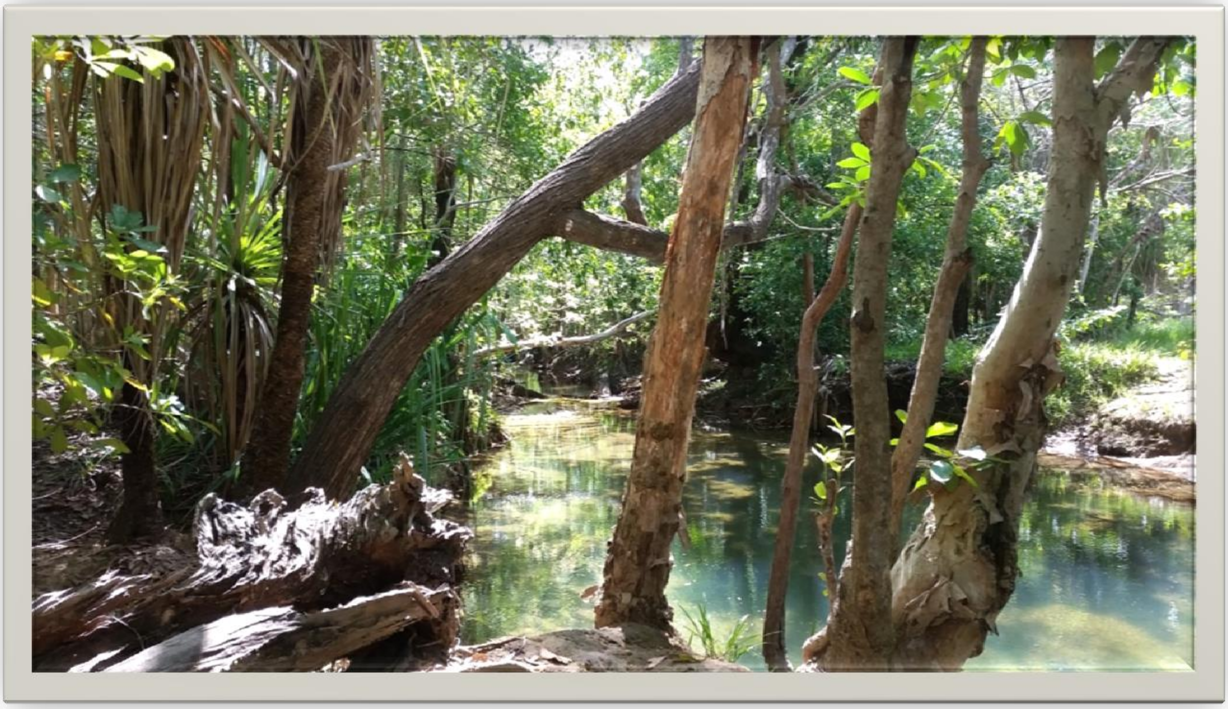
Environmental Chemistry and Microbiology Unit (ECMU)
Research Institute for the Environment and Livelihoods
Darwin, NT 0909 Australia

Summary of Technical Report

Analysis of Faecal Indicators & Abiotic Water Parameters from upper Rapid Creek, Feb 2009 – Feb 2016



Report prepared for

Northern Territory Airports Pty Ltd

June 2016

Dr Mirjam Kaestli

This report is the summary of the technical report "Analysis of Faecal Indicators & Abiotic Water Parameters from upper Rapid Creek, Feb 2009 – Feb 2016". Please consult the technical report for details.

Aim of Report

The primary objective of this work was the analysis of bacterial faecal indicators of 191 surface water samples collected between 2009 and 2016 by EcOz Environmental Consultants from 3 creek sites and 5 stormwater drains along upper Rapid creek in urban Darwin. Bacterial faecal indicators included enterococci, *Escherichia coli (E.coli)*, faecal and total coliforms.

Main Findings

- There were large variations in bacterial faecal indicator levels between the dry and wet season:
    - Indicator levels peaked after the first major rainfall in the build-up.
    - Indicator levels were lowest in the dry season with no land runoff, less turbidity and more sunlight i.e. UV irradiance.
- Water of the stormwater drains which only flowed after rainfall had significantly higher indicator levels than water from Rapid Creek.
- The enterococci levels in the drain behind Darwin Airport Resort were markedly higher when compared to other sites.
    - Possible reasons could be linked to sewage but the contribution from faecal contamination by wildlife, dogs & cats frequenting this area, and the enterococci multiplication in nutrient-rich water covered by leaves and debris are also likely to be key contributors.
    - Drain works in late 2015 were followed by a drastic drop in enterococci levels. Future sampling will show whether these levels stay low.
- The more rainfall beyond the first flush of the wet season, the more faecal indicators in the creek but not in the drains.
    - Possibly also due to resuspension of bacteria from the creek bed with increased stream flow.
- The higher the water turbidity, the more faecal indicators.
- The more TKN (organic nitrogen + ammonia) in the water, the more enterococci.
- The more total phosphorus in the water, the more faecal and total coliforms.

