Tribulations and triumphs of landscape scale biodiversity assessment with eDNA

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Key Points

- A landscape-scale eDNA monitoring program was designed and implemented to evaluate the 10-year Healthy Waterways Strategy.
- Challenges were identified early in the first of four sampling campaigns and improvements made that enhanced species detection in streams, wetlands and estuaries.
- The significant spatial, temporal, and taxonomic coverage of the dataset holds immense promise for identifying optimal investment for waterway health improvement across Melbourne and for applied research.

Abstract

Understanding the distribution and diversity of organisms within a region is fundamental for environmental managers tasked with developing and implementing effective strategies to protect biodiversity. Over ~10 years, Melbourne Water has contributed to research developing and testing eDNA techniques suitable for assessing aquatic vertebrate biodiversity at a landscape scale.

In 2021, Melbourne Water implemented a biodiversity monitoring project covering 69 subcatchments, resulting in the collection of ~8000 samples across 1,862 unique sites in wetland, estuarine and stream habitats over 24 months (sites were sampled multiple times; Spring and Autumn only). The program was specifically designed to baseline distributions of platypus, fish and frogs for monitoring long-term biodiversity change.

The logistics of running such a large-scale project in compressed timelines necessitated uplift in several key areas; 1) staffing capacity to ensure necessary sampling intensity, 2) sample throughput, 3) Project Management effort, and 4) site selection processes.

The program delivered a comprehensive baseline of aquatic vertebrate biodiversity for the region: 328 taxa were detected, including 117 target taxa (i.e., aquatic-dependent vertebrates; 100 fish taxa, 16 frog taxa, platypuses) and 211 off-target taxa (largely terrestrial or semi-aquatic). Birds (132 taxa) and mammals (64 taxa) comprised the majority of off-target taxa.

The generated dataset holds promise for extensive analyses relating predictor variables such as land use, hydrologic modification, landscape connectivity, and climate change to species distribution. The dataset is already proving a valuable complement to traditional sampling methods. A remaining challenge is how to best incorporate expanded knowledge into business processes and reporting requirements.

Keywords

Environmental DNA, vertebrate biodiversity, landscape scale monitoring, instream barriers, strategy effectiveness.

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Introduction

Background

For the first time, Melbourne Water's Healthy Waterways Strategy (HWS, 2018) embedded a Monitoring, Evaluation, Reporting and Improvement (MERI) framework at the beginning of the strategy to ensure suitable data was gathered to assess the effectiveness of management interventions. Mid-term and end of strategy reporting were critical tasks that the MERI was designed to facilitate over the 10-year strategy life.

The MERI, and associated Monitoring and Evaluation Plans (MEP's) for streams, estuaries and wetlands (healthywaterways.com), included a new program that sampled 'environmental' DNA (eDNA) to baseline biodiversity across the region. 10 years of Research and Development to build a complete library of barcodes and qPCR tests for all known target species in the region provided confidence that large-scale deployment across the region would be successful. eDNA also held promise for non-target purposes, such as qualifying the impact of pollution events on detectable species.

Program design

To aid success, the program was heavily informed by Habitat Suitability Model (HSM) predictions (Chee *et al.* 2020), which were used to approximate species occupancy. eDNA detection probabilities were derived from a previous study (McColl-Gausden et al., 2021) or from expert knowledge. Species-specific probabilities of occupancy and detection were used as inputs to power analyses for each subcatchment (see Tingley, Wu & Weeks, 2020 for further details). These power analyses provided a range of program options with various levels of confidence to detect occupancy change, as a surrogate for condition (Figure 1a), through time (nominally the first 5 years of strategy (baseline) and a future program in the second five years of strategy). Ultimately, approvals were granted for a program that sampled approximately 40 sites (sampled twice per five-year block) in each subcatchment. This equated to 1862 sites being sampled across two years (Spring 2021, Autumn 2022, Spring 2022 and Autumn 2023) in the first half of the HWS. The power analysis included a target funding ceiling of $24,000 for each HWS subcatchment, noting that eDNA will represent the only monitoring in many subcatchments.

