LP190100555.
- **Dr Nick Murphy** – Senior lecturer, Dept., of Ecology, Environment and Evolution at La Trobe University. Nick has an extensive collection of GAB spring fauna to facilitate the mitochondrial genome sequencing for this project, if required.
- **Dr Mieke van der Hyde** – ARC Research Associate, TrEnD lab at Curtin University. Mieke is the Research Associate on LP190100555 and supervised by Nicole and Michelle. She is involved in all the eDNA development work required for both projects with a focus on the Pilbara fauna.
- **Kimberley Solly** – Key contact, Specialist Environment, BHP Olympic Dam in South Australia. Kim was introduced to this project by Stephen White (BHP Perth) and has been instrumental with coordinating field sample collection and transport to Adelaide Uni. In addition, Kim has coordinated the invertebrates to be collected (BHP Field Team) and sorted by consultant GHD (Zac Billingham).
- **Tanya Carroll** – Key contact, BHP Perth. Tanya is the key contact for organising fieldwork, accommodation, maps, site safety and inductions with Alison Fairfax, Logistics Coordinator at BHP Perth.

Note: Running parallel to this project is ARC Linkage Project LP190100555 – "Taking eDNA Underground: Transforming assessment of subterranean ecosystems" with Michelle Guzik and Mieke van der Heyde. Goals are to ensure the research objectives for both projects are complementary to each other with the overall aim of improving and enhancing the molecular toolkit required for rapid and accurate biodiversity monitoring tools for subterranean systems in Western Australia and South Australia.

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
Sequencing and assembly of the mitochondrial genome of the blind cave gudgeon	The mitochondrial geonomes of the blind cave gudgeon, Milyeringa veritas and M. justicea were sequenced at the TrEnD lab and validation of genus specific eDNA markers is underway at Adelaide University with PhD student Milad Khosravi supervised by Dr Michelle Guzik.	Ongoing
Sequencing and assembly of invertebrate mitochondrial genomes	67 complete or near complete invertebrate mitochondrial genomes from the GAB and Ethel Gorge were sequenced. In addition, and working in collaboration with Dr Michelle Guzik ARC LP190100555, 83 subterranean fauna were sequenced from the Pilbara and 20 with Guilia Perina under ABRS grant.	Complete
GAB and Pilbara fieldwork, DNA extraction and metabarcoding of groundwater	Fifty-five aquifer eDNA samples and 125 spring water eDNA samples were collected from the GAB and metabarcoded with two assays (18S and Bact16S). The third COI assay will be sequenced at the end of May 2024 to complete this dataset.	Completed and ongoing
Test new eDNA markers on groundwater (Pilbara and GAB)	The mitochondrial genomes are under bioinformatic assembly and eDNA marker development, lab and field validation are underway.	Commenced and ongoing
Manuscript preparation and submission	Nine manuscripts (some overlap with eDGES Project 3) with Dr Mattia Sacco, ARC LP190100555 with Dr Michelle Guzik and Dr Mieke van der Heyde and ABRS Grant with Dr Giulia Perina on subterranean fauna research.	Ongoing

Overview of outputs and outcomes

- **Sequencing of mitochondrial genomes with bioinformatic assembly is Complete** – A total of 170 complete or near complete mitochondrial genomes have been sequenced for subterranean vertebrates and invertebrates from WA and SA.
  - ▷ Sixty-five complete or near complete mitochondrial genomes were successfully sequenced from the GAB.
  - ▷ **New insights into genetic differentiation:** Seventeen complete or near complete Amphipoda Chiltrioniidae mitochondrial genomes from four GAB Spring complexes showing 1.7% – 4.4% intraspecies diversity within a spring complex for this taxon group. Sixteen complete or near complete Isopoda Amphisopidae mitochondrial genomes from four GAB Spring complexes showing 0% – 0.8% intraspecies diversity within a spring complex for this taxon group.
  - ▷ **Deep splits in Ostracods:** Fifteen complete or near complete Ostracoda mitochondrial genomes were from four GAB Spring complexes show two highly distinct lineages with overlapping distributions.
  - ▷ One *Fonscochlea aquatica* and seven *Fonscochlea accepta* mitochondrial genomes were sequenced from two GAB Spring complexes showing 0.7% intraspecies variation for *F. accepta* within the Hermit Hills spring complex.

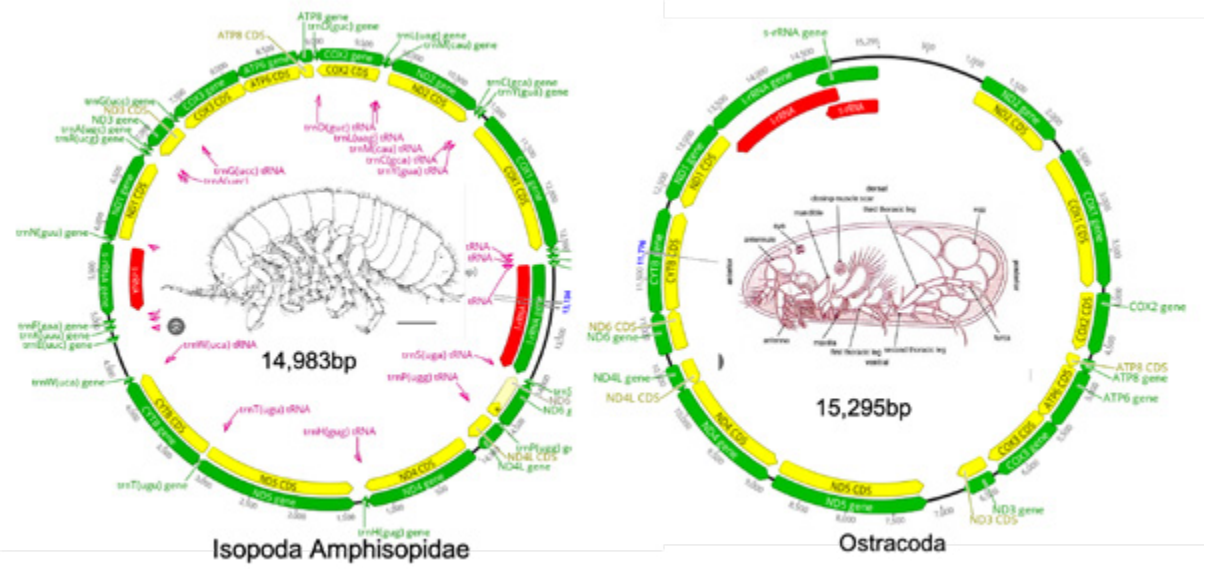


Figure 12. Complete mitochondrial genomes for two GAB spring fauna (Isopod and Ostracod) are shown. Differences in mitochondrial gene order have been identified which can provide valuable insights into the evolutionary relationships, molecular evolution and mechanisms of genomic rearrangement.

- Further, nine mitochondrial genomes were sequenced from Ethel Gorge including *Chydaekata acuminata*, Chydaekata AMP005, *Diacyclops cockingi*, *Pygolabis humphreysi*, *Brevisomabathynella pilbarensis* and *Pilbaranella ethelensis*.
- Two blind cave gudgeon mitochondrial genomes *Milyeringa veritas* (Cape Range) and *Milyeringa justitia* (Barrow Island) were successfully sequenced for eDNA marker development.

- **eDNA marker development with lab validation is underway** – Species-specific eDNA marker development for *M. veritas* and *M. justicea* is underway is underway at Adelaide University with PhD student Milad Khosravi. PhD candidate Milad Kosravi holds a PhD scholarship funded by ARC Linkage Grant LP190100555. Dr Nicole White is an external supervisor of Milad's PhD research program together with Dr Michelle Guzik and Professor Steve Cooper at Adelaide University. The mitochondrial genomes from the GAB fauna are under bioinformatic analyses, gene annotation and quality checking. New eDNA assays have been tested *in-silico* and COI metabarcoding with the new assay has been tested on GAB spring water. The mitochondrial genome reference database will be used to compare the metabarcoding data for field validation of the assay. Results of field validation are expected to be complete by mid-June 2024.
- **GAB fieldwork and DNA extraction of groundwater is complete. Pilbara fieldwork and DNA extraction of groundwater is delayed** – Field sampling for 2022 in the Pilbara (Ethel Gorge Stygobiont Threatened Ecological Community) focused on stygofauna specimens for mitochondrial sequencing. Communications with BHP staff and eDNA Frontiers is underway with the possibility of obtaining the previously collected and DNA extracted groundwater from Ethel Gorge Stygobiont Threatened Ecological Community to test new eDNA markers once the mitogenomes from this community have been produced.
- Ground and spring water (*n*=192) spanning 130km across the Lake Eyre GAB springs have been completed with the universal 18S assay, bacterial 16S assay, and soon to be complete COI assay. The combined dataset on the GAB ground and spring water will be analysed and published.

- **Testing of new eDNA markers on groundwater (Pilbara) is delayed and GAB has commenced –** Fifty-five aquifer eDNA samples (Wellfields A&B) and 125 spring water eDNA samples were collected from the GAB and metabarcoded with two assays (18S and Bact16S) showing springs contain a higher diversity of organisms than aquifers (Wellfield A & B; Figure 15).
    - ▷ New eDNA assays have been tested in-silico and COI metabarcoding with the new assay has been tested on GAB spring water. The mitochondrial genome reference database will be used to compare the metabarcoding data for field validation of the assay. Results of field validation are expected to be complete by mid-June.

Manuscript preparation and submission is ongoing

Nine manuscripts from eDGES Project 2&3 with Dr Mattia Sacco, ARC LP190100555 with Dr Michelle Guzik and Dr Mieke van der Heyde and ABRS Grant with Dr Giulia Perina on subterranean fauna research.

van der Heyde, M., **White, N.E.**, Nevill, P., Austin, A.D., Stevens, N., Jones, M. and Guzik, M.T., (2023). Taking eDNA underground: factors affecting eDNA detection of subterranean fauna in groundwater. *Molecular Ecology Resources*. 0: 1-18. https://doi.org/10.1111/1755-0998.13792

Saccò, M., Guzik, M.T*., van der Heyde, M.; Nevill, P., Cooper, S.J.B. Austin, A.D, Coates, P.J., Allentoft, A. E.; **White, N.E.** (2022) eDNA in subterranean ecosystems: applications, technical aspects and future prospects. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2022.153223

Saccò, M., Mammola, S., Altermatt, F., Alther, R., Bolpagni, R., Brancelj, A., Brankovits, D., Fišer, C., Gerovasileiou, V., Griebler, C., Guareschi, S., Hose, G. C., Korbel, K., Lictevout, E., Malard, F., Martínez, A., Niemiller, M. L., Robertson, A., Tanalgo, K. C., Guzik, M.T., ... Reinecke, R. (2023). Groundwater is a hidden global keystone ecosystem. *Global Change Biology*, 30, e17066. https://doi.org/10.1111/gcb.17066

Perina, G., Camacho, A.I., **White, N.E.**, Callan, S.K, Abello, J., Morgan, L. Guzik, M.T. (Submitted) An integrated study of *Atopobathynella* (Parabathynellidae, Bathynellacea) species reveals restricted distributions in a complex hydrogeological setting: two new species from the Pilbara (Australia). Contributions to Zoology.