Background

Rapid Creek is the largest freshwater system in the urban Darwin area [1]. It originates in the Marrara swamp area in the NE of Darwin Airport and flows for 9.8 km until it discharges as a tidal mangrove-lined creek into Darwin Harbour [2].

In recent years, elevated levels of *E.coli* and Enterococci have been reported in Rapid Creek [1]. In the Darwin Harbour Beaches Report Card 2010 four of seven water samples collected in the tidal downstream area of Rapid Creek between June-Sept 2010 exceeded the “warning” trigger values set by the NRETAS 2010 Darwin Harbour Water Quality Objectives for Rapid Creek water [1].

A similar percentage of samples exceeding the trigger values were also found in other monitored urban Darwin creeks such as Vesteys Creek and a higher percentage in Mindil Creek [1].

High *E.coli* counts in Rapid Creek water were reported in a NT Government report in 1982 before the major airport development. The report stated wildlife in Marrara swamp was a possible source of elevated counts in upper Rapid Creek [3].

Data Collection & Analysis

Surface water collection and initial reporting was by EcOz Environmental Consultants for the Northern Territory Airports.

Standard monitoring by EcOz consisted of average four sampling rounds per year with one in the wet season (Jan-Mar), one between April to May, one in the middle of the dry season (July-Aug) and one after the first major rainfall during the build-up (Sept-Dec).

Water was collected from three sites along upper Rapid creek south of McMillans road, four stormwater drains in the upper Rapid creek catchment area and one stormwater drain at Bunnings carpark at the western end of the Darwin International Airport in the Ludmilla creek catchment (Figure 1).

