SOURCE TRACKING OF FAECAL INDICATORS IN NON-SEWERED CATCHMENTS

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1 Abstract

There are approximately 127,000 onsite wastewater treatment and disposal systems in South East Queensland (SEQ). A number of site based audits and surveys undertaken in SEQ and throughout Australia have found that a significant proportion of onsite systems show signs of failure, presenting a potential risk to human and receiving water health. However while audits of onsite systems may point towards systemic performance problems, direct and unambiguous evidence of widespread onsite system discharge to receiving waters is difficult to source.

A number of methods are now emerging to enable the differentiation of pollutants sourced directly from onsite systems in receiving waters to those from other diffuse sources. This paper describes an application of two of these emerging technologies, biochemical fingerprinting and faecal sterols analysis, to unambiguously identify catchment faecal sources from non-sewered catchments in SEQ.

Biochemical fingerprinting and faecal sterols methods have been applied to three non-sewered catchments in Pine Rivers Shire in SEQ. The initial phase of this investigation, dubbed the 'Smoking Gun Study', was designed to detect onsite system derived pollutants in stormflows. Rising stage samplers have been installed and hydrograph recession samples from small rainfall events have been collected and analysed for C_{27} and C_{29} sterols, faecal coliforms, *Escherichia coli* and enterococci. The results of this study pave the way for more detailed investigations aimed at quantifying pollutant loads and export processes from onsite systems and thus enable the quantification of health risk posed by non-sewered catchments.

Keywords: Onsite systems, pollutant source tracking, faecal sterols, biochemical fingerprinting, stormwater quality

2 Introduction

Of the 127,000 onsite wastewater treatment and disposal systems in South East Queensland (SEQ), approximately 102,000 are septic systems and approximately 25,000 are aerated wastewater treatment (AWTS) and other systems (Beal et al 2003). **Figure 1** shows a location map of SEQ shires and the proportion of domestic dwellings serviced by onsite wastewater treatment and disposal systems.

The level of management of onsite systems in SEQ varies from shire to shire and anecdotal evidence suggests widespread system failures (Beal et al, 2003). However a review of the evidence of pollutant export from onsite systems by Gardner (2005) led to the conclusion that a 'smoking gun' (unambiguous evidence of pollutant export) was not evident. This apparent inconsistency between anecdotal evidence of system failure and lack of resultant water quality evidence has prompted the authors to develop a study with the aim of investigating the methods to *identify and quantify* pollutant exports from onsite systems. This study is called the 'Smoking Gun' Scoping Study (officially known as: '*Using faecal sterols and biochemical fingerprinting to*

estimate off-site impacts of onsite wastewater treatment and disposal systems in Pine Rivers Shire').