The program targeted fish, frogs and platypus key values across stream, estuarine and wetland habitats in 69 subcatchments (Figure 1b) and complimented other programs designed to track population health at long-term monitoring sites. Distribution information for many off-target animals, (e.g. reptiles, deer, pig, etc.) that could prove useful for management purposes was incidentally collected as part of eDNA sampling and analysis. Additional benefits include the ability to produce rapid and accurate program effectiveness and strategy progress reports to improve opportunities for rigorous adaptive management.

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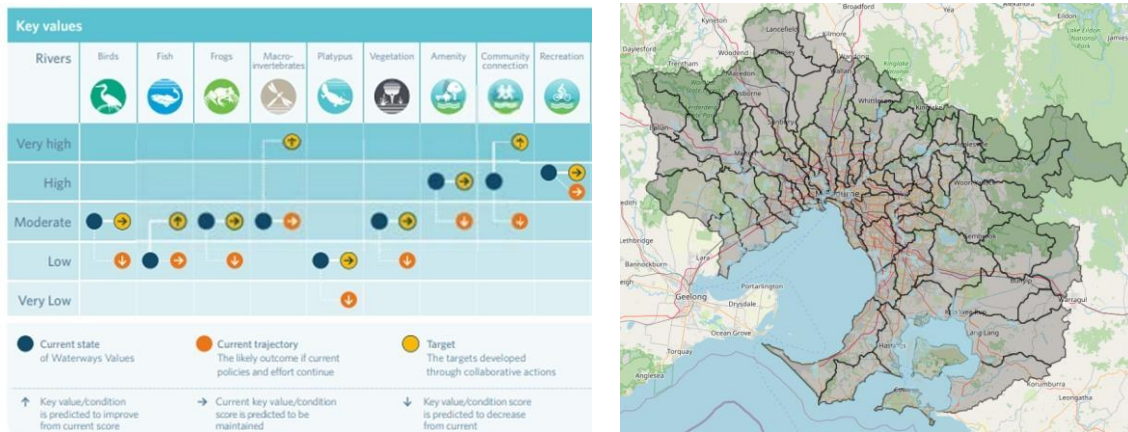

Figure 1 (a) Example of condition categories and expected trajectory of key values in the 2018 Healthy Waterways Strategy. Summaries of each of the five major catchments can be found in the strategy, but explicit targets are known for each of the 69 sub-catchments. The eDNA program was designed to detect changes in condition categories through time for fish, frogs and platypuses. River, Estuarine and Wetland habitats were targeted with the eDNA program. **(b)** the 69 subcatchments of the MW region monitored with eDNA.

Challenges of large-scale monitoring

To date, Melbourne Water's eDNA program is the biggest known of its type in the world. A total of 1,862 sites were sampled across different habitat types relatively issue free, despite strict, lengthy COVID lockdowns and several substantial rainfall events delaying sampling. Sample throughput in the laboratory was initially a pinch-point but robotisation of processes and outsourcing of sequencing smoothed analysis greatly while introducing only small additional time-cost. Outsourcing the sequencing process was necessary for such large sample numbers and only delayed the availability of results in Spring 2022.

One month after project approval, a part-time Project Manager at Enviro DNA was dedicated specifically to the MERI program. This was necessary as a point of contact for the program for both field and technical staff and Melbourne Water staff. Field staff had the most challenges finding sample sites with access through public land. Future access was paramount for site consideration and the reason why public land was greatly preferred for access to sites. Very few subcatchments were unable to meet sampling requirements (required number of sites) due to restricted sampling locations (e.g. mostly privately held land and/or access being refused due to biohazard risks during cattle foot and mouth outbreak concerns).

Wetland sampling required some development after the first season of sampling, as detections were not as high as anticipated from previous trials. Small changes to the sampling method and tweaks to sequencing techniques (e.g. Unique Dual Indexing) combined with greater knowledge of seasonal shifts in detection strength (particularly frogs) resolved concerns for the rest of the sample period.

Due to delays in program approvals and the necessary time to collect adequate sample numbers, the results of the baselining program were not available for mid-term review reporting purposes. This did not constitute a failure of the program because the baseline for future comparison had been established and a large amount of additional management benefit had been extracted.

