BHP | Curtin University

The eDNA for Global Environment Studies (eDGES) Program Round 2

Annual Report 2024

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Executive Summary

The eDNA for Global Environment Studies (eDGES) announced the second round of an exciting research portfolio at the 2024 eDGES annual showcase meeting on the 12th November. Research in this round grows to nine projects that continue to expand upon the research completed within the original six projects of the first eDGES program (running 2020–2024).

The nine projects described here will make significant contributions to biodiversity monitoring in terrestrial, subterranean, wetland and marine environments, whilst advancing the utility of eDNA datasets through their application to ecological abundance metrics and natural capital accounting. The portfolio includes five terrestrial and inland aquatic-based projects that push eDNA well into new territory from both an analytical perspective (merging eDNA and genomics) and an environmental perspective (subterranean ecosystems, urban wetlands, and hypersaline lakes). There are two marine based projects, one developing new molecular tools to address gaps in detecting invasive marine species and the 2nd aiming to map the undescribed coastal marine life to better define native taxonomy which in turn assists with managing invasive incursions.

In this first annual report of the eDGES round 2 program, despite the program being very much in its infancy, we can report a strong presence at the 2025 Southern eDNA Society Conference, in Wellington, New Zealand, with 10 Curtin University scientists and 2 environmental scientists from BHP in attendance. Furthermore, the eDGES program supported the conference through a gold-level sponsorship highlighting to the field the BHP-Curtin University strategic alliance and leadership in this technology. To date 26 scientific papers have been submitted, accepted or published as part of our continuing eDGES program and 9 of those in the past year, since the initiation of eDGES round 2.

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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

- eDGES delivers more than just innovative science. Key outcomes include international PhD training, peer-reviewed publications, new funding opportunities (e.g., ARC Linkages), contributions to environmental policy, and community education.
- We engage the public through seminars, awareness events, annual program meetings, and a final symposium to share outcomes and foster dialogue.
- eDGES contributes to the strategic BHP-Curtin University Alliance (launched in 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. Round 1 (round 1) concluded in June 2024, and Round 2 launched in July 2024, running through to December 2028.

Project Summaries

The nine projects described here focus on improving, validating, applying, and contextualising eDNA techniques to benefit the use of eDNA analysis by all its proponents.

The projects represent new application areas of eDNA and extensions to get key biodiversity and sustainable management data that have previously been unavailable or expensive to obtain and also help translate this into functional biomonitoring tools for industry and governments.

The eDGES programme will also generate fundamental new scientific knowledge on the evolution, distribution and threats to biodiversity in ecosystems worldwide, as well as investigate novel methods of enhancing interpretation of the eDNA datasets including species abundance estimates and natural capital accounting.

Project 1A (eDGES round 1)

eGenomics of the Pilbara olive python

This project was initiated as part of eDGES round 1 and aims to merge environmental DNA monitoring with whole genomic analyses to study the Pilbara olive python (*Liasis olivaceus barroni*), enhancing our capability for targeted biomonitoring and obtain deep population genomic insights and evolutionary ecology information to guide conservation priorities.

Project 1B

Improving reptile monitoring in the Pilbara

This project will commence in mid-2025 and aims to expand reference databases, develop new metabarcoding assays and merge cutting-edge genomic technology with hands-on field biology to improve eDNA biomonitoring of reptiles. A specific focus will be given to the Pilbara where mining activity is overlapping with one of the most diverse reptile assemblages in the World.

Project 2

eDNA and 'moving beyond the barcode' for subterranean fauna detection and conservation

This project aims to develop new high-resolution eDNA tools for monitoring genetic diversity and community structure in subterranean habitats across different sites to effectively manage their conservation and obtain new ground-breaking information into the evolutionary processes in this hidden world.

Project 3

Tracking environmental restoration of Salar de Punta Negra

This project will use eDNA, groundwater data and ecological models to provide guidelines for long-term rehabilitation of Chile's Punta Negra salt flat – environmentally damaged due to water extraction.

Project 4

Detecting invasive marine species

This project will produce new, advanced eDNA detection technologies for Invasive Marine Species (IMS) that will provide managers results associated with confidence levels to enable informed decisions on IMS management. IMS are one of the most damaging consequences of globalised movement of marine resources and of changing ocean environments. Environmental DNA provides the best available detection system for IMS, yet this technology is still evolving.

Project 5

Mapping marine life in the Northern Pilbara

The Pilbara region contains a great diversity and abundance of marine life. Many of the marine species in this area are not described, which causes problems in identifying which species are invasive to the region, and which are native. This project will describe new marine species from the Pilbara and will compile these with existing knowledge in a book, Marine Life of the Pilbara.

Project 6

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

This project will develop and 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 7

Living waters of Western Australia (LiWA)

This project will enhance the ability to monitor wetland health using DNA-based survey methods, crucial for tackling wetland loss in WA. Ancient DNA from sediments will be used to examine past biodiversity changes. The project will include a citizen science component and collaboration with Traditional Owners to increase the societal value and help us interpret the impact of past and present biodiversity challenges.

Project 8

Measuring species abundance with eDNA

This project will produce a new method for deriving relative species abundance from environmental DNA sampled from any environment. Environmental DNA analysis methods currently produce information on the presence or absence of species in an environment, but do not provide measures of abundance.

Project 9

Using environmental DNA in natural capital accounting

This project aims to develop a framework for implementing eDNA indicators of biodiversity into natural capital accounting. By merging environmental and economic information, businesses can track their impacts and make more informed, sustainable decisions.

Project Summaries in Detail

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.

TERRESTRIAL CONSERVATION

Project 1A (eDGES round 1)

eGenomics of the Pilbara olive python

Summary

This project aims to merge whole-genome shotgun sequencing with environmental DNA (eDNA) monitoring for the study of the Pilbara olive python (*Liasis olivaceus barroni*). We will develop an eDNA qPCR assay for the targeted detection of the Pilbara olive python and determine the substrates best for biomonitoring this cryptic reptile.

We will also assemble complete reference genomes for the Pilbara olive python and its sister subspecies, the Kimberley olive python, forming the foundation for cutting-edge research on population genomics and evolutionary ecology. Additionally, the development of a reference genome for the Pilbara olive python has potential to enable whole-genome-based biomonitoring, improving detection sensitivity and allowing the retrieval of population-level analyses from eDNA samples.

Figure 1.1 Pilbara olive python (*L.o. barroni*). Photo: R.J.Ellis

Implementation

This project formed part of the eDGES round one portfolio but is still ongoing due to initial delays in recruiting a PhD student. This remains the only ongoing project from eDGES round one.

Staffing and management

- **Prof Morten Allentoft**
  Principal investigator, head of TrEnD lab and part of the advisory board 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**
  Lecturer associated with TrEnD Lab, Working on snake genomics and bioinformatics. 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.

External 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
Assembly of two genomes	The Pilbara genome is fully assembled. The Kimberley genome is in the final stages.	Ongoing
Pilbara olive python qPCR assay	Complete, manuscript accepted	Complete
Identification of best substrates	qPCR and metabarcoding work has been completed. Shotgun sequencing to commence soon.	Ongoing
eDNA decay experiments	Finalising planning. To commence in April	Ongoing
2025 Australian & New Zealand Environmental DNA Conference	Attended the Southern eDNA Society 2025 conference in Wellington, NZ	Complete
Population genomics	In-house olive python samples will soon undergo shotgun sequencing for preliminary data generation. Remainder being planned.	In planning
Development and implementation of community engagement and awareness program	In planning as part of the fieldwork	In planning

Overview of outputs and outcomes

A key output from the past year is the development of a highly sensitive and subspecies-specific qPCR assay for the detection of Pilbara olive python. This is a natural continuation from our paper published in 2022, showing that python eDNA can be obtained directly from rock pools (Mousavi-Derazmahalleh et al., 2023). Substrates from Perth Zoo and Caversham Wildlife Park have undergone qPCR and metabarcoding analyses, highlighting the most effective substrates to target. These will soon go through shotgun sequencing to validate the eGenomics methodology.

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

Still waiting on VGP to complete the assembly of the Kimberley olive python genome. This delay should not affect progress of the project as there is still some time until it would be utilised, and we now have the Pilbara olive python genome that can be used for most of these analyses.

Leveraged funding and value-add activities

None to report.

Challenges

No major challenges to report for the period of July 2024 to December 2024.

Learnings

Shotgun sequencing data from Mr Heyward's honours project has acted as a test dataset and allowed for the further development of bioinformatics pipelines to be used during the substrate shotgun sequencing step.

Communication

- Manuscript accepted: Kathryn L. Dawkins, Benjamin L. Heyward, Mahsa Mousavi-Derazmahalleh, Nicole E. White, Morten E. Allentoft (accepted) A qPCR assay for the threatened Pilbara Olive Python (*Liasis olivaceus barroni*). *Environmental DNA*.
- This project has been referenced on the new eDGES program website https://www.edgesprogram.org
- Ben Heyward attended and presented at the 2nd Australian and New Zealand environmental DNA (eDNA) conference at Wellington (NZ) in February 2025. His talk was titled “eDNA biomonitoring for the Pilbara olive python – insights and challenges”.