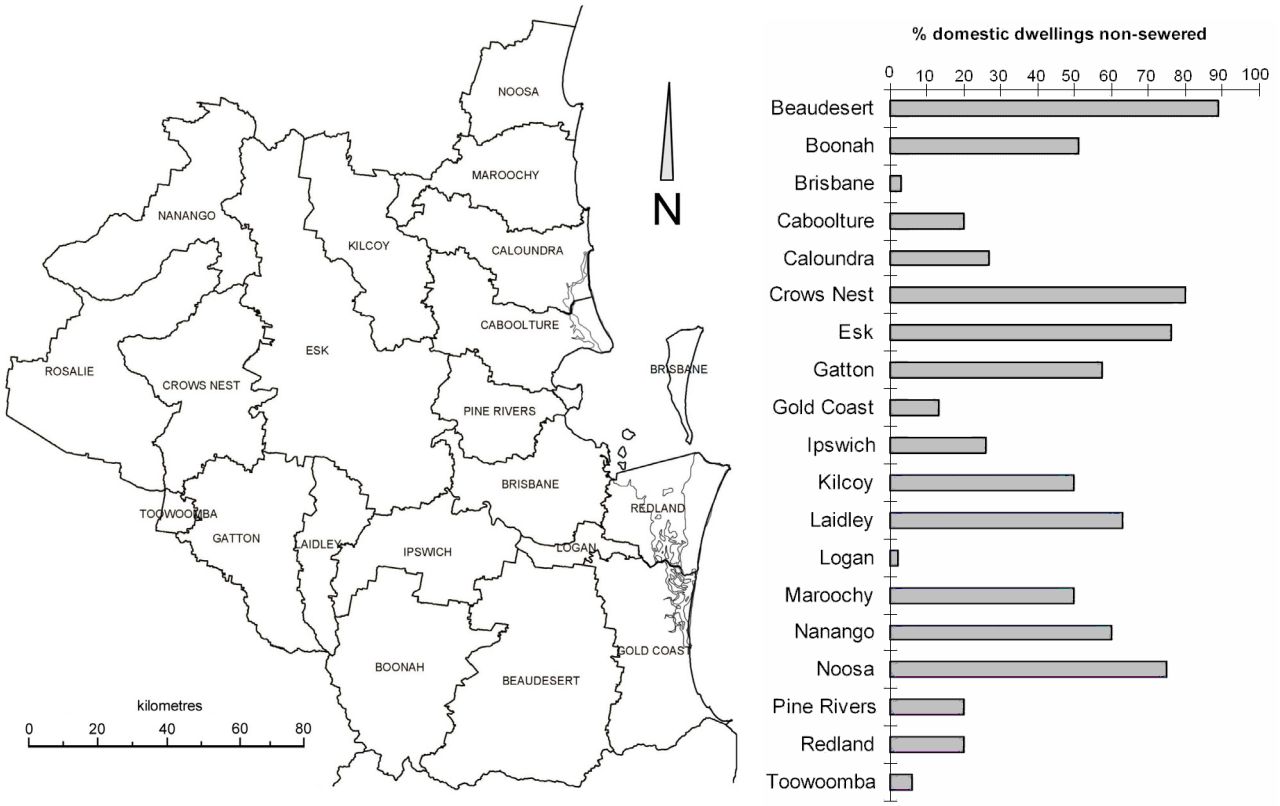

Figure 1. South East Queensland Local Government Shires and proportion of domestic dwellings serviced by onsite treatment systems (Beal et al 2003).

The Smoking Gun Scoping Study is jointly funded by the Moreton Bay Partnership and South East Queensland Catchments and is being undertaken in collaboration between the Queensland Department of Natural Resources, Mines and Water and the University of the Sunshine Coast. The Study is being undertaken in Pine Rivers Shire. A 'sister study', utilising the same analytical techniques, is currently being undertaken in Maroochy Shire by the University of the Sunshine Coast.

Two faecal source tracking methods have been selected to identify human specific faecal contamination in stormwaters from non sewered areas. These methods include Faecal Sterols Analysis (FSA) and Biochemical Fingerprinting (BF) (Ahmed et al 2005a, b). FSA was chosen as it had been widely reported in the literature as a pollution source tracking technique, is reported to have stable source specific ratios (Leeming et al 1996) and can be detected across many orders of magnitude (mg/L-ng/L), thus potentially enabling detection of source specific pollutants even after significant dilution. BF has been selected as it has been previously used in SEQ and enables the source identification of faecal indicators, such as *E. coli* and enterococci, thus potentially enabling the identification of onsite system derived pollutants.

This paper reports on the preliminary findings of this investigation and highlights some of the problems encountered when using pollutant source tracking techniques for catchment monitoring.

3 Materials and Methods

3.1 Catchment selection

Catchment selection for monitoring points was undertaken following the process outlined in **Figure 2**. The catchment short listing process involved a selection of 14 sub-catchments in Pine Rivers Shire using GIS desktop analysis and assessing:

- The number of onsite systems located with a catchment,
- The number of onsite systems located within 100m of a mapped drainage lines
- The weighted compound topographic index CTI (a wetness index) for each mapped onsite system within 100m of a drainage line
- The size of each catchment.

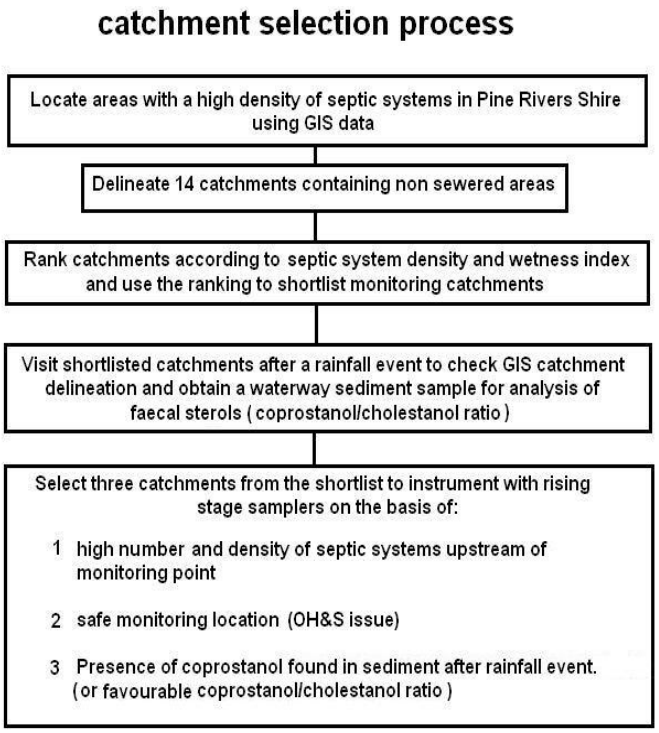

Figure 2. Catchment Selection

We used the density of onsite systems within 100m of a mapped drainage lines in each catchment (onsites per catchment area) and the average CTI of septics within 100m of a drainage line to rank each of the 14 catchments. The highest combined ranking indicates catchments more likely to have failing septic trenches that export pollutants to creeks. This method resulted in the smaller catchments with a large number of onsite systems ranking higher. Six catchments were chosen for further investigation based on rank and catchment size (capturing large numbers of onsite systems) and limited diversity of land uses (catchments dominated by only one or two land uses). Ground-truthing of catchments, including sediment sampling for coprostanol, access and monitoring practicalities resulted in the selection of the final three monitoring catchments.