Perina, G., Camacho, A. I., Danks, M., **White, N.**, & Guzik, M. T. (2023). Two new species of *Atopobathynella* (Parabathynellidae, Bathynellacea) from the Pilbara region, Australia. *Systematics and Biodiversity*, 21(1), 2228326. https://doi.org/10.1080/14772000.2023.2228326

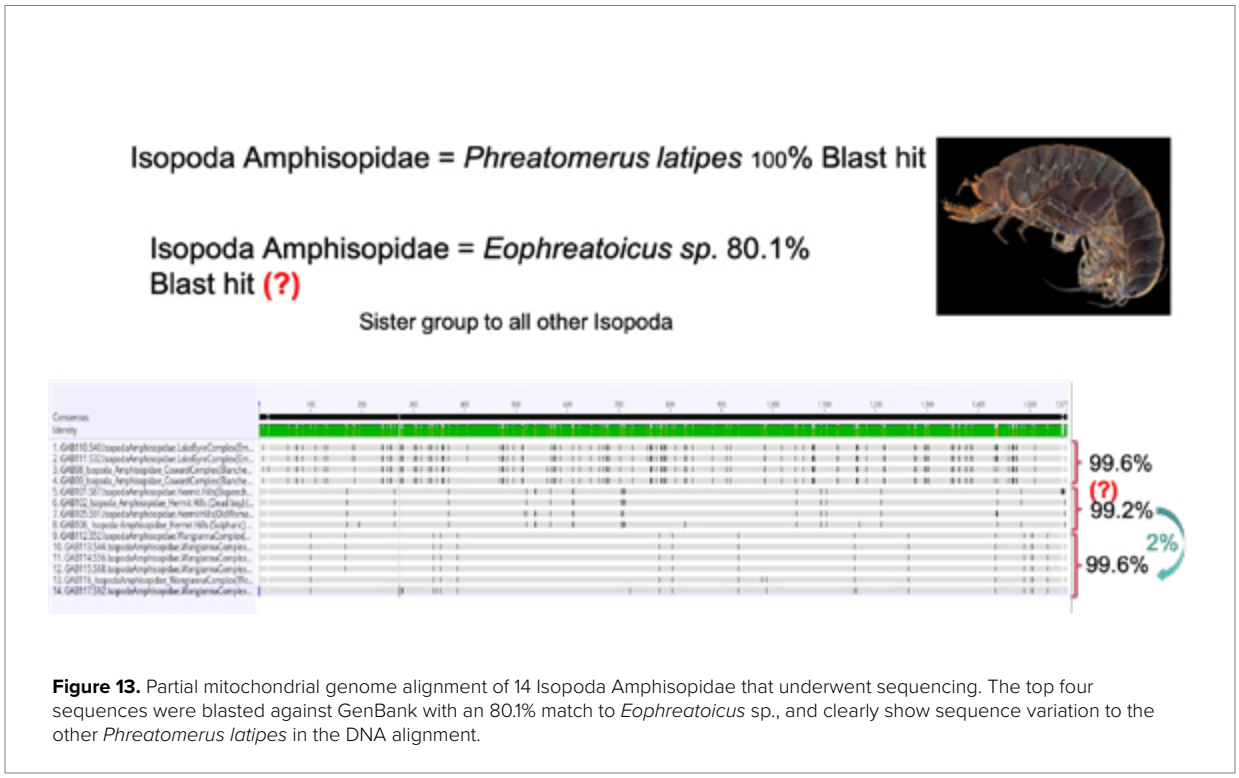


Figure 13. Partial mitochondrial genome alignment of 14 Isopoda Amphisopidae that underwent sequencing. The top four sequences were blasted against GenBank with an 80.1% match to *Eophreatoicus* sp., and clearly show sequence variation to the other *Phreatomerus latipes* in the DNA alignment.

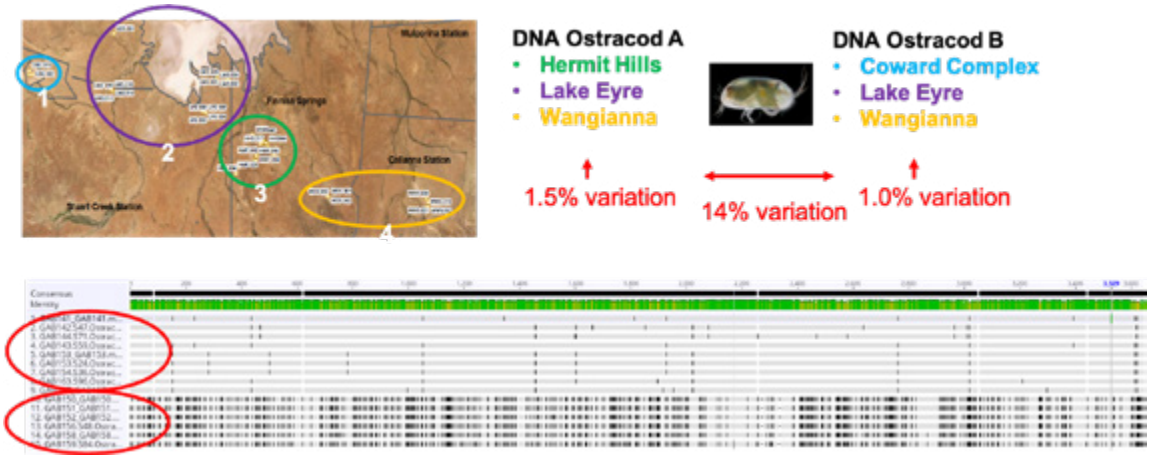


Figure 14. Intraspecies variation found amongst Ostracod mitochondrial genomes which were collected from four spring complexes (Coward, Lake Eyre, Hermit Hills, Wangianna) and shows two distinct genetic lineages with 14% genetic variation between the lineages.

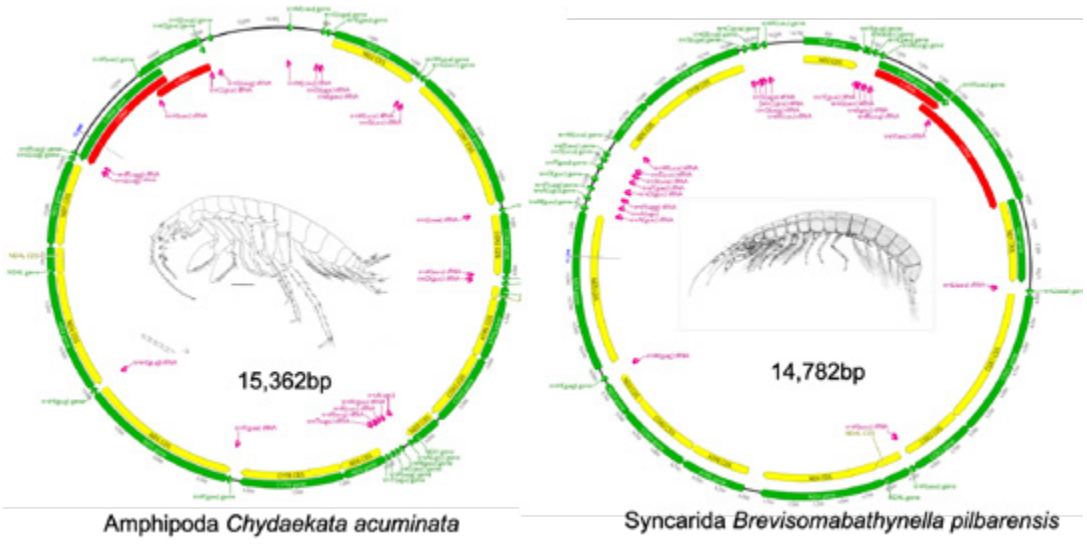


Figure 15. Complete mitochondrial genomes for two Ethel Gorge subterranean fauna (Amphipod and Syncarid) are shown. Differences in mitochondrial gene order have been identified with subterranean fauna inhabiting Ethel Gorge. Such data offers the opportunity to explore diverse aspects of evolutionary biology, molecular genetics, and ecological adaptation in these animals.

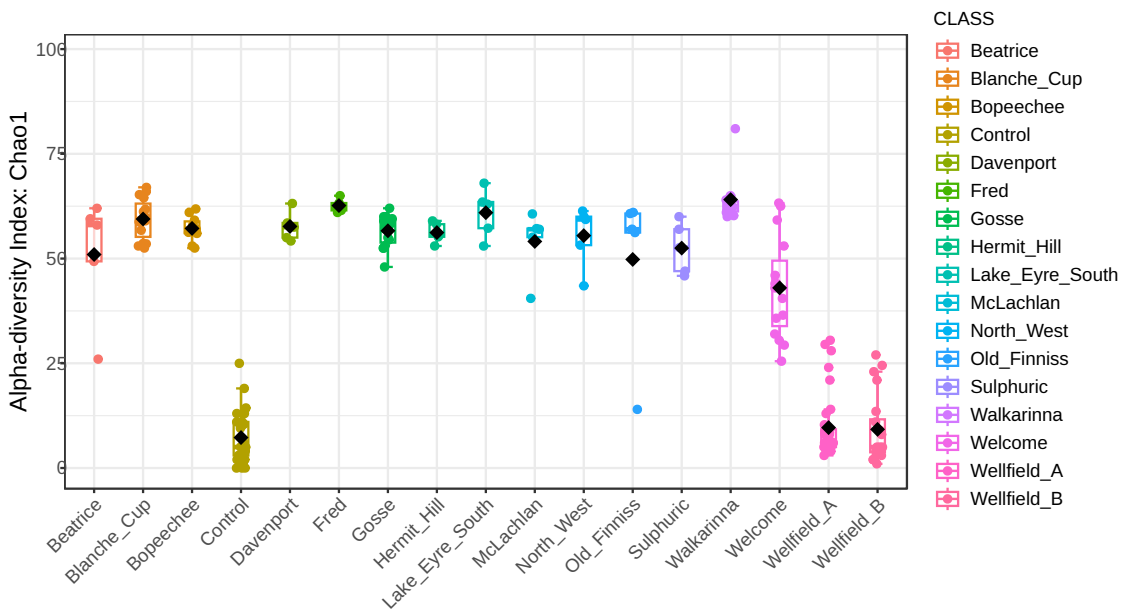


Figure 16. Alpha-diversity index showing GAB springs contain a higher diversity of organisms than that contained in the deep aquifers (Wellfield A & B) of the Great Artesian Basin.

Guzik, M.T., Stringer, D.N., Thornhill, J.C., Coates, P.J., Humphreys, W.F., van der Heyde, M., **White, N. E.**, Sacco, M., Besaley-Hall, P., Hillyer, M.J., Wilson, N.J., Hosie, A.M., Kirkendale, L., Alexander, J., Huey, J.A., King, R.A, Cooper, S.J.B., Austin, A.D. (Submitted) Best practices for curating eDNA custom barcode reference libraries: an Australian case study. *Limnology and Oceanography*.

Guzik, M.T., Thornhill, J.C., van der Heyde, M., Stringer, D.N., **White, N. E.**, Sacco, M., Beasley-Hall, P., King, R.A, Cooper, S.J.B., Nevill, P., Austin, A.D. (Submitted) Towards a global barcode reference library for subterranean fauna. *Science of the Total Environment*.

Beasley-Hall, P.G., Murphy, N.P., King, R.A., **White, N.E.**, Hedges, B.A., Cooper, S.J.B., Austin, A.D., Guzik, M.T (2023) Time capsules of biodiversity: Future research directions for groundwater-dependent ecosystems of the Great Artesian Basin. *Frontiers in Environmental Science*. https://doi.org/10.3389/fenvs.2022.1021987

Takahashi, M., Sacco, M., et al. (2023) Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. *Science of the Total Environment*, Vol 873, 162322. https://doi.org/10.1016/j.scitotenv.2023.162322

Overview of any unexpected impacts on the Project and/or beneficiary group

Field samples and subterranean specimens for mitochondrial sequencing from the Ethel Gorge Stygobiont Threatened Ecological Community in the Pilbara may have to rely on BHP contracted consultants with site access granted as part of regular monitoring of BHP activities in this WA region.

Challenges

No major challenges with lab work, despite COVID delays with travel and obtaining samples having had an impact on eDGES project 2.

Learnings

Genomic datasets are foundational for understanding species evolution and mitogenome assemblies provide a valuable resource for efficient mapping of additional sequences. This will enable characterisation of mitogenomic diversity and differences across individuals, populations, and species groups. The amount of time required for mitogenome quality control,

annotation and analyses was under-estimated, partly due to completing 4x more data than was originally proposed in this project. But a valuable resource, has emerged from this.