Sustainability

Collation of olive python tissue samples is currently ongoing, focusing on those available in our in-house tissue sample database, through collaborators, or through the Western Australian Museum, with collection during field work as a secondary option. Doing so saves considerable logistics and reduces unnecessary human-wildlife interaction, work time, and associated emissions.

Next Steps

Key activities planned for the next period:

- Collecting and processing of samples for the DNA decay tank experiment
- Shotgun sequencing of Perth Zoo and Caversham Wildlife Park substrates
- Analysis of all substrate data and writing up for publication.

Figure 1.2 PhD student Benjamin Heywood conducting water sampling.

TERRESTRIAL CONSERVATION

Project 1B

Improving reptile monitoring in the Pilbara

Summary

By designing new sampling methods, expanding reference databases, and developing new metabarcoding assays, this project will merge cutting-edge genomic technology with hands-on field biology to improve eDNA biomonitoring of reptiles. A specific focus will be given to the Pilbara where mining activity is overlapping with one of the most diverse reptile assemblages in the World.

This project is set to begin by mid-2025 (July or August) with the recruitment of a postdoctoral researcher. A candidate for that role has already been identified. There are ongoing developments to align this project with Prof Allentoft's recent ARC Discovery Grant (eGenomics) that seeks to implement whole genome sequencing in eDNA biomonitoring of reptiles. The exact project strategy will solidify over the next 2-3 months in discussions with eDGES BHP stakeholders and the wider project team.

SUBTERRANEAN CONSERVATION

Project 2

eDNA and ‘moving beyond the barcode’ for subterranean fauna detection and conservation

Summary

Australia’s subterranean environment is internationally renowned as a global biodiversity hotspot for groundwater fauna. It is also recognised for the large number of short-range endemic invertebrate species, in addition to having one of the highest stygofauna densities in the world. This diversity remains largely undescribed due to the limited access to these underground environments. This project aims to develop new high-resolution eDNA tools for characterising, monitoring and conserving the genetic diversity and community structure in these habitats. This project will continue to generate a high-resolution mitogenome reference database for selected taxa across geographical sampling points from the Pilbara to facilitate the investigation of intraspecific genetic diversity and differentiation in greater detail than has been attempted before.

Figure 2.1 Image of an Amphipod.

Implementation

This project commenced July 2024 and Dr Nicole White was on long-service leave from mid-July to the end of September 2024. Upon return the preparation for the final eDGES v1 annual symposium and stakeholders meeting commenced, in addition to obtaining the final batch of stygofauna animals from Ethel Gorge in preparation for shot-gun sequencing.

Staffing and management

- **Dr Nicole White**
  Principal investigator and Project lead, Senior 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 part of the advisory board in eDNA Frontiers at Curtin University.
- **Dr Mieke van der Heyde**
  Research Fellow, TrEnD lab at Curtin University. Mieke was the Research Associate on LP190100555 and supervised by Nicole and Dr Michelle Guzik. She was and still is involved in all the eDNA development work required for both projects with a focus on the Pilbara fauna.

External 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.

Note: Running parallel to this project was ARC Linkage Project LP190100555 – "Taking eDNA Underground: Transforming assessment of subterranean ecosystems" that is now compete with Michelle Guzik and Mieke van der Heyde. The goals were 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
Obtain animals for mitogenome work (Ethel Gorge)	Last batch of animals have now been collected from Stantec which covers the diversity of animals that inhabit this aquifer.	Complete
Library preparation and genome sequencing	Currently in progress.	In progress
eDGES v1 Final annual symposium and stakeholders meeting	Nicole presented the research finding from the Great Artesian Basin study, in addition to the work done with the numerous collaborations associated with this project. Dr Michelle Guzik and Dr Mieke van der Heyde – ARC Linkage "Taking eDNA underground: transforming assessment of subterranean ecosystems"; Jake Thornhill (Hons) "A molecular characterisation of the stygofauna of Barrow Island"; Dr Guilia Perina – ABRS Grant "Biodiversity and systematics of Australian Bathynellacea"; PhD candidate Milad Khosravi – Blind cave fish assay; and Dr Jason Alexander – Troglofauna air eDNA project.	Complete
2025 2nd Australian & New Zealand Environmental DNA Conference	Attended and provided support to the Southern eDNA Society in Wellington, NZ.	Complete
Support Jake Thornhill PhD research program	Jake successfully obtained over 275+ groundwater samples in January for the development of a taxonomy-free groundwater status index.	Complete

Figure 2.2 Nicole in the lab examining stygofauna under the microscope.

Overview of outputs and outcomes

- Field samples and subterranean specimens for mitochondrial sequencing from the Ethel Gorge Stygobiont Threatened Ecological Community were obtained by BHP contracted consultants with site access granted as part of regular monitoring of BHP activities in this WA region.
- A total of 170 complete or near complete mitochondrial genomes have been sequenced for the mitogenome reference database from the Great Artesian Basin and two locations in the Pilbara region. Ongoing mitogenomic work will focus on two other locations in the Pilbara region to capture the breath of intraspecies diversity for these short-range endemic species. Over 100 animals from Ethel Gorge are currently being prepared for shot-gun sequencing and eDNA assay design and validation
- eDGES v1 Final annual symposium and stakeholders meeting was a success and fantastic opportunity to share research findings.

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

- The delayed period in obtaining stygofauna from the consultancy group Stantec was unexpected.

Leveraged funding and value-add activities

Nicole supervises five PhD student projects focused on the development and application of eDNA methods in conservation and management. These projects cover a diverse range of topics, including Pilbara olive pythons, sea snakes, Blind Cave Gudgeon, crop disease and soil health, and groundwater ecosystems. Additionally, Nicole is actively involved in eDGES Project 1, round 1, and provided in-kind support to ARC Linkage Project LP190100555, “Taking eDNA Underground: Transforming Assessment of Subterranean Ecosystems” in collaboration with Dr Michelle Guzik and Dr Mieke van der Heyde. Nicole, Michelle, Mieke and Dr Paul Nevill are currently writing the next ARC Linkage grant application for submission in June 2025.

Challenges

No major challenges to report for the period of July 2025 to December 2025.

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 bioinformatic quality control, annotation and analyses were under-estimated.

Communication

- Manuscript currently under review: Mieke van der Heyde, Michael Curran, Stephanie Floeckner, Paul Nevill, Nicole E. White, Andy D. Austin, Michelle T. Guzik (in review) Validating COI eDNA metabarcoding primers for detection of subterranean fauna. *Molecular Ecology Resources*.
- This project has been referenced on the new eDGES program website https://www.edgesprogram.org
- 'Meet the researcher' video filming completed March 2025.

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

- Finalise manuscript writing for the GAB springs metabarcoding dataset.
- Design and validate metabarcoding markers for Amphipoda and Isopoda.
- Validate gudgeon control region metabarcoding markers for the two species.
- Continue with shot-gun library builds for Ethel Gorge subterranean fauna.

WETLAND CONSERVATION

Project 3

Restoration ecology via integrative eDNA approaches at the Salar de Punta Negra

Summary

This project focuses on the Salar de Punta Negra, where a restoration plan is in place which will require monitoring over a long temporal period. Environmental DNA (eDNA) approaches will be combined with routinary hydrogeological data to assess the ecological recovery of the Salar. eDNA samples will be collected from the water column of the Salar, allowing ecosystem-level assessment of the ecological restauration of the lake through comparison with reference pristine ecosystems in the region. The molecular data gathered through the project will also help explore the designing of STICI (Salar Taxon Independent Community Index) and refine current kits designed for freshwater environmental biomonitoring. The broader program, led by the eDGES team, will involve BHP staff, associated workgroup representatives and local Chilean academics to participate in the sample collections, biomonitoring and data analysis to create meaningful connections to the restoration's progress.

Implementation

The project commenced in July 2024 in continuity with the previous project on the use of eDNA techniques in hypersaline lakes of the Chilean Precordillera. Led by a diverse team composed by academic researchers and industry partners, this study focuses on ecological restoration of the Salar de Punta Negra via eDNA approaches. This project involves close interaction with eDGES Project 7 (LiWA) and its lessons learned in terms of wetland molecular biomonitoring. Multiple strategical meetings with managers on site and collaborators from academia have been carried out to frame an effective and robust design for the study, which will be implemented in 2025 and 2026.

Figure 3.1 Aerial view of the Salar de Punta Negra (left) and photo from the Salar in Summer (right).

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 Angus Lawrie**
  Chief investigator, research associate at TrEnD and expert in hypersaline ecology.
- **Dr Matthew Campbell**
  Chief investigator, research associate at TrEnD and expert in molecular biology.
- **Prof Morten Allentoft**
  Principal investigator, head of TrEnD lab part of the advisory of the eDNA Frontiers group at Curtin University.

BHP:

- **Magdalena Fernandez**
  Principal Biodiversity & Land at BHP. She is providing conceptual and logistical support to the project.
- **Marcia Faermann**
  Manager Environmental Closure at BHP. She is providing management and logistical advice to the project.

