Estimating Non-Point Source Contaminant Loads using Faecal Sterols, Bacterial Indicators and Hydrology

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Abstract
Non-Point Source (NPS) faecal pollution is a major concern for the management of drinking water reservoirs and other receiving waters. Two major types impacting Australian surface waters are septic tank/absorption trench overflows from un-sewered urban areas, and domestic animal faeces. Management is difficult because by its nature NPS pollution is diffuse, hard to quantify and associate with its possible source(s), and difficult for the public to conceptualise. For example while on-site systems are known to frequently 'fail' on strict technical grounds, the scale of this failure is not well documented and the need for remediation is not well defined. This impasse in now being addressed by the development of 'source tracking' technologies. This paper describes the results of one promising approach trialled using data from two Australian drinking water catchments under high and low flow conditions. By combining concentration and flow data total contaminant load flux rates were estimated. Next, based on faecal sterol ratios, 'fingerprint' profiling and land use information human and/or herbivore faecal pollution was identified and relative proportions determined.

Under dry conditions the quantities of septic tank liquid and manure discharged to the two streams were minor. Under high run-off event conditions (50 and 30 mm rainfall in 24h) however the loads of bacterial indicators and faecal sterols indicated contamination of run-off with *ca* 1 megalitre of settled septic tank liquid (urban) and *ca* 4 tonnes of manure (agricultural catchment). Reconnaissance sampling indicated the septic tank liquid probably originated from across the urban catchment, while the majority of the manure came from a 1 km reach of the agricultural catchment's main stream.

The data generated confirmed the need/value for reticulated sewage in the urban catchment. The agricultural catchment data indicated that future management should be focused on properties in the vicinity of the emission zone. In respect to source tracking technologies, our work suggested there is a need for such analyses to be more closely integrated with other catchment profiling approaches.

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Introduction

Non-Point Source (NPS) faecal pollution is a major concern for the management of source drinking waters and other receiving waters. Two significant types impacting Australian surface waters are septic tank/absorption trench overflows from un-sewered urban areas, and domestic animal faeces. Management is difficult because by its nature NPS pollution is diffuse, hard to quantify and associate with its possible source(s), and difficult for the public to conceptualise. For example while on-site systems are known to frequently 'fail' on strict technical grounds, the scale of this failure is not well documented and the need for remediation is not well defined.

A range of water analysis technologies currently exist with which the source of such pollution can in theory be characterised which are collectively known by such names 'Microbial Source Tracking and Tracing Tools' (Rochelle and De Leon, 2005; Seurinck *et al.* 2005; Scott *et al.* 2002). Recommended tools include library-based (meaning very large local database required [e.g. Wiggins *et al*., 2003]) for ribotyping or PCR targeting repetitive genes of bacterial indicators, antibiotic resistance analyses, and biochemical fingerprinting of indicator isolates. Non library-based approaches recommended include PCR identification of the Bacteroidales group of anaerobic gut bacteria (Shanks *et al.*, 2006), faecal sterols (Leeming *et al.*, 1998) and bile acid characterisation (Bull *et al.* 2002). The concept is that by using one or more of these microbiological, genotypic, phenotypic, and chemical analytes "the origin of fecal pollution can be traced" (Scott *et al.* 2002*)*. Outputs of this process are usually a 'count' of target bacteria (e.g. antibiotic resistant *E. coli*) or a source contribution breakdown e.g. %duck, %cow, %human etc. statistics.

What is less clear are how these output measurements:

1. can account for water quality variability and measurement uncertainty;
2. relate to the underlying problems of unacceptable pathogen risks and faecal matter loads;
3. can quantify the absolute scale and circumstances of contamination;
4. can work where there are multiple point or non-point sources; and
5. support better environmental management and remediation and address the question of whether in a given situation remediation is actually warranted.

With the rapid growth of Queensland's urban population the State's Department of Natural Resources and Management is interested in these latter issues and is investigating a number of potential tracking technologies with a view to quantifying NPS pollution. The aim is to identify one or more methods which will not only be quantitative, but affordable and feasible for local government use, generate analysis outputs easily communicated to catchment stakeholders, and contribute to an overall catchment profiling that can underpin allocation of resources to faecal emission control.

With the above in mind the integration of source tracking and tracing technology with catchment land use and hydrological data was investigated on two catchments as part of a larger drinking water study (Roser *et al.* 2003). Our contention was that source tracking technologies should not be used in isolation but should be integrated with other catchment assessments. Operationally the approach trialled was to adapt the concept of contaminant loadings (USEPA, 2001). Faecal sterol (a source tracking technology) and microbial indicator measurements were sequentially

combined with flow data and land used information to estimate the scale and convincingly identify likely sources of contamination and hence the need for remedial intervention.

