The eDNA for Global Environment Studies (eDGES) Program

Annual Report 2025

Executive Summary

Environmental DNA research is changing how we understand and interact with ecosystems in all parts of the globe. We can sample seawater, air, soil and freshwater for eDNA that tells us what lives in any place that we want to know about. The knowledge we get from this is already changing how we manage these environments. The research project results presented here demonstrate how we can get the best information from eDNA, and how the information can improve how we interact with the natural world for sustainable economic activity, for social value, and for conservation.

The eDGES programme in its second round (eDGES2) supports nine projects which focus on a range of ecosystems from deserts in South Australia and the Pilbara, wetlands in southwest Western Australia, salt lakes in Chile, subterranean aquatic ecosystems, oyster farms in Thailand, and the busiest industrial harbour in Australia. The projects cover mine site biodiversity, the habitat of the iconic Pilbara Olive Python, computational methods for making eDNA more quantitative, and new approaches for using eDNA in Natural Capital Accounting. The portfolio of projects in eDGES reflects the broad utility of eDNA research in improving social, economic, and natural values in Australia and the wider world.

In this second annual report of the eDGES2 programme, we report significant progress on all nine projects. Since we initiated our research in eDGES2 in June 2024, the results of our work have led to 12 publications in scientific journals, and 17 presentations at conferences. Over this period, we have established new collaborations with community groups engaged in wetland habitat management, economists specialising in environmental valuation, and both government and BHP personnel working to maintain the ecological integrity of Port Hedland’s waters by monitoring for invasive species.

In addition, a draft manuscript of a forthcoming book on Marine Life in the Pilbara has been completed, aiming to highlight the remarkable diversity of marine organisms inhabiting this remote and biologically diverse region to a broader audience.

This second round of eDGES projects has entered its most productive chapter. All projects have developed beyond the establishment phase where staff and students are recruited and choices on initial directions have been made. Over the coming year, we anticipate that the eDGES team will produce a wide range of exciting results illustrating how eDNA can help us as a society work with the natural world.

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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 formally commenced in July 2020 with Round 1 which concluded in June 2024 and Round 2 was launched in July 2024 which will run through till December 2028.

Project Summaries

The nine projects described here focus on improving, validating, applying, and contextualising eDNA techniques. The overall outcomes of these projects will be to strengthen social connection and improve management of the environments being studied.

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 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 Introductions

Project 1A (eDGES round 1)

eGenomics of the Pilbara olive python

This project was initiated as part of eDGES v1 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 commenced 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.

Program Publications from eDGES2 Projects

Dawkins, K.L., Heyward, B.L., Mousavi-Derazmahalleh, M., White, N.E., Allentoft, M.E. (2025). A qPCR assay for the threatened Pilbara Olive Python (Liasis olivaceus barroni). Environmental DNA, 7:e70114. https://doi.org/10.1002/edn3.70114

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. https://doi.org/10.26107/RBZ-2025-0002

Guzik, M.T., Stringer, D.N., Thornhill, J., Coates, P.J., **van der Heyde, M.**, Hillyer, M.J., **White, N.E., Saccò, M.**, Beasley-Hall, P.G., Humphreys, W.F., Harvey, M.S., Huey, J.A., Wilson, N.G., Alexander, J., Humphreys, G., King, R.A., Cooper, S.J.B., Pinder, A., Perina, G., Hosie, A.M., Kirkendale, L., **Nevill, P.**, Austin, A.D (2025). What are the best practices for curating eDNA custom barcode reference libraries? A case study using Australian subterranean fauna. Biological journal of the Linnean Society. Linnean Society of London146:blaf053. https://doi.org/10.1093/biolinnean/blaf053

Guzik, M.T., Thornhill, J.C., **van der Heyde, M.**, Stringer, D.N., **White, N.E., Saccò, M.**, Beasley-Hall, P.G., King, R.A., Cooper, S.J.B., Pinder, A., Perina, G., **Nevill, P.**, and Austin, A.D. 2025. Towards a global barcode reference library for subterranean fauna. Science of The Total Environment, 1011, 181078. https://doi.org/10.1101/2024.09.20.613981

Power, Haylea, Mitchell J. O'Brien, Miwa Takahashi, Henry Hui, Kathy Fuller, Darren Korbie, Oliver Berry, and **Simon Jarman**. "Isolation and Genetic Identification of Metazoan Cells From Seawater." Environmental DNA 7, no. 4 (2025): e70154. https://doi.org/10.1002/edn3.70154

Saccò, M., Elmasri, A., Tawal, M., Alther, R., Altermatt, F., Martínez, A., … & Guzik, M. T. (2025). Advancing subterranean conservation through Global Research on eDNA in Groundwaters (GReG). Subterranean biology, 53, 31-40. https://doi.org/10.3897/subtbiol.53.165710

Saccò, M., Huggins, X., Martínez, A., & Reinecke, R. (2025). Collaborative Science for Groundwater Biodiversity Conservation. Ground water. https://doi.org/10.1111/gwat.13495

Saccò, M., **Campbell, M.A.**, Aguilar, P., Salazar, G., Berry, T.E., Heydenrych, M.J., **Lawrie, A.**, **White, N.E.**, Harrod, C., **Allentoft, M.E.** (2025). Metazoan diversity in Chilean hypersaline lakes unveiled by environmental DNA. Frontiers in ecology and evolution 13:1504666. https://doi.org/10.3389/fevo.2025.1504666

Smits, A. P., Hall, E. K., Deemer, B. R., Scordo, F., Barbosa, C. C., Carlson, S. M., … & **Saccò, M.** (2025). Too much and not enough data: Challenges and solutions for generating information in freshwater research and monitoring. Ecosphere, 16(3), e70205. https://doi.org/10.1002/ecs2.70205

Tan SK, **Wells FE**, Tan KS, **Lukehurst S**, Morgan M & Fotedar S. (2025). Identity of the enigmatic oyster Saccostrea cuccullata (Bivalvia: Ostreidae). Journal of Molluscan Studies. 91: 007 https://doi.org/10.26107/RBZ-2025-0002

Teufel D, **Wells FE.** (2025) Notes on the northern baler shell Melo amphora. The Malacological society of Australasia, 193.

Thompson, **Samuel**, **Simon Jarman**, Kingsley Griffin, Matthew Heydenrych, Julian Partridge, and Tim Langlois. "In Vitro Passive eDNA Sampling Provides a Cost-Effective Alternative for Large Scale Sample Collection." Environmental DNA 7, no. 3 (2025): e70101. https://doi.org/10.1002/edn3.70101

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 v1)

eGenomics of the Pilbara olive python

Summary

This project, extended from eDGES v1, integrates whole-genome shotgun sequencing with environmental DNA (eDNA) monitoring to advance the study of the Pilbara olive python (*Liasis olivaceus barroni*). A quantitative PCR (qPCR) assay will be developed and validated for subspecies-specific detection from environmental samples, and substrate types will be systematically evaluated to identify those most suitable for biomonitoring this cryptic reptile.

In parallel, high-quality reference genomes will be generated for both the Pilbara olive python and its sister subspecies, the Kimberley olive python (*Liasis olivaceus olivaceus*), establishing the genomic foundation required for future research into population structure and evolutionary ecology. The availability of a reference genome for the Pilbara olive python will also enable the development of whole-genome-based biomonitoring approaches. This "eGenomics" framework will increase detection sensitivity and support population-level inference directly from eDNA, transforming biomonitoring from a presence-absence tool into a means of obtaining genomic information from the environment. Additionally, to characterise the persistence of eDNA signals under controlled conditions, a tank experiment will be conducted to assess the decay of Pilbara olive python eDNA over a four-month period, assessed using both qPCR and shotgun sequencing.

Implementation

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://vertebrategenomesproject.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.
- **Assoc Prof Shyam Gopalakrishnan**
  Globe Institute, University of Copenhagen
  A leading computational biologist with expertise in genome analysis.
- **Assoc 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 and Kimberley olive python genomes are fully assembled.	Completed
Pilbara olive python qPCR assay	Complete, manuscript accepted.	Completed
Identification of best substrates	All lab work complete. Currently analysing data.	In progress
eDNA decay experiments	Tank experiments complete. Currently processing samples.	In progress
Review on the potential of shotgun sequencing for eDNA biomonitoring	Currently drafting.	In progress
Combined Biological Sciences Meeting 2025	Attended the CBSM 2025 conference at The University of Western Australia, Perth.	Completed
Population genomics	Currently sourcing samples for olive python population genomics.	In progress

Overview of outputs and outcomes

All lab work and sequencing have been completed for the Perth Zoo and Caversham Wildlife Park reptile enclosure substrate samples. Sequencing data is currently being analysed to determine the performance of substrates when it comes to detecting reptile DNA.

The eDNA decay tank experiments have finished, with 19 samples collected from 12 tanks (6 natural water, 6 pure water) over 4 months. This study will provide a key perspective on the temporal scale of eDNA in natural aquatic systems. Samples will undergo qPCR and shotgun sequencing to validate the potential of the eGenomics framework in a controlled environment.

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

Tank experiments had to be re-run after the initial method of water filtration proved inadequate for picking up small-scale changes in DNA quantity.

Leveraged funding and value-add activities

None to report.

Challenges

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

Learnings

Multiple bioinformatics tools are being tested to determine a comprehensive and scalable pipeline for the analysis of modern-day shotgun sequenced eDNA. Through refinement of the pipeline, we have seen how the choice of tool at various steps can produce different results.

Communication

- This project has been referenced on the eDGES program website www.edgesprogram.org.
- Ben Heyward attended and presented at the Combined Biological Sciences Meeting (CBSM) 2025 at The University of Western Australia in September 2025. His talk was titled “eDNA biomonitoring for the Pilbara olive python”.
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, December 2025.

Sustainability

We shall continue reaching out to collaborators to obtain olive python tissue samples, saving considerable logistics and reducing unnecessary human-wildlife interaction, work time, and associated emissions.

Next Steps

Key activities planned for the next period:

- Selection of samples from the tank experiment to undergo shotgun sequencing.
- Analysis of qPCR, metabarcoding, and shotgun sequencing substrate data.
- Writing up papers for publication.
- Collection of olive python tissue samples and subsequent shotgun sequencing for population genomics analyses.

TERRESTRIAL CONSERVATION

Project 1B

Improving reptile monitoring in the Pilbara

Summary

Reptiles are declining at a global scale with >20% of the worlds' species threatened by extinction. Efficient biomonitoring methods are crucial and because reptiles are often elusive and difficult to observe in the field (e.g. burrowing snakes and skinks), an eDNA approach could seem like an obvious solution. Unfortunately, the general notion is that eDNA biomonitoring is not suited for reptiles due to their low DNA shedding rate. This presents a significant limitation in Australia where ~40% of Australia's terrestrial vertebrates are reptiles, many of which are both endemic and endangered.

This project, which commenced in August 2025, aims to address this challenge by developing new sampling methods specifically targeted at capturing reptilian eDNA in terrestrial environments. This work will be combined with the development of new metabarcoding assays and expand existing reptile sequence databases for improved detection. Together, these efforts will generate a suite of optimized methods and reference resources designed to advance reptile eDNA biomonitoring to a much-needed next level.

Implementation

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 Joshua Newton**
  Research Associate in TrEnD lab, working on optimisation of reptile eDNA.
- **Dr Nicole White**
  Research Fellow in TrEnD lab at Curtin University.
- **Dr Mahsa Mousaviderazmahalleh**
  Lecturer associated with TrEnD Lab, Working on snake genomics and bioinformatics.
- **Prof Paul Nevill**
  Leads the Curtin Minesite Biodiversity Monitoring group (MBioMe), which specialises in the development of eDNA methods for the resources sector.
- **Assoc Prof Bill Bateman**
  Leads the Curtin Behavioural Ecology group and expert in reptile ecology.
- **Dr Matthew Campbell**
  Research associate in TrEnD and expert in molecular biology.
- **Mr Jake Dempsey**
  PhD student (2026-2029) studying the improved biomonitoring of reptiles using environmental DNA.

External collaborators

- **Dr Paul Doughty**
  Head of Terrestrial Zoology and Curator of Herpetology (reptiles & amphibians) at the Western Australian Museum.
- **The National Biodiversity DNA Library (NBDL)**
  DNA reference data initiative within the National Research Collections Australia at CSIRO.

Achievements

Status of progress against Project Milestones

Expected Project Deliverables	Progress/Comments	Status
Reference databases	Sequencing of 160 Western Australian reptile species scheduled to commence March 2026.	In progress
Improved assays	Will form the first data chapter of Jake Dempsey's PhD, with work to commence following generation of the expanded reptile reference database.	Commencing soon
Improved sampling	Initial substrate testing using samples collected from the western tiger snake enclosures is currently underway.	In progress
Pilbara fieldwork	Not scheduled till mid 2027.	

Overview of outputs and outcomes

The project commenced in August 2025 with the employment of Dr Joshua Newton and has since recruited PhD candidate Jake Dempsey. The first phase of the project included the construction of six custom-built reptile enclosures at Curtin University, designed as a controlled model system to refine and evaluate environmental DNA sampling methods for reptile detection (Figure 1b.1). Our initial target species, the western tiger snake (*Notechis scutatus*), was temporarily housed in the enclosures for three weeks before being safely returned to their original locations. After the removal of snakes, eDNA samples were collected across multiple substrate types and time points, resulting in the collection of more than 1,000 samples to date (Figure 1b.2). These samples will be used to assess the persistence of reptile DNA in terrestrial environments and to determine optimal sampling strategies for future fieldwork in the Pilbara region.

Figure 1b.1 Experimental setup used to investigate environmental DNA sampling methods for reptiles. (a) Capture of a western tiger snake (*Notechis scutatus*) prior to placement in experimental enclosures. (b) Individual tiger snake within an enclosure (c) Overhead view of the enclosure setup showing the range of substrates and structures (e.g., shelter boxes, cover boards, soil, and water) used to test different environmental sampling approaches for detecting reptile eDNA.

Figure 1b.2 Enthusiastic eDGES staff collecting environmental DNA samples within reptile enclosures following the removal of tiger snakes.

In parallel, we are currently collaborating with CSIRO's National Biodiversity DNA Library initiative and the Western Australian Museum to coordinate the generation of mitochondrial genomes for approximately 160 Western Australian reptile species with sample collection commencing in mid-March. This effort aims to fill key reference gaps resulting in a near-complete mitochondrial reference database for the state's reptile fauna that will directly support the design and validation of new reptile eDNA primers.

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

None to report.

