WATERMICRO23
Darwin, Australia 4-8 June 2023

Faecal source tracking for catchment vulnerability assessments—an Australian perspective

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Abstract: A national project was undertaken bringing together water utilities and testing laboratories to demonstrate how molecular faecal source tracking (FST) technologies can improve the efficiency of catchment monitoring and better clarify risk. It developed and validated a protocol for FST, including sampling requirements, quality standards and guidelines for data interpretation of these molecular technologies—demonstrating how limitations around the use of the current microbial indicator *E. coli* in the Health-Based Targets (HBTs) approach can be overcome. Primarily, it presented how such technologies could be integrated into a HBTs approach, providing the additional risk discrimination required when catchment vulnerability assessments are in doubt.

Keywords: Faecal Source Tracking (FST); Catchment Vulnerability Assessment; Health Based Targets (HBTs)

Water utilities must consistently deliver safe drinking water. To facilitate this, the National Health and Medical Research Council introduced the concept of using microbial Health Based Targets (HBTs) in the Australian Drinking Water Guidelines and the HBT manual was subsequently developed (NHMRC, 2009; WSAA, 2015). It prescribes using a sanitary survey and vulnerability assessment which can be used in conjunction with microbial indicators—namely *E. coli*—as part of the mandatory Tier 1 assessment of source water. This is used to place the source challenge into one of four broad vulnerability assessment categories. The pathogen reduction requirements for reference bacteria, viruses and protozoa are described for each of these source categories and assist water utilities in understanding treatment requirements and where on the Water Safety Continuum they reside.

However, *E. coli* has limitations as an indicator of faecal contamination—in particular, the presence of environmental blooms that do not arise from faecal contamination. Subsequently, catchment vulnerability assessments are often not supported by *E. coli* data. While an optional Tier 2 assessment using pathogen monitoring data can also be used, monitoring for pathogens is expensive and results often show 'nil detects' which may not be a true assessment of the risk. This casts doubt on the assessment undertaken—potentially resulting in costly yet unwarranted additional treatment processes, or conversely, supplying water that may not be safe to drink. Developments reported within the scientific literature have highlighted molecular techniques that offer alternative approaches to better characterise risk and improve the efficiency of monitoring. This project developed and validated a protocol for vertebrate faecal source tracking (targeting mitochondrial DNA), including sampling requirements, quality standards and guidelines for data interpretation, with the objective to integrate this new profiling technique with existing FST techniques to support vulnerability assessment and the identification of faecal host sources.

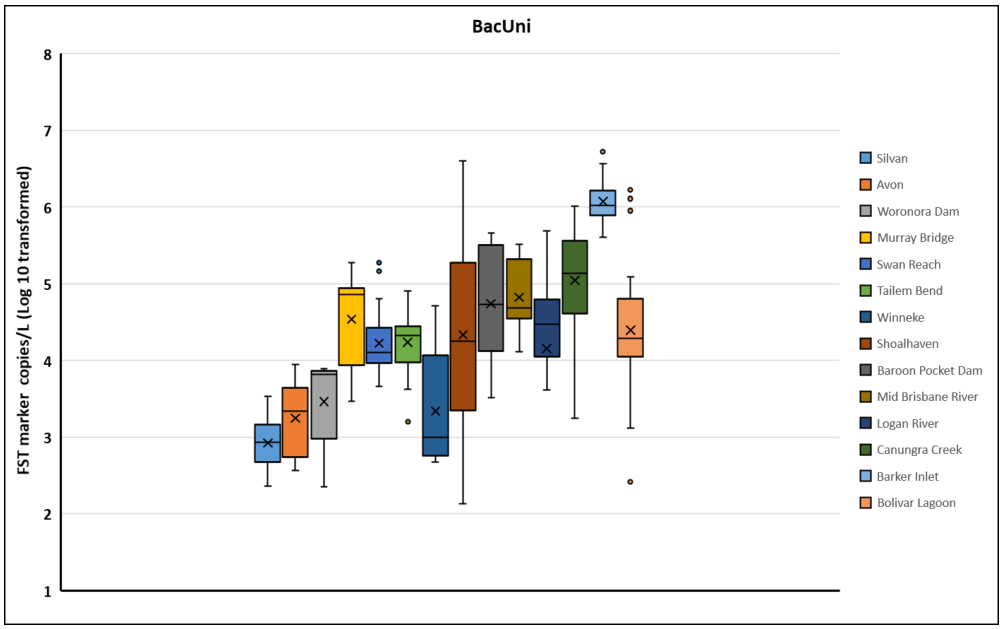
To achieve this goal, qPCR (quantitative Polymerase Chain Reaction) markers targeting DNA from vertebrate mitochondria and Bacteroidales were selected and evaluated. Microcosm experiments were conducted to understand the decay of the