Communication

- Grant award application (BHP HSEC Award for Environment and Climate Change) co-written by Nicole and Kim Solly was submitted November 2022.
- eDGES project 2 was presented at the 1st Australian & New Zealand eDNA Conference in Hobart, 14–17th February 2023.
- eDGES project 2 was presented in Adelaide at an ARC Linkage grant writing workshop in March 2023.
- This project has been referenced on the eDGES website (https://www.edgesprogram.org).
- eDGES project 2 was presented at the eDGES Annual Stakeholder meeting in Perth, 15th November 2024
- eDGES project 2 was presented at the final ARC Linkage meeting LP109100555 “Taking eDNA underground: transforming assessment of subterranean ecosystems” in Perth, 27th February 2024.
- eDGES project 2 was presented at the WABSI workshop “Integrating eDNA into bioassessment and monitoring of subterranean fauna in WA”, 28th February 2024.

Sustainability

The pooling of resources and expertise between this project and the ARC Linkage Project (LP190100555) has enabled less travel and field work duplication while enabling greater anticipated outputs for the development of a comprehensive eDNA molecular toolkit for describing and monitoring subterranean diversity and conservation in Australian groundwater ecosystems.

Next Steps

Key activities planned for the next period:

- Obtain next batch of stygofauna animals from Ethel Gorge for mitochondrial genome sequencing.
- Obtain additional data from GAB water samples for analyses and publication.
- Continue with eDNA marker validation for SA and WA groundwater systems.

Wetland Conservation

Project 3

Functional ecology of Chilean precordillera lakes: towards a Wetland Health Index for birds

Figure 17. Flamingo flocks at the Salar de Tara. Credit: Pablo Aguilar.

Summary

In this project we used eDNA technology (and isotope analysis) to monitor biodiversity and characterize ecosystem functioning of hypersaline lakes within the Chilean precordillera. These environments provide habitats and resources that are key to the survival of several species of high conservation concern, including the Chilean James's and Andean flamingos. However, the current knowledge of the ecological dynamics that sustain these hypersaline wetlands is poor. This study incorporated eDNA monitoring for a wide range of prokaryotic and eukaryotic taxa in water and sediments. This approach establishes a solid knowledge baseline in understanding the ecosystem functioning that is shaping key water bird habitats such as the high-altitude hyper-saline environments of the Chilean precordillera. By consistently widening current knowledge on diversity and functions in hypersaline lakes, the analytical toolkit developed through this project has the real potential to improve the coexistence of economic (i.e., mining) and non-economic (i.e., ecosystem) services into the future.

Implementation

The project commenced in July 2020 incorporating the back then postdoc (and now Lecturer) Dr Mattia Saccò who has led the project since this time under the guidance and advice from Prof Morten Allentoft and Dr Nicole White, and the collaboration of Dr Matthew Campbell. Throughout this reporting period, regular meetings with collaborators at the University of Antofagasta have been organised to structure the international partnership, recruit the PhD student and develop the PhD project and refine the scientific agenda of the study.

Staffing and management

Curtin University:

- **Dr Mattia Saccò**
  Principal investigator and Lecturer at TrEnD. He is leading fieldwork organisation, laboratory analyses, data elaboration and writing of research articles.
- **Dr Nicole White**
  Principal investigator, research fellow and former TrEnD laboratory manager with expertise in conservation genetics, wildlife forensics and eDNA research. Nicole assisted with the coordination of the project, including field work implementation, student and staff training, including laboratory work and data analysis.
- **Dr Matthew Campbell**
  Chief investigator, expert in molecular ecology and biogeochemical analysis. Matthew assisted with the development of the Rottnest Island satellite project and the data analysis derived from the samples collected in the Chilean precordillera.
- **Prof Morten Allentoft**
  Principal investigator, head of TrEnD lab & Chief Scientist of the eDNA Frontiers group at Curtin.

University of Antofagasta:

- **Professor Chris Harrod**
  Project coordinator from the Chilean institution (University of Antofagasta) and director of the Universidad de Antofagasta Stable Isotope Facility (UASIF). He is managing the Chilean component of the study, providing space, equipment and expertise, as well as analytical capacities (laboratory and statistical).
- **Dr Pablo Aguilar**
  Microbial ecologist and limnologist. His contribution to the project is related to field work, molecular analyses and the contribution of microbial taxa to higher trophic levels using molecular techniques.
- **Gonzalo Salazar**
  Full time PhD student based at the University of Antofagasta and supervised by Prof Prof Chris Harrod.
- **Dr Pablo Pérez**
  Analytical chemist and laboratory manager of UASIF. His contribution to the project focuses on the laboratory development of multi-isotope tracers to identify the flow of energy and nutrients through salar food webs.

External collaborators:

- **Dr Alex Laini**
  Senior researcher at the University of Parma (Italy). He is an expert in freshwater ecological modelling and provided statistical and methodological support.

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
2023–2024		
Continuation with the education objective (schools, universities, CONAF)	Due to the difficulties encountered in engaging with local collaborators, regulators and communities, educational initiatives have not been delivered. Funding for this deliverable has been redirected to analytical and publication costs (e.g., open access article fees) to increase the outreach and impact of the research associated with the project.	Funding redirected
Further research article submitted	A manuscript on the combination of conventional and eDNA techniques in hypersaline lakes has been published in the *Journal of Oceanology* and *Limnology*. TrEnD researchers were also involved in the writing of an aquatic eDNA decadal review published in *Science of the Total Environment*. An additional article on the methodological comparison between molecular platforms for environmental studies will be submitted by July 2024.	Completed

Expected Project Deliverables	Progress/Comments	Status
2023–2024		
Contribution to a public seminar on eDNA and hypersaline ecosystems	An oral presentation was delivered at the eDNA Conference in Hobart in February 2023. Mattia has also been invited to the participate to the 'Revisiting Freshwater Imperative' Workshop at Colorado State University in July 2023. He also participated in the writing of an article on the use of eDNA in environmental research for *Bushland News*.	Completed
Project finalised and PhD thesis completed (according to the timeline dictated by the University of Antofagasta and Curtin University)	PhD student Gonzalo Salazar started his PhD in 2021 and he is expected to finalise his degree in March 2026.	Behind schedule
Phase 4 report with final results	This report contains the final results in correspondence of the finalisation of the project.	Current report

BHP managerial support:

- **Magdalena Fernandez** Principal Environment Non Ops Joint Venture at BHP. She provided conceptual and logistical support to the project.

Leveraging opportunities

- Unused analytical (only one batch of samples out of the two initially planned) and staff funds (Mattia twice on 0.5 FTEs for a few months for caring duties over 4 years) were redirected for the employment of Dr Angus Lawrie (March-June 2024) and Matthew Heydenrych (January-June 2024) as Research Associates to collect further samples, analyse data and test the applicability of the design for eDGES v2. The incorporation of Angus and Matt to the team has provided great value in terms of logistical support and scientific expertise, allowing the collection of a remarkable dataset on hypersaline lakes in WA.

Overview of outputs and outcomes achieved during the 4-year project

PhD project at the University of Antofagasta carried out by Gonzalo Salazar Riquelme

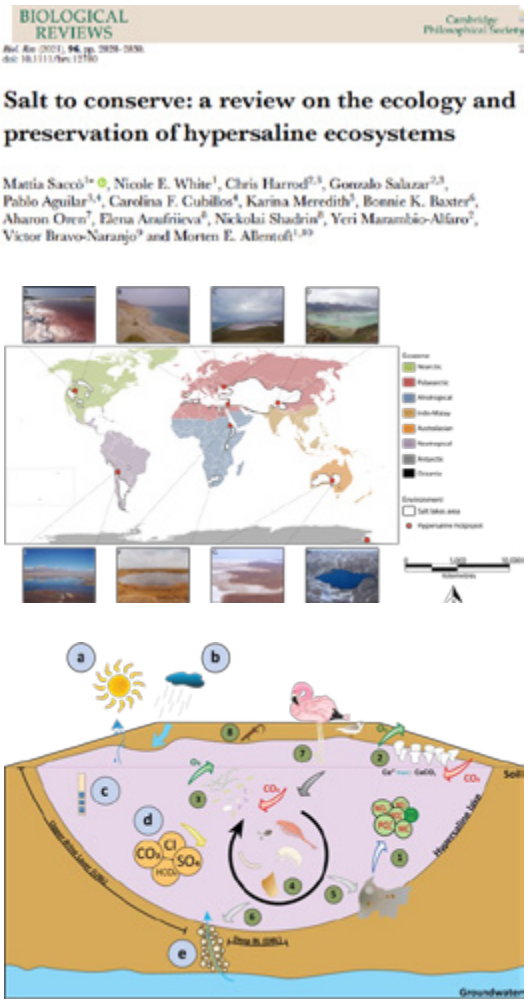
The PhD student Gonzalo Salazar was officially recruited at the University of Antofagasta in 2021. Both his salary and research has been funded by current project, and completion is due in 2026. During the interval 2021-2024, he has actively participated in the sampling plan definition, fieldtrip and sorting of specimens, and writing of manuscripts. He has proved to be a proactive student with remarkable skills in morphological identification of aquatic invertebrates. He was also involved in the presentation at the eDGES stakeholder meeting in

Figure 18. Photos of Gonzalo Salazar in the filed with the CONAF and the UoA Team collecting samples for the project, and presenting at the eDGES stakeholder meeting in 2023.

2023, and will submit his PhD Thesis by March 2026 due to internal delays associated with logistical (e.g., sampling permits) and bureaucratical (e.g., PhD contract agreement) issues.

International multidisciplinary literature review on the ecology of hypersaline lakes

A literature review on the ecology and preservation of hypersaline wetlands was published in the prestigious journal *Biological reviews* in 2021 (https://doi.org/10.1111/brv.12780). So far, the manuscript has been cited 64 times in less than three years, demonstrating the high impact of the research carried out by our team. The work developed through the review has also led to international collaborations with experts in Russia, China and Europe.


BIOLOGICAL REVIEWS

Salt to conserve: a review on the ecology and preservation of hypersaline ecosystems

Mattia Saccò, Nicole E. White, Chris Harrod, Gonzalo Salazar, Pablo Aguilar, Carolina F. Cubillos, Karina Meredith, Bonnie K. Baxter, Aharon Oren, Elena Anufriieva, Nickolai Shadrin, Yeri Marambio-Alfaro, Víctor Bravo-Naranjo and Morten E. Allentoft

Figure 19. Front page and two graphs on the distribution and ecology of hypersaline lakes from the published manuscript.

Satellite investigations at Rottnest Island

A pilot study targeting the hypersaline lakes at Rottnest Island was initiated in 2020 with the goal of testing the state-of-the-art molecular techniques that were planned to be implemented in the Chilean precordillera. Several fieldtrips were carried out to assess the sampling techniques and finally collect samples from five hypersaline lakes on the island. The research associated with this satellite investigation has led to the publication of two manuscripts in *Water* (https://doi.org/10.3390/w13141899) and *Journal of Oceanology and Limnology* (https://doi.org/10.1007/s00343-022-2151-9), respectively:

Metabarcoding under Brine: Microbial Ecology of Five Hypersaline Lakes at Rottnest Island (WA, Australia)
In this study we tested the use of eDNA techniques to assess the microbial communities of five hypersaline lakes at Rottnest Island. Our findings confirm the high effectiveness of DNA metabarcoding analysis in elucidating differential microbial patterns (diversity and functions) under different environmental conditions.