Leveraged funding and value-add activities

This project leverages infrastructure established under the ARC Discovery Project LP190100555, "eGenomics – Next generation biomonitoring of threatened species". In particular, it utilises the purpose-built reptile enclosures funded through the ARC Discovery grant, along with the tiger snakes temporarily housed within them, to generate additional data under controlled conditions. This integration strengthens the development of a comprehensive terrestrial reptile eDNA molecular toolkit.

Challenges

No major challenges to report.

Learnings

This project provided training in safe and ethical reptile handling, including completion of a venomous snake handling course by those involved. It also provided practical experience in the capture and temporary holding of living snakes while adhering to animal ethics requirements and ensuring animal welfare.

Communication

- This project has been referenced on the eDGES program website (Project 1: Improving reptile monitoring in the Pilbara) and has been presented at the annual eDGES symposium.
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, December 2025.

Sustainability

The pooling of resources and expertise between this project and the ARC Discovery Project (DP240100966) has reduced duplication of field and laboratory effort while maximising research outputs.

Next Steps

Key activities for the next period are:

- Design and validation of new reptile-specific eDNA primers.
- Processing and analysis of samples collected from the western tiger snake enclosures.
- Introduction of an additional reptile species into the enclosure system to continue substrate testing and evaluate consistency of eDNA detection across species.
- Development and testing of methods for the detection of arboreal reptiles, including evaluation of appropriate substrate types.
- Supporting PhD candidate Jake Dempsey in establishing his research project, including experimental planning and laboratory training.

SUBTERRANEAN CONSERVATION

Project 2

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

Summary

eDNA and ‘moving beyond the barcode’ has successfully advanced the field of environmental DNA research by sequencing 270+ complete or near complete mitochondrial genomes from 350 animals received since the commencement of this project. In addition, 180 ground and artesian spring water eDNA samples from the Great Artesian Basin (GAB) have been metabarcoded. This project focussed on a diverse range of subterranean taxa collected from the GAB and Pilbara, encompassing several invertebrate groups and two conservation priority vertebrates. Invertebrate genome sequencing included Chiltrioniidae (Amphipods), Amphisopidae (Isopods), Ostracoda (Ostracods), Hydrobiidae (Snails), Copepoda (Copepods), Tainisopidae (Isopods), Cyclopidae (Copepods), Parabathynellidae (Syncarids) and Bathynellacea (Syncarids). Vertebrate genome sequencing included *Milyeringa veritas* and *M. justitia*, known as blind cave gudgeon. The establishment of this extensive mitochondrial genome dataset marks a significant technological advancement, providing a critical reference for future eDNA detection efforts. This dataset will facilitate the development and validation of eDNA markers, enhancing the ability to monitor and conserve subterranean and groundwater fauna. This project sets a foundation for continued innovation in environmental genomics, supporting conservation strategies and ecological studies by enabling more precise and efficient detection of subterranean biodiversity through eDNA methodologies.

Implementation

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 Michelle. 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**
  Adjunct of Australian Centre for Evolutionary Biology and Biodiversity at the University of Adelaide.
- **Stantec Australia Pty Ltd**
  226 Adelaide Terrace, Perth WA 6000
  www.stantec.com/en.

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.	Completed
Library preparation and genome sequencing	A total of 103 animals received from Stantec with 93 stygofauna prepared for genome sequencing.	Completed
eDNA marker development and validation	As part of ongoing efforts to improve monitoring and conservation of *Milyeringa* (subterranean fish species), a genus-specific eDNA metabarcoding assay has been developed and validated. Mitochondrial genomes were sequenced and assembled to inform PCR assay design and lab validation included *in-silico, in-vitro*, and *in-situ* studies.	Ongoing
Bioinformatics – Mitogenome annotation	All mitogenome are being checked for quality and characterisation of protein-coding genes, tRNAs, and rRNAs prior to submission to a public database.	Ongoing
Gudgeon assay and mapping distribution through the Pilbara	Application of the gudgeon assay to screen existing DNA groundwater extracts from the Pilbara that are held in the TrEnD lab.	Ongoing
Obtain animals for mitogenome work (Welli Wolli)	Sourcing animals from this region is underway.	Ongoing

Overview of outputs and outcomes

- Genome library preparation and sequencing (Ethel Gorge) resulted in 67 complete to near-complete reference mitochondrial genomes for stygofauna covering 11 taxonomic Families and include: *Phreodrilidae* (annelids), *Pezidae* (mites), *Paramelitidae* (amphipods), *Microcerberidae* (isopod), *Tainisopidae* (isopod), *Bathynellidae* (crustacean), *Parabathynellidae* (crustacean), *Cyclopidae* (copepod), *Ameiridae* (Harpacticoid), *Candonidae* (ostracod), *Limnocytheridae* (ostracod) (Figure 1). This resulted in a 72% success rate from DNA extraction to genome sequencing.
- eDNA marker development and validation for blind cave gudgeon (*M. justitia and M. veritas*) was designed on the mitochondrial genomes sequenced and resulted in a modelled Limit of Detection (LOD) of 1.36 copies/reaction for *M. justitia* and a LOD of 12.8 copies/reaction for *M. veritas* (Figure 2). The manuscript has been submitted to the journal Environmental DNA.

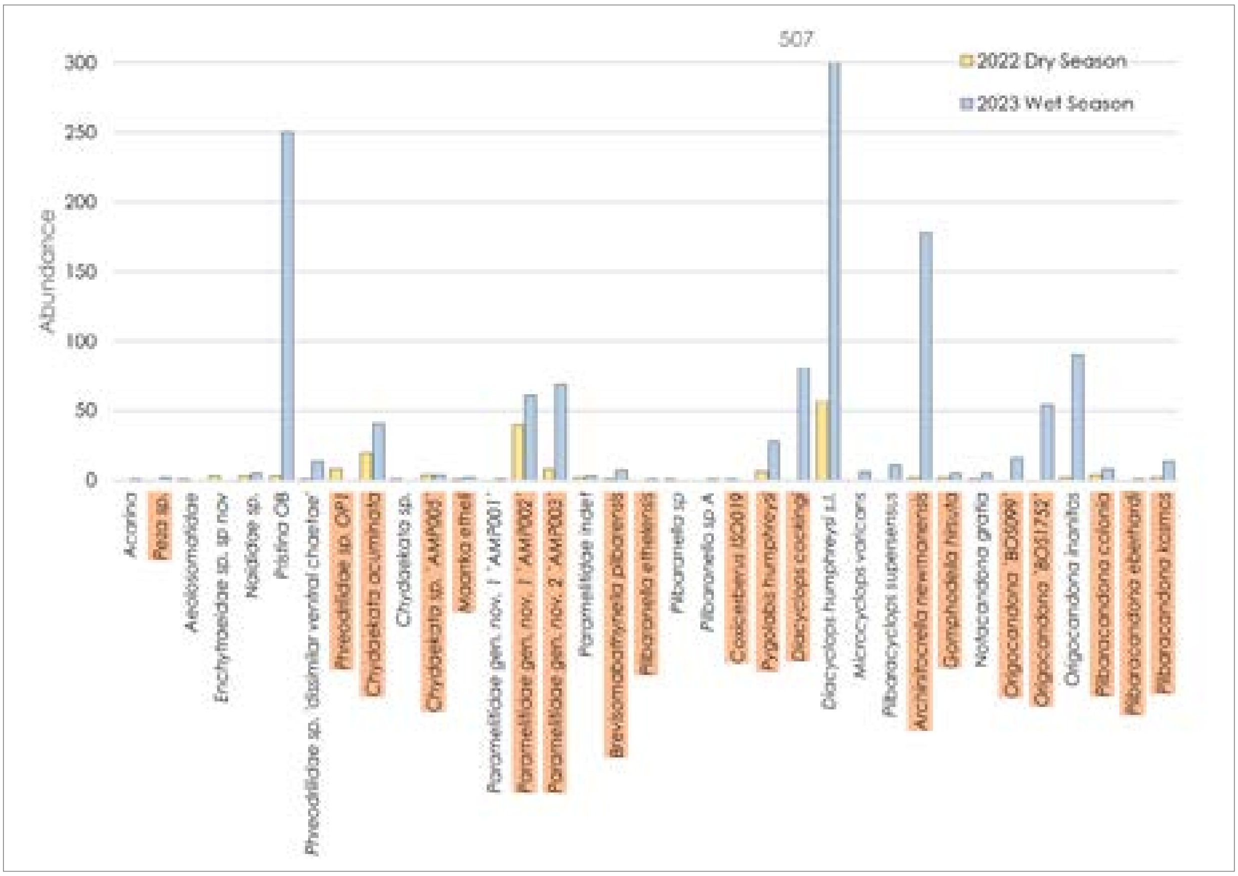


Figure 2.1 Total abundance of each stygofauna taxon in 2022 Dry (Yellow) and 2023 Wet (Blue). Image taken from Ethel Gorge TEC Stygofauna Monitoring 2022/2023 report by Stantec with species shown for which reference mitochondrial genomes have been sequenced (highlighted orange).

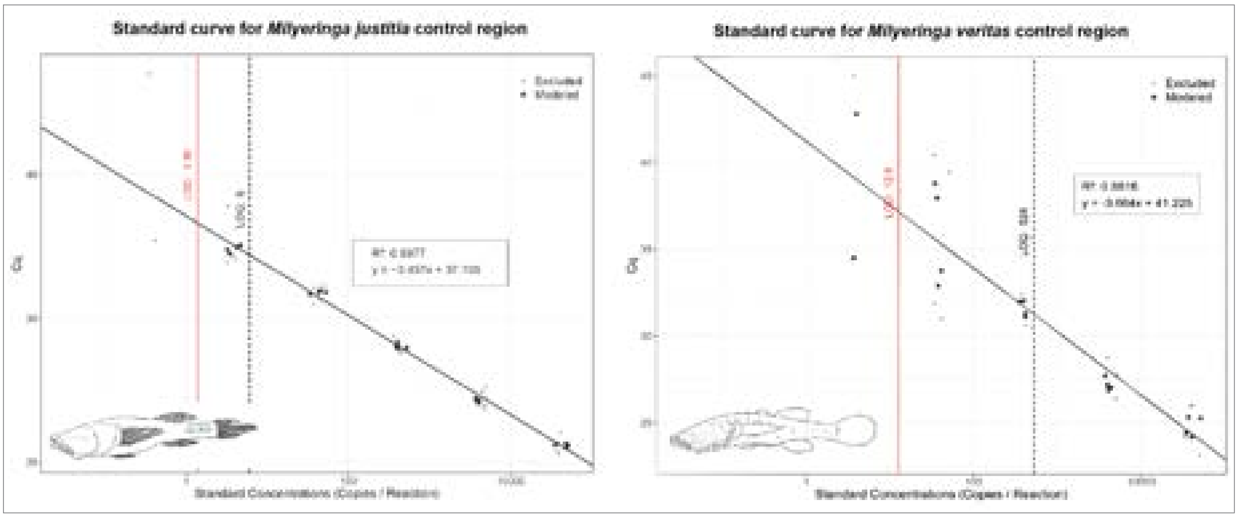


Figure 2.2 Standard curve showing the modelled limit of detection (LOD; solid red line) and limit of quantification (LOQ; dotted black line) for the MilyeringaCR assay per Klymus *et al.* (2019). Cq is the cycle number at which the fluorescent signal crossed the defined threshold above background fluorescent levels in the PCR reaction. LOD and LOQ were calculated to be 1.36 copies/reaction and 6 copies/reaction, respectively for *Milyeringa justitia*. LOD and LOQ were calculated to be 12.8 copies/reaction and 524 copies/reaction, respectively for *Milyeringa veritas*. Left insert - illustration of the holotype (male) of *Milyeringa justitia* - WAM P.33166-001. Right insert - illustration of a female Cave Gudgeon, *Milyeringa veritas* - WAM P.33157-001. Source: Helen K. Larson in Fishes of Australia, https://fishesofaustralia.net.au/home/species/1172 and species/4857.

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

- Field samples and subterranean specimens for mitochondrial sequencing from Weeli Wooli in the Pilbara may have to rely on BHP contracted consultants with site access granted as part of regular monitoring in this WA region.
- The amount of time required for genome quality control, annotation, analyses and upload to GenBank was under-estimated, partly due to completing 4x more data than was originally proposed in this project. Although a valuable resource, nonetheless.

Leveraged funding and value-add activities

Under this project umbrella Nicole supervises 5 PhD students with 3 students working on subterranean groundwater systems and are supported by eDGES Project 2:

- Jake Thornhill (PhD candidate, Adelaide University): Thesis title *“New methods in monitoring groundwater-dependent ecosystems using environmental DNA”.*
- Milad Khosravi (PhD candidate, Adelaide University): Thesis title *“Improving eDNA Methods for Groundwater Biomonitoring”.*
- Mahima Tawal (PhD candidate, Curtin University): Thesis title *“Unveiling the Ecosystem Dynamics of Three Western Australian Groundwater Systems Using Food-Web Approaches”.*

Further value-add activities include submission of ARC Linkage Grant application (LP250200539) “Developing advanced biomonitoring technologies for subterranean fauna” that received favourable reviews and rejoinder submitted on 1 October 2025. Successful grant applications will be announced sometime between March – June 2026.

Challenges

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

Learnings

Need to develop bioinformatic tools and pipeline for genome annotation and submission to public database.

Communication

- This project is referenced on the eDGES program website www.edgesprogram.org, in addition to video content.
- Two publications were generated from this project (refer page 6).
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, December 2025.

Sustainability

Continue ongoing collaborations to obtain subterranean animals, that have been identified by taxonomic specialists, for mitochondrial genome sequencing and assay design.

Next Steps

- Obtain next batch of stygofauna animals for genome sequencing.
- Design amphipod eDNA assay for lab and field validation.
- Continue ongoing support and supervision of PhD student projects.
- Commence writing of metabarcoding manuscript for GAB spring samples.
- Awaiting outcome of recent ARC linkage grant (LP250200539).

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 long-term monitoring. 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 restoration 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 study 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 strategic 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 2026.

Staffing and management

- **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 advice to the project.
- **Silmara Costa**
  Principal Environmental Closure at BHP. She is providing logistical help 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.
- **Dr Raquel Rodriguez**
  Researcher at the University of Antofagasta. She is an expert in hypersaline protists, particularly in relation to the lakes' communities of the Chilean Precordillera.

