

Improved biomonitoring of urban freshwater ecosystems using DNA barcodes



Healthy Waterways
Strategy 2018-2028
Port Phillip & Westernport, Victoria



Melbourne
Water



Strategic alignment

Regional Performance Objectives (RPOs):

- RPO 45: Research partnerships with universities and other research institutions are in place to address the key research areas and build our knowledge and capacity to efficiency and effectively achieve the HWS performance objectives and targets.

Key Research Areas:

- Other Aquatic Biodiversity: Improving our understanding of critical ecological processes and the ecology of key species to improve our conceptual and quantitative models
- Streamside Vegetation and Instream Habitat: Developing decision support tools to support improved investment in riparian and instream habitat activities and locations

Summary

Freshwater macroinvertebrates are commonly used by water managers to assess ecosystem health and set environmental protection and improvement objectives. Family-based macroinvertebrate metrics are legislated biological objectives in the Environmental Reference Standards and are widely used by water managers for 'state of the environment' reporting (Government of Victoria, 2018). However, these family-based metrics fail to quantify macroinvertebrate species loss from human induced impacts and can provide limited information about what actions are most likely to improve stream health (Walsh & Webb, 2016, Tsyrlin et al. 2023). By using DNA technologies, rather than morphological examination, macroinvertebrate species rather than families can be more easily and accurately identified (e.g. Ball et al., 2005; Shackleton & Rees, 2015) allowing for assessment of species biodiversity. Species information allows development of new species-based metrics and models with the potential to better understand and characterise impacts on stream health which can integrate the stressor responses of individual species (i.e. Baird & Hajibabaei, 2012; Kuemmerlen et al., 2014). DNA-barcoding (metabarcoding) is a molecular technique that enables identification of multiple different types of animals within a sample, as opposed to other molecular techniques that target a single species within a sample.

A proof-of-concept study (Improved biomonitoring of urban freshwater ecosystems using DNA barcodes) was conducted in 2018/2019 by the University of Melbourne and Melbourne Water to examine whether DNA metabarcoding could be used to routinely identify macroinvertebrate species for Melbourne Water's biological monitoring programs. The studies' aims were to determine:

- if family level macroinvertebrate identifications made through DNA metabarcoding produce similar site assessments or 'bioassessment fidelity' as traditional methods (morphological identification), when tested more broadly across greater Melbourne;
- if bioassessments conducted through DNA metabarcoding could be done more cost-effectively using a 'rapid' DNA extraction method where picked macroinvertebrate samples are directly homogenised (or blended) with little or no sorting;
- what current costs are involved in processing samples using traditional methods and how these compare to DNA metabarcoding;
- how many additional DNA barcodes are needed to identify the full suite of macroinvertebrate species found across the greater Melbourne area;
- what macroinvertebrate species biodiversity exists across the greater Melbourne area using DNA metabarcoding.

The study successfully demonstrated the cost-effectiveness and feasibility of performing broad-scale 'species' identification of macroinvertebrate samples with DNA metabarcoding. It showed that macroinvertebrate biodiversity was accurately detected using rapid DNA extraction approaches. Considering this, two further studies were developed, to address the limitation in the reference library of barcodes for individual species and to explore the use of DNA metabarcoding of macroinvertebrates for ecological modelling.

Recommendations:

- All DNA-based macroinvertebrate identification relies on creating DNA reference databases/libraries where DNA barcodes are linked to identified voucher specimens. We recommend further investment in DNA barcoding of individual species to fill 'gap' in DNA barcode reference libraries for greater Melbourne, especially in areas such as the upper Yarra and closed catchments which appear to contain many locally endemic species.
- Biodiversity measured at a family level is a poor predictor of species biodiversity which was supported by this study. We recommend using species level identification to understand and conserve invertebrate biodiversity rather the families.
- Rapid DNA extraction methods reduce the manual sample handling and therefore cost. For macroinvertebrate diversity assessment that strongly overlap with traditional methods we recommend development of methods that use net collected macroinvertebrates that are not sorted from net

debris prior to DNA metabarcoding.

- The DNA metabarcoding presented here provides cost-effective, rapid and accurate means to identify macroinvertebrate species diversity in situ. We recommend (which will occur via Project A4) that this study be broadened to include hundreds of sites so species responses can be elucidated using Habitat Suitability Models (HSM's) and other modelling approaches, approaches adopted in the subsequent project. This will assist MW to develop and assess management of streams and rivers.