3.2 Stormwater sampling

The relatively small catchments selected appear to discharge only during storm events and after extended wet periods, therefore water sampling to characterise pollutant exports was focussed on storm events, in particular the rising and falling stages of discharge hydrographs. Rising stage samplers (2.5 L bottles) and hydrograph recession grab sampling were chosen for this scoping study over automatic refrigerated pumping samplers to obtain maximum information gain on a modest budget.

The stormwater sampling approach was to construct rising stage samplers (Hydrological Services, 2004) and install them such that the sample collected is representative of the flow rate during the rising stage. This was achieved by locating the sample intake tubes at set heights determined through creek survey and hydraulic modelling using HECRAS (HEC 2001) to obtain stage/discharge relationship. This approach was integral to the sampling strategy for two reasons:

- A flow weighted composite sample, *representative* of the water quality during the rising stage of a hydrograph was required to attempt to remove ambiguity in results obtained,
- A large volume of sample was required to enable faecal sterols analysis as the concentration of sterols in water are generally very low - the larger the volume collected, the more faecal sterols able to be extracted and lower detection limit achieved

A depth/velocity probe and 0.2 mm tipping bucket rain gauge was installed in one catchment to better understand catchment hydrological behaviour, however vandalism of both pieces of equipment have prevented further use to date.

3.3 Faecal sterol extraction method

Water samples (5-10 L) were filtered through 0.45 micron glass fibre filters. The whole filter with particulates were extracted between methanol (50 mL)/hexane(50 mL) containing cholestane internal standard (2 µg) and sodium carbonate (0.5 g), with tumbling for 24 h. The solvent mixture was transferred to a separating funnel and 50 mL of water added. The hexane phase was retained, and washed with saturated brine (10 mL). The hexane was dried over anhydrous sodium sulphate, then concentrated under nitrogen. The residue was transferred to a 2 mL vial with hexane, and evaporated. The residue was dried in vacuo, (0.1 mBar). The residue was dissolved in hexane (1 mL), and 20 µL trimethylsilylimidazole added. After 3 h incubation, the sample was analysed by GC-MS. Quantitation of sterols was against cholestane IS, and expressed as ng/L water.

3.4 Faecal Sterol analysis

FSA was undertaken by a combination of techniques including the decision support system as presented by Leeming et al (1998), visual inspection of the 'faecalprints' as described by Roser et al (2003) and the more general C27:C29 and 5B:5A ratio comparison (see Section 4.5). Source identification of faecal sterols relies on interpretation of not only the presence/absence of particular sterols but also the ratios between the concentrations of different sterols.

3.5 Isolation of faecal indicators

Serial dilutions were made for all samples and filtered through a 0.45µm pore size (47mm-diameter) nitrocellulose membranes (Advantec, Japan) and placed on m-enterococcus (Difco, UK) and RAPID' *E. coli* 2 (REC 2) with supplement (Bio-rad, USA) agar plates. Plates were then incubated at 37ºC for 48h (for faecal streptococci) and at 44ºC for 24h (for faecal coliform and *E. coli*). The REC 2 medium, used for isolation of *E. coli* is based on the detection of 2 enzyme activities; β-D-glucuronidase (β-gluc) and β-D-galactosidase (β-gal). The hydrolysis of chromogenic substrates results in purple *E. coli* (β-gluc positive/β-gal positive) and blue faecal coliform colonies (β-gluc negative/β-gal positive). The supplement added to the medium inhibits interfering Gram-negative flora, which can be found in wastewater and natural waters. Single purple colonies from this medium were streaked on McConkey agar (Oxoid, USA) for purity and also tested for indole production and citrate cleavage. Indole positive and citrate negative isolates were identified as *E. coli*. All isolates from m-enterococcus plates were also tested for esculin hydrolysis on to Bile Esculin Agar (Oxoid, UK) and incubated at 45°C for 1 h to confirm their identification as enterococci (i.e. black coloration) (Manero and Blanch, 1999).

3.6 Biochemical fingerprinting

The biochemical fingerprinting method (known as 'biochemical fingerprinting with the PhPlate system') has only recently been applied for microbial source tracking in Australia (Ahmed et al 2005a,b). The method involves the isolation of bacteria, such as *E. coli* and enterococci, which are then grown in micro plates containing 8 different growth media and the growth rate is observed over time. The unique pattern of growth is the metabolic fingerprint of the isolated bacteria. The Biochemical fingerprinting method involves the development of a database which characterises the growth rates of thousands of isolated bacteria from known sources, identifying unique bacteria strains from known sources and using the metabolic fingerprints of these unique bacteria to compare with bacteria found in environmental samples.

In this study, we have used two types of micro plates specifically developed for typing of enterococci (PhP-RF plates) and *E. coli* strains (PhP-RE plates) (PhPlate system, PhPlate AB, Stockholm). The growth medium for PhP-RF and RE was prepared according to the manufacturer instructions. To maximize the metabolic fingerprint, from each sample up to 39 colonies of enterococci and 39 colonies of *E. coli* were picked with sterile toothpicks from the agar plates and tested with the PhPlate system (PhPlate AB, Stockholm) (Kühn et al.1991).