Achievements

Status of Progress against Project Milestones

Expected Project Deliverables	Progress/Comments	Status
Submission of a manuscript led by PhD student Gonzalo Salazar (eDGES Project 3 V1) on the use of stable isotopes in hypersaline Precordillera lakes	Paper titled "Stable isotopes indicate consistent trophic structures but marked spatial variation in isotopic values across contrasting high-altitude Andean salars" and submitted to the Journal Isotopes in *Environment and Health Studies*	Completed
Submission of manuscript led by PhD student Gonzalo Salazar (eDGES Project 3 V1) on the use of multidisciplinary approached in hypersaline ecology	Paper titled "Diversity of the aquatic invertebrate community in high-altitude ecosystems in northern Chile" and submitted to the journal *Freshwater Biology*	Completed
Publication of the additional study to validate the use of eDNA as a biomonitoring tool for microorganisms at the Precordillera Chilean hypersaline lakes (eDGES Project 3 V1)	Draft almost ready for submission, pending a re-analysis for protists give the release of updated and improved databases	Behind schedule
Framing of the sampling design for 2026	Due to internal restructuring at the BHP Chile team, design of the final sampling plan has been slightly delayed. One sampling campaign is now confirmed for April 2026.	Completed
Sampling campaign to collect eDNA samples from Salar de Punta Negra and reference sites.	Samples will be collected from the Salar de Punta Negra (target) and Salar de Imilac (reference) by a local team composed by consultancies and BHP collaborators.	Behind schedule

Overview of outputs and outcomes

The microorganismal dataset from the eDGES Project 3 V1 has been thoroughly analysed in collaboration with Dr Aguilar and Dr Rodrigues at the University of Antofagasta (Figure 1), with interesting results to be ultimately framed in a second site-specific publication that will be submitted by July 2026. The PhD Student Gonzalo Salazar has submitted his PhD Thesis and will deliver a *viva voce* on the 26th of March 2026. This is an important milestone, given the entire PhD project was funded by the eDGES programme as part of the international collaboration.

As a result of the research activities led by Mattia Saccò, a total of ten manuscripts has been published, with other ten currently under review, including global studies on the use of eDNA for detecting fish diversity patterns, investigations on the biogeochemical cycles from the biggest river in Italy (Figure 2) (within the framework of the bilateral collaboration between Curtin University and the Universita' di Parma), and several studies on the implementation of eDNA approaches in subterranean ecosystems.

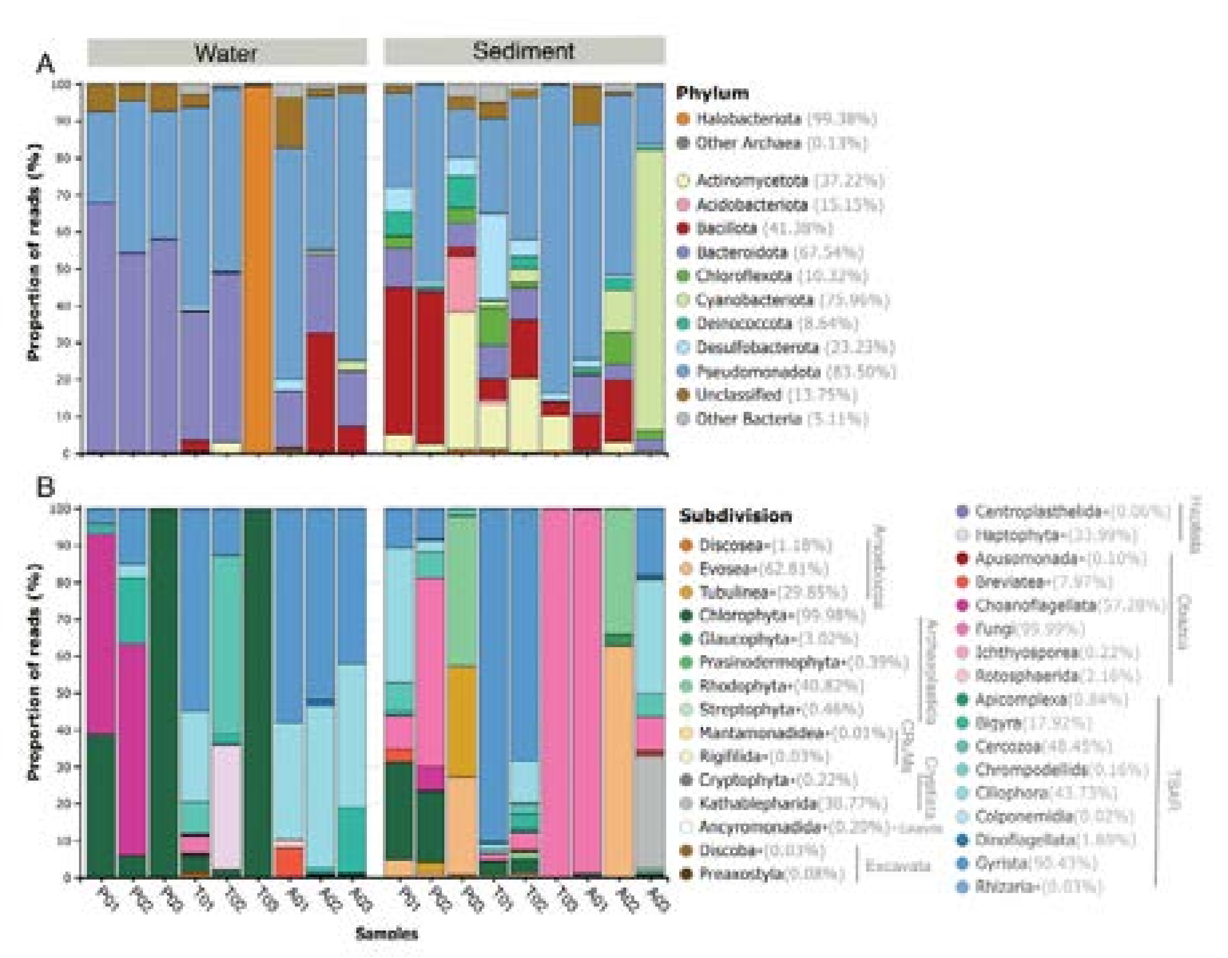


Figure 3.1 Microbial community composition based of 16S rRNA (A) and 18S rRNA (B) gene amplicon sequencing in samples from Salar de Pujsa (P), Salar de Tara (T) and Puilar lagoon (A; Salar de Atacama).

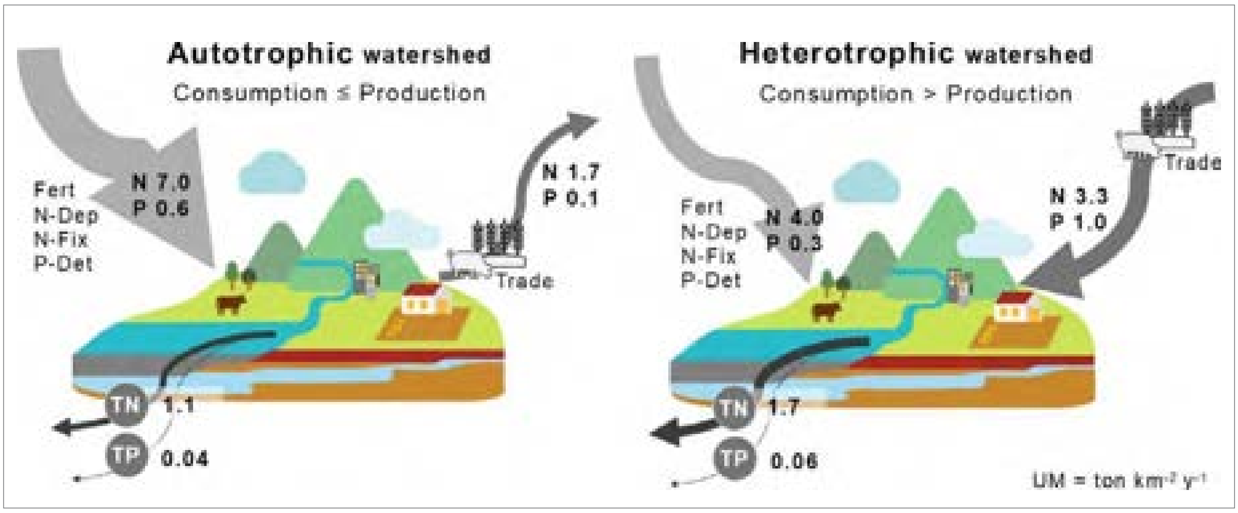
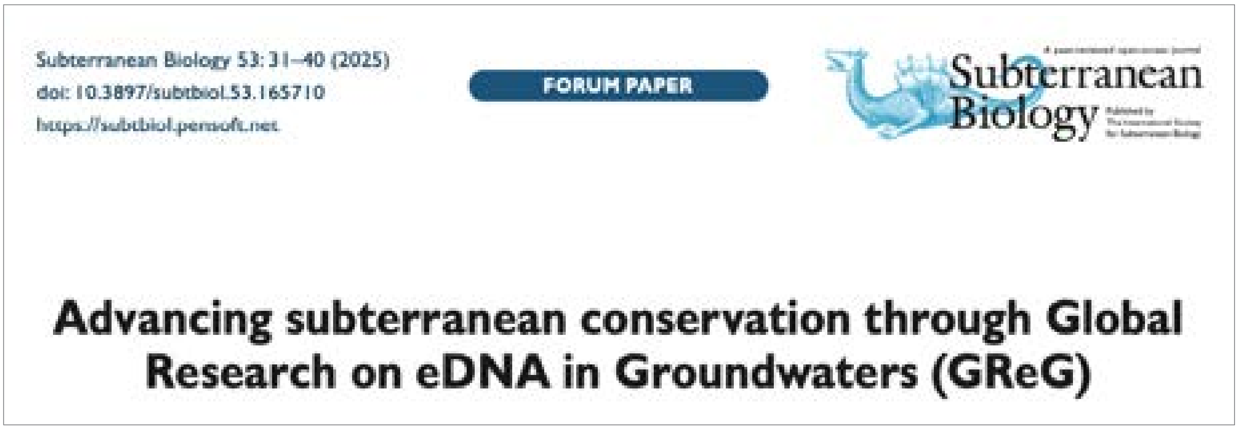


Figure 3.2 Graphical abstract from the publication titled “Anthropogenic feed and food trade and hydrological factors influence river N and P export and stoichiometry from heavily exploited watersheds” in *Science of the Total Environment*.

Mattia Saccò has led an eDGES satellite research initiative called Global Research on eDNA in Groundwaters (GReG) - https://www.gregproject.com/. GReG started in June 2025 and it has gathered over 80 collaborators over the world to bring to reality the first intercontinental study on eDNA in groundwaters. Within the framework of the visiting researcher held by Mattia Saccò at the Natural History Museum of Paris, a workshop involving 40 GReG collaborators will be organised in June 2026. Results will be presented to collaborators and an application to a large-scale grant will be prepared. A Forum paper to describe the initiative has been published in Subterranean Biology (Figure 3).


Subterranean Biology 53: 31–40 (2025)
doi: 10.3897/subtbiol.53.165710
https://subtbiol.pensoft.net

FORUM PAPER

Subterranean Biology

Advancing subterranean conservation through Global Research on eDNA in Groundwaters (GReG)

Figure 3.3 Screenshot highlighting the title of the GReG manuscript publishes in *Subterranean Biology*.

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

The first sampling campaign has been delayed due to internal changes within the BHP team in Chile. As a result, given the time left for the project (finalising in July 2027), only one sampling campaign will be carried out in April/May 2026. However, the updated sampling design will allow to assess the diversity and ecology of Salar de Punta Negra and compare these patterns to other wetlands in the area with similar hydrogeological conditions (e.g., “laguna-veiga” system). As a result, the lack of seasonality is compensated with more comprehensive comparative studies across systems in the region.

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, a Memorandum of Understanding (MoU) between Curtin University and MNHN has been signed to further ease interchange of knowledge and researchers. As part of the organisation and coordination of the sampling campaigns planned in 2026, involvement of indigenous communities has been discussed with BHP partners. Mattia Saccò has applied for an ARC Discovery Early Career Research Award (DECRA), with results to be expected in the second quarter of 2026.

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

- Seminar titled: “Hypersaline ecology and new frontiers “at the series “Life at its limits” organised by Sorbonne University in Paris in May 2025 and presented by Mattia Saccò.
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, December 2025.
- This project has been referenced on the eDGES program website 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 2026 sampling campaign at Salar de Punta Negra and at the reference site Salar de Imilac. This will involve detailed coordination with BHP collaborators around the shipping of kits, tutoring on the collection of samples, and fieldwork procedures overall.
- Analysis of the dataset generated through the fieldwork planned in April 2026, followed by preparation of manuscripts and outreach to disseminate results.

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 tool 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

Staffing and management

- **Prof Simon Jarman**
  Principal investigator, Simon is a geneticist and an expert in developing genomic methods for studying natural environments. Simon leads coordination of the overall project, communications and scientific output.
- **Sherralee Lukehurst**
  Investigator, Sherralee is a research officer at TrEnD and is responsible for field work organisation, field collections, molecular 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.
- **Prof Euan Harvey**
  Investigator, Euan possesses extensive expertise in using eDNA for large-scale biodiversity surveys of both natural and artificial structures, particularly in sampling design and statistical analysis.
- **Adj Prof Fred Wells**
  Investigator, Fred has considerable skills in the taxonomy and ecology of marine invertebrates and extensive experience in IMS detection and the issues involved. He contributes to field collections, analysis and scientific writing.
- **Dr Justin McDonald**
  Investigator, Justin leads the policy, science and surveillance areas of Aquatic Biosecurity within DPIRD. He provides current policy and management advice to the project.

External collaborators

- **Claire Wellington** and **Matthew Hewitt**
  Aquatic Biosecurity DPIRD.
- **Dr Seema Fotedar**
  DPIRD Diagnostic and Laboratory Services.
- **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.
- **Dr Richard Willan**
  Museum and Art Gallery of the Northern Territory.
- **Dr Simon Grove**
  Tasmanian Museum and Art Gallery.
- **Dr John Healy**
  Queensland Museum.