Materials and Methods

The scheme used to collect and interpret the faecal contamination data is summarised in Figure 1.

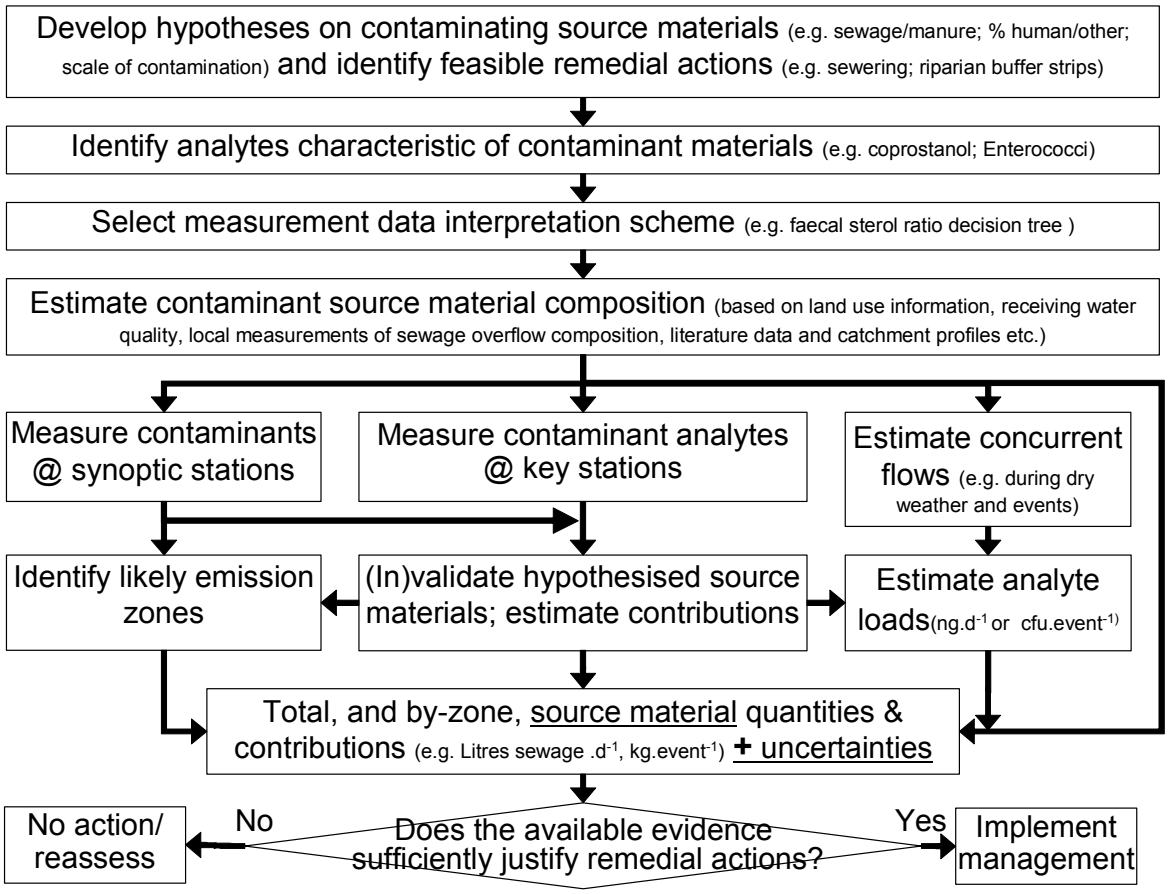

Figure 1. Scheme for Collecting and Interpreting NPS Data

Two surface source water systems were selected from six available for analysis on the basis of they're being likely to be impacted by the two NPS contamination forms of interest. They were a predominantly urbanised catchment of 1000-2000 dwellings in the early stages of having a full reticulated sewer installed, and an intensively utilised agricultural catchment. The urban catchment's wastewater disposal was still dominated by septic tanks. The agricultural catchment was dominated by cattle grazing and dairy (86%) and had no towns or villages above the main water quality monitoring station. The study catchments drained areas of 8 km^2 (urban) and 77 km^2 (agricultural) respectively above their key monitoring stations.