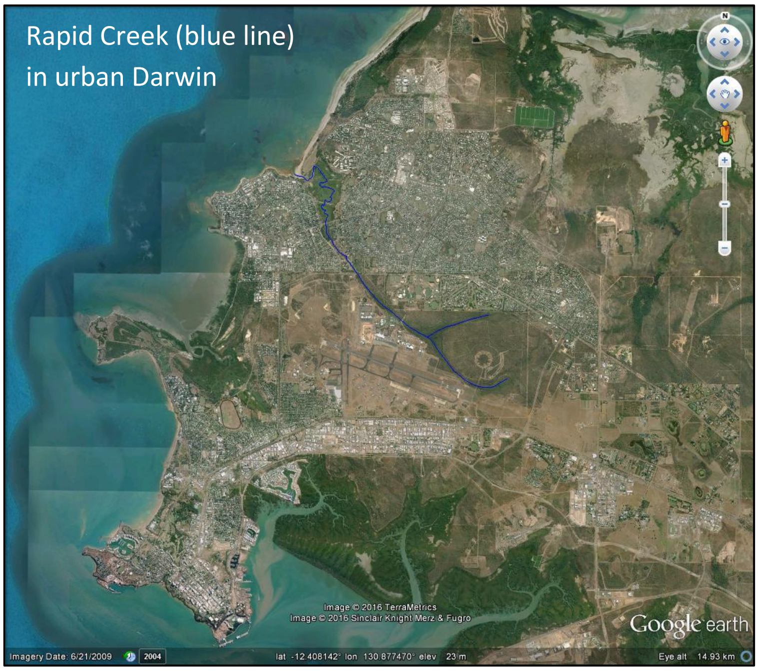


Figure 1: *Water collection sites along upper Rapid Creek*

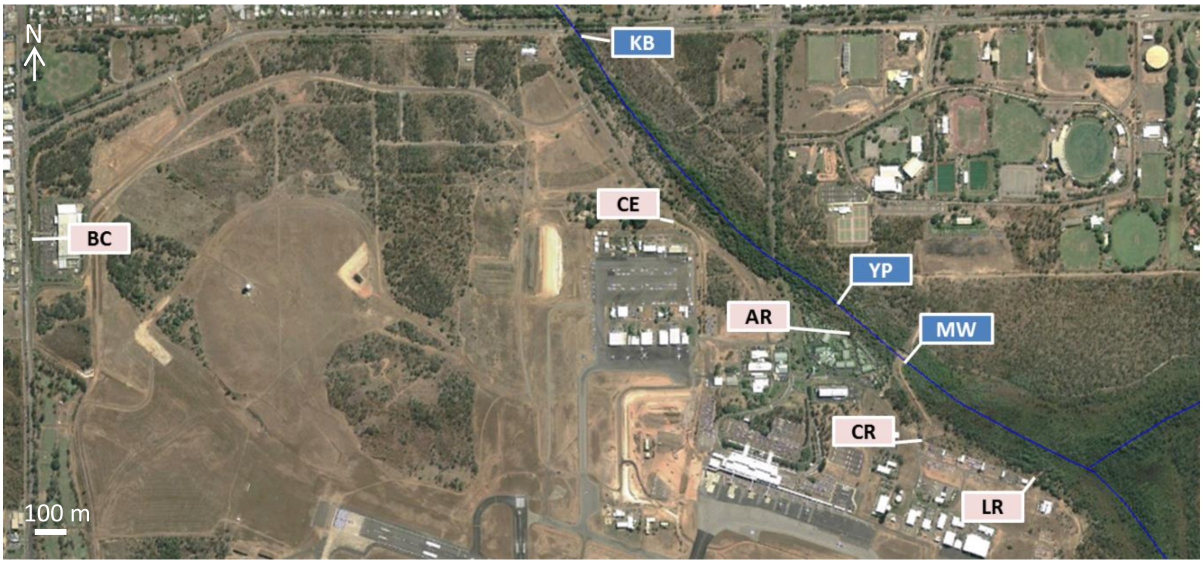
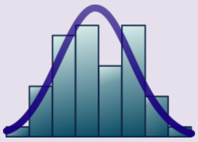


Figure 1 Legend: *The blue line indicates upper Rapid creek. The blue shaded sites are creek sites with light red indicating stormwater drains. "BC" Bunnings carpark drain, "KB" Kimmorley Bridge, "CE" Charles Eaton Dr drain, "AR" Darwin Airport Resort drain, "YP" Yankee Ponds, "MW" Mitigation Wall, "CR" Car Rental Yard drain, "LR" Larkin Road drain*



The Statistical Analysis

Data were checked for outliers, log transformed if needed, the raw data was visualized with box, dot and scatter plots and a multivariate negative binomial model with a robust variance clustered for sites was applied to study the association between faecal indicators, sites, seasons, years, rainfall and water parameters.

Results

Faecal indicators in the creek and drains

There were large variations in faecal indicator counts between the creek sites and drains (Figure 2). Enterococci was the only indicator which consistently increased from the most upstream site "Mitigation Wall" to Yankee Ponds to Kimmorley Bridge at McMillans Road. Indicator levels were significantly higher in the stormwater drains which only contained water for short periods after rainfall.