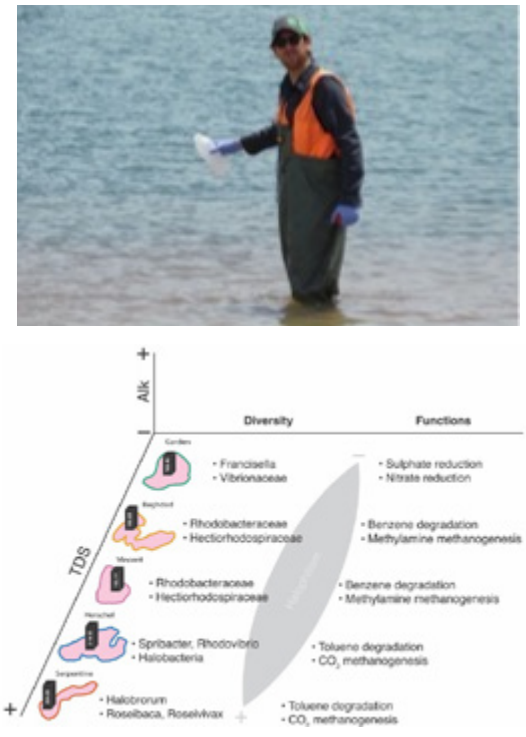


Figure 20. Photo from the fieldwork activities and summary graph from the published manuscript (https://doi.org/10.3390/w13141899) showing the diversity and functional patterns unveiled through eDNA techniques.

A third manuscript titled "Looking under the mat: benchmarking computational methods for analysing microbial datasets" is currently in progress, and it is expected to get submitted by the end of July 2024. In this study we compare the performance of several functional genomics platforms with the main goal to validate the use of DNA metabarcoding techniques for environmental monitoring through time by using microbial mats as model systems.

Chilean precordillera study

This was the main component of the project and involved the design, collection and analysis of samples from three hypersaline lakes located at different altitude in the Chilean precordillera: Puilar lagoon (Salar de Atacama), Salar de Tara and Salar de Pujsa. Fieldwork was carried out in October 2021 and both sediment and water samples were collected from each salar. The vast dataset generated through DNA metabarcoding approaches has been divided in two manuscripts in preparation (and both to be submitted by July 2024 to *Frontiers in Environmental Science*):

Environmental DNA as a tool to unveil metazoan diversity of Chilean Precordillera hypersaline lakes
In this work (short communication) we investigate the use of eDNA analysis as a tool to unveil metazoan hypersaline diversity. Our results indicate that our multi-assay approach is able to unveil a vast group of endemic taxa (from microscopic to wading birds) across the three salars targeted. Some examples of charismatic and hypersaline resident taxa detected through eDNA include flamingos (*Phoenicopterus*), gastropods (*Heleobia atacamensis*), amphipods (Hyalella), copepods (Diaptomidae) and ostracods (*Heterocypris salina*).

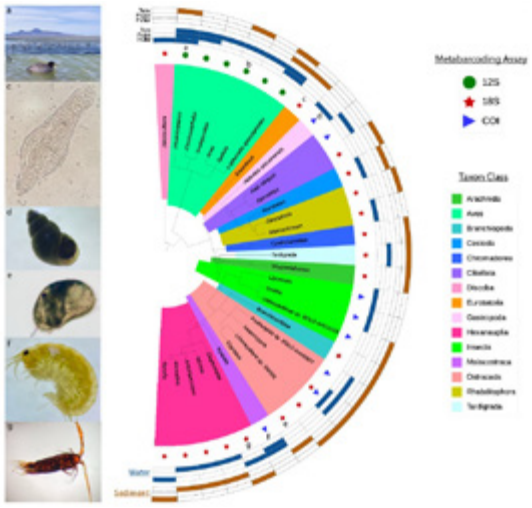


Figure 21. Graph from the manuscript (in preparation) showing the array of species found in water and sediment samples across the three salars via DNA metabarcoding techniques.

Diversity of resident prokaryotic and eukaryotic microorganisms in three hypersaline lakes via DNA metabarcoding
In this work we provide an in-depth characterisation of the microorganismal diversity patterns of the three salars investigated. A multi-assay approach (Bact16S - microbes, COI – fungi and protists, 18S – fungi and protists, and rbcl – algae). Our results indicate that the salars are shaped by high intra- and inter-lake microorganismal diversity patterns, highlighting the uniqueness of these ecosystems in terms of functional adaptations to high altitude and UV ranges.

Isotope work – The analysis of food web interactions and diet preferences of macroinvertebrates and flamingos has been led by Prof Chris Harrod and his team at the Universidad de Antofagasta Stable Isotope Facility (UASIF). Transfer of funds for analytical purposes was included in the fieldwork allowance (initially planned to cover two campaigns, but eventually allocated for just one sampling event in October 2021). Results are expected to be generated over 2024 and 2025, and data will be analysed and presented in Gonzalo's Thesis and through publications in collaboration with the eDGES team of researchers.

Other published manuscripts derived from the project

PI Mattia Saccò has co-led the writing of a literature review on the last ten years of aquatic eDNA research that was published in the high impact factor (IF: 10.753) scientific journal *Science of the Total Environment* on the 15th of May 2023. PIs Nicole White and Morten Allentoft and CI Matthew Campbell were also involved in this manuscript titled "Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution" (https://doi.org/10.1016/j.scitotenv.2023.162322), which reviews for the first time the last 10 years of aquatic eDNA research by thoroughly assessing techniques involved and standardisation patterns. The review has been cited 61 times in just over one year, confirming once again the high impact value of the publications led by our team.

Over the last 4 years and as a result of the collaborations derived from the research developed in the eDGES project, Mattia was involved in the following two manuscripts on macroinvertebrate ecology:

Laini, A., Stubbington, R., Beermann, A.J., Burgazzi, G., Datry, T., Viaroli, P., Wilkes, M., Zizka, V.M.A., **Saccò, M.** and Leese, F., 2023. Dissecting biodiversity: assessing the taxonomic, functional and phylogenetic structure of an insect metacommunity in a river network using morphological and metabarcoding data. *The European Zoological Journal*, 90(1), pp.320-332.

Burgazzi, G., Laini, A., Ovaskainen, O., **Saccò, M.**, Stubbington, R. and Viaroli, P., 2020. Communities in high definition: Spatial and environmental factors shape the micro-distribution of aquatic invertebrates. *Freshwater Biology*, 65(12), pp.2053-2065.

Outreach

Scientific outreach to conservation agencies (CONAF): involvement of the local conservation agency CONAF has been consolidated through the first sampling fieldtrip in October 2021.

The manuscript published in *Water* (https://doi.org/10.3390/w13141899) has been portrayed in the following blog: https://emg.reallyhelps.org/.

PI Mattia Saccò has been involved in the writing of the article titled “New emerging technology – Environmental DNA (eDNA)” for *Bushland News*.

PI Mattia Saccò has been invited to comment an article on lithium extraction and conservation of flamingos for Science News (https://www.sciencenews.org/article/lithium-mining-flamingo-technology-climate-change)

Overview of any unexpected impacts on the Project and/or beneficiary group

The international collaboration was severely impacted by COVID-19 situation in the early-middle phases of the project. However, collaborators at the University of Antofagasta have continued lab work activities and biogeochemical analyses linked to the project such as sorting of specimens and pre-treatment of samples.

Planning of fieldtrips and the logistical aspects of the project was impacted, and only one fieldtrip occurred in October 2021. Shipping of samples also got consistently delayed and caused further delay to the metabarcoding analyses. Workflow in place at TrEnD has allowed processing of samples and generation of molecular data well within completion of the project.

Under these complicated circumstances, bureaucratical procedures such as the completion of the PhD contract agreement for transferring funds between the two entities (Curtin University and the University of Antofagasta) have been delayed. Final signatures from both institutions (Curtin University and University of Antofagasta) happened in the second half of 2022. This eased the transfer of money both for the PhD salary and to cover the logistical and analytical costs of the project.

Leveraged funding and value-add activities

The incorporation of the two Research Associates Dr Angus Lawrie and Matthew Heydenrych to the team has allowed the testing and validation of analytical approaches that will be employed in eDGES v2 Project 3. In essence, the expansion of the team enabled two streams of work during the last few months of the project: writing of manuscripts (led by PI Mattia Saccò) and collection of samples from saline/hypersaline lakes to test TICI approaches.

Challenges

Due to the COVID-19 circumstances, the 2021 fieldtrip initially planned for March/April was delayed to October 2021. Lack of permits from the local communities caused cancelling of the second fieldtrip.

Bureaucratical issues and delays have also kept emerging. The signing of the PhD contract agreement prepared by Curtin and Antofagasta has faced major legal review delays, and it was finally approved in 2022 and money to cover the PhD salary got transferred almost three years after the start of the project. This has had an impact on the PhD student, who has had to pay out of pocket for the annual fees of his PhD program.

Key strategies

As commented above, the first fieldtrip was delayed to October 2021. This change did not affect the scientific value of the project given it is the first time that the targeted hypersaline lakes are studied with eDNA techniques.

Given the closure of international borders in Australia, all the fieldtrips needed to be carried out by the collaborators in Chile. Funds covering all the costs were transferred to the researchers at the University of Antofagasta and one fieldtrip was successfully carried out. Shipping of the first and unique batch of samples happened in August 2022 and samples have been quickly analysed thanks to the established TrEnD workflow for eDNA studies. In-parallel planned

biogeochemical analyses are being carried out by our collaborators at the University of Antofagasta.

Bureaucratical roadblocks emerged regarding the PhD contract agreement which have been mainly in due diligence and simply resolved, but the workloads of the institutions have slowed this process considerably. Regular check-ins have assisted to keep this on priority and now signature and transfer of funds for the PhD are completed.

Learnings

This project has presented several challenging situations, however some key learnings from the project have been:

Flexibility under COVID-19 scenario: consistent and constant readjustment of the initial plan has fostered resilience and strong forward thinking, strengthening the collaborations between the research groups and the stakeholders involved in the project.

Permits and local community involvement: given this project involves a complex and multi-layered framework (international partnership, local communities, etc.), we have gathered a deep knowledge on the internal dynamics shaping science projects in Chile. This learning process will be extremely useful for eDGES Project 3 v2 on the ecological restoration at Salar de Punta Negra.

Communication

The project has been presented through several on-line workshops, initially to collaborators at the University of Antofagasta and BHP Chile, and later also to stakeholders from Minerals America.

An oral presentation on the published manuscript and the eDGES project has been presented at the 14th International Conference of inland salt lakes and salinas (ICSLR – International Conference on salt lake research).

A short presentation on the eDGES project has been delivered by Dr Mattia Saccò and Dr Nicole White in quality of guest speakers at the 18th Annual WA Wetlands Conference 2022 in Cockburn, Perth.

An oral presentation at the 1st Australian and New Zealand Environmental DNA (eDNA) Conference titled “Looking under the mat: benchmarking computational methods for analyzing microbial datasets”.

This project has been referenced on the eDGES website (https://www.edgesprogram.org).

Sustainability

Our COVID-19 contingency plan has involved no flights to Chile (i.e., researcher from Curtin did not go on site for sample collection) and therefore reduced fossil fuel emissions is a sustainability upside.

High quality results have been achieved through the preliminary (and very productive in terms of publications) pilot study at Rottnest Island, allowing more efficient field and laboratory procedures.

Next Steps

Key activities planned for the next period:

- Finalise the writing of the manuscript in preparation ad submission to high impact factor scientific journals

Marine Management

Project 4

Improving the sensitivity and specificity of eDNA metabarcoding tools for invasive marine species detection

Summary

During this project we have enhanced and validated the use of metabarcoding of environmental DNA as a sampling tool for detecting Invasive Marine Species (IMS). This research project fits into a larger state-wide array surveillance program (SWASP) that was developed by the Department of Primary Industries and Regional Development (DPIRD) in consultation with the port authorities, shipping industry and the oil and gas sector.

Environmental DNA has some advantages over conventional sampling in that it can detect cryptic, rare and early life stage species in water and biofouling that a person may not readily observe. However, eDNA is a relatively new tool that needs further validation, particularly in the IMS area. It is recognised that the confidence in IMS detections is only as good as the ability of the tests and the genetic reference sequences that they are based on.