An identity (ID) level of 0.96 was established based on the reproducibility of the system after testing 20 isolates in duplicates. Isolates with similarity higher than the ID-level were regarded as identical and assigned to the same biochemical phenotype (BPT). The phenotypic similarity between different bacterial populations in two or more samples was calculated as population similarity (Sp) coefficient. The Sp coefficient calculates the proportion of isolates that are identical in two or more compared bacterial populations. It is high (maximum of 1) if two populations contain similar BPTs and is low (minimum of 0) if the populations contain different BPTs.

3.7 Source tracking database

The host specific metabolic fingerprint database used in the study was developed by testing 4,833 enterococci isolates and 4,508 *E. coli* isolates from 10 host groups. These host groups included humans (via septic tanks and STPs), cattle, horses, chickens, dogs, Kangaroos, waterfowl (including ducks and birds), deer, sheep and pigs.

The representativeness and stability of the fingerprints were assessed prior to this application in a previous cross catchment study and the database was successful to categorize the sources of dominant faecal indicator bacterial contamination in a coastal lake (Ahmed et al. 2006). Since then, the database is being regularly updated by adding more bacterial isolates from different farms from different catchments within the region.

The biochemical fingerprints used in the database were categorized on the basis of occurrence in host groups. The database consists of 308 unique enterococci BPTs and 303 unique *E. coli* BPTs. These unique BPTs were specific to host groups. In contrast, 286 enterococci shared BPTs and 295 *E. coli* shared BPTs were also found among host groups. These BPTs were shared among animal host groups. The latter was also used to identify non-specific animal contribution to the studied creeks.

3.8 Biochemical fingerprinting data analysis

All data handling, including optical readings, calculations of correlations and coefficients, diversity indexes as well as clustering and printing dendrograms, was performed using the PhPlate software version 4001 (PhPlate system, PhPlate AB, Stockholm).

4 Results

4.1 Selected catchments

The three catchments selected through GIS analysis and sediment sampling for the Scoping Study include Bergin Creek, Four Mile Creek and River Oaks Drive in Pine Rivers Shire. The dominant land use in all catchments is residential non-sewered and bushland/open space. Maps of the monitoring catchments showing locations of sampling sites, drainage lines, locations of septic and AWTS systems and allotment densities are shown in **Figures 3-5**.

The catchments range in size from 96 ha to 429 ha. All three catchments are located in undulating landscape and allotments generally feature typical suburban homes on large blocks. Very few allotments have been observed keeping stock animals such as horses or cattle. The drainage lines in all three catchments are characterised with dry creekbeds with occasional natural pools and constructed dams.