Figure 2: *The range of enterococci counts at the sites*

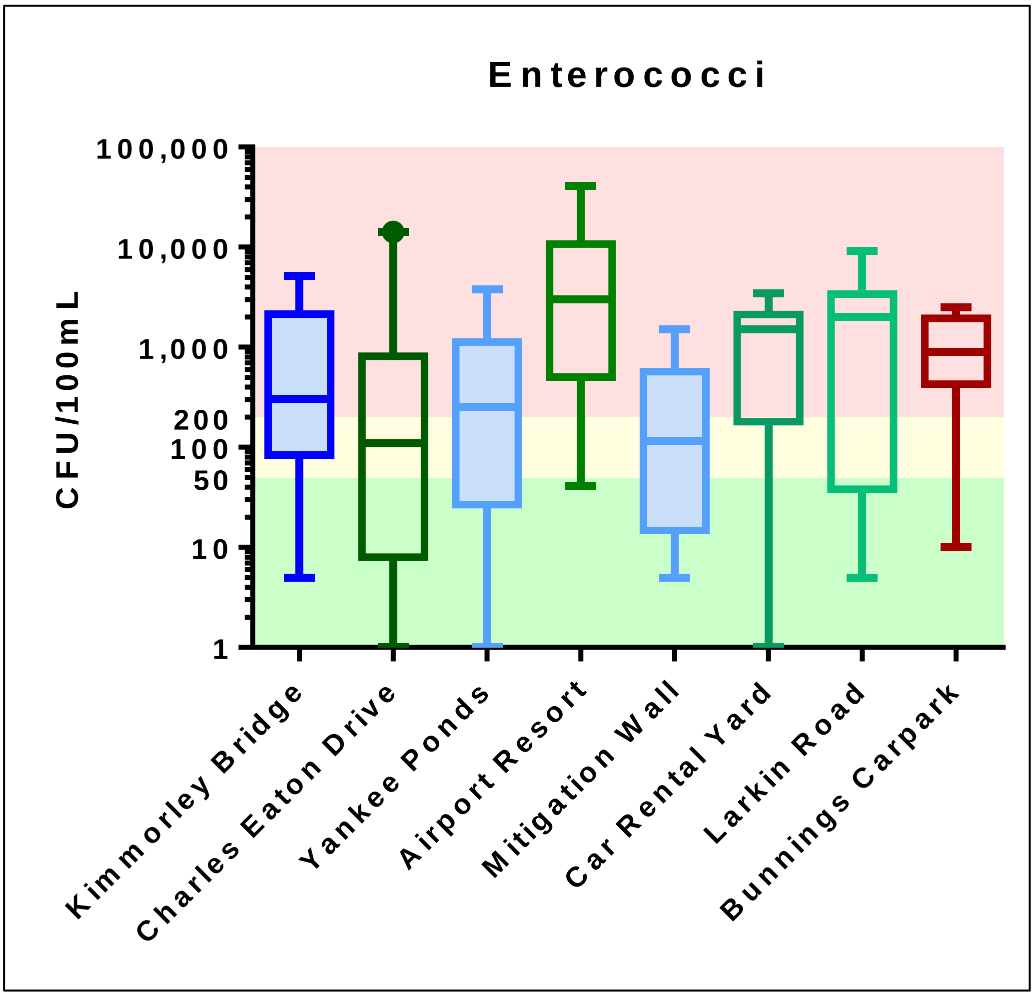


Figure 2 Legend*: The boxes mark the middle 50% of the enterococci counts per site (i.e. 25th-75th percentile), the horizontal line the median (50th percentile) and the vertical line extends from the 5th to the 95th percentile of the enterococci counts. Blue boxes indicate creek sites. There are 9-10 samples per site. The y-axis is in log-10 scale. CFU/100mL stands for "colony forming units per 100 mL" i.e. number of enterococci in 100 mL of water. The shaded areas mark the accepted (green, <50 CFU/100mL), warning (yellow, 50-200 CFU/100mL) and exceeded (red, >200 CFU/100mL) concentrations for enterococci as per NTG Recreational Water Quality Guidance Notes 2010.*

There were large variations in faecal indicator counts between seasons. While counts were low in the dry season, all indicators peaked in the build-up (Sept-Dec) after the first major rainfall of the wet season (Figures 3 & 4).