This project has contributed to the global reference databases through barcoding target IMS and their native congeners. We also developed and validated new metabarcoding assays that are able to assign species identity to genetic sequences with a high level of confidence, allowing the detection of target IMS from a range of biological substrates.

Implementation

Project 4 focuses on using environmental DNA for detecting IMS as part of an ongoing collaboration with DPIRD. This project was developed in consultation with DPIRD and BHP. Project 4 had a delayed start of three months. We have been fortunate to make up on time in that during planning and implementation of this project post COVID it has been possible to identify and access existing specimens, genomic sequences and data from DPIRD.

We have also been able to leverage existing DPIRD funding that had been committed to developing sequences of some of the key target species. We have employed Sherralee Lukehurst who has been working closely with DPIRD staff, assessing their whole specimen collections and determining which are suitable for DNA extraction and what sequence data collections already exist. We also appointed Dr Fred Wells (an adjunct staff member at Curtin) as an associate (being remunerated through an honorarium). Dr Wells is actively participating in the project and brings many taxonomic skills and taxonomic contacts to the project which are vital.

This project has involved remote field collection, museum sample acquisition, DNA sequencing of DNA metabarcode regions, and data curation. The field work has been completed successfully by Lukehurst and Wells. DNA sequence generation and archiving in international databases has been completed by Lukehurst. Scientific papers outlining the work have been written either by Wells or international collaborators as lead author, and the rest of the team shown here have contributed. The project has produced five publications so far.

Staffing and management

- **Professor Euan Harvey** (Curtin University) and **Dr Justin McDonald** (DPIRD, Aquatic Biosecurity) co lead this project with Professor Harvey responsible for the coordination of overall project management at Curtin. Professor Harvey and Dr McDonald have been collaborating since 2001 on a number of marine biodiversity assessments.
- **Professor Euan Harvey** is a marine ecologist with 30 years of experience in developing, testing and

validating techniques for non-invasively sampling marine biodiversity. He has been working with eDNA since 2014 comparing it to standard sampling tools and applying eDNA to broadscale biodiversity surveys of natural and manmade structures.

- **Dr Justin McDonald** is a benthic ecologist and biosecurity scientist. Justin leads the policy, science and surveillance areas of Aquatic Biosecurity within DPIRD for all Western Australia. His current research focusses heavily on the early detection and identification of aquatic species of national concern. He also leads projects on aquatic pest eradication and habitat restoration with native aquatic species. His contribution to the project centres around identification and targeting of species of concern, ensuring robust QA and QC with regards sample collection and taxonomic verification. He also contributes to field-based surveillance, collections, analysis and writing.
- **Sherralee Lukehurst** recruited into Research Officer position on eDGES project in September 2020. She has worked with DPIRD in the past and is responsible for laboratory work, data analysis and liaison with DPIRD staff to understand what research they are currently doing which is complementary or overlapping and to access samples and data.
- **Adjunct Professor Fred Wells** has considerable skills in the taxonomy and ecology of marine invertebrates gained during 30 years at the Western Australian Museum. He then went to WA Fisheries where he established and managed for four years the marine pest research program now headed by Justin. Following this Fred worked as a marine environmental consultant for 10 years concentrating on IMS issues. He designed and undertook IMS monitoring programs for major LNG projects through the cycle of planning, construction and operations and has assessed and inspected over 100 vessels for IMS. Fred has extensive experience in IMS detection and a thorough knowledge of the issues involved. He has written a number of papers on IMS and serves on the editorial boards of three international journals specialising in IMS issues, including three years as editor in chief of Aquatic invasions.

External collaborators

- **Dr Tina Berry** – eDNA Frontiers (Curtin University).
- **Claire Wellington** and **Matthew Hewitt** – Aquatic Biosecurity DPIRD.
- **Dr Seema Fotedar** – DPIRD Diagnostic and Laboratory Services.
- **Anita Ramage** and **Jayden Zieth** – Biosecurity Queensland, Department of Agriculture and Fisheries.
- **Nicola Stokes** – North Queensland Bulk Ports Corporation.
- **Dr Tan Koh Siang** and **Tan Siong Kiat** – National University of Singapore.
- **Dr Teerapong Duangdee** and **Dr Kittithorn Sanpanich** – Kasetsart University and Burapha University (Thailand).
- **Dr Tiffany Simpson** and **Melissa Morgan** – Conservation and Fisheries Directorate, Ascension Island Government
- **Dr Andrew Hosie** – Western Australian Museum (WAM).

Overview of outputs and outcomes achieved to date

The first stage of Project 4 aimed to fill gaps in the molecular data available for priority IMS and their native taxa, in some cases there are limited, or no sequence data from taxonomically confirmed specimens.

- Finalised GenBank submission of 31 full mitochondrial genome sequences, 7 partial mitochondrial genomes and five chloroplast genome sequences.
- Compiled a custom 16S rRNA reference database of native Western Australian congeners to improve taxonomic assignment from eDNA sampling. This was achieved by sequencing the 16S rRNA

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
2020		
Accessing samples for developing assays	Identification of required sister taxa. Collection of gDNA or tissue samples from DPIRD & Queensland Museum.	Complete
Desktop study for assay validation	Suitable gene region selected and available sequence data obtained from GenBank, DPIRD & WAM databases. Multiple DNA sequence alignments and analysis. Primers designed and ordered.	Complete
2021		
Laboratory testing for validation of assays for multiple biological substrates	Two phyla specific assays tested on single source tissue samples and mock communities. New assays used to screen eDNA samples from Dampier using the full TrEnD workflow- Metabarcoding PCR fusion tag, MiSeq run and sequence analysis.	Complete
Evaluation of limits of detection and replication	Synthetic double-stranded DNA fragments for use as controls have been purchased for IMS. Standard curves of synthetic positives and spiked samples have been tested.	Complete
2022		
Multiplex assay development and testing	Mock communities have been screened using multiplexed assays and have been taken through the TrEnD workflow.	Complete
Publication of journal articles, guidelines and sequences	*Talonostrea* n. sp information to be included in publication by overseas colleagues. Reference sequences uploaded to GenBank Manuscript in preparation	In Progress
2023-2024 Additional		
Pilbara oyster survey	Additional field collecting was undertaken with surveys in the Dampier Archipelago, Balla Balla and Port Hedland. Sequences uploaded to GenBank Manuscript published	Complete
Singapore oyster survey	Field sampling in Singapore (19th-28th Feb), 27 sites and 90 tissue samples of oysters and mussels brought back for processing. All DNA extractions, PCRs and sanger sequencing, has been completed. Manuscript in preparation	In Progress
Brachidontes status project	Specimens from three different locations have been sequenced for 5 gene regions and added to an extensive data set. Sequences uploaded to GenBank Manuscript accepted.	Complete
Thailand *Mytella strigata* survey	Sherralee and Fred completed field sampling in Thailand (1st-9th Aug 2023). Manuscript published	Complete
Thailand oyster survey	Sherralee and Fred completed field sampling in Thailand (1st-9th Aug 2023). All DNA extractions, PCRs and sanger sequencing, has been completed. Sequence upload to GenBank in progress Manuscript in preparation	In Progress
Passive device field experiment	North Queensland Bulk Ports Corporation deployed at devices at Mackay Port QLD, between Sept-Dec 2023 and DPIRD deployed devices at Australian Marine Complex and Kwinana Grain Jetty between Feb-April 2024. Samples are currently being processed by eDNA Frontiers for three metabarcoding assays.	In Progress

gene region of samples acquired from DPIRD's collections and specimens collected during field trips to Dampier (West side of the Burrup Peninsula, Oct 21), Onslow (Aug 22), Dampier (East side of the Burrup Peninsula and islands in the Dampier Archipelago, Oct 22) and Port Hedland (Jan 23). Available sequence data obtained from GenBank, DPIRD and WAM databases was also included in this reference database.

- A previously unknown species of *Talonostrea* has been found in Hooley Creek, the Dampier Archipelago and at Balla Balla. The species is small, about 3 cm in diameter and occurred in three intertidal habitats: on sand in a creek, attached to the underside of mangroves and on a steel structure. The Pilbara DNA sequences match a new species of *Talanostrea* recorded by Dr Carmel McDougall of Griffith University between Brisbane and Cairns. Dr Carmel McDougall is describing the new species with Dr Graham Oliver of the National Museum of Wales. The Pilbara data will be included in this species description.

The second stage of the project was to develop novel metabarcoding assays, to enable discrimination between IMS and closely related native species within phyla Arthropoda and Mollusca.

- Two assays, targeting the 16SrRNA region, were designed to target both Arthropoda and Mollusca. Assays were initially tested on single source tissue gDNA extracts from IMS of interest and native congeners. This single source gDNA was also combined to create mock communities, which were used as positive controls for the metabarcoding workflow. The IMS of interest were successfully detected in these mock communities using the relevant assay.
- We tested the assays using eDNA extracted from biofouling from settlement arrays deployed at Dampier port. In the environmental samples, the Arthropoda assay detected 12 genera from Malacostraca, three genera from Hexanauplia and one from Insecta. The Mollusca assay detected 15 genera from Bivalvia and 10 genera from Gastropoda. Even though the Mollusca assay was able to detect 25 genera, 97.4% of sequence reads were unassigned, this indicates the importance of reference sequences and updated databases.
- The Arthropoda assay was used to screen eDNA extracted from water and plankton tow samples collected during DPIRD's routine surveillance for *Charybdis japonica* in the Swan River, between 2019 and 2022. The assay had detections of *Arenallianassa arenosa*, *Macrobrachium intermedium*, *Panulirus cygnus*, *Penaeus latisulcatus*, *Portunus armatus*, *Thalamita sima*, *Balanus trigonus*, *Armatobalanus sp.*, *Tetraclitella purpurascens*. All common species found in the area, with a greater number of sequence reads found in the plankton tow samples.
- To determine the sensitivity of the assays, two synthetic oligonucleotides were designed to be used as a positive controls. These positive controls were based on IMS 16S gene regions with a few changes to allow discrimination from actual *Balanus*

Figure 22. Newspaper article discussing the newly identified *Talonostrea* sp. This article was also featured in the online *West Australian* and the *North West Telegraph*, May 2024.

glandula and *Perna viridis* DNA. The synthetic positives were spiked into DNA extracts from three different environmental substrates (settlement array, water filter, plankton tow) at three different concentrations. The Mollusca assay was more sensitive than the Arthropoda assay with 4 copies/µL being detected in spiked samples when assay was run as either a single- or multi-plex. The lowest concentration of 4.7 copies/µL was detected in 66% of spiked samples when the Arthropda assay was performed in a multiplex, otherwise 47 copies/µL was the limit of detection when the assay was performed alone.

Additional projects

- Collaboration with Dr Tan Koh Siang from the National University of Singapore working on *Brachidontes* species, to see where the WA native species belong within the genus. DNA extractions and sequencing of five gene regions for specimens from three sites in WA (Dampier, Woodman Point and Albany) have been completed and submitted to GenBank.
- Collaboration with Dr Tan Koh Siang and Tan Siong Kiat on Singapore oyster survey is progressing. Lukehurst and Wells completed field sampling in Singapore (19th-28th Feb 2023), 27 sites and 90 tissue samples of oysters and mussels were brought back for processing. Shells and remaining tissue of all specimens were retained by Lee Kong Chian Natural History Museum and have been vouchered. DNA extractions, PCRs, sanger sequencing and sequence editing has been completed for all specimens. A manuscript reporting on species of the oyster genus *Magallana* in Singapore and their biosecurity risk to Australia has been accepted.
- Collaboration with Dr Teerapong Duangdee (Kasetsart University) and Dr Kittithorn Sanpanich (retired from Burapha University) on Oysters in Thailand and the status of the invasive charru mussel *Mytella strigata* five years after first being identified in the Gulf of Thailand. One manuscript published (Figure 24) and manuscript on the oysters of Thailand currently in preparation.
- Research into possible assays that can be used to distinguish IMS of macroalgae from closely related native species. Macroalgae specimens have been acquired from DPIRD and DBCA Herbarium collections. DNA has been extracted and the 23S chloroplast gene region sequenced to create an algae reference database for this region. For the genus *Sargassum* there was not enough variation between species, therefore other gene regions were considered. Primers for the space between the rbcL and rbcS chloroplast gene regions have been designed and are awaiting testing.