What did we do?

This proof-of-concept study used macroinvertebrate sweep samples from 47 sites. At each site two macroinvertebrate sweep samples were collected, and macroinvertebrates were picked using a standard laboratory sub-sampling approach (see below). The duplicate samples from each site were then

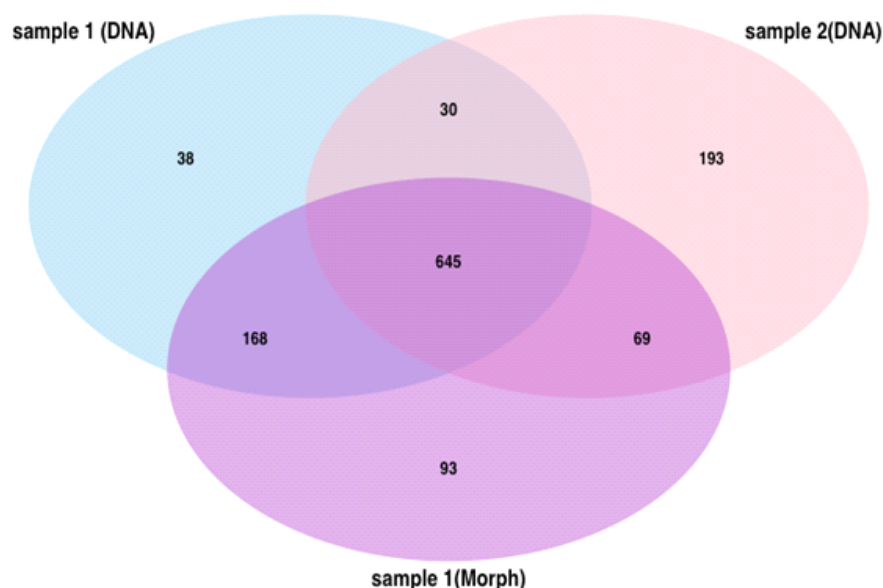


Figure 1. A Venn diagram showing the overlap in families identified with DNA metabarcoding (sample 1 (DNA)) and those identified from the same sample with morphology (sample 1 (Morph)) compared to those identified from rapid DNA metabarcoding (sample 2 (DNA))

processed differently:

- 'Sample 1s' were retained by the consultants for standard morphological identification to family (the traditional method) then transferred to the University of Melbourne (UoM) where they were subjected to non-destructive DNA extraction followed by DNA metabarcoding to identify families and species.
- 'Sample 2s' were transferred straight to UoM and directly homogenised for rapid (destructive) DNA extraction followed by DNA metabarcoding to identify families and species.

Macroinvertebrate identifications based on morphology (completed by Ecology Australia and AQUEST at RMIT University) were directly compared to those from DNA metabarcoding (completed by UoM and MW), to determine if morphologically observed taxa were also detected with DNA metabarcoding. However, the morphological identifications from the consultants were not shared prior to DNA metabarcoding. Families detected in the sample 2s with DNA metabarcoding were also compared to those in the sample 1s from morphological and DNA metabarcoding family identification. To examine how DNA metabarcoding performed for a suite of standard bioassessment

biological metrics, we considered: number of families, number of key families, SIGNAL2 (Chessman, 2003) and number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa. All metrics were calculated and/or compared to Victorian environmental protection objectives (Government of Victoria 1999; 2018). We also compared the species found using the two DNA metabarcoding approaches from samples 1s and 2s from each site to examine patterns of species biodiversity.

What did we find?

- That DNA metabarcoding provides a cost-effective option for qualitative assessments of macroinvertebrate biodiversity
- DNA metabarcoding produced similar identifications to morphological examination with strong overlap between datasets (figure 1) This resulted in few differences in site assessments when compared to environmental objectives (figure 2). Most differences between morphological and DNA metabarcoding identifications at the family level arose from: an inability to detect some macroinvertebrate families due to a lack of reference DNA barcodes needed to make identifications and the detection of extra macroinvertebrate families with DNA metabarcoding. The latter likely resulted from amplification of DNA from immature specimens, eggs, body parts, gut contents or environmental DNA also present in macroinvertebrate samples.
 - Freshwater macroinvertebrate DNA barcode library coverage for greater Melbourne was high, with on average, ~70% of species found at each site having DNA barcodes. Further, targeted DNA barcoding has improved this to 90% of macroinvertebrates with reference DNA barcodes for the study sites (figure 3).
 - Cost analysis showed that DNA metabarcoding has become less costly to perform in recent years and costs slightly less than morphological family identification but with the additional benefit of providing species level identification.