External collaborators

- **Prof Nicolas Rabet**
  Deputy Director of the Laboratory of Biology of Aquatic Organisms and Ecosystems (BOREA) at the National Natural History Museum (MNHN) in Paris. With over 25 years of experience, he is an expert in saline wetland biology and taxonomy.
- **Dr Pablo Aguilar**
  Assistant Professor at the University of Antofagasta. He is an expert in hypersaline molecular biology, particularly in relation to the lakes of the Chilean Precordillera.

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
Publication of the cornerstone study to validate the use of eDNA as a biomonitoring tool for metazoans at the Precordillera Chilean hypersaline lakes	Collaborative manuscript published in the journal *Frontiers in Ecology and Evolution:* Saccò, M., Campbell, M. A., Aguilar, P., Salazar, G., Berry, T. E., Heydenrych, M. J., ... & Allentoft, M. E. (2025). Metazoan diversity in Chilean hypersaline lakes unveiled by environmental DNA. *Frontiers in Ecology and Evolution*, 13, 1504666.	Completed
Publication of the additional study to validate the use of eDNA as a biomonitoring tool for microorganisms at the Precordillera Chilean hypersaline lakes	Analysis of the microbial dataset delayed by service leave of one of the external collaborators. Updated plan involves submission of the manuscript by July 2025.	Behind schedule
Framing of the seasonal sampling design for 2025 and 2026	Plan solidified through multiple meetings with BHP operators on site. Plan involves multiple sampling campaigns in 2025 and 2026. Salar de Punta Negra (target) and other more pristine Salars (reference) such as Salar de Pajonales or Salar de Aguas Calientes IV will be monitored.	Completed
First sampling campaign at Salar de Punta Negra	Project task slightly delayed by the lack of confirmation on the availability of collaborators on site, which had an impact on the shipping of eDNA kits. Sampling campaign intended to be carried out within the first half of 2025.	Behind schedule

Figure 3.2 Poster presentation at the international Conference on Salt Lake Research (left) and first page of the manuscript published in Frontiers in Ecology and Evolution in 2023.

Overview of outputs and outcomes

The first six months of the project have been invested in finalising the work on the dataset generated through the previous eDGES project (eDGES Project 3). This work has led to the publication of a manuscript (Saccò et al. 2025) of crucial importance for validating the use of eDNA as a sound tool to assess hypersaline metazoan biota. In parallel, the dataset has also been analysed for microorganismal diversity, with interesting results to be ultimately framed in a second site-specific publication. In addition, with the goal of increasing the outreach of our research, a poster has been presented at the 15th International Conference on Salt Lake Research in Turkey (4th-8th of November 2024).

As a result of the research activities led by Mattia Saccò, a manuscript on data disparity titled “Too much and not enough data: Challenges and solutions for generating information in freshwater research and monitoring” has been recently published in the journal Ecosphere, and five other manuscripts/chapters are currently under review – including a study on the use of eDNA to detect fish diversity in Italy derived from his bilateral collaboration with the University of Parma in Italy in 2024. In addition, three manuscripts are in preparation as part of the PhD project (Gonzalo Salazar) of the student funded through the first round of the eDGES programme. He expects to finish his final Thesis report by mid-August 2025.

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

The first sampling campaign, originally planned in April 2025, has been delayed due to a substantial underestimation of the time necessary for coordinating industry partners, given the complexity of the network of stakeholders involved in the project. This aspect has had a minimal impact on the study, as the samples collection will equally go ahead seasonally as originally planned. In addition, Dr Saccò’s temporary relocation to the National Natural History Museum in Paris has partially delayed the coordination of the project. Nonetheless, as this relocation happened in January 2025, the impact has been significantly reduced since January and February represent the most complex months of the year for organising research project in Chile due to the extended Christmas break and the holiday period in the country.

Leveraged funding and value-add activities

The collaboration in place with Prof Nicola Rabet (BOREA, Natural History Museum) and his team provides a value-add initiative for the project. Their expertise in wetland ecology, once combined with the TrEnD's expertise in molecular biomonitoring, can noticeably increase the scientific value of the study. Within this context, the completion of a Memorandum of Understanding (MoU) to further ease interchange of knowledge and researchers is in progress between Curtin University and MNHN (planned to be signed by both parties by June 2025). In addition, to increase the outreach of our research, BOREA website has been used as a platform to showcase the latest published manuscript. As part of the organisation and coordination of the sampling campaigns planned in 2025 and 2026, involvement of indigenous communities has been discussed with BHP partners.

Challenges

Meeting industry needs with academic research has required a thorough analysis of all the aspects involved. As this project is heavily focusing on tracking the ecological status of one ecosystem that is under semi-natural hydrological regimes, detailed hydrogeological analyses were required. On one side, this had an impact in the initially planned timeline, but on the other side a comprehensive understanding of the environmental condition has been achieved.

Learnings

Very useful insights on how to combine industry views and research approaches have been gathered through the multiple strategical meeting organised over the last few months. Since Salar de Punta Negra is a semi-natural environment, we also learned that expectations around complete ecological restoration outcomes should be (and will be) thoroughly considered..

Communication

- A poster presentation at the International Conference on Salt Lake Research in Turkey (4th-8th of November 2024) titled "Environmental DNA as a tool to unveil metazoan diversity of Chilean Precordillera hypersaline lakes".
- An oral exposition led by the PhD student Gonzalo Salazar at the International Conference on Salt Lake Research in Turkey (4th-8th of November 2024) titled "Identifying knowledge gaps in hypersaline systems driving the global electrical transition: invertebrate community composition in salars from the lithium triangle".
- Seminar titled "What is going on down (under) there? Multidisciplinary approaches to unveil functional ecology of aquatic ecosystems" delivered by Mattia Saccò at the BOREA seminar series on the 5th of March 2025.
- This project has been referenced on the new eDGES program website https://www.edgesprogram.org

Sustainability

The absence of flights to Chile by members of TrEnD Lab consistently reduced the carbon footprint of the project. Leveraging on BHP collaborators has also allowed to minimise the cost for fieldwork, as some of the sampling campaigns will overlap with routinary monitoring on site and at the reference wetlands.

Next Steps

Key activities planned for the next period:

- Finalise the last stage of the writing of the manuscript on the microorganismal diversity at Salar de Tara, Pujsa and Atacama. This is a study linked to the previous eDGES round, but it has an impact on the robustness of the approach designed for this new study.
- Carry out the 2025 sampling campaigns at Salar de Punta Negra and at the reference sites. This will involve detailed coordination with BHP collaborators around the shipping of kits, tutoring on the collection of samples, and fieldwork procedures overall.
- In parallel to the fieldwork activities, we will foster interaction with indigenous communities to increase the participatory component of the project. This will have multiple benefit for the outreach of the project from a research and industry perspective.

- - Deliver a seminar on hypersaline biota at the seminar series "Life at the limits" at the Sorbonne University on the 12th of May 2025.

MARINE MANAGEMENT

Project 4

Invasive marine species detection by a new generation of environmental DNA survey methods

Summary

Invasive marine species (IMS) are a major driver of the global decline of marine ecosystem health. The establishment of IMS on marine infrastructure can result in negative environmental, social and economic consequences for affected regions. Previous work on IMS funded through eDGES achieved the development of a new metabarcoding multiplex assay that can be used to detect potential high risk IMS molluscs and crustaceans. We will build upon the previous research in this new project. Molecular methods for detecting IMS using environmental DNA (eDNA) can offer decreased cost, improved efficiency, sensitivity, and accuracy compared to traditional visual surveillance. However, the quality and type of information that eDNA analyses can provide is constrained by the genetic databases and sampling methods. Furthermore, the processing of eDNA samples is predominantly lab-based which can slow detections by the time samples are transported to a laboratory, processed and reported upon. There is a requirement for a field-deployable "point-of-need" identification for high-risk species where quick decisions are essential.

This project consists of four work packages, the first package Improving reference sequence databases will continue the work started in eDGES round 1. Working with taxonomic experts we hope to resolve the taxonomic identifications of taxa of interest using genetic data, in particular the Saccostrea oysters and Brachidontes mussels. Packages two and three, will be the development and validation of new molecular tools that will be of benefit to Australian biosecurity regulators. The final package will use LiWA data to map invasive aquatic species (IAS) in some of the regions that the taxonomy-free approach will target, allowing interactions between IAS and overall ecosystem health to be measured.

Implementation

The project commenced in July 2024 in continuity with the eDGES round 1 version of Project 4.