molecular markers and determine their utility as faecal indicators. Experiments established that Bacteroidales markers are robust indicators of recent faecal contamination while mitochondrial markers can indicate more persistent contamination. Selected markers were incorporated into synthetic DNA standards designed in collaboration with the National Measurement Institute (NMI) and calibrated using digital droplet PCR to allow precision quantification of DNA in samples. Furthermore, a vertebrate mitochondrial 12S ribosomal DNA diversity profiling platform was established to assist with the identification of host faecal inputs. The capability of the FST technologies in capturing the spatial and temporal variation of a system and how this can influence sampling strategy were defined before field testing of the technologies was undertaken.

The general Bacteroidales marker, BacUni, showed the greatest power to discriminate between the catchments representing different source water vulnerabilities and may provide a significant improvement over *E. coli* as a marker indicating general faecal contamination (Figure 1). When source water target DNA was above a threshold concentration value, the vertebrate mitochondrial diversity profiling platform was shown to be extremely powerful in establishing a comprehensive profile of the water (Figure 2). Conversely, when the DNA target was below this threshold, mitochondrial 12S vertebrate DNA diversity profiling may not be appropriate due to sampling and technical biases, needing a host-specific mitochondrial marker used in conjunction with a general mitochondrial marker to verify the vertebrate diversity profile. Finally, an inter-laboratory trial conducted to examine the robustness of these methods demonstrated that the techniques are highly transferable with congruent results between the laboratories.

The techniques and approach for source water characterisation in this project show substantial promise in being able to provide additional risk discrimination, as is required when assessment of source water vulnerability categories is not supported by *E. coli* data. Furthermore, they may provide an alternative to embarking on a Tier 2 assessment which can be expensive and may still result in no further discrimination of risk. Nevertheless, the work presented in this project identified several limitations that need careful consideration in application of this approach. Thus, a simple conceptual model of how the molecular tools developed can be used in catchment monitoring was developed that will assist with decision making for when a Tier 1 re-assessment needs to be undertaken or before deciding on whether a Tier 2 approach is warranted.