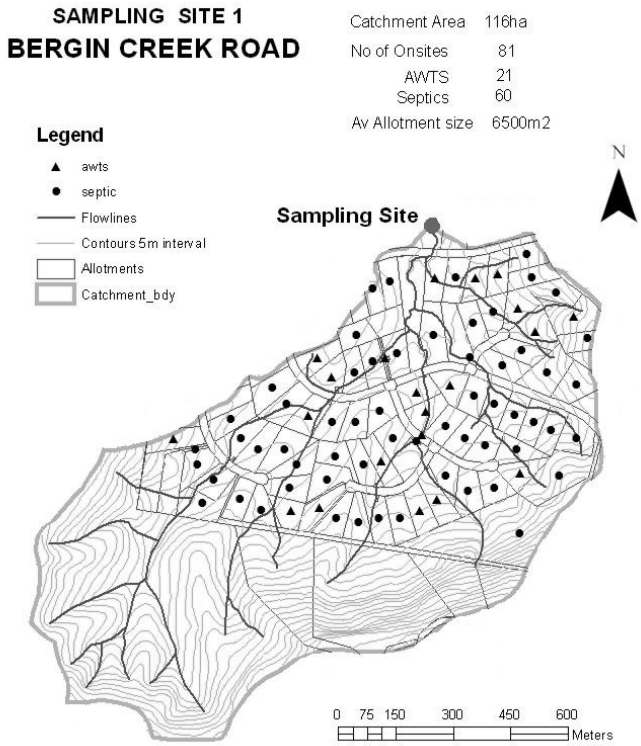

Figure 3. Bergin Creek catchment

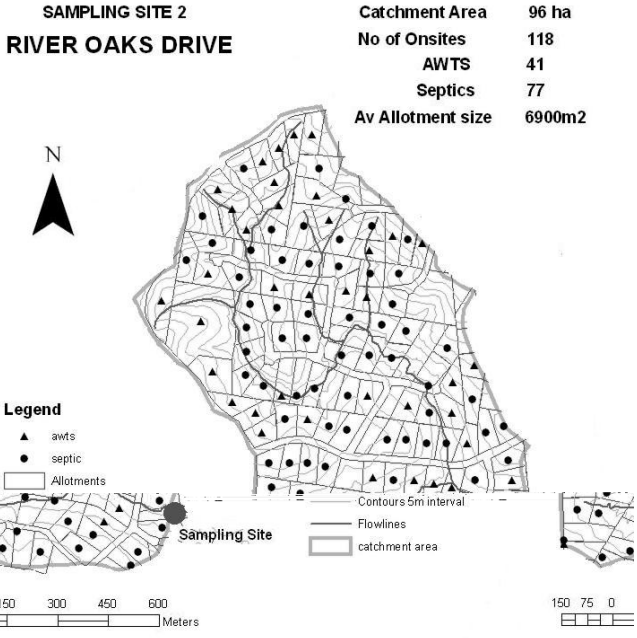

Figure 4. River Oaks catchment

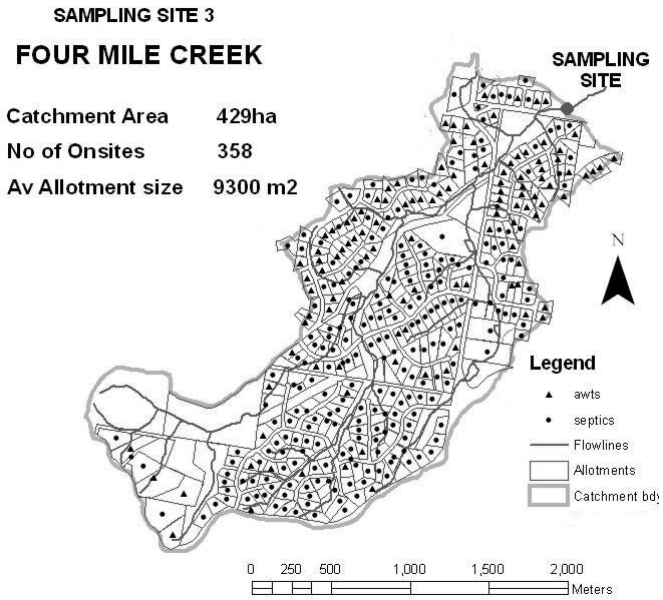

Figure 5. Four Mile Creek catchment

4.2 Water sampling

To date, 10 water samples (grab samples) representing up to 4 separate rainfall events per catchment have been taken. Although rising stage samplers have been installed, no rainfall events have been large enough to trigger them. Without significant rainfall (>15mm), each of the three monitoring catchments do not show signs of significant runoff due to low proportion of impervious surfaces in the catchments. The dates when water samples were collected from each catchment and corresponding rainfall depths from the nearest rain gauges are shown in **Table 1.**

Table 1. Sampling dates and rainfall.

Date	Rainfall*	catchment
20/1/2006	95mm	Four Mile Creek
	62mm	River Oaks Drive
	59mm	Bergin Creek
Rising stage samplers installed		
22/2/2006	15mm	Four Mile Creek
	30mm	River Oaks Drive
28/2/2006	18mm	Four Mile Creek
	19mm	River Oaks Drive
	32mm	Bergin Creek
4/3/2006	26mm	Four Mile Creek
	22mm	River Oaks Drive

*rainfall depths are approximate, were not quality controlled and are from the nearest gauge to the catchment (typically 1-4km distant).

4.3 Water quality

Table 2 contains the biological and faecal sterol results for the 4 storm events.

Table 2: Water quality data obtained from hydrograph recession grab sampling

Faecal indicators	Four Mile Creek				River Oaks				Bergin Creek	
Sampling Date	20/1/06	22/2/06	28/2/06	4/3/06	20/1/06	22/2/06	28/2/06	4/3/06	20/1/06	28/2/06
Faecal Coliforms (cfu/100ml)	23,020	10,413	14,516	3,050	43,060	31,060	9,600	4,880	26,700	24,550
E. coli (cfu/100ml)	2,680	480	1,250	1366	3,529	4,360	1,200	1675	3,700	3,550
Enterococci (cfu/100ml)	4560	750	3,320	1306	3280	1,950	3,700	866	3520	5,950
Faecal Sterol (ng/L)										
Coprostanol C27, 5B	14	<10	<10	<10	13	<10	<10	<10	<10	33
Cholesterol C27	3627	503	301	222	805	394	254	222	702	209
5a-Cholestanol C27, 5A	94	34	28	23	73	56	25	23	47	21
24-Ethyl coprostanol C29, 5B	<10	17	<10	<10	<10	<10	<10	<10	<10	<10
24-Ethyl epicoprostanol	<10	15	10	<10	<10	<10	<10	<10	<10	<10
Stigmasterol	3418	320	275	150	1336	617	254	154	622	163
Sitosterol C29	638	279	297	162	792	746	245	62	668	208
Sitostanol C29, 5A	152	45	57	29	21	130	36	29	<10	47
Sterol Ratios										
ΣC27/ ΣC29	4.73	1.60	0.92	1.27	1.10	0.52	0.98	2.59	1.12	1.00
Σ5B/ Σ5A	0.06	0.33	0.15	0.17	0.14	0.09	0.16	0.17	0.00	0.62