Staffing and management

- **Professor Simon Jarman** is a geneticist and an expert in developing genomic methods for studying natural environments. Simon has 25 years of experience in working on extracting information from the natural world with genetic analyses. His research on genomic methods for biodiversity measurement has transformed approaches for studying animal diet and measuring biodiversity in aquatic environments.
- **Sherralee Lukehurst** is responsible for project management, 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. Sherralee is an experienced molecular biologist, who has previously worked at DPIRD in the areas of fish health and aquatic biosecurity.
- **Professor Euan Harvey** is a marine ecologist with 30 years of experience in developing, testing and validating techniques for non-invasively sampling marine biodiversity. Euan has been working with eDNA since 2014 comparing it to standard sampling tools and applying eDNA to broadscale biodiversity surveys of natural and artificial structures.
- **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

Achievements

Status of progress against project milestones

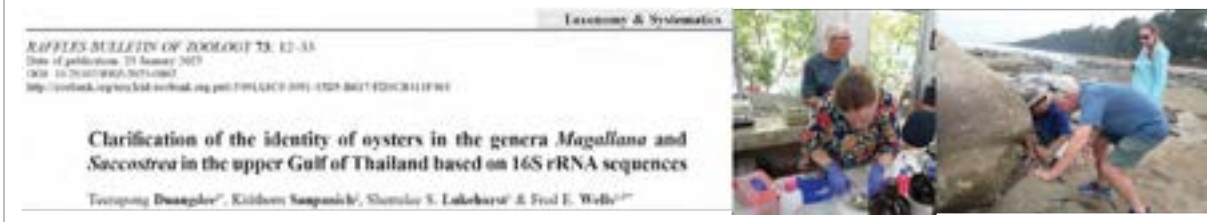
Expected Project Deliverables	Progress/Comments	Status
Assay design new methods	Identified the candidate species for the new IMS panel. Acquired additional DNA extracts from DPIRDs collection for the first priority species for testing. Email discussion with government biosecurity officers from different states regarding point of need detection tools.	In Progress
Lab testing new methods	Not scheduled till late 2025	
Reference sample collection WA	Field trip to Carnarvon and Exmouth, completed 17th–20th October 2024 to collect specimens of oysters and mussels.	In Progress
Thailand travel	Agreement reached with Dr Duangdee on scope and timing of 2025 research survey of the Andaman coast. This was scheduled for 2026 but will now occur November 2025.	In Progress

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.

- **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 of 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.

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 Chinthaka Hewavitharane**
  Fisheries Aquaculture and Marine Ecosystems (FAME) Division/Division Pêche, Aquaculture et Ecosystèmes Marins, Pacific Community (SPC) Communauté du Pacifique, Nabua, Suva, Fiji
- **Jasha Dehm**
  School of Agriculture, Geography, Environment, Ocean, and Natural Science, Laucala Campus, University of the South Pacific, Suva, Fiji


Taxonomy & Systematics

RAFFLES BULLETIN OF ZOOLOGY 73: 12–33

Clarification of the identity of oysters in the genera *Magallana* and *Saccostrea* in the upper Gulf of Thailand based on 16S rRNA sequences

Figure 4.1 Upper Gulf of Thailand survey that took place during August 2023.

Overview of outputs and outcomes achieved to date

The first six months of the project have been spent finalising the data generated through the previous eDGES programme (eDGES Project 4). This work has led to the preparation of three manuscripts, with one being accepted for publication.

Duangdee T., Sanpanich K., Lukehurst S.S., Wells F.E. (2025). Clarification of the identity of oysters in the genera Magallana and Saccostrea in the upper Gulf of Thailand based on 16S rRNA sequences. *Raffles Bulletin of Zoology*, 73: 12–33.

The remaining eDGES round 1 projects are in the data analysis stage and draft manuscripts are being prepared.

- Assay design and validation manuscript – we were fortunate to have the opportunity to organise roller sampling of a tug that was in docked in Singapore (Tuas Basin) in November 2024. These samples were amplified with the new multiplex assay and data will be included in the manuscript.
- Passive device field experiment-North Queensland Bulk Ports Corporation deployed 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. At both sites, devices were attached to settlement arrays that are deployed during routine monitoring. As part of this experiment, settlement plates were also swabbed with a roller. Samples were processed by eDNA Frontiers for three metabarcoding assays and bioinformatics was finalised by Sherralee, August 2024.

Work undertaken as part of eDGES round 2:
Oysters are widespread in tropical oceans worldwide where they are both ecosystem engineers and commercially important aquaculture species. Some species are also significant IMS, including the Pacific oyster *Magallana gigas*. The species supports a thriving aquaculture industry in southeastern Australia but is also listed as a serious IMS pest. *M. gigas* has been recorded in the scientific literature from Singapore and Thailand. With the Pilbara construction boom of the 2010s there was considerable concern that construction vessels arriving from the two countries could distribute the species to WA. In eDGES1 we surveyed key Pilbara localities and demonstrated that the species has not been introduced to the Pilbara. We then used modern DNA techniques to demonstrate the Singapore and Thai identifications of *M. gigas* were based on outdated taxonomy. *M. gigas* is not present in the northern Gulf of Thailand or in Singapore. Both countries have populations of two related species, *M. bilineata* and *M. belcheri*.

The other widespread intertidal genus, *Saccostrea*, is a taxonomic nightmare and our research for eDGES round 2 has turned to this genus. A 1985 analysis of the group concluded there is a single widespread Indo-Pacific species, *S. cucullata*, that also occurs in the South Atlantic Ocean. However, recent genetic work on *Saccostrea* has demonstrated that there are several species that can be distinguished genetically but are not readily separated based on shell characters.

S. cucullata was described from Ascension Island in the South Atlantic Ocean. Working with Ascension Island staff and our colleagues at the National University of Singapore we were able to obtain specimens of *S. cucullata* from the type locality. DNA sequences demonstrated that the true *S. cucullata* is restricted to the South Atlantic, and possibly southern Africa, but does not occur in the Indo-West Pacific. All identifications of *S. cucullata* from the Indo-West Pacific, including Western Australia, are wrong. This work has recently been accepted for publication.

Tan, S.K., Wells, F.E., Tan, K.S., Lukehurst, S.S., Morgan, M., & Fotedar S. (2025). Identity of the enigmatic oyster *Saccostrea cucullata* (Bivalvia: Ostreidae). *Journal of Molluscan Studies*: in press.

The other *Saccostrea* occurring in Western Australia is *S. scyphophilla* which was described from Bernier Island in Shark Bay. A number of authors have considered *Saccostrea mordax* from Fiji to be the same species. We hope by obtaining specimens from the type localities and redescribing the species using shells, tissue anatomy and DNA sequences we can determine whether or not the two species are in fact conspecific.

Oyster specimens collected during Carnarvon and Exmouth field work will be used in the redescription of *Saccostrea scyphophilla*. We have also been able to source topotype material of *S. mordax* from Fiji, with the help of Dr Chinthaka Hewavitharane (The Pacific Community) and Jasha Dehm (The University of the South Pacific). DNA extractions and PCRs for the 16S gene regions have been completed for all specimens.

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

Nothing to report.

Leveraged funding and value-add activities

We have been fortunate to have specimens supplied for DNA work, allowing us to expand our work on *Saccostrea* oysters.

Challenges

Additional target pest specimens will likely need to be sourced from native locations overseas, since museum collections are often unsuitable. We are reliant on overseas partners to send specimens. Potential issues include incorrect identification, poor preservation and transport company delays.

Learnings

The importance of working with biosecurity regulators to gain current knowledge of which species have become a greater risk to Australia. This information is important when designing new molecular tools that we hope industry will be excited to use for routine monitoring.

Communication

- The projects work on oysters was presented at the Triennial Conference of the Malacological Society of Australasia, November 2024 “Assessing the threat of the oyster genus *Magallana* (Bivalvia: *Ostreidae*) in Singapore to the Australian marine environment” by Prof Fred Wells.
- This project has been referenced on the new eDGES program website https://www.edgesprogram.org

Figure 4.2 Oyster Bed at Point Quobba, Carnarvon WA. *Saccostrea scyphophilla* was described from Shark Bay, Western Australia. We are redescribing the species and examining its relationship with *S. mordax* from Fiji.

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 assay design manuscript and GenBank submission.
- Data analysis and manuscript preparation for passive device/roller/settlement plate experiment.
- Working with DPIRD and Deakin University to complete the annotations and GenBank submissions of 32 mitochondrial genomes remaining from the molecular reference collection project.

MARINE MANAGEMENT

Project 5

Mapping marine life in the Northern Pilbara

Summary

The Pilbara is widely acknowledged as the powerhouse of the Australian economy. The extensive economic activity and widespread media reports on global warming, marine heatwaves and invasive species give the impression that the Pilbara marine environment is degraded. It contains diverse habitats, including mangroves, rocky and coral reefs, mudflats, and estuaries that each support highly localised species endemism. The marine life biodiversity of the Pilbara is thought to be largely intact, despite being the region containing major facilities for mineral extraction industries. The marine biodiversity of this region has been well-preserved through effective management practices, and likely through ports and other industrial sites being adjacent to high biomass ecosystems of native species that provide little opportunity for invasive species to establish. Despite the economic importance of the Pilbara and its high levels of endemic biodiversity, many species remain undescribed. New biodiversity of the Pilbara marine area will be described for the first time. An example group, the air-breathing marine slugs (Onchidiidae) will be studied in detail and the undescribed species from the Pilbara will be formally described. The huge biodiversity of marine life in the Pilbara will be summarised in a book to bring this knowledge into one place that showcases the natural variety that the Pilbara coast supports.