Figure 3: *Enterococci at creek sites at different times of the year*

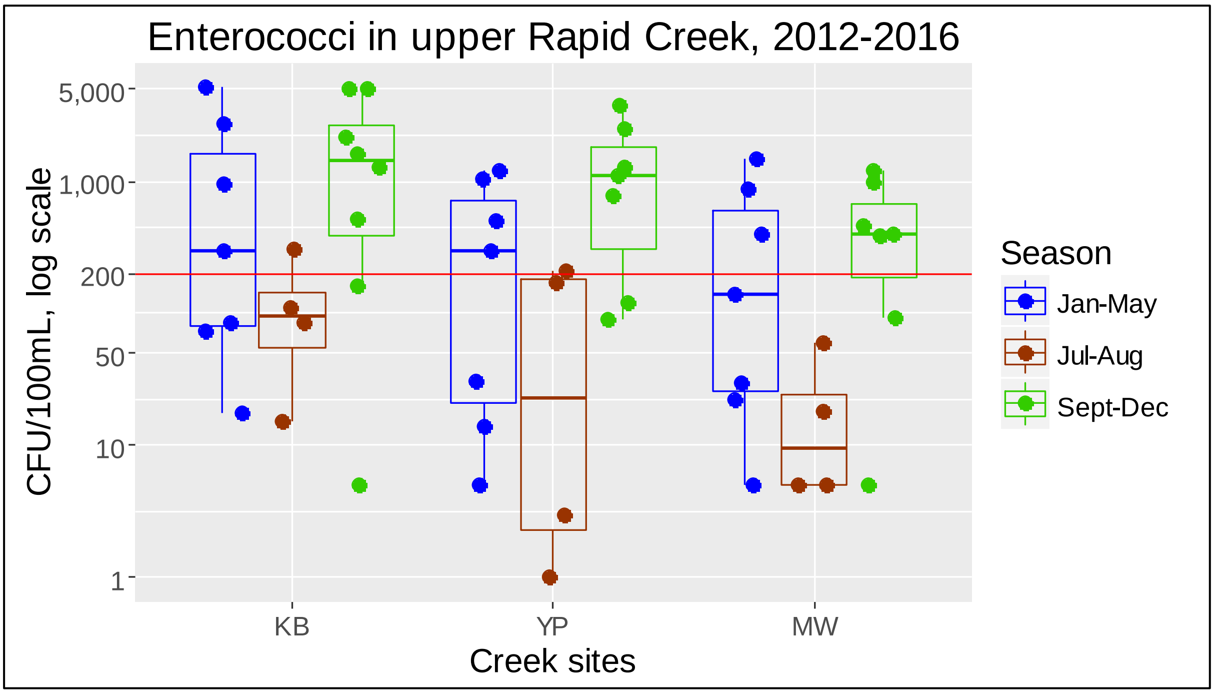
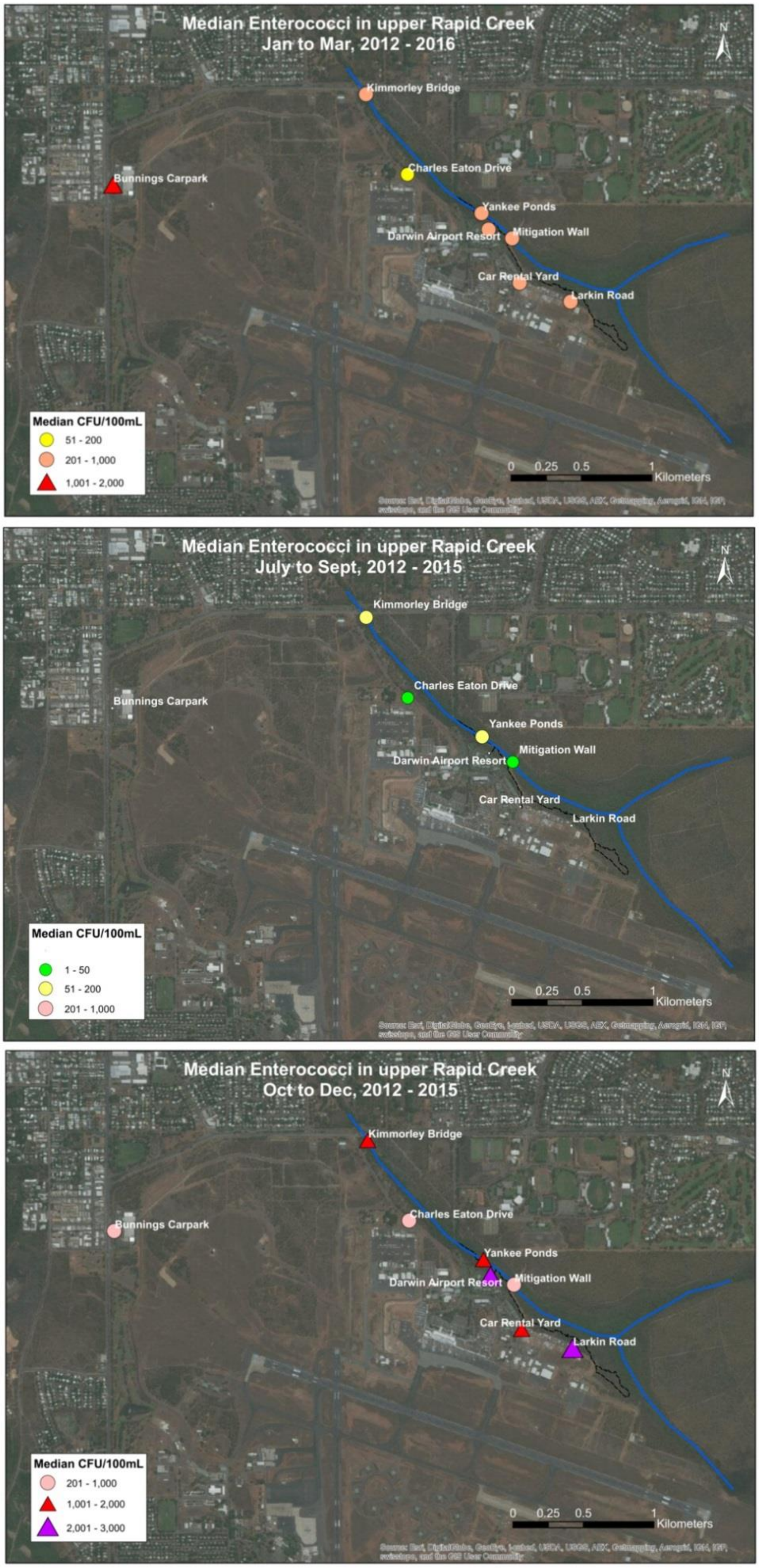