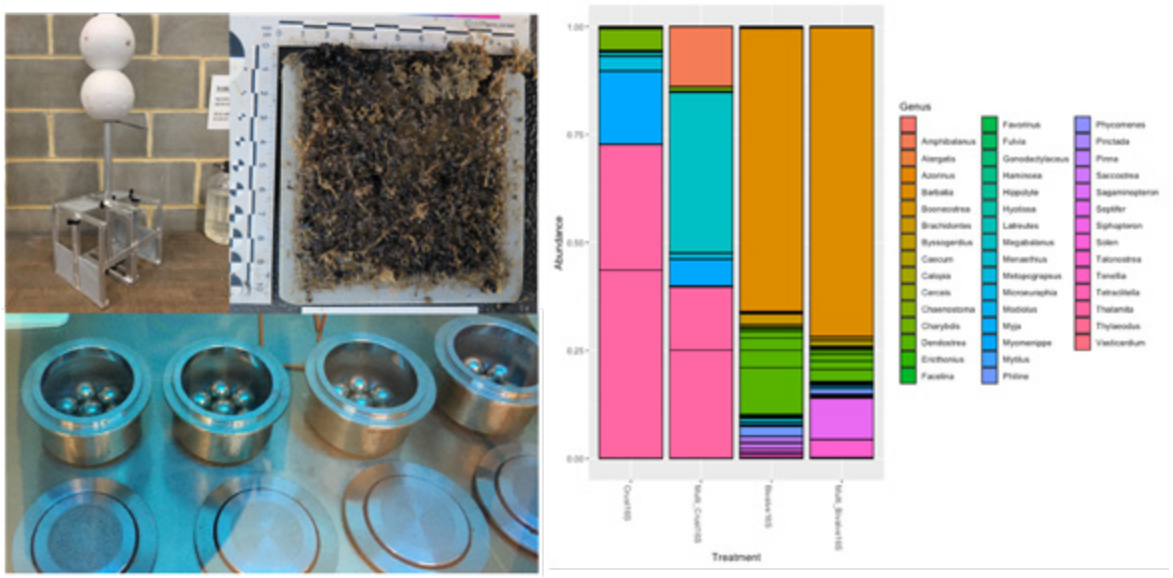


Figure 23. DNA extracted from biofoul that settled onto plates deployed at Dampier port was screened with the new assays in singleplex and multiplex.

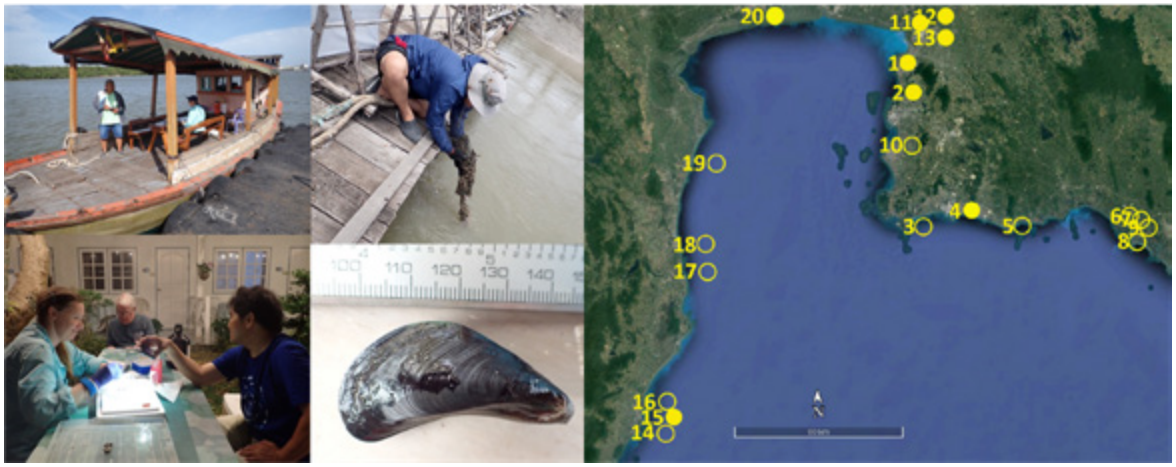


Figure 24. *Mytella strigata* survey in the upper Gulf of Thailand, August 2023. Yellow circles represent sampling sites, with closed circles indicating the sites where the species was present at the time of the survey.

Publications

Simpson T.J., Wellington C.M., Lukehurst S.S., Huerlimann R., Veilleux H., Snow M., Dias J., McDonald J.I. (2023). Development of a real-time PCR (qPCR) method for the identification of the invasive paddle crab *Charybdis japonica* (Crustacea, Portunidae). *PeerJ* 11: e15522 https://doi.org/10.7717/peerj.15522

Tan K.S., Tan S.K., Lukehurst S.S., Wells F.E. (2024) Assessing the threat to the Australian marine environment of the oyster genus *Magallana* in Singapore. *Raffles Bulletin of Zoology*. (accepted)

Tan S.H.M., Wells F.E., Lukehurst S.S., Strong E., Sanpanich K., Duangdee T., Ambarwati R. and Tan K.S. (2024). Unravelling the *Brachidontes variabilis* species complex (Bivalvia: Mytilidae) of the Indo-Pacific region. *Journal of Molluscan Studies*. (accepted)

Wells F.E., Duangdee T., Sanpanich K., Lukehurst S.S. (2024). Status of the invasive charru mussel *Mytella strigata* (Hanley, 1843) in the upper Gulf of Thailand five years after it was first surveyed. *BioInvasions Records* 13(1): 69–82, https://doi.org/10.3391/bir.2024.13.1.07

Wells F.E., Lukehurst S.S., Fullwood L.A.F., Harvey E.S. (2024). Distribution of intertidal rock oysters in the Pilbara, Western Australia. *Management of Biological Invasions* 15(1): 131–143, https://doi.org/10.3391/mbi.2024.15.1.08

Overview of any unexpected impacts on the Project and/or beneficiary group

- Staff changes and recruitment of Sherralee Lukehurst resulted in a delayed start to the project in 2020/21.

Leveraged funding and value-add activities

- The DPIRD Marine Pest Taxonomic and Molecular Reference Collection was recently identified by the National Biosecurity Committee as part of their 'strategic biosecurity asset and infrastructure stocktake' as a collection of national significance. It is likely to be the only dedicated marine pest library in Australia and one of few dedicated libraries globally.
- The cost of generation and therefore value of leveraging was:
  - Phase 1 – Initial development (with commonwealth funding) $35,200
  - Phase 2 – Second round of mitogenome sequencing $33,242

Challenges

- COVID and travel restrictions during 2020-2022 caused challenges with obtaining suitable tissue samples or DNA extracts of some of the IMS and even some of the native congeners of interest.
- We have found that WA Museum collections do not have some of the native specimens of suitable quality for molecular work, necessitating our collection directly.
- The priority IMS for this project are also organisms that rapidly degrade, so freshly preserved samples are needed. Additional specimens will need to be sourced from native locations overseas. Unless we can travel to collect, we are reliant on overseas partners to provide specimens and are dependent on their knowledge to accurately identify and preserve. COVID has also complicated this issue, with limited travel allowed in some areas leading to reduced field work opportunities.
- Tank experiments with a native species from the genus *Charybdis*, were planned for Feb 2022. It was not possible to run these experiments as DPIRD was unsuccessful in catching any live Charybdis during their routine surveillance and extra trapping days.

Key strategies

- Since initially collecting our own specimens was not possible, we have relied on publicly available sequence data and data from WAM and DPIRD to investigate within species variation for the 16S rRNA gene region.

Learnings

- An agile management approach during COVID restrictions has allowed the project to adapt, and yet still remain on target. The adaptation and re-prioritisation of the project has also allowed data to be uploaded onto a public database sooner than may have happened otherwise.
- Knowledge sharing to key stakeholders was accomplished by submission of sequence data to a public database (NCBI GenBank). The data are full mitochondrial genomes and separate gene regions. In some instances, these are sequence data from multiple individuals of the same species from various locations.

Communication

The project has been listed on the eDGES website (https://www.edgesprogram.org) where a summary video can be viewed.

Sherralee Lukehurst attended and presented an ePoster at the 1st Australian and New Zealand Environmental (eDNA) Conference in Hobart, February 2023. The poster was titled “Development of a multi-assay approach for detecting invasive marine species (IMS) for use in biosecurity applications.” Further, at the same conference a presentation titled “An overview of eDGES program 4 and 5” was to be delivered by Prof Euan Harvey. However, due to personal circumstances Prof Morten Allentoft delivered this in his place.

Sustainability

Submission of sequence data to GenBank makes it available to other end users and enables the avoidance of duplicated efforts that can lead to unnecessarily destroyed specimens and the cost of time and reagent wastage.

Investigating the use of passive devices as an alternative method to deploying settlement arrays, could reduce the need for dry ice and cold transport options for government biosecurity teams and port authorities.

Next Steps

- Complete three publications on the oyster work that is currently underway.
- Complete data analysis of sequence data from the passive device, roller, settlement array samples that are currently being processed by eDNA Frontiers team. Prepare a manuscript on the three different sampling methods.
- Collaboration with Dr Andrew Hosie from WAM on the species *Megabalanus occator*. This barnacle was the dominant invertebrate fouling species found on channel markers within the Ashburton port during field collections in August 2022. Sherralee Lukehurst has full mitochondrial genome sequences for three specimens.
- The development of point of need tools for the rapid in field detection of specific taxa, that have a high risk of being an IMS in Australia.

Figure 25. Lee Kong Chian Natural History Museum X (formally twitter) post, Feb 2023. During field work in Singapore, Fred and Sherralee were based at the Lee Kong Chian Natural History Museum.

Marine Management

Project 5

Benthic communities associated with port and harbour infrastructure in the Pilbara: Targeting invasive marine species

Summary

Biological diversity of the Pilbara coast is one of the most poorly characterised coastal regions in the developed world. This region is particularly interesting for biodiversity assessment because it is known to have high levels of endemism, and it is relatively undisturbed by human activities relative to tropic coastlines internationally. The region also supports substantial industrial activity, concentrated on the bulk shipping facilities at Port Hedland.

Environmental DNA (eDNA) is a valuable tool for biodiversity assessment, with particular strengths for generating lists of taxa resident in a region. eDNA is also particularly suitable for aquatic biodiversity assessment because DNA itself is water soluble, and much of the biological material that eDNA is bound to such as cellular debris and biofilms is present in particulate organic matter in the water column. Aquatic eDNA samples provide a useful aggregate assessment of species that have lived in the water body being sampled if they are analysed appropriately. Detecting eDNA of invasive marine species (IMS) is valuable for management of any coastal marine environment, as this is currently the most sensitive method available for IMS detection.

This project is built around sampling surveys of aquatic eDNA in Port Hedland and adjacent environments that do not currently harbour industrial activity. The close proximity of high conservation value coastal habitats to regulated and intensively managed industrial activities at Port Hedland provides an opportunity to investigate the biodiversity differences between relatively untouched coastal reserves and a busy port. Port Hedland is thought to be free of established IMS. This is intriguing given that it is one of the world's busier bulk transport ports, and the shipping is concentrated in a very small area. The sampling survey that we have undertaken will measure biodiversity links between the areas of industrial activity and reserved areas nearby to investigate the possible links between native biodiversity and protection from IMS establishment.