Methods for water sample collection, in particular high flow event sampling have been previously described in Roser *et al.* (2002), and sterol analysis in Leeming *et al.* (1998) and Roser *et al.* (2003). Analyses of *Escherichia coli,* Enterococci and *Clostridium perfringens* were undertaken using standard methods under contract by the Australian Water Quality Centre Bolivar South Australia and Pathcentre Western

Australian. Land use surveys were undertaken by the South Australian Environment Protection Agency. Hydrological data were provided by the South Australia Department of Water Resources.

Results

The rationale for our urban catchment analysis was that septic systems in the area were likely to be leaking, overloaded or in need of replacement but the extent and hence need for a reticulated sewer was not clear. In the case of the rural catchment it was suspected that cattle faeces or manure were entering the main water course but the scale of contamination was not clear.

Contaminant Source Materials

The first step in quantifying faecal contamination loads was to obtain estimates of the source material composition. Estimates of the faecal sterol and indicator concentrations in cow manure were available from a previous study (Leeming *et al.*, 1998 in Table 3). However, the composition of the septic tank overflows was unclear. Accordingly analyses were undertaken of the composition of liquid which could be drawn from the access ports of septic tanks after careful removal of floating solids. Septic systems sampled were located in South Australia with the study catchment and in urban Western Australia. A third source class of septic tank liquid analysed was the contents of a small bore sewerage system servicing a community located within 10 km of both the study catchment study catchments. This community had been serviced initially by septic tanks connected to leachfields. But subsequently the leachfields had been replaced by a small bore common sewer which transported the settled septic tank liquid to an oxidation pond some kilometres distant. Within this system there were three pumping stations where septic tank liquid, composited from 120, 303 and 665 dwellings, could be collected.

The composition of three septic tank liquid sample sets is shown in Table 1. The individual septic tank liquid composition varied enormously with analyte concentrations being lognormally distributed and ranging typically over three orders of magnitude. The reason for this variability was probably the varying extent of accumulation of solids in individual tanks. The communal discharge material in contrast was remarkably consistent in composition especially in respect to faecal sterols. Despite this variability in 28 of 29 samples there was a coprostanol:cholestanol ratio >0.5, coprostanol:cholesterol ratio >0.1 and a coprostanol:24ethyl-coprostanol ratio >0.8 consistent with the human source. Most importantly the composited samples, representing the averaged contents of 2016 septic tanks (Data set 3) had a faecal profile consistent with 100% human faecal matter, consistent with the non-library requirement, i.e. no need to calibrate with site-specific sterol data.

Of the three data sets the communal discharge pipeline samples were viewed as likely to be the most representative of overflow material and because of the large number of households in effect sampled. This said it is important to recognise that this material is still not strictly identical to septic liquid reaching a stream during a storm as failure can also involve filtration by grass swales following surfacing, accelerated transport through the surficial groundwater, or mobilisation of some solids from leachfield pipes.

Table 1. Faecal Sterol and Indicator Content of Septic Tank Liquid

Analyte	Units	Septic Tank Liquid Source		
		Access Chambers SA (data set 1, n=17)	Access Chamber WA (data set 2, n=5)	Communal discharge pipeline SA (data set 3, n= 3 to 6)
E. coli [1]	mpn. 100 mL^{-1}	1,700,000 [0.41]	980,000 [1.29]	370,000 [0.55]
Enterococci [1]		3200 [1.13]	18,000 [1.27]	51,000 [0.79]
Clostridium perfringens [1]	cfu. 100 mL^{-1}	280,000 [0.89]	2200 [1.84][3]	25,000 [0.18]
Coprostanol [1]	ng.L^{-1}	4,100,000 [0.90]	52,000 [0.99]	60,000 [0.11]
24-ethyl Coprostanol [1]		1,400,000 [0.95]	8100 [0.90]	29,000 [0.19]
Cholesterol [1]		1,400,000 [0.93]	46,000 [0.90]	100,000 [0.07]
Sitosterol [1]		480,000 [1.01]	4000 [1.41]	22,000 [0.15]
Cholestanol [1]		110,000 [0.92]	3400 [0.85]	5300 [0.26]
Sitostanol [1]		56,000 [1.00]	1200 [1.34]	2000 [0.19]
Coprostanol: cholestanol[2]	ng.ng^{-1}	38 [0.35] (6.6)	14 [0.8] (0.86)	11.4 [0.19] (6.0)
Coprostanol: cholesterol[2]		3.0 [0.47] (0.60)	0.98 [1.5] 0.004[4]	0.60 [0.08] (0.46)
Coprostanol: 24ethylcoprostanol[2]		3.0 [0.25] (0.88)	7.1 [0.41] (1.9)	2.1 [0.09] (1.58)