Preliminary results

A total of 7,909 water samples from 1,862 unique sites were processed for eDNA analysis as part of the MERI eDNA program. This effort resulted in 80,214 faunal detections of 328 taxa across all sampling seasons combined. Of these taxa, 237 (72.3%) were resolved at the species level, with an additional 73 (22.3%) resolved to the level of genus.

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Terrestrial (non-target) taxa (classes Aves, Reptilia, and Mammalia, excluding the platypus) were over-represented in the data, both in terms of species richness (211 taxa, 64.3% of the total), and number of detections (44,813 detections, 55.9% of all detections). Birds were the most common terrestrial group (132 taxa), followed by mammals (64), and reptiles (15). However, this is perhaps unsurprising, given avian diversity relative to that of other groups.

Aquatic taxa, including members of the classes Amphibia, Chondrichthyes, Hyperoartia, and Actinopteri, as well as the platypus, were less frequently detected than their terrestrial counterparts. Most aquatic taxa (94 taxa, 88.5% of aquatic detections) belonged to the class Actinopteri (ray-finned fish).

Comparing species-level eDNA detections to previous Melbourne Water observations revealed strong positive correlations between the number of subcatchments occupied by each species. Nonetheless, 70 fish species (98.6%) and all 13 frog species (100%) were detected via eDNA sampling in one or more new subcatchments in which they had not previously been detected. Melbourne Water has therefore been able to generate a more complete picture of key values across all 69 subcatchments.

Management benefits

Flow-on benefits of access to current, high coverage vertebrate site occupations (akin to presence/absence) data were substantial. Turnaround times for data request and the accuracy of data has improved greatly, investment decisions are better informed and substantiated and citizen scientists are also very interested in the dataset as it allows community groups (e.g. 'Friends of', Landcare groups etc.) to redirect resources from monitoring, to more on ground work or education programs etc. A biodiversity baseline also proved useful for pollution event responses, with only minimal post event sampling needed to have confidence in the observed environmental impact.
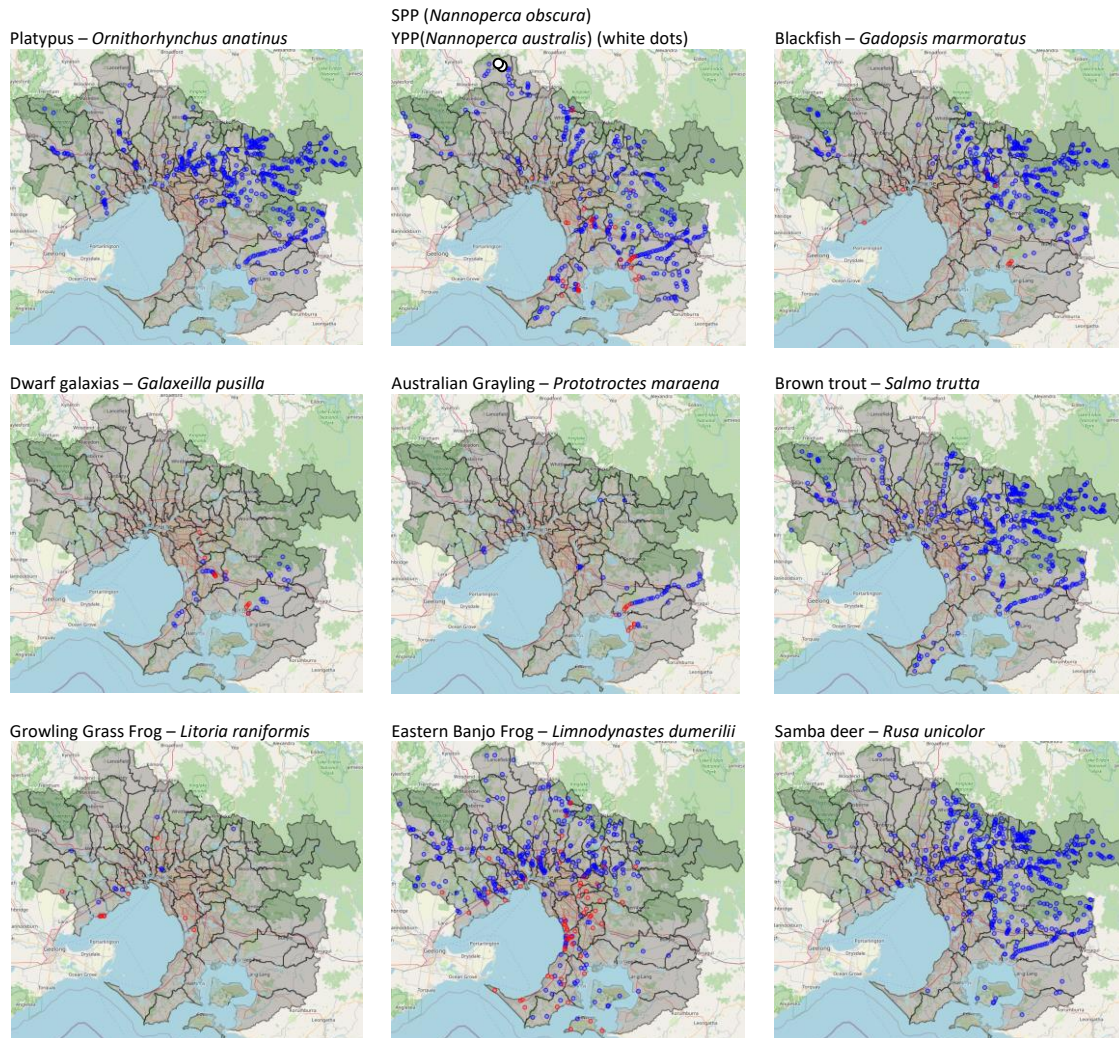
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Figure 2. Distribution of some species of interest as measured with eDNA over four sampling campaigns (Spring 2021, Autumn 2022, Spring 2022 & Autumn 2023). Red circles are sampled from wetland or estuarine habitations and blue circles are stream habitats. Detections of amphibians in Autumn samples were consistently very weak, but strong in Spring samples. SPP and YPP refer to Southern Pygmy Perch and Yarra Pygmy Perch.