Figure 3 Legend: *"KB" Kimmorley Bridge, "YP" Yankee Ponds, "MW" Mitigation Wall.*
The line in the box indicates the median while the box marks the 25th to 75th percentile of the data.
The whiskers show the data range excluding outliers. The dots mark the actual data points.
The red line indicates the enterococci *exceedance value at 200 CFU/100mL.*

This is consistent with reports of stormwater runoff containing high levels of suspended particles, bacteria and nutrients. All indicators were positively associated with turbidity reflecting suspended solids and bacteria in runoff. In particular, faecal and total coliforms increased during the build-up, which is consistent with land runoff. Faecal and total coliforms are known to contain bacteria which naturally occur and multiply in the environment.

An increase in rainfall beyond the first flush of the wet season was associated with an increase in indicators mainly in the creek itself. This may be attributed to further land runoff not limited to the tested drains, and resuspension from indicators from the creek bed due to increased stream flow.

Figure 4: *Maps of* enterococci *levels in upper Rapid Creek across the seasons*



Enterococci in the Airport Resort stormwater drain

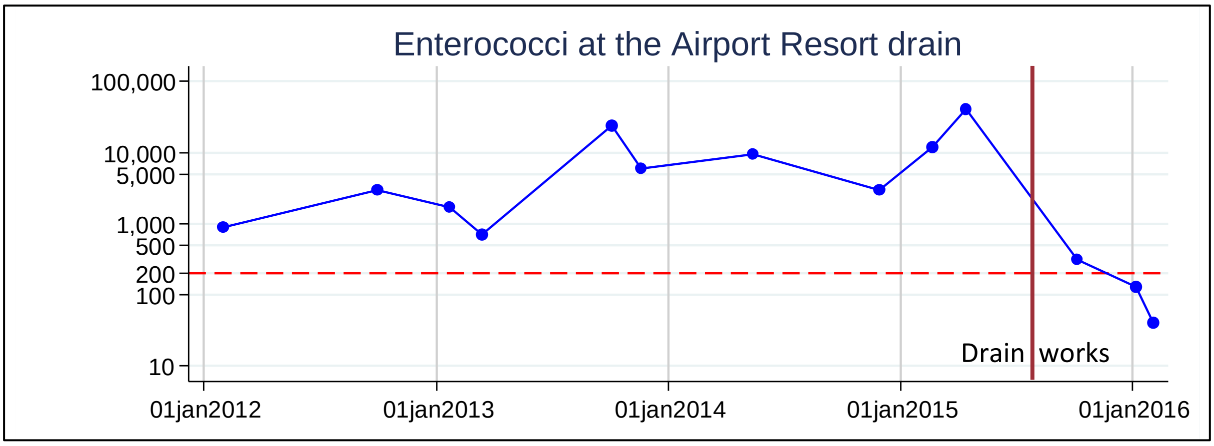
With a geometric mean of 2,041 CFU/100mL, the enterococci levels in the stormwater drain behind the Airport Resort exceeded the enterococci exceedance value of 200 CFU/100mL for recreational water in 10 out of 10 samples collected between 2012 and 2015 (Figure 5). Drains are not considered recreational water and therefore strictly speaking the guideline value does not apply, however these drains empty into Rapid Creek which is a natural waterway and is subject to recreational use.

While human sewage could not be excluded as a cause for these high counts, this could also point to regular visits and faecal matter from dogs, cats, native wildlife including birds, bats or even frogs or potential organic fertilizers or manure used in the Resort gardens.

The water in this drain also had the highest total Kjeldahl nitrogen (organic nitrogen & ammonia) and phosphorus concentration of all sites which might be due to fertilizers or natural sources. Enterococci counts were positively associated with nitrogen, and enterococci might have multiplied in the nutrient-rich water which was also covered with debris and leaves, and protected from sunlight. It has been shown that enterococci can multiply outside the mammalian gut under optimal tropical conditions [4, 5].

Drain works in late 2015 desilting the channel and installing silt traps coincided with a drastic drop in enterococci and *E.coli* counts and future sampling will show whether these works consistently lowered the levels (Figure 5).

Figure 5: *The resort stormwater drain before and after the drain works in late 2015 and the timeline of enterococci counts from 2012 to 2016.*



Enterococci in the Bunnings carpark stormwater drain

Indicator levels incl. enterococci were also elevated in the stormwater drain of Bunnings carpark which is 2-3 km away from all other sites and feeds into Ludmilla creek.