Implementation

This project involved one of the largest aquatic eDNA sampling surveys ever completed. We used high throughput, portable peristaltic pumps to reduce collection times for sampling the water column. We also sampled surface eDNA in parallel to the samples from the water column by using mini paint rollers to collect benthic eDNA from surfaces such as pylons in the port area, and mangroves in the non-port areas. Our implementation of rapid, high-performance eDNA sampling methods allowed us to collect more than 1,000 samples from the port area and surrounding habitats at Port Hedland over a one week sampling campaign. The sampling sites are shown in Figure 26 below.

This project has involved field collection of aquatic eDNA samples, DNA purification, and sequencing of DNA metabarcode regions, data curation, and initial data analysis. The field work has been completed successfully by Harvey, Lukehurst, Wells, and Jarman. The lab components of the work have been completed at Curtin University's eDNA Frontiers facilities. DNA sequences have been generated for all samples for one DNA metabarcode region representing all eukaryotes. DNA sequences are being generated for two more DNA regions, one for fish identification, and one for molluscs and crustaceans. High-throughput eDNA sampling methods using drill-powered peristaltic pumps for water and paint rollers for surface eDNA were used to great effect (Figure 27).

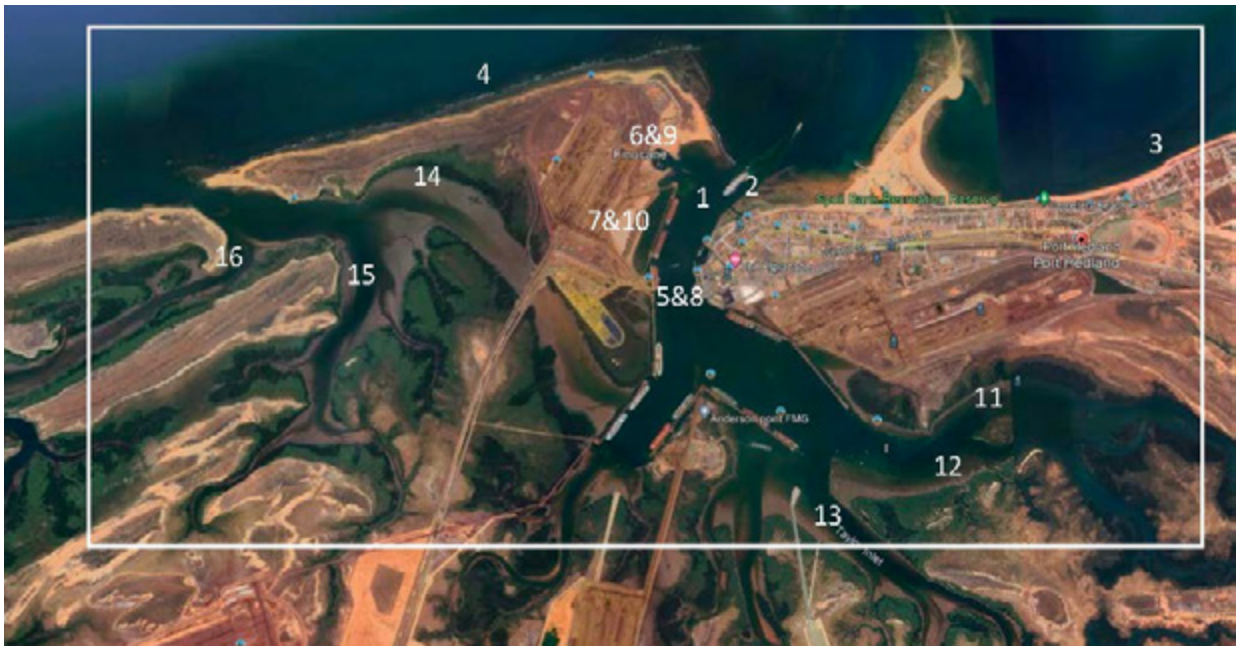


Figure 26. Map of Port Hedland, showing sampling sites. The white box marks the limit of the survey area.

Our work involved collaboration with the Pilbara Port Authority and BHP. We planned the survey in partnership with both organisations. A Pilbara Port Authority staff member, Mark Logue, assisted with sampling, and Zoe Keller assisted with planning and permitting. Dave Arthur of Northwest Marine supplied a boat and logistics support.

Staffing and management

- **Professor Euan Harvey** is a marine ecologist with 30 years of experience in developing, testing and validating techniques for non-invasively sampling marine biodiversity. He has been working with eDNA since 2014 comparing it to standard sampling tools and applying eDNA to broadscale biodiversity surveys of natural and manmade structures.
- **Sherralee Lukehurst** recruited into Research Officer position on eDGES project in September 2020. She has worked with DPIRD in the past and is responsible for laboratory work, data analysis and liaison with DPIRD staff to understand what research they are currently doing which is complementary or overlapping and to access samples and data.
- **Adjunct Professor Fred Wells** has considerable skills in the taxonomy and ecology of marine invertebrates gained during 30 years at the Western Australian Museum. He then went to WA Fisheries where he established and managed for four years the marine pest research program now headed by Justin. Following this Fred worked as a marine environmental consultant for 10 years concentrating on IMS issues. He designed and undertook IMS monitoring programs for major LNG projects through the cycle of planning, construction and operations and has assessed and inspected over 100 vessels for IMS. Fred has extensive experience in IMS detection and a thorough knowledge of the issues involved. He has written a number of papers on IMS and serves on the editorial boards of three international journals specialising in IMS issues, including three years as editor in chief of Aquatic invasions.
- **Professor Simon Jarman** is geneticist and an expert in developing genomic methods for studying natural environments. He has 25 years of experience in working on extracting information from the natural work with genetic analyses. His research on genomic methods for biodiversity measurement has transformed approaches for studying animal diet and measuring biodiversity in aquatic environments.

External collaborators

- **Dr Mahsa Mousaviderazmahalleh** – eDNA Frontiers and TrEnD Lab (Curtin University).
- **Dr Kat Dawkins** – eDNA Frontiers (Curtin University).
- **Dr Shane Herbert** – eDNA Frontiers (Curtin University).
- **Dr Shaun Wilkinson** – Wilderlab pty ltd, NZ.
- **Dave Arthur** – Northwest Marine
- **Mark Logue** – Pilbara Port Authority
- **Zoe Keller** – Pilbara Port Authority

Figure 27. Sampling eDNA from water on inter-tidal rock platforms (top) and taking surface eDNA samples from pylons in the port area (bottom left) and mangroves (bottom right) using paint rollers on a pole.

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
2024		
Experimental design	A sampling strategy was developed to allow highly replicated comparisons among port and non-port habitats at different depths for biodiversity patterns.	Complete
Testing of new sampling methods	High-throuhgput water column eDNA sampling and paired samples of benthic eDNA were tested and demonstrated effective.	Complete
Port Hedland sampling campaign	We took 1,110 samples of aquatic eDNA and surface eDNA from the Port Hedland region between 22nd and 29th October 2023.	Complete
DNA extraction	All samples collected at Port Hedland have had bulk eDNA purified using the Kingfisher Flex magnetic bead platform.	Complete
DNA metabarcode amplification	PCR amplification of three DNA metabarcode regions: 18S eukaryote marker, 16S fish marker, and 16S crustacean and mollusc marker have been completed.	Complete
DNA sequencing	DNA sequencing of the 18S eukaryote marker and 16S crustacean and mollusc marker is complete.	⅔ complete

Overview of outputs and outcomes achieved to date

- A uniquely large-scale eDNA sampling survey has been completed. This survey included over 1100 samples, which is the most comprehensive that we know of, on a world-wide scale.
- New, high throughput eDNA sampling procedures have been implemented.
- DNA markers have been generated from all samples collected in the 2024 survey, and sequencing of two of these markers has been done for the entire sample set.

Other projects

- This project links to eDGES 4, which is producing new DNA sequences for identification of species found in the Pilbara, and potential invasive species found in SE Asia. The data archived from eDGES 4 will enhance the quality of identifications of both native species and any IMS in this project.
- Data from this project will underpin the work proposed on Natural Capital Accounting in eDGES 9 (round 2). Port Hedland provides an excellent example of an environment that has value for industry, biogeochemical cycling, and inherent natural values.
- This project will also provide data that will be re-used for the quantitative eDNA assessment methods that will be developed in eDGES 8 (round 2).

Overview of any unexpected impacts on the Project and/or beneficiary group

- The bioinformatician who worked on the intial phases of this project, Mahsa Mousaviderazmahalleh, has moved to a new role. She has been managing the transition to a new DNA sequencing platform, and disruption to her work as a result of her new job has slowed progress slightly.

Leveraged funding and value-add activities

- The sampling undertaken in this project will provide eDNA data and baseline biodiversity information for future eDGES projects 8 and 9.

Challenges

- This project is the first one to use eDNA Frontiers new, BHP-funded DNA sequencing platform. This new NextSeq 2000 produces an order of magnitude more data per run, which has necessitated a new software architecture. This is in place now but has slowed progress.
- The new DNA sequencing technology also had an incompatibility with the 16S crustacean/mollusc primer set that we are using. Troubleshooting with Illumina tech support has allowed progress to be made and the assay has now been successfully sequenced.

Learnings

We deployed new DNA sequencing technology for this project. This resulted in unexpected delays because the software framework that we had in place through eDNA Frontiers needed to be re-worked for the much larger data throughput that the NextSeq requires. The learning is that we need to manage staffing with our collaborators actively when advancing into new technological territory.

Communication

The project has been listed on the eDGES website (https://www.edgesprogram.org) where a summary video can be viewed.

Sustainability

The data that we generate from this project will be archived in public repositories on publication to ensure reusability and transparency.

Our development of more efficient eDNA sampling systems reduces costs and wastage, while increasing data output and levels of replication. The overall effect is more scientific output per dollar spent.

Next Steps

- Once we have DNA sequence data from the remaining two of the three DNA markers being used in the study we will progress to biological interpretations of the DNA sequences, with preliminary results being produced by the end of 2024.
- A paper on the overall results of this biodiversity survey will be produced early in 2025.
- A paper on temporal sampling to investigate diurnal variation in eDNA measures of biodiversity in the port area will also be produced in 2025.

Terrestrial Biomonitoring

Project 6

Terrestrial ecosystem biomonitoring with eDNA across the tree of life: the Olympic Dam case study

Summary

Large scale projects aimed at conserving or restoring landscapes can positively impact biodiversity. However, assessing the success of landscape scale conservation or restoration programs is crucial and monitoring is conducted for several interconnected reasons. First, without monitoring, it is impossible to assess whether conservation or restoration efforts are successful. Second, monitoring can indicate whether future interventions may be necessary to achieve targets, Third, monitoring provides evidence to stakeholders that targets, goals and objectives of the project are met.

While biological monitoring is crucial for assessing ecosystem health, it's challenging due to the labour-intensive nature of biodiversity data acquisition. Arid regions, with their remote and rugged terrain, present even greater difficulties for fieldwork and sample collection. Non-invasive eDNA-based surveys are transforming biomonitoring by generating vast amounts of biodiversity data, but concerns persist about data validity, especially in terrestrial ecosystems like Australia's. More research is needed to understand eDNA based biomonitoring's effectiveness in arid environments and to develop simpler sampling protocols that do not require specialized equipment or cold storage. User-friendly methodologies are essential for broadening the reach of eDNA-based monitoring, enabling diverse stakeholders, including citizen scientists and indigenous rangers, to contribute to biodiversity conservation efforts.

This project aims to validate DNA-based tools for assessing terrestrial biodiversity and work towards development of an eDNA-based metric for ecosystem evaluation. Initial steps involve streamlining sampling methods to make them more accessible and efficient, followed by assessing spatial and temporal variability in biodiversity signals from eDNA to establish robust monitoring protocols. Ultimately, these efforts will empower a diverse group of stakeholders, from citizen scientists to indigenous rangers, to contribute to biodiversity conservation around the Olympic Dam Mine and beyond.