A



B

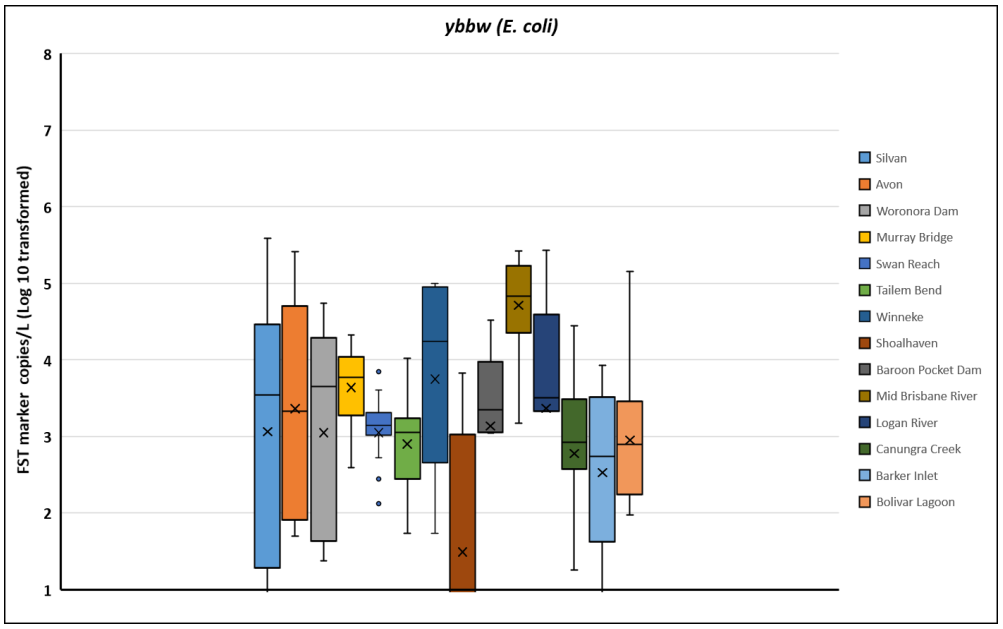


Figure 1. Box and Whisker Plots representing the (**A**) BacUni (general Bacteroidales) and (**B**) *ybbw* (E. *coli*) (copies/L [log 10 transformed) marker concentrations for data sets across several catchments of various source water vulnerability categories, grouped sequentially left to right on Y-axis from low to high vulnerability.

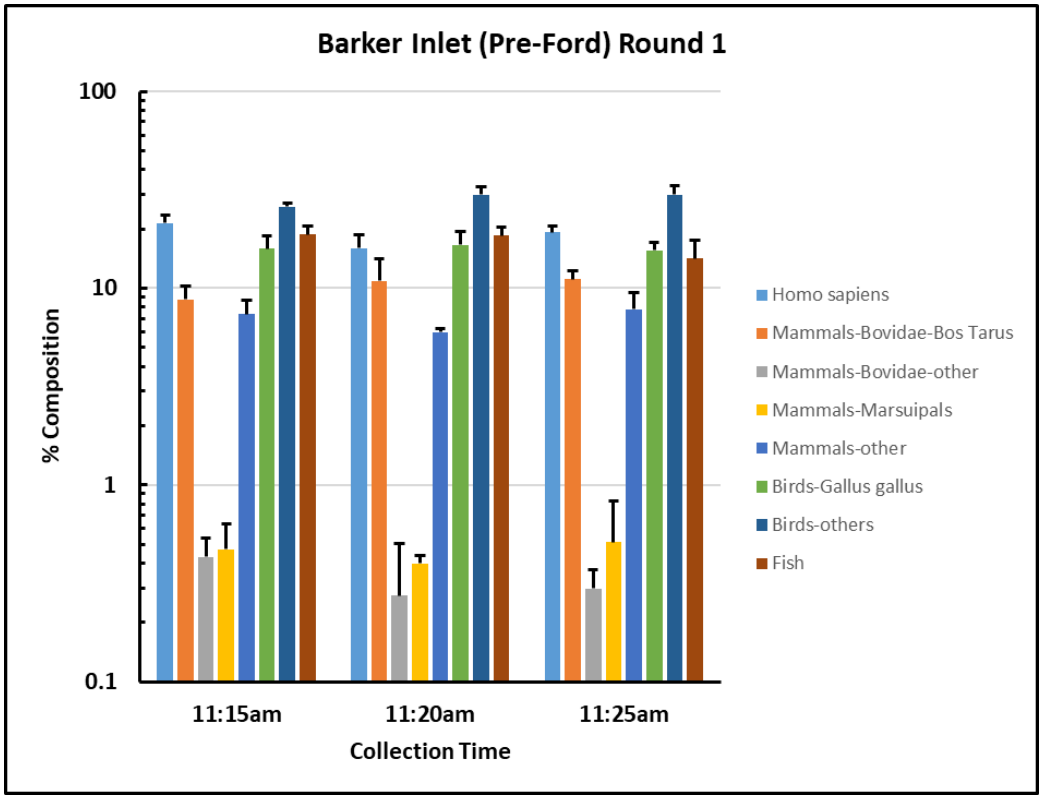


Figure 2 Vertebrate diversity at Barker Inlet quantified using Next Generation Sequencing (NGS). The error bars represent +/- standard deviation based on three independent samples.

REFERENCES

NHMRC, 2009. *Health Based Targets for Microbial Safety of Drinking Water Supplies Draft Discussion Paper*.

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