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 tools. Reagents and lateral flow devices chosen and purchased for point-of-need assay development.	In progress
Lab testing new methods	26 primer pairs have been designed and tested. Multiplex optimised, containing 11 assays and sequencing of first test samples (32) has been completed.	In progress
Reference sample collection WA	*Brachidontes* collections of the Natural History Museum London, Australian Museum Sydney (AMS), Museum Victoria and South Australian Museum have been examined during private trips by Fred Wells. *Brachidontes* specimens have been obtained from the AMS, Northern Territory Museum and Tasmanian Museum for DNA sequencing. Further field trips will be planned to collect mussel specimens if necessary.	In progress
Thailand travel	This was originally scheduled for 2026 but occurred in November 2025. The survey of the Andaman coast consisted of 28 sites and resulted in the collection of 194 tissue samples; these samples were brought to Curtin for DNA extraction, PCR and sequencing. Oysters collected were deposited in the Kasetsart University Fisheries Museum.	In progress
Field collections for IMS panel testing	Simon Jarman collected 10 roller eDNA samples in Tasmania (Oct 2025). Sherralee Lukehurst collected 30 roller eDNA samples from two sites in Thailand (Nov 2025). Other samples will be obtained from eDNA Frontiers archived material or other eDGES projects.	In progress
Field testing point-of-need assays	Not scheduled till 2026.	
LiWA - IAS data integration	Although this package was initially planned for 2026, its start date was moved earlier to coincide with the LiWA sampling period. Dr Angus Lawrie collected 6 replicate water samples from 5 DPIRD priority Perth metro lakes in Dec 2025.	In progress

Overview of outputs and outcomes

The remaining eDGES version one projects:

- **Assay design and validation manuscript** - Development of a 16S rRNA metabarcoding multiplex for detecting invasive marine species for use in biosecurity applications. Submitted to Management of Biological Invasions. Submitted reference sequences to GenBank:16S (73) and COX1 (53).

- Completed the annotations and GenBank submissions of 22 complete and 10 partial mitochondrial genomes and 50 x 18S sequence data remaining from the molecular reference collection project a collaboration with DPIRD and Deakin University.

- **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. Writing of the manuscript is underway.

Oysters

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 version 2 has turned to this genus. A 1985 analysis of the group concluded there is a single widespread Indo-Pacific species, *S. cuccullata*, 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. cuccullata 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. cuccullata* from the type locality. DNA sequences demonstrated that the true *S. cuccullata* is restricted to the South Atlantic, and possibly southern Africa, but does not occur in the Indo-West Pacific. All identifications of *S. cuccullata* from the Indo-West Pacific, including Western Australia, are wrong. This work was published in 2025.

Journal of Molluscan Studies

The Malacological Society of London

Published online 26 August 2025

Identity of the enigmatic oyster *Saccostrea cucullata* (Bivalvia: Ostreidae)

Siong Kiat Tan[1], Fred E. Wells[2,3], Koh Siang Tan[4], Sherralee S. Lukehurst[2], Melissa Morgan[2] and Seema Fotedar[5]

Figure 4.1 Publication on the identity of *Saccostrea cuccullata* that would not have been possible without the help of the Ascension Island government and DPIRD staff.

Tan, S.K., Wells, F.E., Tan, K.S., Lukehurst, S.S., Morgan, M., & Fotedar S. (2025). Identity of the enigmatic oyster *Saccostrea cuccullata* (Bivalvia: Ostreidae). *Journal of Molluscan Studies* 91: eyaf007.

The other *Saccostrea* occurring in Western Australia is *S. scyphophilla* which was described from Bernier Island in Shark Bay. Several authors have considered Saccostrea mordax from Fiji to be the same species. Oyster specimens collected during Carnarvon and Exmouth field work were used in the redescription of *Saccostrea scyphophilla*. We have sourced 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, PCRs and sequencing of COX1 and 16S gene regions were completed for all specimens. We demonstrated the two are in fact separate species. A paper describing this work has been accepted for publication:

Lukehurst SS, Tan SK, Tan KS, Dehm J, Hewavitharane C & Wells FE (2026) Clarifying the identity of *Saccostrea scyphophilla* (Péron & Lesueur, 1807) and *S. mordax* (Gould, 1850) (Bivalvia: Ostreidae) from the Indo-Pacific. *Zootaxa*: in press.

Mussels

Like oysters, mussels are diverse. Some species are environmental engineers, forming habitats that are then home to other species. Some species become invasive. Mussel taxonomy is controversial, but DNA sequencing is now helping to establish generic and species boundaries. As part of eDGES1 we teamed with Singaporean, Thai, Indonesian and American colleagues to delineate the species of the *Brachidontes variabilis* complex and published a paper on the group in the Indo-west Pacific.

Tan SHM, Wells FE, Lukehurst SS, Strong E, Sanpanich K, Duangdee T, Ambarwati R and Tan KS (2024) Unravelling the *Brachidontes variabilis* species complex (Bivalvia: Mytilidae). *Journal of Molluscan Studies* 90: eyae037, 23 pages.

This paper included limited information on the group in Australia. We are now working with Dr Tan Koh Siang of the National University of Singapore and scientists from Australian museums to explore the relationships and biodiversity of the group in Australia. The goal is to develop sufficient information on Australian species by late 2026 to work on a manuscript with Dr Tan and others when Dr Tan comes to Curtin for a research visit.

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 and *Brachidontes* mussels.

Figure 4.2 Pictures from Thailand fieldwork which included an oyster survey of the Andaman coast and eDNA sampling using rollers at two locations where mussels considered invasive to Australia can be found.

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.

- This project has been referenced on the eDGES program website www.edgesprogram.org.
- Two manuscripts have been published this year and another two have been submitted.
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, December 2025.

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.

Receiving specimens from museum collections has reduced the amount of field trips required to collect our own specimens, hence reducing the carbon footprint of the project.

Next Steps

- Complete manuscript preparation for passive device/roller/settlement plate experiment.
- DNA extractions, PCRs and sequencing to be completed for the Thai oyster specimens and Australian mussel specimens.
- A research visit to Curtin by Dr Tan Koh Siang of the National University of Singapore is planned for late 2026. The work will include a field trip to examine mussels in their natural habitat.
- Further IMS panel testing is planned with extra eDNA samples obtained from our field collections and eDNA Frontiers archived material.

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 northwestern Australia will be summarised in a book to bring this knowledge into one place that showcases the natural variety that the Pilbara coast supports.

Implementation

Staffing and management

- **Adj Prof Fred Wells**
  Principal investigator, Fred leads coordination of the overall project management, communications and scientific output.
- **Sherralee Lukehurst**
  Investigator, Sherralee is responsible for field work organisation, field collections, molecular laboratory work and data analysis.
- **Prof Euan Harvey**
  Investigator, Euan is a marine ecologist with experience in developing, testing and validating techniques for non-invasive sampling of marine biodiversity.
- **Prof Monique Gagnon**
  Investigator, Monique possesses extensive knowledge of marine environmental issues and excels as a photographer, editor, and scientific writer.

External collaborators

- **Dr Tim Cooper**
  BHP
- **Associate Professor Benoît Dayrat**
  Pennsylvania State University
- **Mrs Doris Teufel**
  Port Hedland

Achievements

Status of progress against Project Milestones

Expected Project Deliverables	Progress/Comments	Status
	Onchidiidae	
Analysis of existing collections	Specimens collected during Port Hedland sampling Oct 2023, 63 specimens from 5 sites. Broome Sept 2024, 29 specimens from 4 sites. Carnarvon and Exmouth, 22 specimens from 2 sites. Western Australian Museum collections also examined.	Completed
New Pilbara onchidiid collections	Field collections were made in Onslow, Dampier and Point Samson in Jan 2025. 101 specimens from 7 sites.	Completed
Work with Benoît Dayrat	Dr Dayrat came to Perth in January 2025 for a month to work on the project. We undertook a field program in the Pilbara. All specimens were photographed and dissected. DNA sequencing of 4 gene regions (COX1, 16S, 28S and ITS) has been completed. Dissected radulae (molluscan teeth), digestive and reproductive systems of all specimens have been sent to Pennsylvania for Dr Dayrat to analyse.	In progress
	Marine Life of Northwestern Australia	
Writing text	A decision was made to broaden the coverage from just the Pilbara to include the region from Shark Bay to Broome. A draft table of contents with 12 chapters has been developed. Preliminary drafts of the text of 9 chapters have been completed. Preliminary work has been undertaken on the 3 remaining chapters.	In progress
Photographs and illustrations	The authors' existing photographs have been searched for relevant photos. Extensive new photographs were made during the fieldwork in Onslow and Dampier and a separate trip to Port Hedland. Additionally, Doris Teufel of Port Hedland has generously provided >1000 photographs of marine life of Port Hedland reef. Dr Lyn Irvine has also provided whale photos from Northwest Cape.	In progress

Overview of outputs and outcomes

Onchidiids live on intertidal sandy, muddy and rocky shores and in mangroves. They secrete themselves in nooks and crannies when the tide is in to escape predation. As the tide goes out onchidiids emerge to feed and reproduce. They are diverse in the Indo-West Pacific but are an extremely difficult family taxonomically. Competent descriptions require detailed studies of the species in life and anatomical studies of their general morphology, teeth, and reproductive and digestive systems. Recent advances in DNA sequencing have greatly enhanced our ability to delineate species relationships in the group.

Dr Dayrat is the world expert on onchidiids. He has studied onchidiids and published on the group in a wide range of countries over the last 15 years, including work in the Northern Territory. We knew there are species in northern Western Australia, but they have never been studied. eDGES2 has provided us with an opportunity to work with Dr Dayrat on this fascinating group. Dr Dayrat came to Curtin in January 2025 for a month to work on the project. We undertook a field program in Onslow, Dampier and Point Samson which allowed him to examine the species alive and for more material to be collected.

A comprehensive collection of onchidiids has been completed from Carnarvon to Broome, 18 sites over four field trips. This has been supplemented by examination of specimens from the Kimberley in the WA Museum collections. A provisional analysis is that nine species from four genera have been recorded, three of which are currently thought to be new to science.

Figure 5.1 Extensive sampling of onchidiids in the northwest of WA, took place between 2023 and 2025.

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

- Writing of marine life to date has highlighted gaps in photographs.
- Several published illustrations will need to be recreated for the book.
- Formatting issues must be overcome.
- Decisions will be required on what internet sites to host the book.

Learnings

Nothing to be reported at this time.

Communication

- The Onslow and Dampier survey resulted in several media outcomes: articles in the SciTech magazine Particle and Pilbara News, and live interviews on ABC radio Karratha and RTR-FM.
- Meetings on the book have been held with BHP Nelson Point, Port Hedland Port Authority, Care for Hedland, Rangelands NRM and the consulting company IMFR. Email contact has been made with DBCA and Karratha and Port Hedland Senior High Schools.
- Working with Mrs Doris Teufel, we have published a few articles in the Malacological Society of Australasia Newsletter.
- This project has been referenced on the eDGES program website 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

We will be working with Dr Dayrat to complete analyses of the molecular data, radulae, and digestive and reproductive systems, as well as to prepare a draft manuscript on the onchidiids of northwestern Australia.

The goal is for a complete draft of the marine environment book to be completed by December 2026.

Pilbara NEWS

Sea slug research could unlock new information about hidden marine biodiversity

Madelin Hayes | Pilbara News

Figure 5.2 Press tour during field work in Karratha (January 2025).

TERRESTRIAL BIOMONITORING

Project 6

Terrestrial ecosystem biomonitoring with eDNA across the tree of life

Summary

Biological monitoring is essential for assessing ecosystem health, yet acquiring biodiversity data is time and 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 specialised equipment or cold storage, that can be implemented at large spatial scales.

This project aims to validate DNA-based tools for assessing terrestrial biodiversity and begin the development of an eDNA-based metric for ecosystem condition evaluation. The initial phase focused on optimising sampling methods to improve accessibility and efficiency, followed by studies examining spatial and temporal variability to establish robust monitoring protocols. The next phase will assess the capacity of eDNA-derived metrics to evaluate ecosystem condition in arid environments. Outcomes from this project will support a wide range of stakeholders—including researchers, industry partners, citizen scientists, and Indigenous ranger groups—in contributing to biodiversity monitoring and conservation in the Pilbara region.

Implementation

Staffing and management

- **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 biodiversity.
- **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.
- **Prof 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.

PhD and honours students

- **Francesca Martino**
  Honours student recruited to investigate options for sampling air DNA (completed 2025)

Participants/Collaborators

- **Prof Morten Allentoft**
  Head of the TrEnD Lab (Curtin University)
- **Dr Mahsa Mousavi Mousaviderazmahalleh**
  Bioinformatician at TrEnD Lab (Curtin University)
- **Dr Angus Lawrie**
  Ecosystem condition (Curtin University)
- **Dr Josh Newton**
  Terrestrial eDNA (Curtin University)
- **Dr Simon Ferrier**
  Remote sensing scientist (CSIRO)
- **Dr Kate Giljohann**
  Remote sensing scientist (CSIRO)
- **Dr Christine Cooper**
  Ecologist/Ecophysiologist (Curtin University)

Achievements

Status of progress against Project Milestones

Expected Project Deliverables	Progress/Comments	Status
Optimise sample collection and assess the spatial resolution of different methods	A total of 360 samples, collected from three substrates (air filters, rollers and soil) and two ecosystems (Dryandra National Park and Roxby Downs), were analysed separately. The manuscript will be submitted in the first half of 2026.	On schedule
Investigation of temporal variability in airborne eDNA samples	Airborne eDNA is being collected bimonthly from five Western Australian ecosystems to understand temporal variation in species detectability. Sequencing to be completed in the second half of 2026.	On schedule
Review paper on eDNA metrics	A workshop involving eDNA and remote sensing specialists was held in the second half of 2025. Outcomes from the workshop are being synthesised. The resulting manuscript is scheduled for submission in the second half of 2026.	Delayed
Audit biodiversity across land uses	Discussions with experts in remote sensing and ecosystem condition assessment are ongoing, alongside the development of synergistic projects. Sampling planning is being conducted in partnership with CSIRO and BHP. Field sampling is scheduled to commence in the first half of 2026.	On schedule
PhD candidate selected	No suitable PhD candidate has been identified and with project end date in 2027, this deliverable has been cancelled.	Cancelled
Postdoc training	Marina de Oliveria replaced Mieke van der Heyde and has been trained in all the processes adopted by TrEnD.	Completed
Optimise methodology for vegetation swabs	We compared vegetation swabs to determine the best method to survey biodiversity in the following steps of the project. This paper was published in February 2026.	Completed
Optimise sample storage methodology for soil eDNA	Samples were collected at Roxby Downs to test three different preservation methods for soil samples. Samples processed and sequenced but delayed due to staff maternity leave.	Delayed

Overview of outputs and outcomes

- Data analyses for 360 samples collected as part of the study on the spatial resolution of different eDNA substrates has been completed. We observed clear differences between eDNA collection methods (air, soil, and vegetation swabs), but these differences were context-dependent, highlighting the difficulty of standardizing methods.
- We have collected 200 samples as part of a temporal study that aims to assess variability in airborne eDNA detection across five Western Australian ecosystems. By July 2026, a total of 300 air samples are expected to be collected.
- The first review synthesising global studies on eDNA in arid ecosystems received positive feedback from peer reviewers. It summarises key insights and highlights emerging applications and methodological innovations relevant to these systems. The paper will be resubmitted in the first half of 2026.
- The paper comparing the efficiency of swabs and rollers in sampling surface-based eDNA was published.
- The paper comparing fixed and vehicle mounted airborne eDNA methods for terrestrial vertebrate detection has been submitted and in now under review.
- We compared the efficiency of air filters, spiderwebs, swabs, and all these collection methods combined in a single tube, to survey plants and vertebrates. The rationale behind this study is that different collection methods would preferably provide information for a set of species, and all methods combined would provide a more comprehensive survey of the biodiversity while decreasing sample processing time.
- A workshop bringing molecular ecologists and remote sensing scientists was held in the second semester of 2025. This event brought together these two disciplines to support this and other EDGES projects.