Implementation

This project commenced activity on a part time basis at the end of 2023 with the employment of Dr Mieke van der Heyde who has co-led the project with Associate Professor Paul Nevill. We have completed a site familiarisation trip to the Olympic Dam area to identify appropriate field sites for the initial goals of the project. Following that trip in February, we have completed sample collection and processing for a study investigating optimal strategies for swabbing vegetation. In May we completed the first field work in Olympic Dam area investigating the spatial resolution of three different sample types: air, soil, and vegetation swabs. An honours student, Francesca Martino, has also been recruited to investigate the possibility of sampling air using vehicles. She completed her field work mid-May and began lab processing immediately.

Staffing and management

Principal Investigators

- **Dr Mieke van der Heyde** – Research Fellow at the TrEnD lab specializing in applying eDNA methods to assess terrestrial and subterranean ecosystems. She is an expert in laboratory processes and bioinformatic analyses of eDNA data. MvdH will co-supervise PhD and honours students, and co-lead the research. This includes project planning, field work implementation, laboratory work, data analyses, and writing research articles.

- **Associate Prof. Paul Nevill** – Paul has substantial experience developing genomic and eDNA approaches for terrestrial environmental research. He leads the Curtin Minesite Biodiversity Monitoring group (MBioMe), which specialises in the development of eDNA methods for the resources sector, and as a member of CU's TrEnD lab, he will co-lead the research and co supervise students.

PhD and honours students

- **Francesca Martino** – Honours student recruited to investigate options for sampling air DNA

Participants/Collaborators

- **Prof Morten Allentoft** – Head of the TrEnD Lab (Curtin University)
- **Dr Mahsa Mousavi Mousaviderazmahalleh** – Researcher at TrEnD Lab (Curtin University)
- **Assoc Prof. Bill Bateman** – Behavioural Ecology Lab (Curtin Unviersity)
- **Dr Christine Cooper** – Ecologist/Ecophysiologist (Curtin Unviersity)
- **Kim Solly** – Principal Sustainability – Biodiversity (BHP)

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
Site familiarisation trip	Completed in Feb 2024, Mieke has all required inductions and access to Olympic Dam area with BHP supervision. Identified Roxby downs as suitable site to test spatial resolution of eDNA sample types.	Completed
Optimize sample methodology for vegetation swabs (Aim 2)	We compared clinical swabs and mini paint rollers to determine which method to use in the spatial study at Roxby Downs.	Completed
Sample collection and processing for spatial resolution study (Aims 1 and 3)	180 samples (60 each of air, vegetation swabs, and soil) were collected from Roxby Downs and surrounding bushland in early May 2024. Sample processing to commence mid May. Second round of sampling scheduled for Q3 2024 at a second site	On Schedule
Optimize sample methodology for soil eDNA – test preservation methods (Aim 2)	Samples were collected at Roxby downs to test three different preservation methods (cold, sarkosyl buffer, LifeGuard -Qiagen) for soil samples. Sample processing to commence mid-May	On Schedule
PhD candidate selected	Modified to honours student and recruited Francesca Martino to investigate air eDNA sampling. All sample collection has been conducted and processing in the lab has commenced	Completed
Proposal for eDGEs v2 complete	Submitted March 2024	Completed
Manuscript preparation and submission	Manuscript preparation begun for swab vs rollers test. Likely to be published as a Research Note – short methods based publication	On schedule

Overview of outputs and outcomes

Sample collection and processing of vegetation swab optimization test is complete– Nine samples each of swabs and mini paint rollers were collected from Henley reserve in Wellard and two metabarcoding assays were applied (12S, 18S). Overall there was no significant difference in the richness detected from each sample type ($P>0.05$); however, the mean richness for rollers was higher than swabs for both assays. Additionally, the proportion of ZOTUs detected by rollers alone was 26.7%, while for swabs it was 10.6%. For these reasons, we decided to use rollers in the spatial study at Roxby Downs. Only about a third of the 12S sequences (a vertebrate specific metabarcoding assay) were assigned taxonomy, indicating greater development of reference databases is required to assess vertebrate diversity.

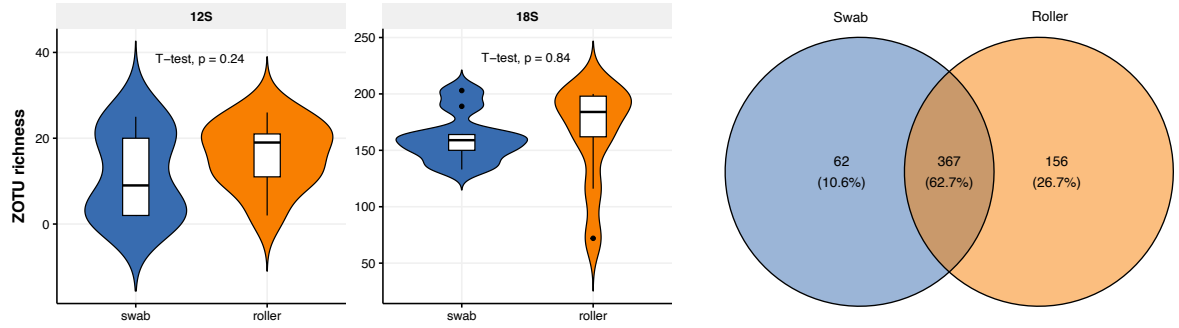


Figure 28. Results of Vegetative swab trial comparing the eDNA collected by clinical swabs and mini paint rollers. Richness of ZOTUs – Zero radius Operational Taxonomic Units – shown above left and the venn diagram of shared vs unique ZOTUs above right.

Figure 29. Images of the rollers (left) and the swabs (right) used in the vegetative swab trial.

Figure 30. Roller swab samples being collected in Olympic Dam area.

Figure 31. Air filter sample collecting eDNA from the air.

Figure 32. Soil samples collected and stored on ice (left) and in preservation buffer (right).

Figure 33. Air DNA sample collection fixed (left) and mobile (right).

Fieldwork at Olympic Dam for Spatial Study is complete – First samples from the Olympic Dam area were collected around Roxby Downs Village and the neighbouring bushland. Samples included air filters, roller sabs of vegetation and rocks, and soil samples. These will be processed shortly by Mieke van der Heyde and eDNAFrontiers.

Fieldwork for air DNA sampling comparison is complete – An honours level project comparing fixed short term, fixed long term and mobile air DNA sampling approaches for terrestrial vertebrate detection is complete. Over 256 km of transects were driven and 160 samples collected. Processing of samples in the lab has commenced.

Overview of any unexpected impacts on the Project and/or beneficiary group

We were able to obtain tissue samples for Plains mouse (Pseudomys Australia) from Arid Recovery to sequence and begin building a custom DNA barcode reference library.

Challenges

- With the South Australia Museum collection closed it has become very difficult to obtain tissue samples from priority plant and animal species to build the DNA barcode reference libraries.

Learnings

Building plant and animal DNA barcode reference libraries will likely require networking and collaborating with many researchers and other groups to obtain tissue samples. We obtained a Plains mouse sample from Arid Recovery and may need to request more samples of species from them and anyone else who may have collections from the area.

Communication

- Short videos have been filmed to describe and promote the project and they will be publically available on the new eDGEs website when completed (https://www.edgesprogram.org).
- We are scheduled to give an oral presentation of the vegetation swab trial results in Canberra, June 3-4, 2024, at the Southern eDNA Society airDNA workshop.
- We are scheduled to give an oral presentation on terrestrial ecosystem eDNA based monitoring in Darwin, June 6, 2024, at a DCCEEW workshop.

Sustainability

This project is investigating sampling methods that do not require cold storage and powered equipment, reducing the need for powered transport to keep samples frozen over long distances. Findings from this project may also reduce the time and manpower costs associated with conventional monitoring methods, consequently minimizing transportation costs of personnel in remote locations.

Next Steps

Key activities planned for the next period:

- We are in the process of identify a second site for the spatial study
- We will conduct sampling at the second study site and process samples in the lab in Q3 and Q4 in 2024
- Narrow down sampling methodologies and commence sampling to investigate temporal variation in eDNA based biodiversity monitoring. This involves collecting ~12 samples (2 hours work) approximately every two months from Roxby Downs area, and this will hopefully be facilitated by someone already on site, to reduce travel costs
- Data analysis and writing of the manuscripts based on data investigating spatial and temporal variation in biodiversity signals from eDNA data
- Review metrics of ecosystem condition and conduct a workshop with the aim of submitting a publication based on findings
- Complete honours project and submit publication.

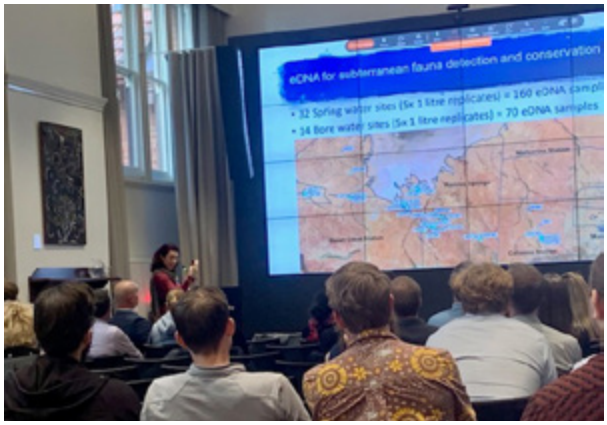
Program Management

Common program meetings and events

Summary

The eDGES program proposal included support for project stakeholder meetings and yearly milestone meetings that were to be shared events for all projects in the program. This support included the annual stakeholder meetings, yearly governance meetings/ seminars, and an open program symposium in the final year of the program. Support for overall promotion activities were included and have helped bring the program to a broader audience.

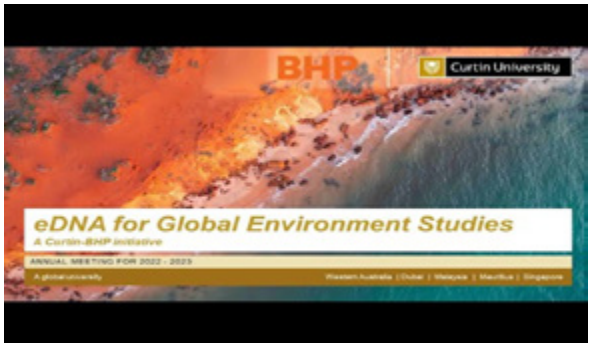
Each of the annual events bring the eDGES researchers and collaborators together with interested stakeholders from across the biodiversity and biomonitoring community including those from industry, government, environmental consultants, community members and researchers. Each of the events host an in-depth seminar of the research streams and their progress, ensuring findings are released to the public domain quickly. Furthermore, it's an excellent opportunity to gather afterwards to discuss science and expand the eDGES network.



Annual Stakeholder meetings were filmed by Curtin University and prepared into a viewable record. These can be found online at the eDGES YouTube channel (youtube.com/@ednaforglobalenvironmentst744) as below.

The eDGES program annual review 2020/2021 recording is viewable at: https://youtu.be/40_xqTcIVU4?si=C6kppCFHDH_fl159

The eDGES program Annual Meeting 2021-2022 recording is viewable at: https://youtu.be/R0PeP1QWoUs?si=uySYHU-TLla9tssv



The 2023 eDNA for Global Environment Studies (eDGES) annual showcase recording is viewable: https://youtu.be/tBdCTiZ-imw?si=mWnMAW8kg-c4ZI2r

We look forward to hosting the final years annual showcase later in 2024.

Further a number of summary videos are in production where the lead researchers of the projects themselves introduce the goals and the outcomes of their work. Stay tuned to the eDGES YouTube site for updates as they are released.

Visit: https://www.youtube.com/@ednaforglobalenvironmentst744

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