4.4 Biochemical fingerprinting

Biochemical fingerprinting of *E. coli* and Enterococci isolates obtained from water samples from the non-sewered areas identified bacteria classified by the database to have *unique* signatures found only in humans. Up to 23 isolates of *E. coli* and enterococci were tested from each water sample. **Table 3** presents the breakdown of total number of biochemical phenotypes found in each sample, those identical and unique to human, animal, shared and unclassified for each sample (at 96% confidence level). Overall, the database used was able to identify approximately 60% of the *E. coli* and enterococci isolates obtained from stormwater samples. Above 50% classification is considered to be a successful application (Warish Ahmed, pers comm.) and the highest obtainable classification rate expected is around 75-80% (Ahmed et al 2006)

Table 3. Biochemical Fingerprinting classification results from stormwater samples

Catchment	Indicator bacteria	Water samples tested	Total isolates tested	Total BPTs found	Unique Human BPTs (%)	Unique Animal BPTs (%)	Shared Animal BPTs (%)	Unknown BPTs (%)
Four Mile Creek	enterococci	4	154	54	1 (2%)	14 (26%)	18 (33%)	21 (39%)
	E.coli		120	68	0 (0%)	16 (24%)	20 (29%)	32 (47%)
River Oaks	enterococci	4	155	68	8 (12%)	19 (28%)	24 (35%)	17 (25%)
	E.coli		130	68	6 (9%)	13 (19%)	21 (31%)	28 (41%)
Bergin Creek	enterococci	2	78	33	6 (18%)	6 (18%)	4 (12%)	17 (52%)
	E.coli		66	37	7 (19%)	5 (14%)	7 (19%)	18 (49%)
Total		10	**703**	**328**	**28**	**73**	**94**	**133**

4.5 Comparing Biochemical Fingerprinting and faecal sterols results

The water quality results show high counts of faecal bacteria, but very low concentrations of some faecal sterols, such as coprostanol. The Biochemical fingerprinting results show unique human biochemical phenotypes, but typically 1/2 of the identified biochemical phenotypes were

found to be of animal origin. With such low concentrations of faecal sterols found, meaningful interpretation via ratio analysis could be highly erroneous, therefore only the most simplistic interpretations have been used for the data collected thus far.

Leeming et al (1998) reports that the main differences between faecal sterol composition from different animal sources are the relative abundance of the 5A and 5B sterols and the C27 and C29 sterols (see Table 3 for which sterols are which). ΣC27: ΣC29 and Σ5A: Σ5B ratios for a number of animals, humans and septic tanks found in previous studies are shown in **Table 4**.

Table 4. C27:C29 and 5B:5A sterol ratios for selected faecal sources

Animal type	Ratio*			Animal type	Ratio*	
	C27:C29	5B:5A			C27:C29	5B:5A
[1]Pig (n=6)	0.88	3.99		[1]Seagull (n=3)	1.39	1.37
[1]Cow (n=6)	0.83	1.42		[1]Cat (n=5)	2.77	3.71
[1]Sheep (n=6)	0.57	2.26		[1]Human (n=6)	2.66	35.98
[1]Possum (n=3)	0.42	13.97		[2]Human (n=13)	3.27	17.62
[1]Horse (n=6)	0.30	1.79		[3]Septic tank with sludge (n=17)	2.25	33.13
[2]Kangaroo (n=20)	0.51	3.58		[3]Septic tank clarified (n=3)	3.12	12.19
[2]Rats (n=23)	0.79	3.12				
[1]Hen (n=6)	0.51	0.67		[1]Duck (n=1)	2.35	0.34
[2]Chicken (n=10)	0.69	0.05		[1]Dog (n=7)	4.96	0.06
				[2]Coastal birds (n=19)	5.13	0.04

Notes: *ratio derived from mean of reported samples, [1]Leeming et al (1996), [2]Leeming et al (1998), [3]Roser et al (2006).

The ratios presented in **Table 4** have been compared to the ratios found in water samples taken from this study (**Table 3**) by presenting them in the quadrants representing the possible ratio combinations greater than or less than 1 (**Figure 6**). This simplistic analysis is based upon the Principle Components Analysis presented in Leeming et al (1998) and differentiates herbivore/human/bird sterol profiles with only a few exceptions (such as seagulls).