Improvement opportunities

Programs of this scale often present improvement opportunities for future programs. Unique Dual Indexing (UDI) greatly improved confidence in detections through the bioinformatic process, reducing false positives that can arise through the laboratory and sequencing process. This method was adopted for the final two sampling seasons. Tweaks to the wetland sampling method also resulted in improved species occupancy detection, but wetland sampling could be further reviewed and developed before the next program roll out. Drones were successfully trialed to sample difficult to access sites and this will be further explored to exploit efficiencies.

Next steps

While a thorough 'sense check' of species distribution was performed against historic distributions, a peer review process for identifying potential false positive site or sample (Darling et al. 2021) detections will likely improve the dataset and will be performed before widespread integration into business systems and processes (e.g. project planning around sensitive species, external data requests, etc.).

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Incorporating the eDNA dataset into improved Habitat Suitability Modelling (HSM's) will improve model accuracy. It is expected that the improved HSM's will feed directly into developing more sophisticated strategies in the future.

Given the original power analysis was informed by HSM's that were developed from historic catch data (Tingley et al. 2020), additional analyses exploring the achieved statistical power are expected to help optimise the next round of sampling. The dataset also holds substantial opportunities for developing sophisticated biodiversity and diversity change metrics, exploring barriers to migration and assessing works effectiveness (e.g. Figure 2; Australian Grayling demonstrate expected distributions against known barriers (Lower Werribee Diversion Weir) and works to remove barriers (Dights Falls, Bunyip River barrier removals)). Asset Management prioritisation processes can be improved with addition of eDNA data which also directly informs funding bids to the Essential Services Commission through Melbourne Waters Price Determination process.

Conclusions

While not perfect, the initial roll-out of this large program went exceptionally well. Improvements to field methods and sequencing/bioinformatics techniques improved sample throughout and accuracy of occupancy detection (as revealed by sample processing time and greater numbers of expected detections). The eDNA generated dataset is already proving a valuable resource to streamline business processes and improve systems. The potential for analyzing the dataset is immense, both from academic and applied management perspectives. The program has provided an effective baseline for vertebrate aquatic biodiversity to test strategy effectiveness and develop investment programs that better target management of waterways, wetlands and estuaries.

Acknowledgments

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