Notes:

1. Data shown are geometric mean, Standard deviation of $\log_{10}$ transformed values [square brackets].
2. Data shown are geometric mean, Standard deviation of $\log_{10}$ transformed values [square brackets] and the minimum ratio value (curved brackets).
3. Due to methodology limitations *C. perfringens* only detected in 2 tanks. However all samples contained high concentrations of sulphite reducing clostridia (geometric mean 180,000 cfu.100mL^{-1}).
4. Minimum value due to a single outlier.
5. In data sets 1 and 2, tanks were each sampled once, and for the communal pipeline the sterol samples were collected on two occasions.

Streamwater Quality and Quantity

The second need for our assessment was for estimates of receiving water quality. Because both catchments were relatively small their hydrographs were well defined and following cessation of rainfall they returned to baseflow within 24 to 48 hours. As a result it was possible to collect sample sets representing dry weather conditions and wet weather events (Table 2). While dry weather sample quality was straightforward to summarise run-off events varied in form considerably. For example the seven events sampled in the urban catchment rainfall ranged from 10 to 50 mm of and total flow from 10 to 65 megalitres over a period of < 24 h. So in the present instance we selected two of the largest events for which data was available when septic overflow or wash off of manure would be most likely and impacts greatest.

It can be seen in both catchments that despite the increased dilution during high run-off periods, the concentration of all contaminants was greatly increased. This increase was observed in all run-off events, emphasising how different contamination patterns under dry and wet weather were. A cursory calculation of Table 2 shows an increase in faecal contaminant loads of some 10^3 to 10^4 fold during high run-off periods, irrespective of the analyte used to assess the increase.

Table 2. Faecal Sterol and Indicator Concentrations in Stream Water

Analyte	Units	Urban Catchment		Agricultural Catchment	
		Dry Weather	Run-off Event	Dry Weather	Run-off Event
E. coli [1]	mpn. 100 mL^{-1}	450	13700	210	28000
Enterococci [1]		200	16200	91	8500
Clostridium perfringens [1]	cfu. 100 mL^{-1}	22	2200	1.0	136
Coprostanol [1]	$ng.L^{-1}$	5.8	890	13	700
24-ethyl Coprostanol [1]		18	640	88	3600
Cholesterol [1]		270	5300	470	2900
Sitosterol [1]		210	18400	490	4900
Cholestanol [1]		10	450	35	450
Sitostanol [1]		9.5	790	57	1900
Coprostanol: cholestanol[2]	$ng.ng^{-1}$	0.58	1.98	0.37	1.57
Coprostanol: cholesterol[2]		0.02	0.17	0.03	0.24
24ethyl Coprostanol: sitostanol[2]		1.89	0.81	1.54	1.91
24ethyl Coprostanol: sitosterol[2]		0.09	0.03	0.18	0.74
Coprostanol: 24ethylcoprostanol[2]		0.32	1.39	0.15	0.19
Source		0% human	62% human	100% herbivore	100% herbivore
Rainfall	mm	0	50	0	30
Flow	m^3.	654 day^{-1}	65,000 . $event^{-1}$	9400 day^{-1}	165,000 $event^{-1}$

Notes:
1. Geometric mean of measurements taken during dry weather.
2. Flow weighted average mean of high flow run-off event.

In addition to providing estimates of general faecal contamination the faecal sterol data showed other important features. Firstly the concentrations of the beta stanols (coprostanol and 24-ethylcoprostanol) were present in concentrations indicating human or herbivore contamination (e.g. coprostanol:cholestanol ratio >0.5 during high flow periods and 24-ethylcoprostanol:sitostanol ratio >0.5 in all samples).

In the urban catchment during the high load high flow period the coprostanol:24-ethylcoprostanol ratio (1.4) indicated a high proportion of human faecal matter was present, consistent with septic tank overflows. Septic leachate is much more infectious for human beings than non-human faecal sources and its presence is the driver for management.

In contrast, the agricultural catchment coprostanol:24-ethylcoprostanol ratio was only 0.15 to 0.19, consistent with herbivores being the primary faecal matter source. In contrast to the urban catchment there was clear faecal matter contamination during both dry and wet weather.

Faecal Matter Loads

The third task was to integrate the source material, water quality and hydrology information to estimate the total loads mobilised during dry and wet weather in the two catchments (Table 3). As management is about controlling the primary waste material the analyte data were transformed into estimates of septic tank liquid and wet manure. The range of analytes measured meant that several combinations of faecal matter and water quality data could be used to estimate contaminant material loads.