Implementation

This project commenced activities as a new project in July 2024.

Figure 5.1 The habitat in which the sea slugs were found during field sampling.

Staffing and management

- **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. 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 Euan Harvey** is a marine ecologist with 30 years of experience in developing, testing and validating techniques for non-invasively sampling marine biodiversity. Euan has been working with eDNA since 2014 comparing it to standard sampling tools and applying eDNA to broadscale biodiversity surveys of natural and artificial structures.
- **Sherralee Lukehurst** is responsible for field work organisation, laboratory work and data analysis. Sherralee is an experienced molecular biologist, who has previously worked at DPIRD in the areas of fish health and aquatic biosecurity.
- **Professor Monique Gagnon** has over 30 years of experience in the field of ecotoxicology, including 25 years in WA. She has a broad knowledge of marine environmental issues, is a skilled photographer, an excellent editor and writer of scientific information.

External collaborators

- **Dr Tim Cooper**
  BHP
- **Associate Professor Benoît Dayrat**
  Pennsylvania State University

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
2024		
Analysis of existing collections	Specimens collected during Port Hedland sampling Oct 2023, 63 specimens from 5 sites.	In progress
New Pilbara onchidiid collections	Field collections: Broome Sept 2024, 29 specimens from 4 sites Carnarvon and Exmouth, 22 specimens from 2 sites.	In progress
Work with Benoît Dayrat	Considerable effort went into the preparation for Benoît's research visit to WA. His passport was renewed and an Australian business visa obtained, then he was appointed as an Adjunct Professor at Curtin for his research.	In progress
Marine Life of the Pilbara book writing and submission	Preliminary work was undertaken but the project was not scheduled for a formal start until January 2025. A decision was made to broaden the coverage from just the Pilbara to include the region from Shark Bay to Broome.	In progress

Overview of outputs and outcomes achieved to date

- Selected specimens from each site had DNA extracted and the COX1 gene region sequenced and sent to Assoc Prof Dayrat Benoît so he could plan for his work in WA.

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

Nothing to report.

Leveraged funding and value-add activities

Nothing to report.

Figure 5.2 Most common genus of sea slug found during field collections, *Peronia*.

Challenges

Obtaining quality photographs for the book will be the biggest hurdle. The authors will go through their own resources to see what is available and additional photos can be obtained during other fieldwork. We may need to source specific photos externally.

Learnings

Nothing to be reported at this time.

Communication

- Several reportable outcomes are imminent, and we expect to be preparing releases in the coming year.
- This project has been referenced on the new eDGES program website https://www.edgesprogram.org

Sustainability

As with Project 4, 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.

The northwestern Australian marine biology book will be on the web so it is free to anyone who wants a copy.

Next Steps

Dr Dayrat will arrive in Perth in late January 2025. We will conduct fieldwork in Dampier and Onslow to fill in the gaps in our onchidiid collection. All specimens will have DNA extracted and the COX1 gene region sequenced. Benoît will dissect the specimens and will make extensive notes. Radulae (teeth), digestive and reproductive systems will be dissected and stored in alcohol. The results of the DNA sequences will dictate which specimens will be studied in detail in Pennsylvania. Other gene regions will need to be sequenced for selected specimens.

Writing of the marine environment book will commence in January 2025. Photographs of intertidal habitats and species present will be taken during the onchidiid survey of Onslow and Dampier.

TERRESTRIAL BIOMONITORING

Project 6

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

Summary

Biological monitoring is essential for assessing ecosystem health, yet acquiring biodiversity data is labour-intensive, especially in remote regions. eDNA-based surveys offer a transformative solution for biomonitoring however, concerns about data validity persist, particularly in arid ecosystems. More research is needed to understand eDNA's effectiveness in harsh environments with large annual fluctuations in biodiversity, and to develop simpler sampling methods that don't require specialized equipment or cold storage, that can be implemented at large spatial scales and can be combined with global satellite sensors.

This project aims to validate DNA-based tools for assessing terrestrial biodiversity and begin the development of an eDNA-based metric for ecosystem evaluation. The initial focus is on streamlining sampling methods to improve accessibility and efficiency, followed by studying spatial and temporal variability to establish reliable monitoring protocols. Ultimately, the goal is to empower various stakeholders, from citizen scientists to Indigenous rangers, to contribute to biodiversity conservation around the Olympic Dam Mine and beyond.

Implementation

Staffing and management

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. Following Mieke's move to a new role at Curtin in December 2024, a new postdoc, Dr Marina de Oliveira was recruited and started in March, 2025.

- **Dr Mieke van der Heyde** is a Researcher 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. She will co-supervise PhD and honours students, and co-lead the research.
- **Associate Professor Paul Nevill** has substantial experience developing genomic and eDNA approaches for terrestrial ecosystem monitoring. 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 Curtin's TrEnD lab, he will co-lead the research and co supervise students.
- **Dr Marina Elisa de Oliveira** is an Associate Researcher at the TrEnD laboratory. She is a molecular ecologist with a focus on applying non-invasive genetic methods to assess vertebrate biodiversity. She has more recently focused her efforts on the application of eDNA, with relevant experience in project design, fieldwork, laboratory workflow and bioinformatic analyses of eDNA data.

PhD and honours students

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

Participants/Collaborators

- **Professor Morten Allentoft**
  Head of the TrEnD Lab (Curtin University)
- **Dr Mahsa Mousavi Mousaviderazmahalleh**
  Bioinformatician at TrEnD Lab (Curtin University)
- **Associate Professor Bill Bateman**
  Behavioural Ecologist (Curtin University)

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
Optimize methodology for vegetation swabs	We compared vegetation swabs of clinical swabs and mini paint rollers to determine which method to use to the spatial study at Roxby Downs. Manuscript is to be submitted for publication in the 2nd quarter of 2025.	Completed
Sample collection and processing for spatial resolution study	360 samples (120 each of air, vegetation swabs, and soil) collected and DNA extracted from two sites, one at Olympic Dam and one in Western Australia. Sequencing to be completed Q2 2025.	On schedule
Optimize sample storage methodology for soil eDNA	Samples were collected at Roxby downs to test three different preservation methods (cold, sarkosyl buffer, LifeGuard -Qiagen) for soil samples. Sample processed and sequenced, but analysis delayed due to staff changes.	Delayed
Review paper on eDNA metrics	Discussions with experts in remote sensing, natural capital accounting, and ecosystem condition assessment are ongoing. A review paper is planned as an outcome from a workshop in the second half of 2025.	Delayed
PhD candidate selected	Modified to honours student and recruited Francesca Martino to investigate air eDNA sampling.	Completed
Postdoc recruitment	Marina de Oliveria replaced Mieke van der Heyde at the full time staff working on the project.	Completed
Sample collection for the investigation of temporal variability in eDNA samples	Samples to be collected starting mid 2025. Delayed because of staffing changes.	Delayed
Audit biodiversity across land uses	Due to commence in the second half of 2025.	On schedule

- **Dr Christine Cooper**
  Ecologist/Ecophysiologist (Curtin University)
- **Kim Solly**
  Principal Sustainability – Biodiversity (BHP)

Overview of outputs and outcomes

- New soil eDNA storage methods were evaluated that will improve sample management in remote, arid regions.
- We compared passive eDNA samplers with 3D printed filters attached to cars that carried out rapid transects through different environments. This simple collection method may be a game changer for large scale, rapid, biomonitoring efforts.
- Sample collection for a large-scale study of the spatial resolution of different eDNA substrates has been done – 360 samples of air, soil, and vegetation swabs were collected at two sites comparing native bushland to human impacted land uses of residential (Olympic Dam) and farmland (Western Australia).
- The first review paper that brings together all global studies using eDNA in arid ecosystems is near

Figure 6.1 Air samples stationed at Dryandra National Park at the border between bushland and farmland.

Figure 6.2 Soil samples being preserved in Lifeguard preservation buffer at Roxby Downs.

finalisation. We summarise key takeaway messages and discuss potential novel applications as well as analytical and methodological innovations on the horizon. We discuss some of the most salient developments with an eye on their importance for arid ecosystems.

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

Staff changes and recruitment of Dr Marina Elisa de Oliveira delayed the project.

Leveraged funding and value-add activities

The establishment of a network of molecular ecologists and spatial scientists will enhance this and other eDGES projects as combining data from global satellite sensors with broad-scale eDNA and machine learning could enable rapid and precise mapping of the distribution of organisms and better assessment of ecosystem condition.

Challenges

- With the South Australia Museum collection closed it has become difficult to obtain tissue samples from priority species to build the barcode reference libraries.
- Recruitment of a postdoctoral researcher will delay the project by ~five months (inclusive of recruitment and training).
- Identifying potential collaborators with remote sensing expertise has been challenging but we have now established a network of people with the necessary expertise from both industry and academia

Learnings

Building up our DNA barcode reference libraries will likely require networking and collaborating with many researchers and other groups to obtaining tissue samples.

Engagement with traditional owners requires significantly more time and is more complex than envisioned but is ongoing.

Communication

- 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.