Figure 6.1 Airborne eDNA filter setup deployed in the Pilbara region as part of the temporal study in collaboration with the BHP Rehabilitation team.

1 paper published

2 papers under review

2 manuscripts in preparation

4 concurrent research projects

560 samples collected

Participation in the Biodiversity Conference

New collaboration with research institutes

Participation in community outreach events

Figure 6.2 Summary of outputs from the EDGES 6 project, highlighting selected communication pieces published on Curtin University's social media channels.

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

- The project focus was shifted from Olympic Dam to the Pilbara region due to logistical considerations, including easier site accessibility and the classification of samples as non-quarantine material.
- The recruitment and training of Dr Marina Elisa de Oliveira delayed the project.

Leveraged funding and value-add activities

- A network of molecular ecologists and remote sensing scientists has been established to support this and other EDGES projects. This collaboration brings together expertise from CSIRO and other national and international institutions, enabling the integration of global satellite sensor data with large-scale eDNA datasets and machine learning approaches to improve the assessment of ecosystem condition. At the same time, the project has strengthened partnerships with BHP's biodiversity and rehabilitation teams, fostering closer collaboration between research and industry for biodiversity monitoring and landscape-scale environmental management.

Challenges

- The change in sampling location has delayed site access and engagement with Traditional Owners.
- The handover and training of a newly appointed postdoctoral researcher have also contributed to project delays.
- Collaboration with multiple national institutions presents administrative challenges, as each organisation has specific requirements that must be met before research activities can proceed.
- The selection of the Pilbara region for the large-scale study introduces logistical complexities due to the diversity of disturbance regimes and natural processes influencing ecosystem condition, as well as the need to access land under different ownership and management arrangements.

Learnings

- Workshop discussions with spatial scientists from CSIRO, Curtin University, University of Melbourne, Cary Institute (USA) indicated that integrating eDNA and remote sensing creates new opportunities for large-scale ecosystem monitoring. Collaboration between molecular ecologists and spatial scientists demonstrated the strong potential of combining eDNA data with satellite-derived environmental information to assess ecosystem condition across large and remote landscapes.
- Sampling design and methodological standardisation are key to scalable eDNA monitoring. Results from the spatial study highlight that substrate choice and sampling design strongly influence species detection, reinforcing the need for optimised and standardised protocols for large-scale biodiversity monitoring programs.

Communication

- Biodiversity Conference, Perth October 2025, Presentation "Spatial resolution of eDNA analyses in arid and semi-arid environments" delivered by Marina E. de Oliveira.
- The Wetlands Centre Cockburn Workshop, Perth November 2025 "Decoding the Helix: Exploring biodiversity and eDNA" delivered by Marina E. de Oliveira and Paul Nevill.
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, December 2025
- The project has been presented at several on-line workshops to collaborators at CSIRO, DWER, University of Adelaide and BHP.
- Paul Nevill appeared in diverse forums promoting eDNA research at Curtin including a TIK TOK on what is eDNA, a television segment revealing Perth's backyard diversity with eDNA for the nationally broadcast prime time program Gardening Australia, a podcast in the Curtin series "The Future of….", and numerous media associated with the BioBlitz both locally and in Singapore (e.g. Facebook, Instagram, X, LinkedIn). A BioBlitz is a race against the clock to record as many species as possible in each area and is a proven model for engaging staff and students with local biodiversity, conservation, hands-on learning, plus the many benefits associated with volunteering.
- This project has been referenced on the eDGES program website www.edgesprogram.org.

Sustainability

- Findings from this project will help reduce sampling costs. The temporal and spatial studies will inform future sampling design, helping to target sampling efforts more efficiently and reducing uncertainty, time, and costs associated with biodiversity monitoring.

Next Steps

- Finalise the sampling design for two studies in the Pilbara region in partnership with BHP and CSIRO. One will employ the mobile collection of airborne eDNA and the second will be based on vegetation swabs and soil samples.
- Strengthen engagement and relationships with Traditional Owners in the Pilbara region.
- Complete and submit the manuscript on the spatial scale of different eDNA collection methods in arid and semi-arid ecosystems.
- Complete and submit the manuscript on the comparison of the efficiency of air filter, spiderwebs, swabs and all these collection methods combined in a single tube to survey plants and vertebrates.
- Finalise data collection and process samples for the temporal study.
- Draft of the review on eDNA integration with other data sources (remote sensing, bioacoustics, camera traps) in collaboration with experts in these areas.
- Review and revise the manuscripts following initial peer-review feedback: “eDNA on the go: A direct comparison of fixed and vehicle mounted airborne eDNA sampling methods for terrestrial vertebrate species detection” and “The past, present and future of eDNA research in arid environments”.

TERRESTRIAL BIOMONITORING

Project 7

Living waters of Western Australia (LiWA)

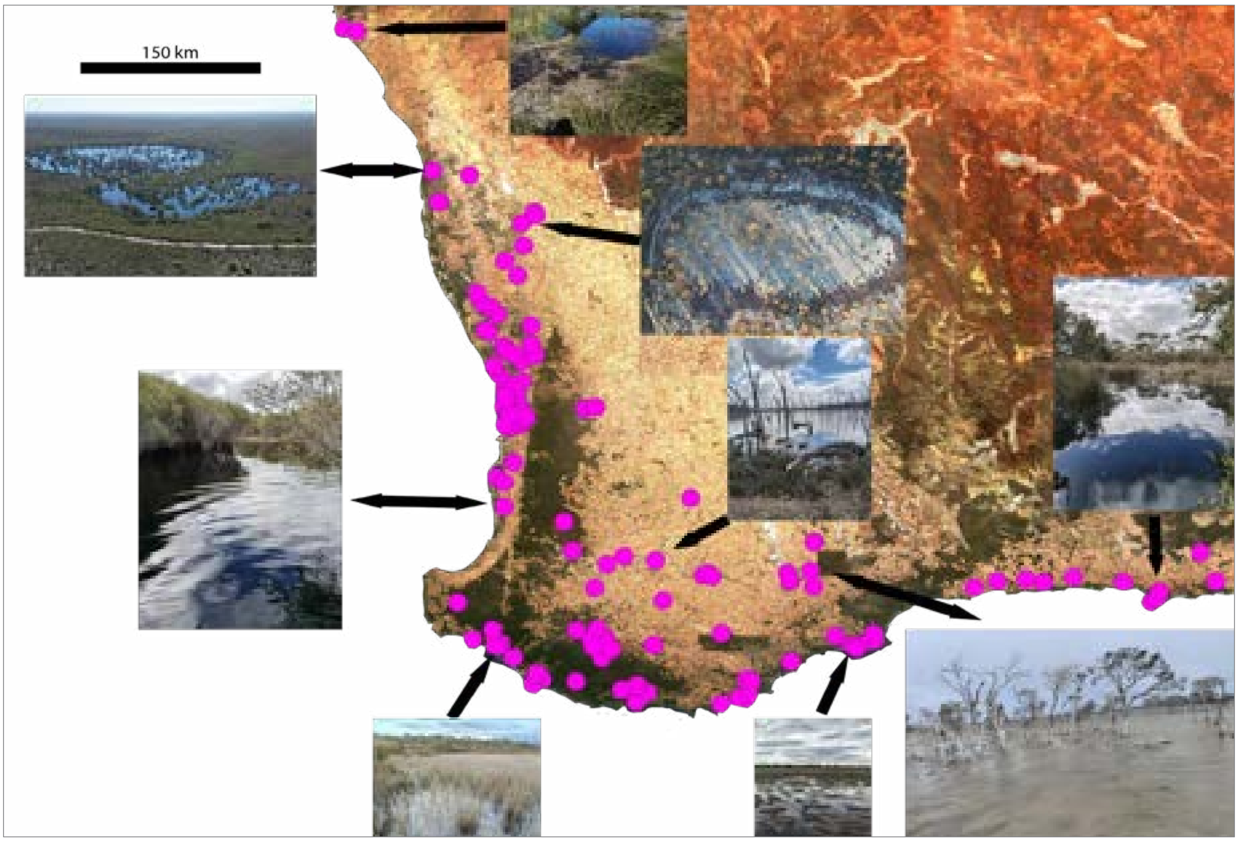


Figure 7.1 Examples of some of the types of wetlands sampled across 2024-2025. Selected wetlands ranged from small and tannin stained to vast and saline. The total survey includes 172 sites across the whole south-western portion of Australia.

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 we 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 Western Australia. 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 for Project 7. 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.
- **Assoc Prof Paul Nevill**
  Chief Investigator who is co-leading the citizen science component of this project.
- **Assoc Prof Bill Bateman**
  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.

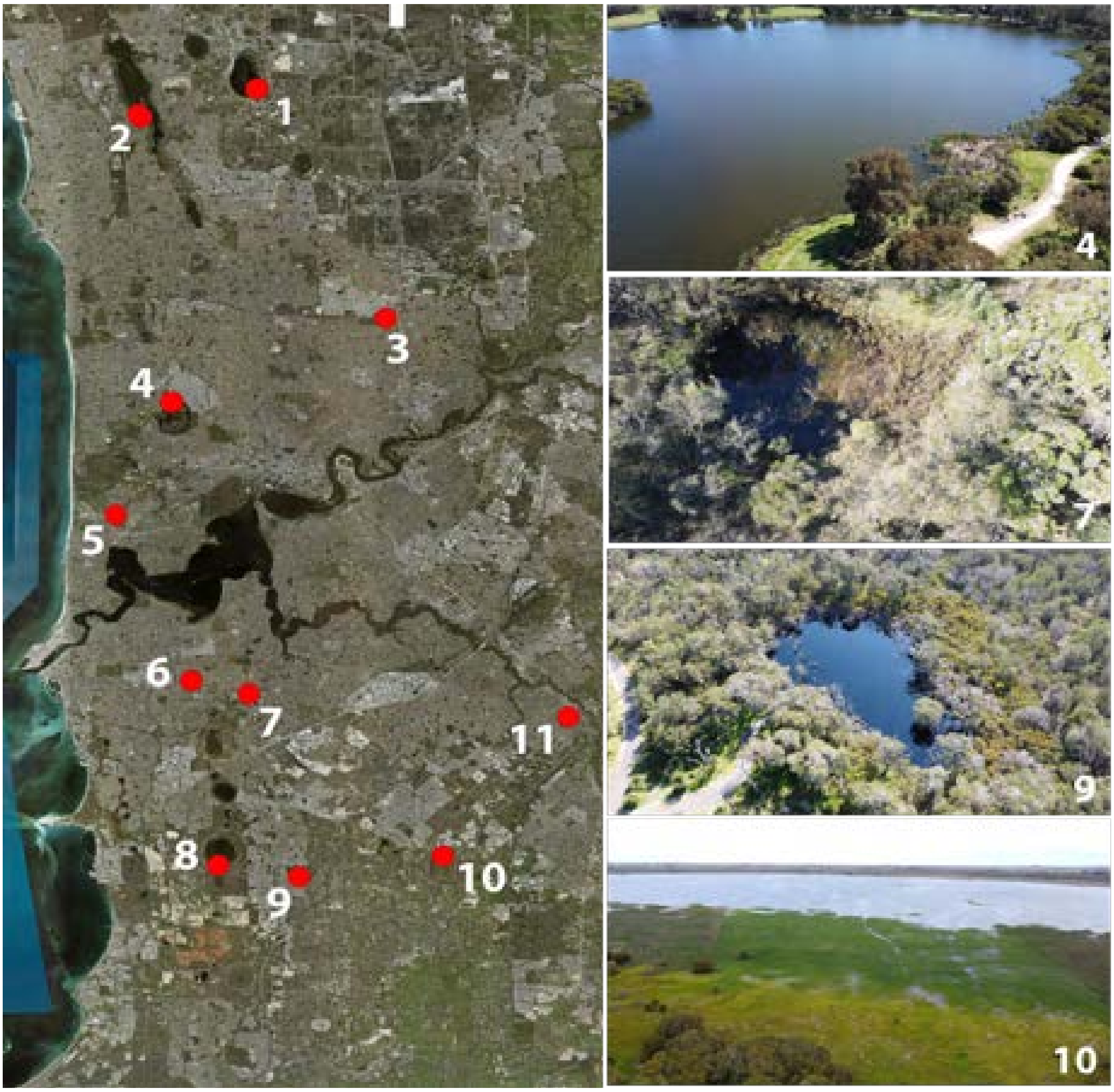


Figure 7.2 Layout of the 11 wetlands sampled monthly between April 2025 – March 2026. Highlighted wetlands include Herdsman Lake, Quenda Swamp, Banksia Lake and Forrestdale Lake.

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
Biodiversity survey samples collected and analysed	We have now completed sampling for the purpose of training the eDNA health model. This dataset currently includes 172 wetlands from across the entire southwest of Australia for a total of 1,123 individual water eDNA samples and paired macroinvertebrate and water quality samples. All 1,123 eDNA samples were amplified with 14 different metabarcoding assays. Generation of AqWATIC models are currently underway that are being validated against various abiotic and biotic indices which show promising preliminary results.	Ongoing
Temporal survey samples collected	We have now completed sampling of 11 wetlands on the Swan Coastal Plain which have been sampled monthly since April 2025. These collections included 4 eDNA water samples per sampling event in addition to a range of water quality data also collected. These data will be very useful in contextualising how the AqWATIC model varies throughout the year.	Completed
Sample collection for wetland eDNA	We have begun collecting samples from wetlands in south-western Australia. We have currently cored two lakes: Thompsons Lake and Forrestdale Lake. We intend to finish our sample collection by mid-2026 and expect that we will begin processing these samples in the laboratory shortly after.	Ongoing
Stakeholder Meetings held	As in 2025, we have had more 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.	Ongoing
PhD candidate selected	Given the lack of suitable candidate, we have decided not to pursue this further.	Completed

Overview of outputs and outcomes

Within the reporting period we have completed a very large biodiversity and water quality survey of 172 wetlands in south-western Australia. We have also completed the data collection for a 12-month sampling program of 11 wetlands on the Swan Coastal Plain. These results are currently being analysed and are demonstrating promising results for the potential use of the AqWATIC index to serve as a reproduceable and effective tool for the environmental management of wetlands. To this end, a significant and continuing effort is being made to communicate these results to the relevant staff of over 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. We have also begun collecting core samples for eDNA analysis which will be processing in 2026. 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 again held a community workshop on the various uses of eDNA at the Wetland Centre, Cockburn in November of 2025 which was attended over 30 members of the public. We also ran a workshop for the "Enviro Engineers" program at the Canning Eco Education Centre which involved demonstrating water sampling and the applicability of eDNA to an audience of primary school aged girls in STEM.