Bunnings carpark stormwater drain after rainfall
Picture by EcOz

This drain had the highest water turbidity recorded of all sites which was likely due to the absence of silt traps and little vegetation groundcover in the vicinity of the drain to trap solids in the stormwater runoff.

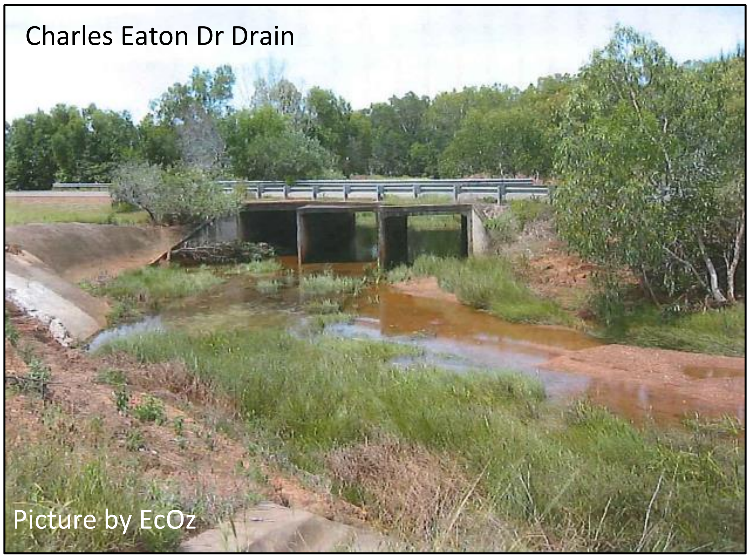
All faecal indicators showed a strong positive association with turbidity and these bacteria are known to attach to dissolved solids [4].

In a multivariate statistical model, the high turbidity explained the elevated enterococci levels in this drain suggesting that mainly land runoff was responsible for the elevated counts at this site.

Enterococci in the Charles Eaton Dr drain

This drain is a large and also partly spring-fed drain with water for most of the year.

Overall, there was no significant difference in enterococci levels in this drain compared to the most upstream control site “Mitigation Wall” in Rapid Creek.


Charles Eaton Dr Drain
Picture by EcOz

Limitations of Bacterial Faecal Indicators

Faecal indicators are routinely used worldwide to monitor microbiological water quality and enterococci are the preferred indicator to date to assess microbiological water quality as per Australian National Health and Medical Research Council (NHMRC) guidelines [6]. However, they are not specific for human faeces. They occur in faeces of warm-blooded animals, they can even multiply in some frogs (our unpublished data) and they can naturalize in tropical freshwater under optimal conditions [5] (Figure 6). Accordingly, the NHMRC guidelines state that its health significance is reduced in waters where animals and/or birds are the primary source of faecal material or in situations where environmental proliferation of indicator bacteria may occur [6].