- This project was presented at the eDGES Annual Stakeholder meeting in Perth, November 2024.
- The project has been presented through several on-line workshops to collaborators at CSIRO and BHP.
- Short videos have been made to describe and promote the project, available on the new eDGES program website when the edits are completed https://www.edgesprogram.org

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 (e.g. vehicle based eDNA sampling), consequently minimizing transportation costs of personnel in remote locations.

Next Steps

- Train Dr Marina Elisa de Oliveira in the eDNA workflow.
- Organise a workshop with CSIRO and BHP collaborators on combining data from global satellite sensors with broad-scale eDNA surveys.
- Complete eDNA in arid zones review publication.
- Complete two manuscripts on methodological develeopment-1. soil storage and 2. vegetation swabbing optimisation.
- Complete generation and analysis of sequence data from the Spatial Resolution study. Prepare a manuscript.
- Complete data collection and analysis for multisampling approach and prepare a manuscript.
- Plan and conduct extensive sampling at OLD site for study combining data from global satellite sensors with broad-scale eDNA surveys.

TERRESTRIAL BIOMONITORING

Project 7

Living waters of Western Australia (LiWA)

Figure 7.1 Examples of some of the types of wetlands sampled in 2024. Two sites (A) Herdsman Lake and (C) Marmion Reserve were identified as in "poor" health while (B) Quenda Swamp and (C) Nambeelup were identified as in comparatively better condition.

Summary

Wetlands are vital ecosystems that support a rich biodiversity and provide several ecosystem services essential for ecosystem and human health, including cycling nutrients, filtering contaminants, and controlling water quality. However, wetlands are globally under threat due to mounting anthropogenic pressures, including in Western Australia (WA) which has many hydro-geologically, taxonomically, and functionally diverse wetland ecosystems. The importance and fragility of these environments is often undervalued by the public and even at a scientific level, knowledge on their biodiversity is insufficient. To this end, in this project are attempting to improve our understanding of the current and historical "health" of wetlands and to educate and inspire the broader community to conserve wetlands in WA. A key contribution of this project will be the development of the novel AqWATIC "health" index derived from eDNA data which has the potential to serve as the basis of ongoing biomonitoring in WA wetlands.

Implementation

Staffing and management

- **Dr Angus Lawrie**
  Chief Investigator and Project Manager. He is primarily responsible for execution of the AqWATIC index and general co-ordination of the other components of this project.
- **Dr Matthew Campbell**
  Chief Investigator and the post-doc leading the ancient DNA component of this project.
- **Dr Mattia Sacco**
  Chief Investigator involved in the execution of the AqWATIC index.
- **Associate Prof Paul Nevill**
  Chief Investigator who is co-leading the citizen science component of this project
- **Associate Prof Bill Batman**
  Chief Investigator who is co-leading the citizen science component of this project
- **Prof Simon Jarman**
  Chief Investigator involved in the execution of the AqWATIC index.
- **Prof Morten Allentoft**
  Principal investigator, head of TrEnD lab & and part of the advisory board in eDNA Frontiers at Curtin University.

External collaborators

- **Dr Kat Dawkins**
  eDNA Frontiers (Curtin University)
- **Mr Shane Herbert**
  eDNA Frontiers (Curtin University)
- **Dr Quinton Burnham**
  Edith Cowan University
- **Dr Shaun Wilkinson**
  Wilderlab NZ Ltd

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
Project Co-ordinator hired	Dr Angus Lawrie was hired to this position and started in May 2024.	Completed
2024 samples collected and analysed	We sampled 65 wetlands mostly on the Swan Coastal Plain for a total of 430 individual water eDNA samples and paired macroinvertebrate and water quality samples. All 430 eDNA samples were amplified with 14 different metabarcoding assays. All macroinvertebrate samples have been sorted and identified to a family level. Preliminary AqWATIC models have been generated from the eDNA data and validated against various abiotic and biotic indices which show promising preliminary results.	Completed
Stakeholder Meeting held	We have had specific meetings where the objectives and goals of the LiWA project have been present to various stakeholders including to the environmental consultancy companies SLR, Biota, Bennelongia and Pheonix and NRM groups including Perth NRM, South-Coast NRM, Peel-Harvey NRM and Rangelands NRM. In addition, all data generated in lakes which are managed by local governments have been provided to and contextualised to the relevance LGA. These meetings have generated thoughtful discussions around the potential for the real-world management implications of this project.	Completed
Logo and Website established	Progress has been made by eDNA Frontiers creating a user-friendly interface to communicate eDNA data to stakeholders. This process is ongoing.	Underway
PhD candidate selected	We have yet to source a suitable candidate for this role.	Not complete

Overview of outputs and outcomes

Within the reporting period we have generated a very large but still preliminary dataset of the biodiversity and water quality of 65 wetlands in south-western Australia. The results of the AqWATIC index to date have demonstrated promising results for its potential use to serve as a reproduceable and effective tool for the environmental management of wetlands. To this end, a significant effort has been made to communicate these preliminary results to the relevant staff of 10 different environmental management organisations to seek feedback and ensure that any tool produced is fit and practical for the needs of the industry it may be applied in. Further, a data sharing agreement with DBCA has been implemented to ensure two-way collaboration and the sharing of relevant information between the LiWA project and DBCA's management objectives. We also held a community workshop on the various uses of eDNA at the Wetland Centre, Cockburn in November of 2024 which was attended by 30 members of the public.

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

With the exception of not finding a suitable candidate to begin a PhD associated with this project, we did not experience any substantial unforeseen challenges within the reporting period.

Leveraged funding and value-add activities

The addition of Dr Quinton Burham from Edith Cowan University (ECU) to the LiWA project team provides ongoing value to the project through his expertise in wetland ecology and access and capacity to share to pre-existing datasets to help contextualise the results being generated by this project.

Challenges

- The time required to collect, sort and identify the aquatic invertebrate samples has taken substantially longer than anticipated.
- In some rare instances, sites of interest were unable to be sampled due to access restrictions e.g. on private property or denied due to cultural significance.
- Engagement with traditional owners has also been challenging but is ongoing.

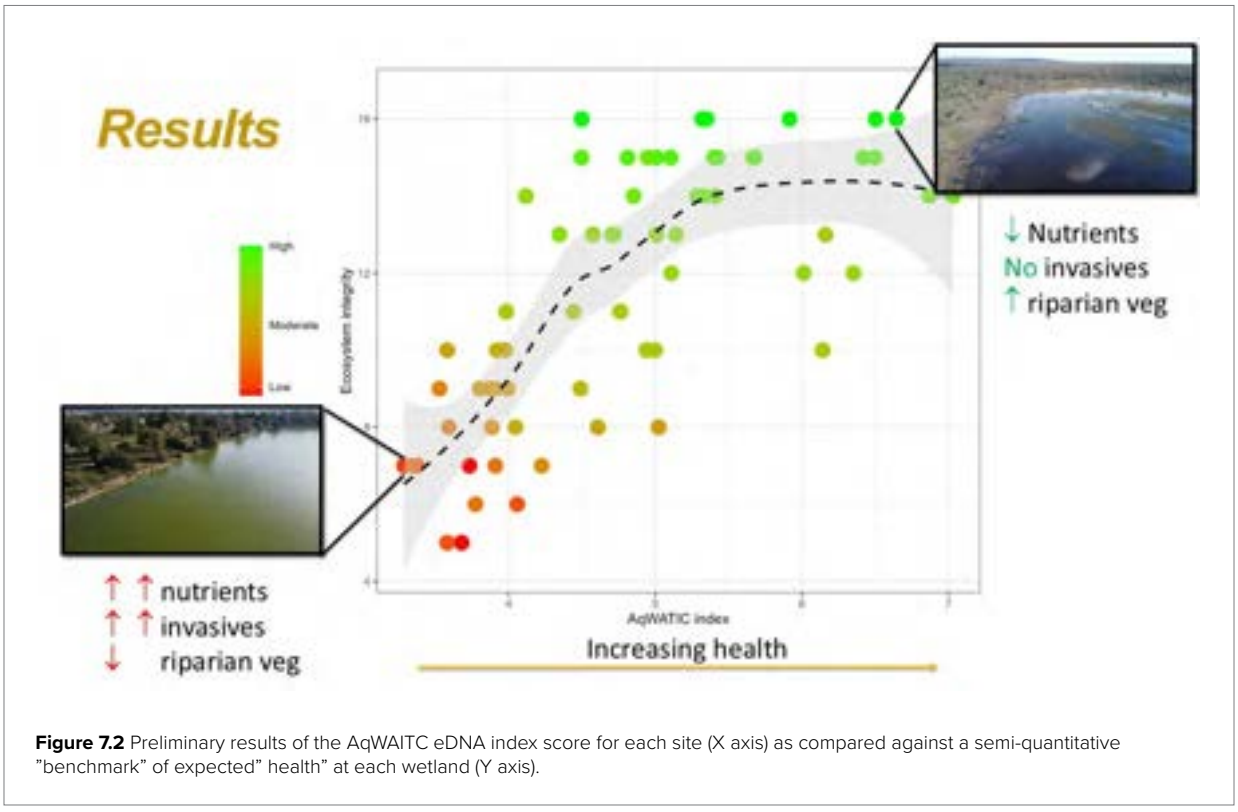


Figure 7.2 Preliminary results of the AqWAITC eDNA index score for each site (X axis) as compared against a semi-quantitative "benchmark" of expected" health" at each wetland (Y axis).