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

We had previously found it difficult to source the correct equipment in order to conduct the coring of the wetlands, however, we have since been able to borrow coring device off a collaborator at Edith Cowan University (Dr Fabian Boesel). The cores taken appear to be suitable for a preliminary assessment of eDNA in south-western Australian wetlands.

Leveraged funding and value-add activities

While collecting samples on Yued country, Dr Angus Lawrie provided some preliminary training in eDNA water collection and processing to four Yued Aboriginal Corporation rangers. Discussions for further collaborative opportunities are on-going.

Figure 7.3 Core being collected with collaborators at Forrestdale Lake.

Challenges

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.

Learnings

No learnings to report currently.

Communication

- The preliminary results of the AqWATIC model were presented as a poster at the 2025 eDNA Conference in Wellington and the 2025 Goldfields Environmental Management Group conference in Kalgoorlie and as a presentation at the 2025 Combined Biological Sciences Meeting in Perth.
- Dr Angus Lawrie ran a seminar workshop with the Wheatbelt NRM to engage with private landowners in the Wheatbelt about the LiWA project.
- We again held a community workshop on the various uses of eDNA at the Wetland Centre, Cockburn in November of 2025 which was attended over 30 members of the public.
- We also ran a workshop for the “Enviro Engineers” program at the Canning Eco Education Centre which involved demonstrating water sampling and the applicability of eDNA to an audience of primary school aged girls in STEM.
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, December 2025.
- This project has been referenced on the eDGES program website www.edgesprogram.org.

Sustainability

The findings from this project may reduce the time and manpower costs associated with conventional monitoring methods of wetlands consequently minimising transportation and labour costs of personnel in management activities.

Next Steps

- Continue to analyse and generate different versions of the AqWATIC model and validate them against water quality information.
- Decide on the optimal series of metabarcoding assays to construct the AqWATIC model.
- Process and analyse the samples from the temporal study to determine how the AqWATIC model varies within and between different seasons.
- Determine whether eDNA can be extracted and analysed from the wetland sediment cores.

ADVANCING eDNA TECHNOLOGIES

Project 8

Measuring species abundance with eDNA

Summary

eDNA metabarcoding datasets contain quantitative signals of the species in the environment where they are sampled. These signals contain a large amount of noise, however, because sampled DNA is not perfectly mixed in the same proportions at which species occur in the environment. This means that any single sample of eDNA will contain DNA molecules that have variable correlation to the ratio of organisms in reality. The deviation from the real ratios is partly random as a result of imperfect mixing and sampling bias, and partly systematic because of consistent technical biases. Analysing multiple eDNA samples allows the random part of the biases to be accounted for. Any approaches for making eDNA analysis quantitative at an ecosystem scale will therefore be improved by high levels of sampling replication.

A major focus of this project has been to develop software for analysing eDNA data with replicated biological sampling to enable relative quantification of species. The software analyses eDNA metabarcoding OTU tables. The primary data it extracts from them are relative read abundance for species per sample, and frequency of occurrence for species in the dataset. This data can be used as the input parameters for classic models of Species Abundance Distributions such as the log-series model developed by R.A. Fisher in the 1940s, although a range of possible models have been made available in the software. Analysis of eDNA data by relative read abundance combined with frequency of occurrence improves estimates of species abundance from eDNA. This analysis type also improves our ability to filter eDNA datasets for low abundance and low frequency sequences that are likely to be contaminants or of low ecological significance.

Another approach to deriving species abundance from eDNA is to use genetic mark-recapture methods to estimate the absolute abundance of single species. This has considered impossible from eDNA in the past because it requires genotypes of individuals to be analysed. In a standard eDNA sample, all of the DNA that goes into it is intermingled, so if multiple individuals from one species are in the sample, genes from those individuals will be mixed up and it is impossible to separate them once the mixture forms. Applying fluorescent activated cell sorting (FACS) to environmental samples allows whole cells to be isolated from the same sample types that eDNA is normally taken from. If an environment is sampled multiple times for cells, then the number of times individuals are found in more than one sample can be used to estimate the absolute number of individuals in the population.

Implementation

Staffing and management

- **Prof Simon Jarman**
  Chief Investigator and Project Manager for Project 8. He is responsible for researching the issue and developing software and field sampling solutions that may address it.

- **Dr Alyssa Budd**
  Dr Budd is an expert bioinformatician and biostatistician who leads eDGES Project 9 on informing Natural Capital Accounting systems with eDNA data.

External collaborators

- **Dr Shaun Wilkinson** Wilderlab NZ Ltd.
  Comparisons with taxon-independent metrics derived from eDNA data.
- **Dr Euan Harvey** Curtin University
  Frequency of occurrence analysis of eDNA datasets as a proxy for abundance.
- **Dr Bruce Deagle** CSIRO
  Metabarcoding data analysis.
- **Dr Haylea Power** CSIRO
  emCell analysis.
- **Dr Olly Berry** CSIRO
  emCell analysis.
- **Dr Miwa Takahashi** CSIRO
  emCell analysis.
- **Dr Katrina West** CSIRO
  emCell analysis.

Overview of outputs and outcomes

There are three main outputs to report at this stage. The first is better quantification from eDNA metabarcoding datasets with high levels of replication. The implementation of Species Abundance Distribution fitting for eDNA data allows this, and a typical output from the software that was produced for this is shown below. The graph in figure 8.1 shows a red line, which is the fitted Species Abundance Distribution, and the blue circles are the species fitted onto it with relative estimated real abundance on the y axis. As expected, a small number of species have a high biomass, and a lot have low biomass. This emphasises why these analyses are important, as standard eDNA work with presence/absence interpretation will treat low-abundance species the same way as high abundance ones.

Achievements

Status of progress against Project Milestones

Expected Project Deliverables	Progress/Comments	Status
Software for species quantification from eDNA	Beta software released	Completed
Paper on software for species quantification in eDNA metabarcoding	In progress	In progress
Paper on emCell isolation and genotyping	First round review successful	In progress
Method for filtering eDNA datasets by combinatiions of read abundance and dataset frequency	Paper to be written	In progress

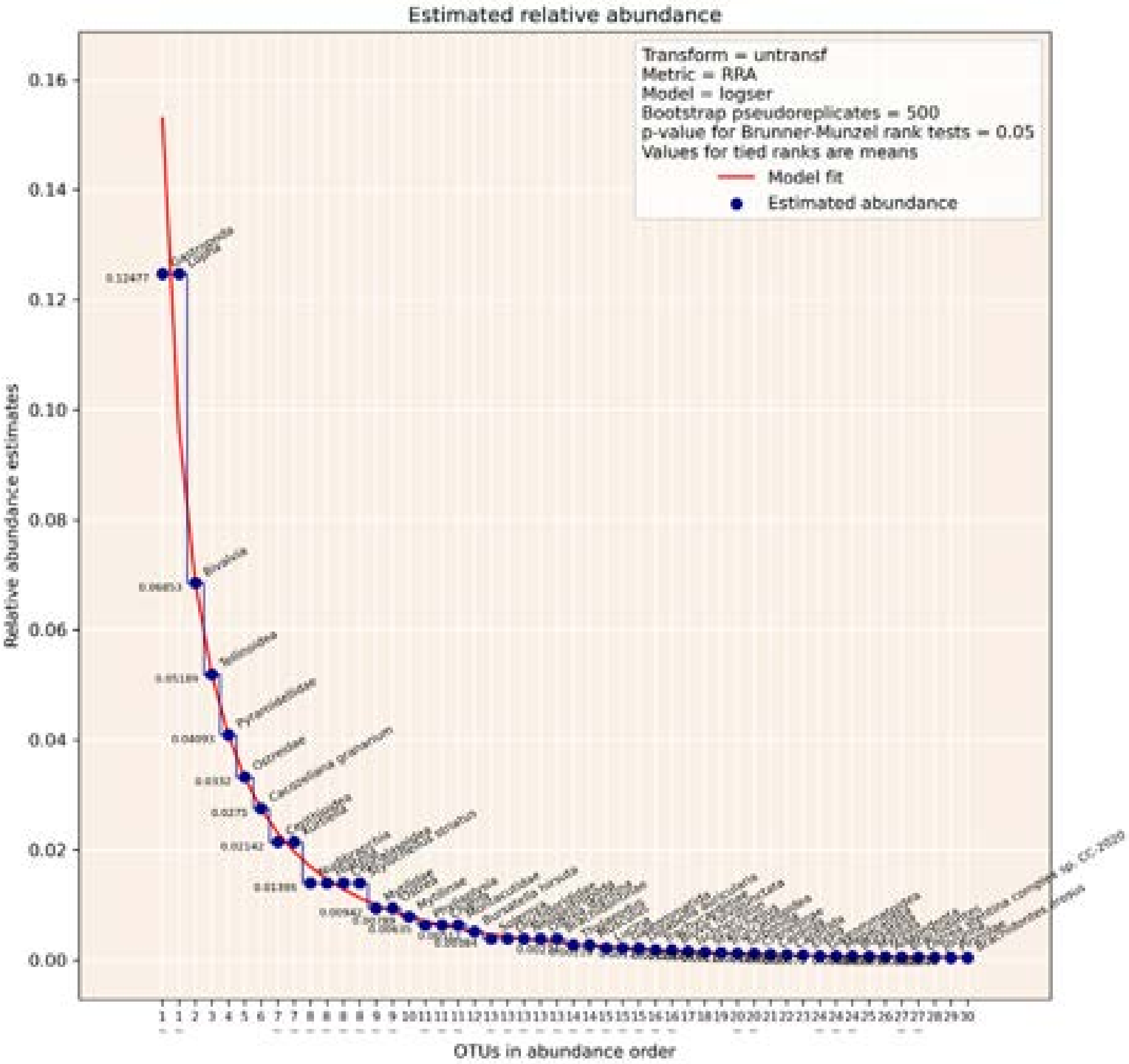


Figure 8.1 An example of predicted relative abundance of species in a highly sampled eDNA dataset. A small number of species dominate the biomass and most species are present only in very small amounts.

A second outcome of the research is the development of new methods for filtering eDNA metabarcoding datasets. The combination of relative read abundance and frequency of occurrence of ZOTUs in metabarcoding data enables different strategies for removing the DNA sequences that are likely to be contamination or technical artefacts (Figure 8.2). The important innovation is that different strategies are appropriate for different ecological questions. The figure below shows four examples. The Maximum Sensitivity example is ideal for detection of invasive species or pathogens, for example. In that case, accepting a small number of false positives is better than excluding and potential hazardous species. The Frequency Based option might be best suited to analyses that focus on the dominant habitat-forming organisms in an eDNA sample. In that case, infrequent identifications are not contributing to the analysis. The other two cases are compromises between these options and would be best used for general ecosystem description from eDNA metabarcoding.

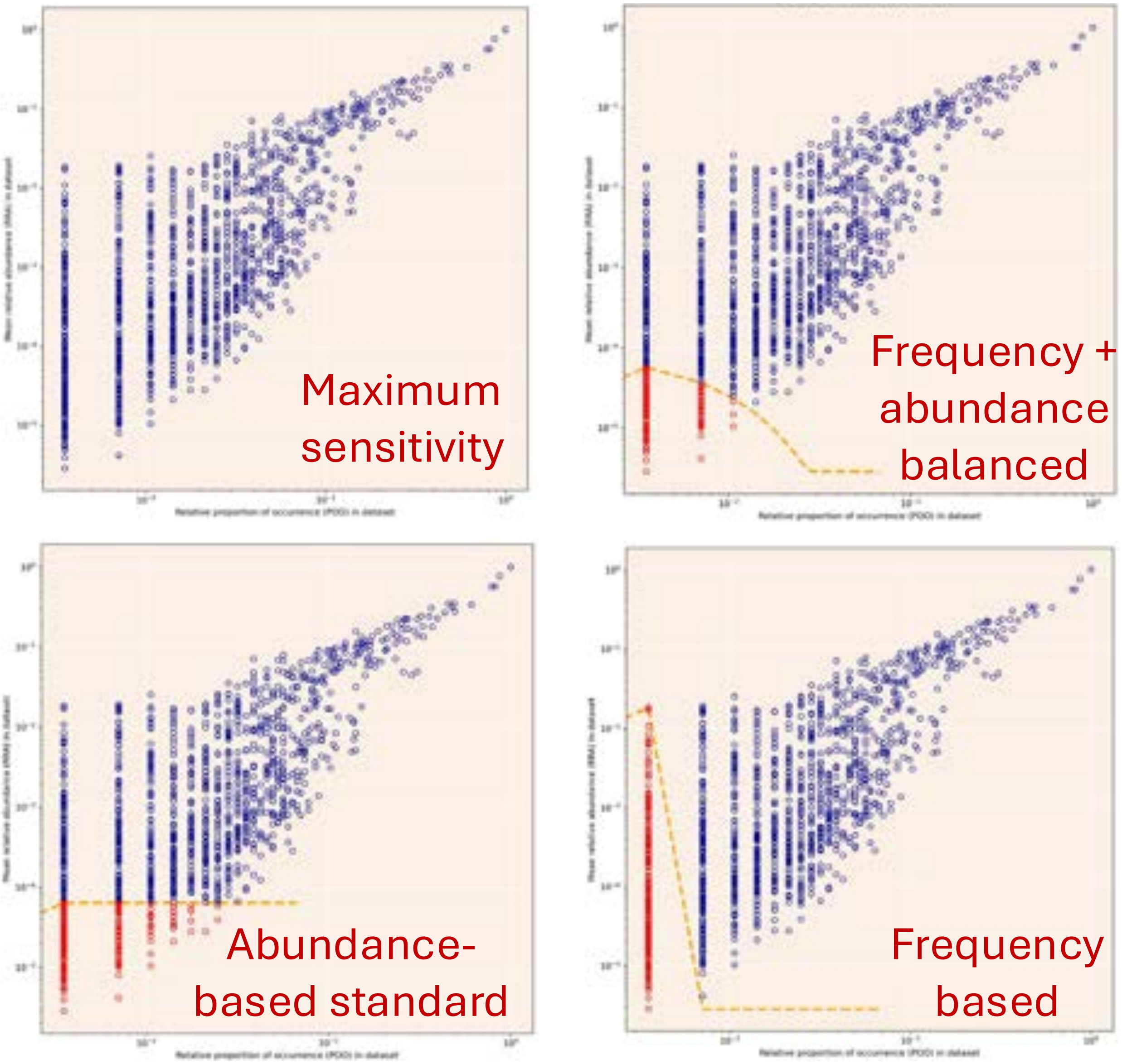


Figure 8.2 Four strategies for filtering eDNA metabarcoding datasets. The x-axis is the relative proportion of occurrence of each OTU in the dataset, the y axis is the relative read abundance, both on log scales. The low abundance and low occurrence sequences can be filtered by different strategies for each ecological question to optimise results.