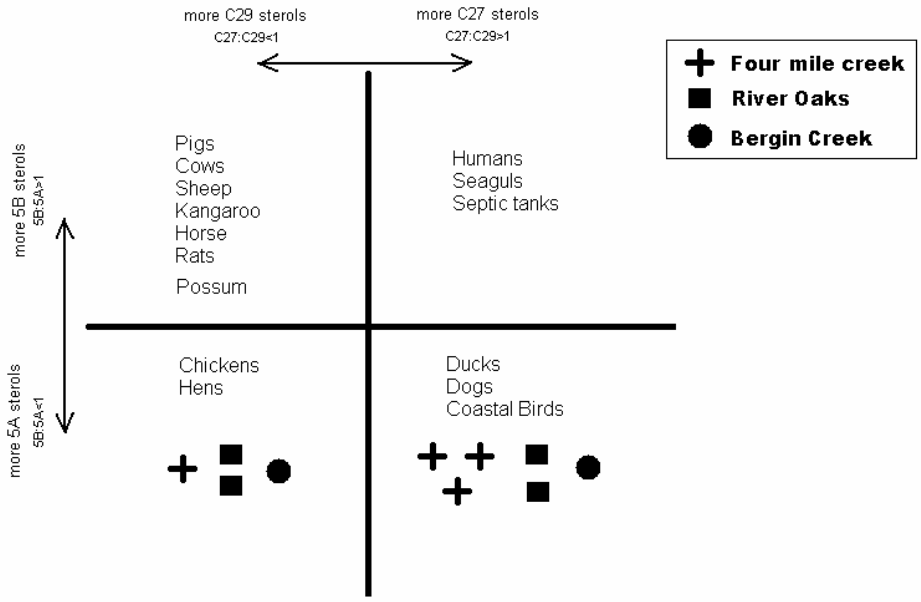

Figure 6: Graphical interpretation of Smoking Gun Scoping Study faecal sterols data using ΣC27: ΣC29 and Σ5A: Σ5B sterol ratios.

Figure 6 shows that using the ΣC27: ΣC29 and Σ5A: Σ5B sterol ratio interpretation, the Pine Rivers water samples indicated herbivores, and humans/septic tanks are *not* the likely source of faecal contamination. The very low concentrations (near the detection limit) of the 5B sterols

also indicated that if human contamination exists it is in very low concentrations. The entire sterol profile from the collected water samples appeared to be more consistent with birds and perhaps dogs being the dominant source of the faecal sterols.

The unique animal biochemical phenotypes found through biochemical fingerprinting accounted for between 15-30% of the total biochemical phenotypes found. A breakdown of these unique animal phenotypes is given in **Table 5**. Animal groups have been presented to be consistent with the general animal groupings of the faecal sterol data (Birds and dogs vs herbivores vs humans).

Table 5. Animal specific classifications found using biochemical fingerprinting

		Phenotypes observed by category					
		Unclassified	Shared animal	Bird and dog	human	herbivore	**Total**
Four Mile Creek	Enterococci	21	18	10	1	4	**54**
	E.coli	32	20	14	0	2	**68**
River Oaks Drive	Enterococci	17	24	13	8	6	**68**
	E.coli	28	21	11	6	2	**68**
Bergin Creek	Enterococci	17	4	4	6	2	**33**
	E.coli	18	7	5	7	0	**37**
Total		**133**	**94**	**57**	**28**	**16**	**328**

For the three non sewered catchments, the biochemical fingerprinting data has generally classified the greatest proportion of biochemical phenotypes to be unknown>shared animal > birds and dogs>humans>herbivores.

When looking at the biochemical phenotypes that were able to be classified, the biochemical fingerprinting data appeared to be consistent with the faecal sterols data in classifying faecal contamination from birds and dogs group. However the two methods did not appear to be consistent in classifying the human component detected using biochemical fingerprinting.

5 Discussion

The authors acknowledge that more samples are required from storm events and catchment faecal sources to draw conclusions as to the faecal pollution contribution to creeks and waterways from onsite wastewater treatment and disposal systems. This study is the first application of the biochemical fingerprinting and faecal sterols source tracking methods on the same catchments.

Biochemical fingerprinting

Of the 10 samples obtained, 8 contained at least 1 biochemical phenotype found to be unique to humans. Of the 328 biochemical phenotypes found through water sampling, a total of 28 unique human biochemical phenotypes were detected. There is always a chance, through further database development, that these unique phenotypes may become shared with another animal type, however with over 9,000 isolates of *E. coli* and Enterococci already catalogued, the chances of this happening for all 28 phenotypes is minimal.

As a control, the biochemical phenotypes found in water samples were also compared to host groups believed not to be present in the catchments (i.e. not identified as a potential contributor during sampling) selected including kangaroos, deer, sheep and pigs (the catchments have limited 'bushland' and block sizes restrict the keeping of many farm animals, other than the odd horse). Only 2 of the 328 biochemical phenotypes found came from the kangaroo, sheep, deer and pig categories, providing confidence that the database does not appear to be misclassifying isolates. A total of 16 unique herbivore phenotypes and 57 bird and dog phenotypes were observed. (**Table 4**)