Learnings

Engagement with traditional owners requires significantly more time and is more complex than initially envisioned but is ongoing.

Communication

- This project was presented at the Australian Freshwater Science Society in Albury, November 2024
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, November 2024
- The preliminary results of the AqWATIC index have been communicated to 10 different environmental management organisations through separate meetings to ensure relevant stakeholders are aware of the potential utility of any outputs from this project
- This project has been referenced on the new eDGES program website https://www.edgesprogram.org

Sustainability

The sampling and sharing of resulting data from of some wetlands by researchers from ECU and Lateral Environmental has enabled less travel and field work duplication by the LiWA team and resulted in a more comprehensive sampling strategy than would have been possible without this collaboration.

Next Steps

Given the promising preliminary results of the AqWATIC index in estimating the environmental “health” of wetlands mostly on the Swan Coastal Plain, the next step for this aspect of the project is to collect samples from a broader geographic area. This will mostly focus on sampling approximately 100 new wetlands across the south-west of Western Australia. Further, we will work with aquatic ecologists from Murdoch University to generate a suite of DNA sequences for select aquatic macroinvertebrates which are presently missing from reference sequence databases to be uploaded to GenBank for public use. In addition, we will collect sediment core samples from select lakes to begin the aDNA component of this project and begin analysis of these samples.

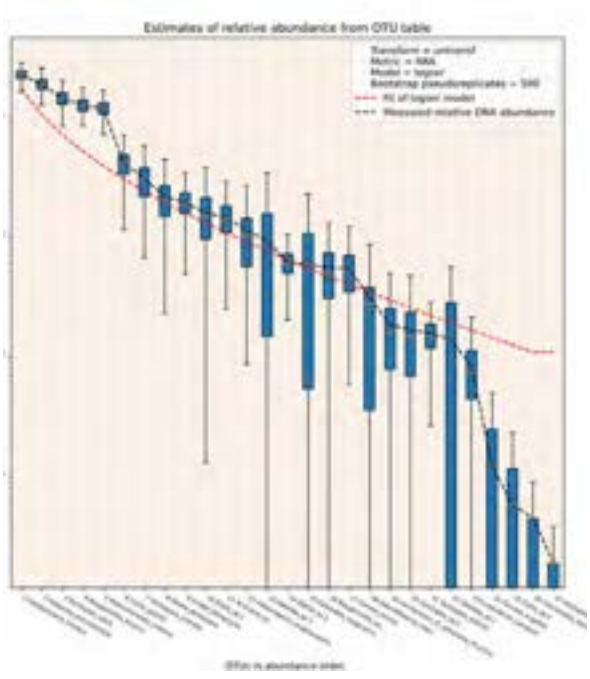
ADVANCING eDNA TECHNOLOGIES

Project 8

Measuring species abundance with eDNA

Summary

eDNA metabarcoding produces results with a quasi-quantitative relationship between molecular abundance and the biomass of organisms represented by DNA barcodes. The relationship is imperfect for two types of reason: 1. Stochastic factors; and 2. Consistent biases. Stochastic factors affecting the relationship between counts of DNA sequence types and the organisms they represent largely stem from eDNA for macro-organisms being sampled from near the organisms, rather than sampling them directly as is done for microbes. Consistent biases include differences in DNA:biomass ratios among organisms, variable rates of eDNA shedding, and systematic failure of PCR to amplify specific barcodes. Stochastic factors can be addressed by increasing sampling replication, whereas consistent factors must be addressed by controlled experiments..



Implementation

A software package for estimating the parameters of SAD models from eDNA metabarcoding data has been written. This programme takes OTU table data as its input, which is a standard format for eDNA metabarcoding. Every OTU in the dataset is ranked by DNA read abundance, and reads are aggregated by one of a range of metrics. SAD curves are fitted to the rank abundance:pseudoabundance values, and the best fitting SAD curve is taken as the model. Species' abundances can then be calculated from the SAD model. The Python package 'SQEMA' has been published on the python package index: https://pypi.org/project/sqema/

Staffing and management

Principal Investigators

- **Prof Simon Jarman**
  Chief Investigator and Project Manager. He is responsible for researching the issue and developing software and field sampling solutions that may address it.

External collaborators

- **Dr Shaun Wilkinson** Wilderlab NZ Ltd
  Dr Wilkinson is an expert on taxon-independent metrics derived from eDNA data.
- **Dr Alyssa Budd** CSIRO
  Dr Budd is an expert bioinformatician and biostatistician and has helped with software development and testing.
- **Dr Bruce Deagle** CSIRO
  Dr Deagle is an expert on the use of relative read abundance from eDNA metabarcoding data in ecological applications

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
Software for species quantification from eDNA	Beta software released	Complete
Paper on software for species quantification in eDNA metabarcoding	In progress	Commenced

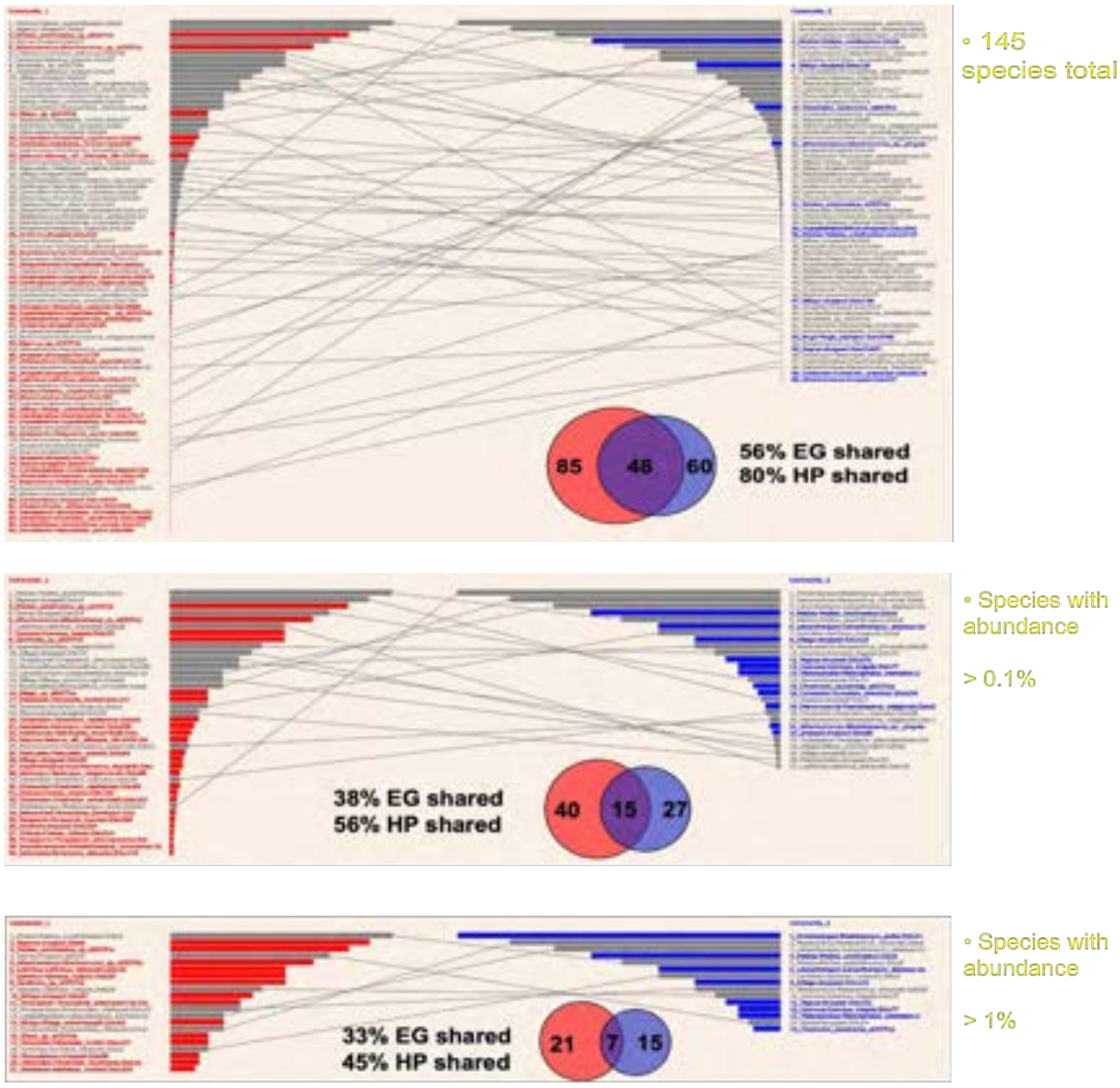


Figure 8.1 The importance of abundance measurement in eDNA metabarcoding. Two communities of fish in adjacent parts of Shark Bay were studied by highly-replicated eDNA sampling. Abundance based on SADs is shown as horizontal bars. Fish unique to their own community are shown as red or blue. Fish present in both communities are grey. If this is analysed with only presence/absence, as given by Venn diagrams, the communities have extensive overlap as raw data. This is because the actual change between communities is in abundance, from high to low, or vice versa. eDNA metabarcoding is very sensitive, and a small quantity of DNA is treated in the same way as a large amount, which is misleading. If the data is filtered to h the most abundant species, the communities look far more differentiated, which is a more accurate description of community differences. This is one application of the software developed in this project, but there are many more.