A third outcome is experiments demonstrating that whole cells can be isolated from eDNA samples. This allows individual genotypes of organisms to be extracted from eDNA. The proof-of-concept experiments were conducted on fish in tanks as a proxy for wild animal populations. Cells from individual fish could be reliably identified with a PCR genotyping panel. The approach can be extended to give genetic mark-recapture abundance estimation of wild animal abundance if combined with the right eDNA sampling strategies.

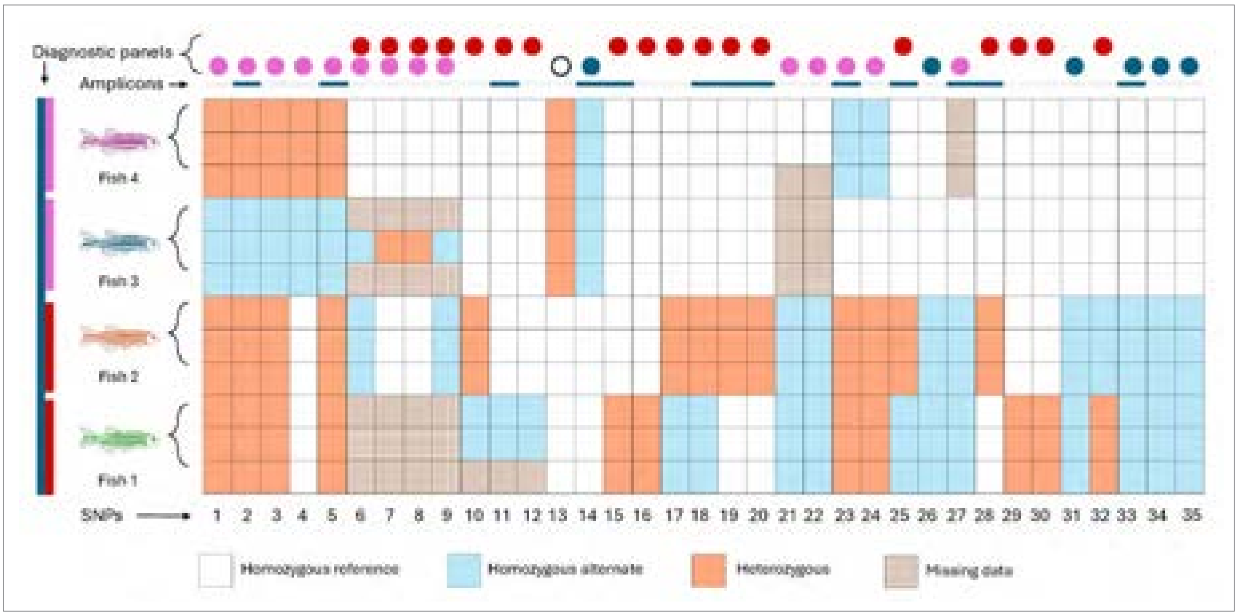


Figure 8.3 Individual fish identification from multiplex SNP genotype panels. Each fish could be differentiated from the others by different combinations of SNP genotypes in each of three multiplex PCR tests.

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

Nothing to report.

Leveraged funding and value-add activities

Work on environmental cell isolation has been incorporated into this project as it is a quantitative application of eDNA that will lead to useful ecosystem indicators for absolute abundance of single species of interest. The cost to this project is Prof. Jarman's time, and the return on this investment is a paper has been produced already, and collaboration with external eDNA researchers strengthened. This research leverages this project's funds as CSIRO resource most of the cell isolation work.

Challenges

Nothing to report.

Learnings

The software package implementing eDNA abundance measurement ideas has had its first release. This keeps growing, with new functions and ideas being incorporated. The main thing to be learnt from this is that finishing the implementation of one set of ideas has led to the possibility of several new ones, and the scope of the software keeps expanding. This is not a problem, except that it leads to delays in completing the paper describing the work.

Communication

- The software package, SQEMA, has been presented at the Australian and New Zealand eDNA Conference in Wellington in 2025. The package is available from the eDGES program website www.edgesprogram.org/software/, and as a standard package for the Python programming environment.
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, December 2025.

Sustainability

The software produced for this project is archived in the Python software repository with an open-source license to make it re-useable and permanently available.

Next Steps

The project is on schedule. Next steps are:

- Decide on whether to conduct experiments on ecological disturbance for testing eDNA abundance-based estimators of it, or whether existing datasets can be used.
- Write a paper on combined frequency ~ abundance methods for eDNA metabarcoding dataset filtering.
- Write a software description paper for the SQEMA eDNA quantification package.
- Write algorithms for comparing species abundance distributions to recognise ecological disturbance.
- Develop a metrics for change in rank abundance among species found in multiple datasets by eDNA and implement this as a comparative metric.
- Revise and resubmit the paper on environmental cell isolation and genotyping.

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 natural capital accounts. This project is developing a framework for implementing eDNA indicators of biodiversity into natural capital accounting.

Implementation

Staffing and management

- **Dr Alyssa Budd**
  Lead investigator. Dr Budd will be responsible for developing a framework for implementing eDNA indicators of biodiversity into natural capital accounting
- **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. Dr 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. Dr Singh is an expert in NCA and will work with Dr Sultana and the rest of the team on eDNA-NCA integration.

Achievements

Status of progress against Project Milestones

Expected Project Deliverables	Progress/Comments	Status
Thought leadership piece on NCA and scope for eDNA-based metrics to inform.	The manuscript was drafted and reviewed by co-authors and a graphic designer was employed to generate a figure. The manuscript was first submitted in December 2025, again in January 2026 and a third time in February 2026.	Awaiting response from *Nature Sustainability*
Conceptual framework linking eDNA to natural capital accounting.	A conceptual framework has been developed outlining how biodiversity information derived from eDNA could be translated into indicators relevant to environmental accounting.	Completed
Assessment of biodiversity indicator requirements in sustainability reporting frameworks.	A review of emerging biodiversity reporting and accounting frameworks has been undertaken to identify the types of biodiversity information required. This work has helped define the project scope and will continue to assist in the identification of specific indicators of use.	Ongoing
Statistical exploration to enhance comparability among eDNA-derived biodiversity data.	Analyses have begun examining how biodiversity metrics derived from different eDNA surveys compare statistically. This work aims to identify approaches for producing biodiversity data that are comparable across accounting periods and geographic regions. These data can then be used to develop meaningful indicators.	Initial analyses underway

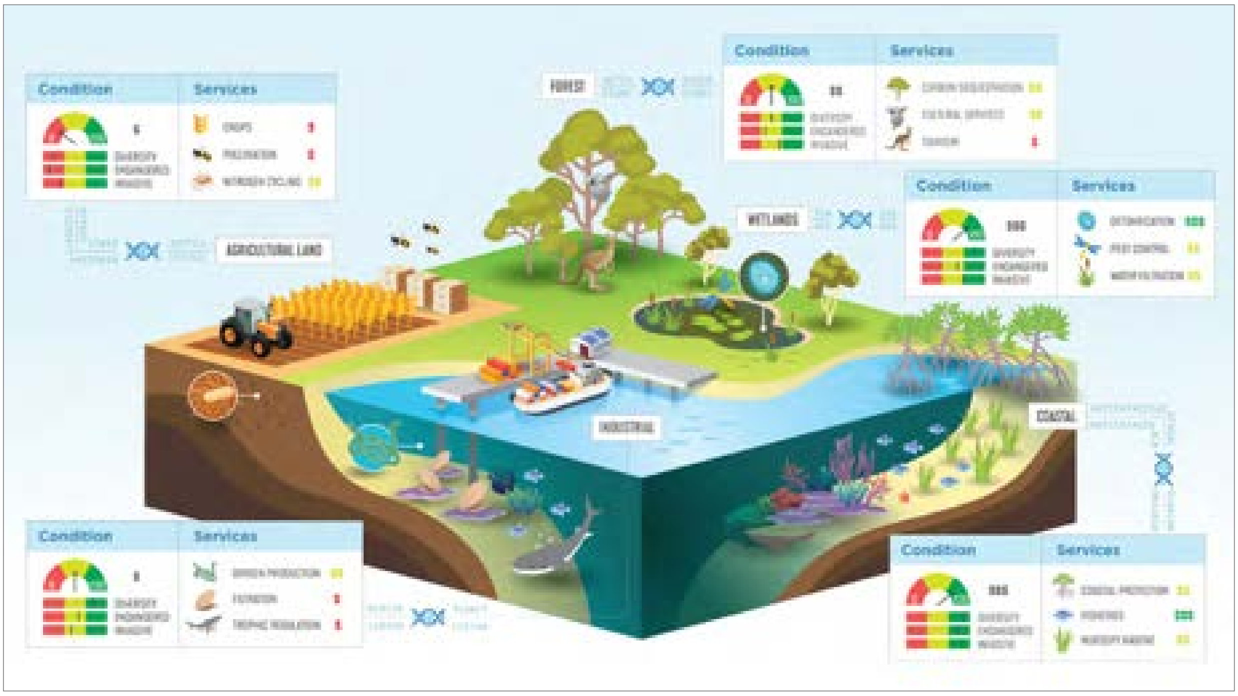


Figure 9.1 Conceptual integration of eDNA into ecosystem accounts. Conceptual diagram showing how environmental DNA (eDNA) biodiversity data could contribute to ecosystem and natural capital accounting across multiple ecosystem types. eDNA sequence data are translated into biodiversity indicators (e.g., species diversity, presence of threatened or invasive taxa, and composite ecosystem-condition scores), which could inform environmental accounts and support evaluation of ecosystem services. Both indicator development and valuation approaches require further empirical validation.

Overview of outputs and outcomes

During this reporting period, the project transitioned from scoping into active conceptual development. The primary output has been a thought-leadership manuscript describing the potential role of eDNA in natural capital accounting frameworks. In parallel, the project team has undertaken substantial background work to understand how biodiversity information is represented in emerging accounting and disclosure frameworks, including SEEA-EA and TNFD. This work has informed the development of an initial conceptual framework describing how eDNA-derived biodiversity indicators could interface with natural capital accounts.

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

Nothing to report.

Leveraged funding and value-add activities

Work undertaken within this project has benefited from collaboration with other eDNA projects within the eDGES program, allowing access to existing datasets and expertise. Conceptual work on biodiversity indicators has also informed discussions with external stakeholders involved in natural capital accounting and biodiversity disclosure frameworks.

Challenges

A key challenge for this project has been the early stage of development of both eDNA biodiversity indicators and natural capital accounting frameworks. While eDNA provides a powerful method for measuring biodiversity, there is currently limited guidance on how biodiversity observations should be translated into indicators suitable for accounting systems or corporate disclosure frameworks. As a result, significant effort has been required to understand the requirements of international accounting frameworks such as SEEA-EA and TNFD as well as Australian sustainability reporting standards S1 and proposed S3.

Learnings

This reporting period highlighted that integrating eDNA biodiversity measures into environmental accounting is a highly interdisciplinary task, requiring integration of ecology, molecular biology, statistics and accounting. Furthermore, it is now understood that both current and emerging sustainability reporting frameworks in Australia emphasise biodiversity measurement and ecosystem condition assessments rather than monetary valuation of nature. Because of this, and because any valuation approach first requires reliable underlying measurements, the most immediate focus of this project will be biodiversity measurement and indicator implementation.

It has also become clear that comparability of eDNA-derived biodiversity data is a major barrier to its use in environmental reporting and accounting frameworks. Biodiversity measurements obtained using different eDNA tools and survey designs can produce substantially different metrics. Work during this period has therefore begun exploring statistical approaches for producing biodiversity metrics that are comparable across methods and sampling designs. This may later extend to comparability across broader survey approaches, enabling eDNA to be integrated with other data types and potentially used to ground truth remote sensing observation.

Communication

- Development of a thought leadership manuscript describing the role of eDNA in natural capital accounting.
- Ongoing engagement with collaborators to align biodiversity metrics with accounting frameworks.
- This project was presented at the eDGES Annual Stakeholder meeting in Perth, December 2025.
- This project has been referenced on the eDGES program website www.edgesprogram.org.

Sustainability

This project has been relying primarily on datasets generated by other eDNA projects within the eDGES program. This approach reduces the need for additional field sampling and laboratory analyses whilst allowing for the efficient development of analytical and conceptual frameworks for biodiversity measurement. The re-use of existing datasets also enhances cohesion across eDGES projects and supports alignment of biodiversity measurement with natural capital accounting objectives.

Next Steps

Priorities for the next reporting period include:

- Finalisation and publication of the thought leadership manuscript.
- Further development of statistical frameworks for generating comparable eDNA-derived biodiversity metrics.
- Identification and testing of candidate biodiversity indicators using eDGES-generated eDNA data.
- Engagement with researchers and practitioners involved in environmental accounting to refine indicator requirements and potential implementation pathways.

Program Management

Common program meetings and events

Figure 10.1 The eDGES annual symposium, held in Perth, November 2025, with Dr Tim Cooper at the podium.

Summary

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 eDNA Frontiers.

An eDGES specific website www.edgesprogram.org, has been developed and made publicly available to provide a source of information for the projects. This will be updated periodically as new information and publications arise from the projects.

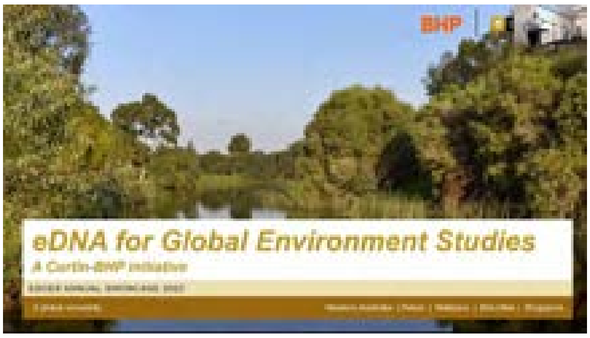


Figure 10.2 Image taken from the video presentation at the eDGES annual symposium, held in Perth December 2025.

eDNA Explorer web application

As part of Dr Samuel Thompson's role as Data Scientist / Bioinformatician jointly for eDGES and eDNA Frontiers, we have developed a full stack of containerised software on AWS cloud infrastructure to securely categorise, store and query our eDNA-related datasets in perpetuity using a PostgreSQL relational database system.