Future direction and Knowledge gaps

This project provided the proof-of-concept needed to pursue the successful ARC linkage funding in Project: Improving stream management using ecological modelling and DNA barcodes (LP190100328). This project commenced in 2021 and aims to address the following areas:

- Design and conduct a comprehensive, unbiased sampling survey that covers environmental gradients of interest (natural, e.g. flow regime, temperature; and human-impact, e.g. urban land, vegetation loss).
- Advance DNA barcode reference libraries and DNA metabarcoding for assessing and describing macroinvertebrate biodiversity for application throughout Australia and internationally.
- Develop HSMs that quantify the relationship between species presence-absence and environmental descriptors for all macroinvertebrate species for which there are sufficient presence records.

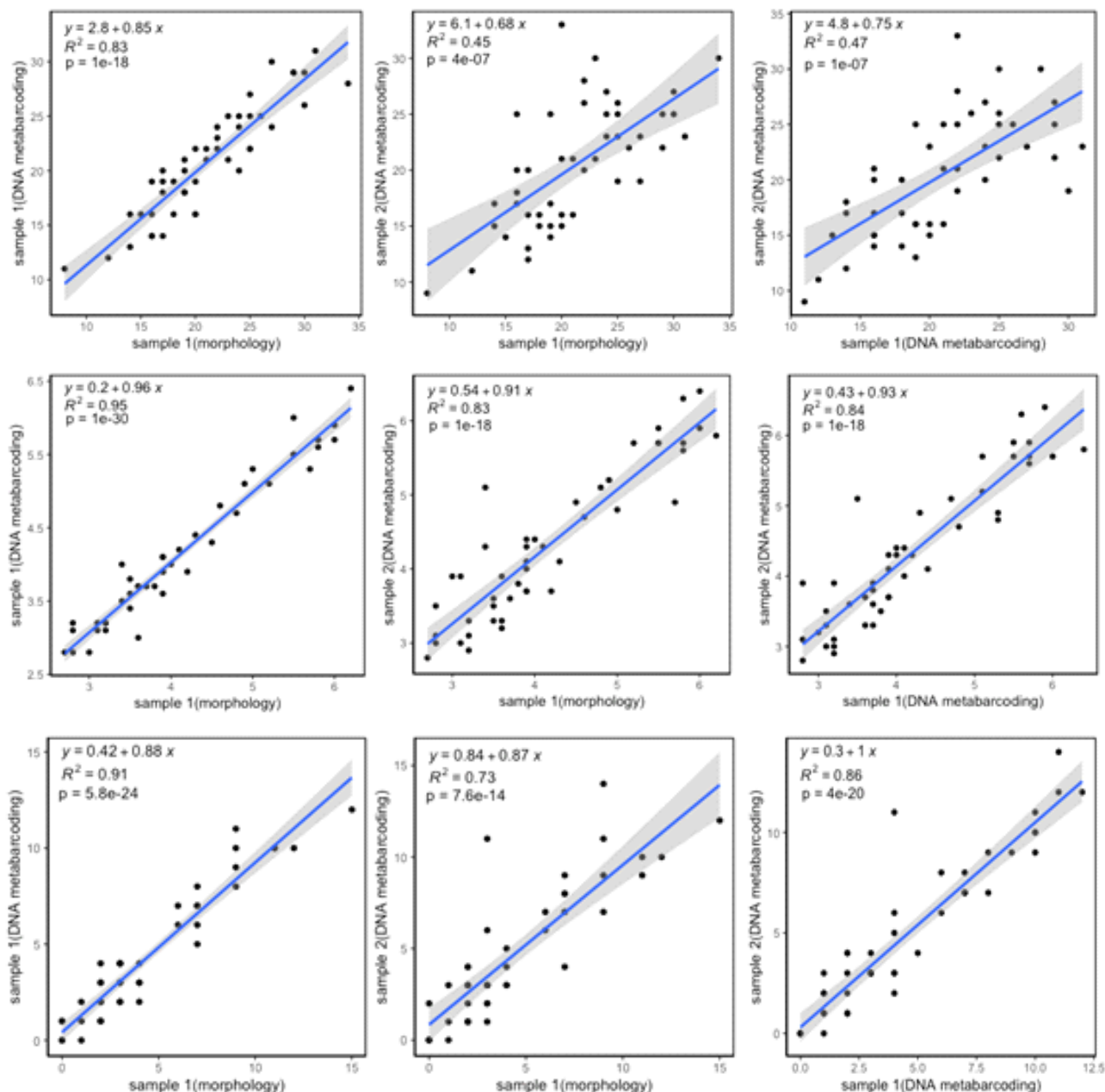


Figure 2. The strong relationship between different bioassessment metrics: a) number of families, b) SIGNAL2 scores and c) number of EPT families for families identified with DNA metabarcoding and morphology (sample 1) and those identified with rapid DNA metabarcoding (sample 2).

- Similar to the family level HSMs used in the development of the HWS, use the new species level HSMs to predict changes in macroinvertebrate species distributions arising from different scenarios of climate change and human impact, including clearing of riparian vegetation, urban stormwater drainage and flow modification.
- Develop a biodiversity priority rank map for the streams in the Melbourne region using all HSMs and the conservation planning software, Zonation.
- Similar to the family level HSMs used in the development of the HWS, use the new species level HSMs and Zonation to analyse and map cost-effective prioritisation of management interventions that are likely to achieve the greatest gains for regional biodiversity.

So far, we have refined our sampling methods to develop standardised processing of samples without first sorting macroinvertebrates from debris – which is expected to save considerable processing time and cost. This is now being rolled