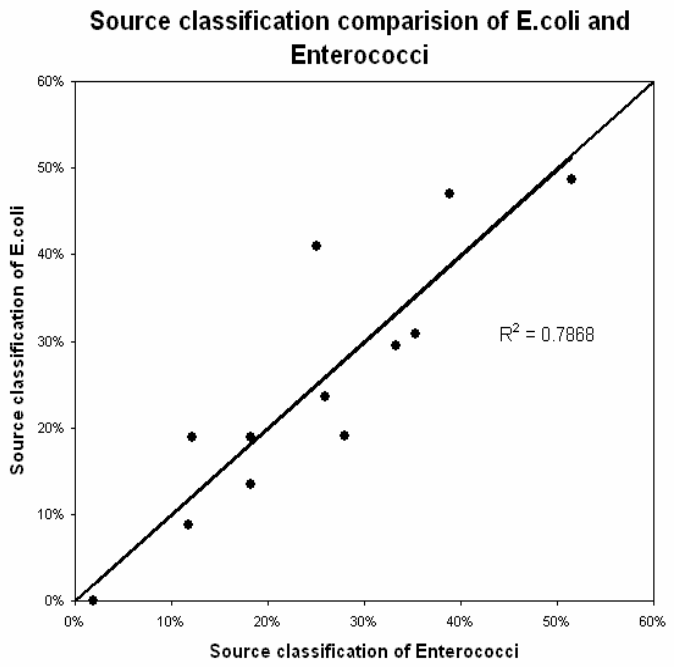

Figure 7. Source classification comparison.

Figure 7 shows agreement (R^2=0.79) in the source identification between the enterococci and *E. coli*. databases. This too provides confidence that the sources of the indicator bacteria are correctly classified.

As **Table 5** highlights, 40% of faecal indicator bacterial types could not be sourced to any host group by the database. Although this identification level is satisfactory, more bacterial isolates from various host groups present in the study catchments could be included in the database to increase the identification level. Undertaking this step is now part of the study plan and may greatly increase the confidence of the technique for the study catchments.

Faecal sterols and comparison of source tracking methods

Only very low concentrations of the faecal sterols found in high proportions in humans (coprostanol) were detected in the water samples, indicating that faecal material from humans was either not present in the water samples or diluted to such an extent as to not be detectible. At very low concentrations, approaching the reporting limit of 10ng/L, interpretation of ratios for faecal source identification becomes problematic, therefore only general ratios have been used for interpretation of the data.

Almost all of the faecal sterol data collected to date points to bird and dog faecal matter, rather than humans, septic tanks or herbivores. This is largely in agreement with the biochemical fingerprinting results, with the exception of the human signature presence.

The inconsistencies between the two source tracking methods may be due to the export, transport, treatment and in-steam retention processes of faecal material from onsite systems being different for faecal sterols and indicator organisms.

Future directions

This study is only in the very early stages and further sampling of non sewered catchments will be continued into 2007. The use of two unrelated faecal source tracking methods has perhaps raised more questions than would have been raised if only one method was selected, highlighting the difficulties surrounding the use and interpretation of faecal source tracking tools. More targeted sampling has been planned to address these questions including:

- Sampling faecal sources within study catchments for *both* faecal sterols and biochemical fingerprints to compare water sample profiles with known catchment sources
- The high population similarity (sp) found between the creeks using biochemical fingerprinting (data not shown) is an indicator that the soil may be a source of the indicator bacteria. Sampling of creekbed sediments to identify faecal bacteria reservoir potential should clarify if this is the case.
- Database independent methods including human specific bacteroides (i.e. HF 183 and HF 134) and esp faecal marker are being explored in conjunction with biochemical fingerprinting methods to confirm human signature.

- Survey/auditing of onsite systems within study catchments has been planned to provide accurate geo-referencing (for distance to drainage line assessment) and condition of onsite systems to indicate pollutant export potential.

6 Conclusions

The Smoking Gun Scoping Study forms part of ongoing investigations by DNRM&W (Gardner et al. 2005, Gardner 2005) to quantify pollutant exports from onsite systems in non-sewered catchments, and forms the first stage in catchment monitoring work to achieve this aim. This study is aimed at developing the techniques to allow the quantification of pollutant loads and export processes from onsite systems and thus enable the quantification of environmental/health risk posed by non-sewered catchments

Faecal sterols and biochemical fingerprinting methods were chosen for this scoping study to identify faecal pollution sources. The combination of these two techniques could potentially identify the faecal bacterial indicator source and the associated pollutant concentration

Of the samples from the three non-sewered catchments collected to date, the bacterial load appears to be relatively high with faecal indicator bacteria *E. coli* and enterococci ranging from 400-6000 cfu/100ml. The biochemical fingerprinting of these bacteria has identified biochemical phenotypes of *E. coli* and enterococci as being unique to human sources accounting for up to 19% of phenotypes identified. Faecal sterol analysis of the same water samples has not shown any significant human/septic characteristics with concentrations of coprostanol found to be below reporting limits on most occasions.

The results of the faecal sterol analysis indicate birds and dogs as potential faecal sources. The biochemical fingerprinting is in agreement with this conclusion finding the largest portion of unique identified phenotypes originating from birds and dogs.

The apparent (in)consistencies found so far with the different faecal source tracking methods demonstrates the need to use a combination of techniques to be sure of the results obtained. Further stormwater sampling and catchment faecal source identification will be continued to address these inconsistencies.

Finally, we found that the application and interpretation of faecal source tracking techniques used in this investigation involved a steep learning curve. With many faecal source tracking techniques not yet out of the research domain, straight forward and consistent interpretations may not always be possible.

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