Overview of outputs and outcomes

Software for species quantification from eDNA released. Project is on track.

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

Nothing to report.

Challenges

Nothing to report.

Learnings

Some good new science has been done. The approach is feasible, more exploration needed to determine where it is most applicable.

Communication

- Presentation at the Australian and New Zealand eDNA conference hosted by the Southern eDNA Society in Wellington, NZ
- Outreach to eDNA researchers to work on finding datasets that we can collaborate on to test the efficacy of the SAD-modelling approach for species quantification from eDNA.
- This project has been referenced on the new eDGES program website https://www.edgesprogram.org

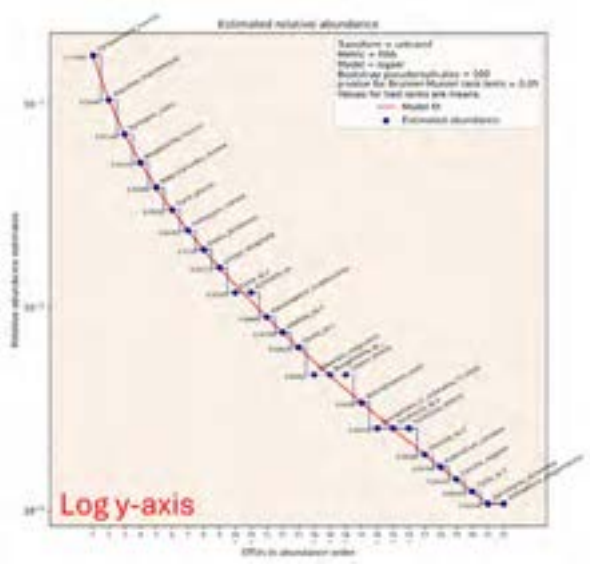
Sustainability

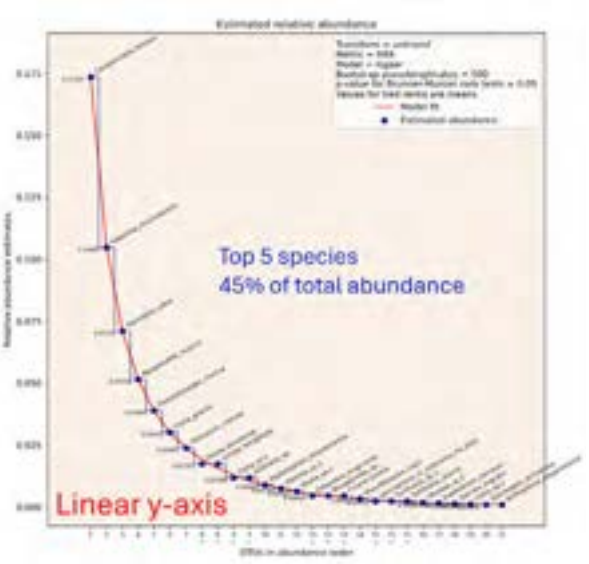
This project focuses on re-using existing data sets. Wherever possible, eDNA metabarcoding data that has already been collected will be used for developing quantitative methods.

Next Steps

Testing of the software framework for species quantification from eDNA will require appropriate datasets from collaborating researchers. There is a need for eDNA data that is collected alongside an independent measure of abundance. Multiple researchers have been contacted to start the process of finding useful datasets for testing the value of the approach.

Ecosystem disruption indicators will be developed next.





ADVANCING eDNA TECHNOLOGIES

Project 9

Using environmental DNA in natural capital accounting

Summary

Natural Capital Accounting (NCA) is a framework for managing natural assets that brings this process into the realm of standard account management. It provides a consistent basis for reporting on the state of natural environments managed by companies, trusts, or other large non-governmental entities. NCA has the potential to drive better environmental outcomes, but this potential will only be met if accurate and appropriate environmental information is used to inform natural asset accounts. eDNA metabarcoding is established as a high-throughput method for obtaining biodiversity information from any environment of interest. This makes eDNA data potentially valuable for NCA, but there are no current analysis frameworks that can interface between the data outputs of eDNA metabarcoding and the data inputs for NCA frameworks. This project is developing a toolbox of eDNA-derived metrics and explore their utility for NCA.

Implementation

Staffing and management

- **Prof Euan Harvey**
  Lead investigator. Prof Harvey will ensure co-ordination with the other marine-focused eDNA projects in eDGES.
- **Prof Morten Allentoft**
  Co-investigator. Prof Allentoft will ensure co-ordination with the other terrestrial-focused eDNA projects in eDGES.
- **Prof Simon Jarman**
  Co-investigator. Prof Jarman will work on integrating the work in eDGES projects 4 and 8 with this project.
- **Prof Nigar Sultana**
  Co-investigator, Curtin University, School of Accounting, Economics and Finance. Prof Sultana is an expert in NCA systems and will work on the later stages of the project when eDNA tools are tested for value in NCA.
- **Assoc Prof Harj Singh**
  Co-investigator, Curtin University, School of Accounting, Economics and Finance. Assoc Prof Singh is an expert in NCA and will work with Prof Sultana and the rest of the team on eDNA-NCA integration.

Achievements

Status of progress against project milestones

Expected Project Deliverables	Progress/Comments	Status
Literature review on NCA and scope for eDNA-based metrics to inform.	Scoped, but will commence in earnest with new staff member in July 2025	Discussion and planning
Assess data gaps for case studies	Will commence in earnest with new staff member in July 2025	Commencing 2026
Model development for integrating ecosystem metrics	A taxon-independent model for comparing ecosystem state based on eDNA abundance has been developed	Initial model complete, requires testing

Overview of outputs and outcomes

Original start date is January 2026 but will be brought forward to July 2025. No major outputs to report to date.

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

Nothing to report.

Leveraged funding and value-add activities

Nothing to report.

Challenges

There have been two challenges in making a solid start to this project. One is that the ideas behind it are so new and that each exploration of them leads to a new set of questions to discuss and consider, but we have worked through that phase. The second challenge has been to find an appropriate employee to drive the progress of the work, which will require a rare combination of technical skills with a broad mind and imaginative approaches to solving large problems. We have found an appropriate employee, who will commence in July 2025.

Learnings

Large, original projects are difficult to scope accurately. We have spent some time working on the scope and have a plan in place now, which will involve bringing forward the start date for a new staff member to July 2025.

Communication

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

Sustainability

This project can in principle operate alongside the other projects in the eDGES programme for field collection of eDNA data. This means that unless a specific reason to acquire new data arises, we can re-use datasets collected in the rest of the programme.

Next Steps

The most important next step will be the commencement of a level C academic to run this project.

Program Management

Common program meetings and events

Summary

The eDGES v2 program proposal includes support for a continuation of project stakeholder and yearly milestone meetings that were to be shared events for all projects in the program. This support includes 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 is included and have helped bring the program to a broader audience.

Each of the annual events will 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. The last of these symposia under eDGES v1 was held on Tuesday 12th November 2024 at Curtin's 137 St Georges Terrace building, where the launch of the eDGES v2 program was formally announced.

To help manage and coordinate the activities of the eDGES program Dr Samuel Thompson was hired as a communications manager (0.2 FTE), alongside his other roles as a Data Scientist and bioinformatician for eDGES projects and eDNAFrontiers.

An eDGES specific website has been deployed and is currently in development to provide a source of information for the projects . www.edgesprogram.org

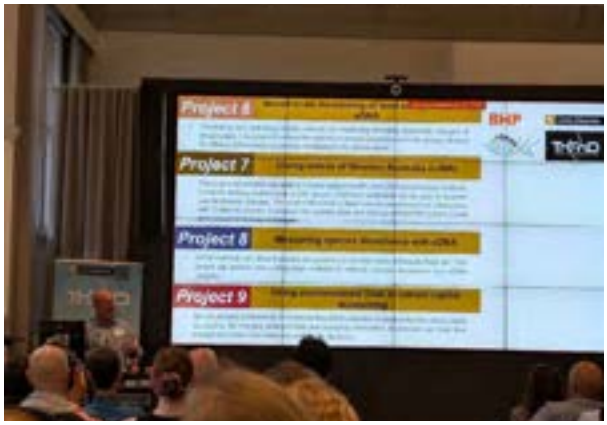


Figure 10.1 Images from the eDGES annual symposium, held in Perth in November 2024.

Curtin University

For more information

Curtin University
Faculty of Science and Engineering
Kent Street, Bentley
Western Australia
Email: director.research@curtin.edu.au

Postal address
GPO Box U1987
Perth, Western Australia 6845

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