Figure 6: *The occurrence of bacterial faecal indicators*

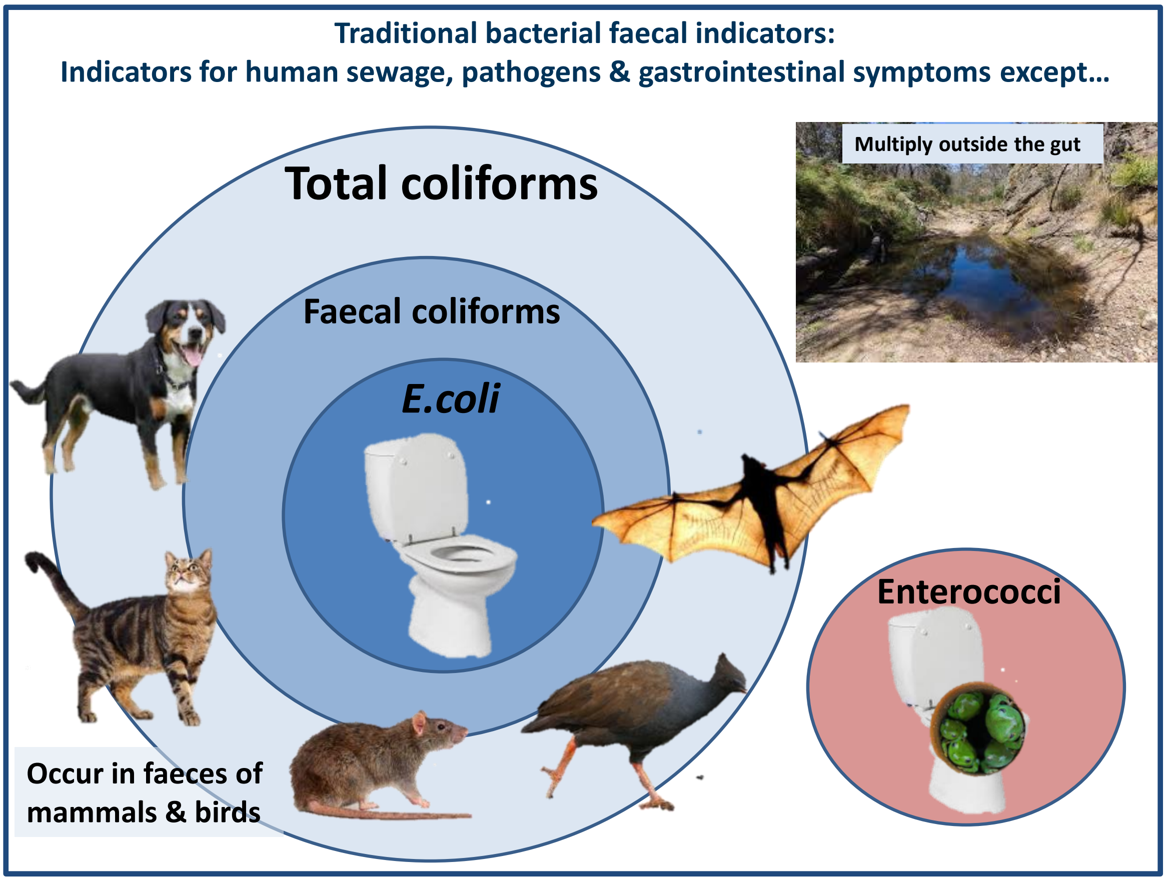


Figure 6 Legend: E.coli *are a bacterial species of the* Enterobacteriaceae *family and a natural part of the gut flora of warm-blooded animals. Only a few strains cause disease. They are a subgroup of faecal (or thermotolerant) coliforms which are part of total coliforms. Enterococci are a bacterial genus of the Firmicutes division. In the past, they have all been used as indicators for human sewage and pathogens, with enterococci now being the preferred indicator by the NHMRC. However, all of these indicators are not human-faeces specific indicators but also occur in faeces of mammals and birds – even some frogs – and are also able to multiply outside the gut under ideal conditions as encountered in the tropics.*

What next?

The large variations in faecal indicator levels between the seasons, the creek sites and stormwater drains match previous reports on faecal indicators in waterways in urban tropical environments [4].

Due to the scarcity of longitudinal data on faecal indicators from other creeks in the Darwin region, it is difficult to assess whether faecal indicator levels in Rapid Creek are significantly higher compared to other urban Darwin waterways.
The sampling of further sites along creeks in the Darwin region could assist in building a reference database of indicator levels reflecting urban land use across different seasons and background levels from areas with less human impact.

To assess the implications of multiple inputs into Rapid Creek such as the impact of stormwater drains from different sources (multiple users), an approach called microbial source tracking using Bayesian statistical techniques is recommended [7].

Human sewage could not be excluded as a cause for elevated enterococci counts. There is a need to include human-specific faecal markers if the real concern is that human sewage is a factor in the microbial quality of Rapid Creek. A combination of indicators likely achieves the best results using conventional enterococci together with molecular detection of human specific faecal markers such as human-associated *Bacteroidales, E.coli* strains, *Bifidobacterium* spp or even other biomarkers such as faecal sterols [8].

Reference List

1. Aquatic Health Unit. Department of Natural Resources, Environment, The Arts and Sport, Northern Territory Government. *Darwin Harbour Region Report Cards 2010*.
2. Northern Territory Government, Darwin Harbour Beach Water Quality Task Force *Sources and pathways of bacteria affecting water quality on Darwin Harbour beaches and waterways: What do we know? What do we need to know.* 2011
3. Northern Territory Government, Technical Report WRD82027: *Report on Rapid Creek Hydrology Studies*. 1982.
4. Rochelle-Newall, E., et al., *A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions.* Frontiers in Microbiology, 2015. **6**: p. 308.
5. Staley, C., G.M. Dunny, and M.J. Sadowsky, *Environmental and animal-associated enterococci.* Adv Appl Microbiol, 2014. **87**: p. 147-86.
6. NHMRC, *Guidelines for Managing Risks in Recreational Water*, Australian Government, 2008.
7. Knights, D., et al., *Bayesian community-wide culture-independent microbial source tracking.* Nature methods, 2011. **8**(9): p. 761-763.
8. Harwood, V.J., et al., *Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes.* FEMS Microbiology Reviews, 2014. **38**(1): p. 1-40.