out throughout our current sampling regime and processing of >300 sites. We anticipate that the data collected will enable us to provide further evidence to support MW using DNA metabarcoding species identification in macroinvertebrate monitoring more broadly and enable the development of species-based metrics for stream health.

How are we sharing findings?

Publications

- Carew, M. E., Nichols, S. J., Batovska, J., St Clair, R., Murphy, N. P., Blacket, M. J., & Shackleton, M. E. (2017). A DNA barcode database of Australia's freshwater macroinvertebrate fauna. *Marine and Freshwater Research*, 68(10), 1788-1802.
- Carew, M. E., Kellar, C. R., Pettigrove, V. J., & Hoffmann, A. A. (2018). Can high-throughput sequencing detect macroinvertebrate diversity for routine monitoring of an urban river? *Ecological indicators*, 85, 440-450.

- Carew, M. E., Coleman, R. A., & Hoffmann, A. A. (2018). Can non-destructive DNA extraction of bulk invertebrate samples be used for metabarcoding? *PeerJ*, 6, e4980.
- Carew, M. E., Yow, W. K., Robinson, K. L., Coleman, R. A., & Hoffmann, A. A. (2022). DNA barcoding and metabarcoding of highly diverse aquatic mites (Acarina) can improve their use in routine biological monitoring. *Marine and Freshwater Research*. 73(7), 900–914.

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- Tsyrlin, E., Carew, M., Hoffmann, A. A., Linke, S., & Coleman, R. A. (2023). Species-level dataset is required for setting biodiversity conservation priorities for freshwater macroinvertebrates in Melbourne streams. *Journal of Environmental Management*, 331, 117186.
- Walsh C.J. & Webb J.A. (2016) Interactive effects of urban stormwater drainage, land clearance, and flow regime on stream macroinvertebrate assemblages across a large metropolitan region. *Freshwater Science*, 35, 324-339.

For more details on the research outcomes of this project, or other projects of the MWRPP, please contact:

Rhys Coleman

Waterways & Wetlands Research Manager (Applied Research)
rhys.coleman@melbournewater.com.au

Slobodanka Stojkovic

Knowledge Broker, Waterways & Wetlands Research
slobodanka.stojkovic@melbournewater.com.au



Figure 3. The proportion of reads that could be assigned to species, genus and family from DNA metabarcoding of 47 sites around greater Melbourne.