The suite involves bioinformatic analysis of sequence data on the Pawsey supercomputing cluster using bioinformatic pipelines such as eDNAFlow (Mousavi-Derazmahalleh *et al.* 2021). Local preprocessing and decontamination of the data is then conducted locally using a new suite of containerised R scripts before upload to the database using a web application accessible to eDNA Frontiers and eDGES staff. Once uploaded, the raw data can be accessed internally using R scripts. Furthermore, to facilitate access for non-SMEs including the public, collaborators and eDNA Frontiers clients, we have developed the eDNA Explorer web application – currently in beta testing and scheduled for release in March 2026, with eDGES datasets being uploaded when available and released publicly if approved.

The application will allow members of the public to view samples and projects that have been made publicly available by their owners, whereas collaborators and clients will be able to log in to access further advanced features such as analysis of relative read abundances, diversity, ordination plotting, taxonomic tree generation and analysis of 'dark' (unassigned) taxa.

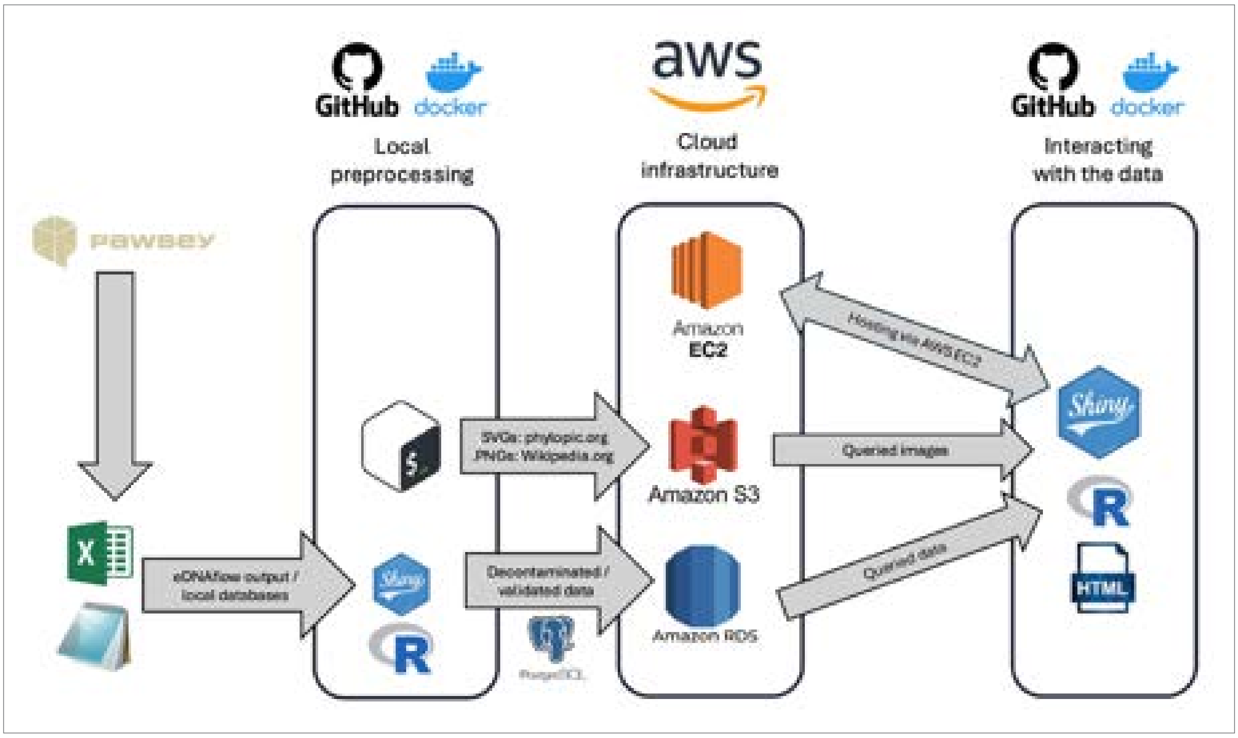


Figure 10.3 Diagram of the full software stack, featuring AWS cloud infrastructure, The Pawsey Supercomputing Cluster, along with R and Shiny for application processing and UI respectively, and docker for application containerisation.

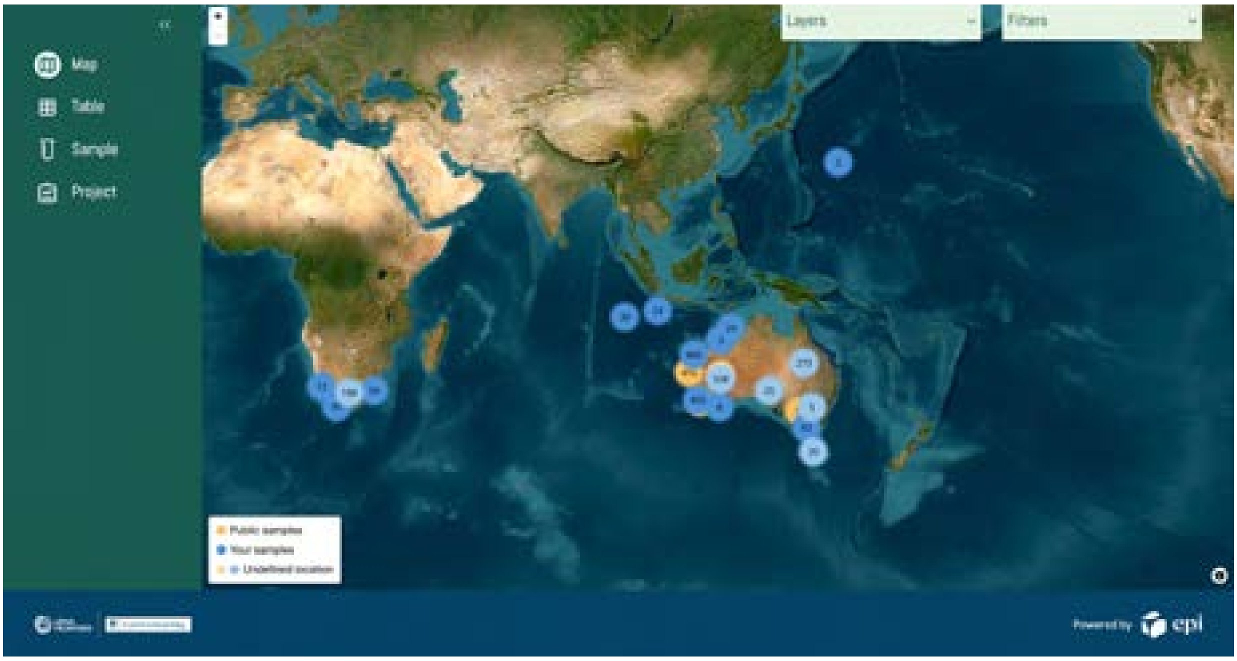


Figure 10.4 Map interface for the eDNA Explorer web application, all samples are being recorded in the app, but not all are shared publicly.

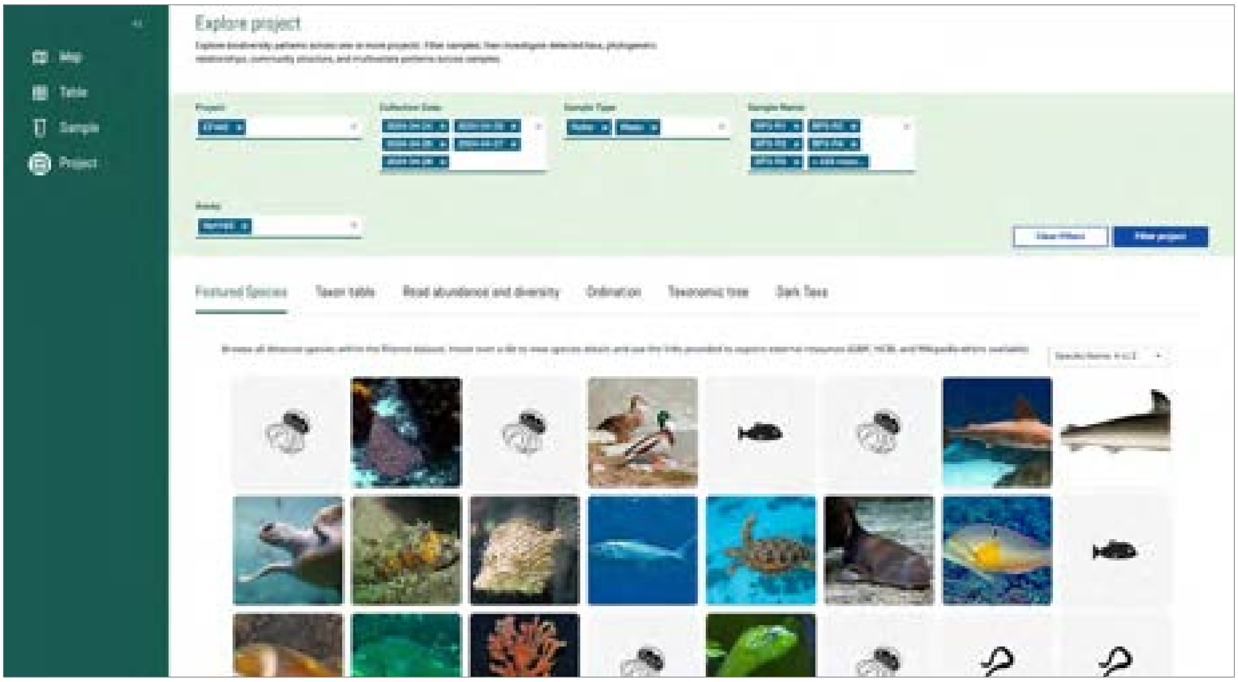


Figure 10.5 Project interface showing the featured species analysis module, containing representative images of each species found in the selected dataset, with links to GBIF, NCBI and Wikipedia pages for each species upon image hover over.

Future additional features for implementation to the database and to the eDNA Explorer application include AIS (alien invasive species) detection / flagging and IUCN listings for endangered species. We will also be implementing a system to automatically share our publicly available data with the global biodiversity information system (GBIF) and the Ocean Biodiversity Information System (OBIS). Architecturally, the database is also designed to allow the realignment of previous datasets to take advantage of the release of new reference sequences, with the capability to store a list of taxonomic assignments for a singular reference sequence, meaning we can track the changes in assignment over time, and facilitate more accurate comparisons between historical and contemporary metabarcoding datasets.

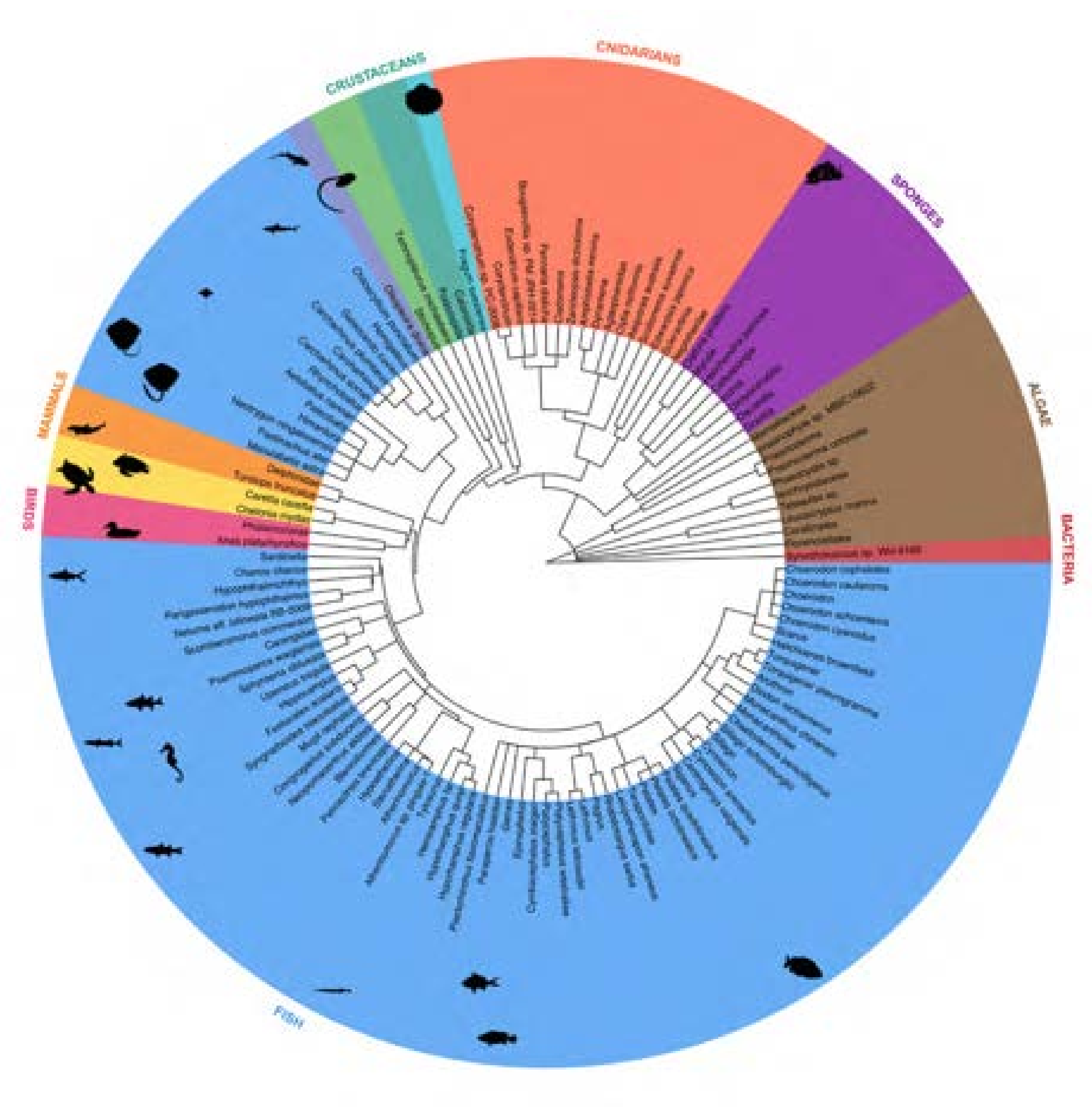


Figure 10.6 Example taxonomic tree generated by the eDNA Explorer application.

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