In the urban catchment during dry weather contamination it was not clear whether the faecal contamination was of human origin at all. In any case it was still very limited (< 300 litres per day or < 0.05% of the daily flow in the creek), considering this liquid was produced by 8 km^2 of urbanisation. During wet weather, however, there was large-scale contamination equivalent to *ca* 1 megalitre of septic tank liquid over the course of the event. The total load of the most definitive analyte, coprostanol, was equivalent to 300-600 litres per household system. This figure is credible given septic tanks hold typically over >1000 litres of wastewater. However it does not imply that each household actually emitted this volume. More likely emissions like individual tank contents vary widely and the contamination reflects in part some very heavily overloaded systems and leachfields. What is more important is that the analysis provided as estimate of the overall scale of contamination which supported the need for remediation e.g. construction of a reticulated sewer.

Table 3. Faecal Contamination Loading Calculations

Calculation Inputs and Outputs		**Urban Catchment**		**Agricultural Catchment**	
		Dry Weather	**Run-off Event**	**Dry Weather**	**Run-off Event**
Analyte units.L^{-1} of run-off	Enterococci	2000	162000	910	85000
	C. perfringens	220	22000	10	1360
	Coprostanol	5.8	890	13	700
	24ethyl Coprostanol	18	640	88	3600
Total Run-off Volume	m^3	654 .d^{-1}	65,000 .$event^{-1}$	9400.d^{-1}	165,000 .$event^{-1}$
% Human or herbivore[3]		Below detection (62%)[1]	62%	100%	100%
Analyte concentration in septic tank liquid or dry cow faeces[2]	Enterococci	510,000 cfu.L^{-1}		$8.2x10^7$.g^{-1} d.w.	
	C. perfringens	250,000 cfu.L^{-1}		$1.7x10^5$.g^{-1} d.w.	
	Coprostanol	60,000 ng.L^{-1}		0.55 mg.g^{-1} d.w.	
	24ethyl Coprostanol	29,000 ng.L^{-1}		1.48 mg.g^{-1} d.w.	
Conversion factors		-		Manure dry matter = 10%	
Quantity of Contaminating Material (septic liquid[4] or wet manure)	Enterococci	<1.59 kL	12800 kL	1.0 kg	1710 kg
	C. perfringens	<0.36 kL	3546 kL	5.5 kg	13200 kg
	Coprostanol	<0.04 kL	600 kL	2.2 kg	2080 kg
	24ethyl Coprostanol	<0.25 kL	890 kL	5.6 kg	4010 kg

Notes:

1. There was no unequivocal evidence of septic tank exfiltration due to the low coprostanol: cholestanol ratio. The wet weather % content value was used to estimate the upper limit of dry weather septic tank discharge volume.
2. Manure content figures from Leeming *et al.* (1998) Table 3 for cattle.

3. % Human or herbivore calculated using the algorithms described in Leeming *et al.* (1998)'s Figure 4.
4. Septic tank liquid figures are for the communal discharge pipeline wastewater described in Table 1.

Similarly in the rural catchment the quantity of manure reaching the monitoring station was relatively small (a few kilograms wet weight per day in a catchment of 77 km^2) when the realities of rural land management are taken into account e.g. broken fences, transfers of stock between fields, poor control of access to water ways, that was minimal contamination. On the other hand the event data suggested that even moderate size run-off events having a recurrence interval of a few months could mobilise tonnes of manure, a scale which might even be detectable visually.

Both the urban and agricultural catchments clearly illustrated that based on faecal loads NPS river contamination was dominated by wet periods emissions, highlighting where the priority for management and auditing should lie. Also the small quantities of faecal material released during dry weather indicated it might be difficult to improve water quality much better than at present.

Identifying Source Material Emission Areas
The final step in the process was to identify the major locality from which the faecal contamination originated. The trialled approach was to undertake reconnaissance or so-called synoptic sampling (USDA/UNRC, 1996) concurrent with key site monitoring described previously (Roser *et al.,* 2003).

From the loading calculations it was evident that the principal main faecal emissions occurred during high run-off periods. So sampling upstream was undertaken concurrently with event sampling to assess whether there were any major emission areas within the catchments. In the case of the urban catchment, three tributaries were sampled at five upstream stations. There was no obviously better or worse zone so it was concluded that better wet weather septic tank management was needed across the whole catchment. With the agricultural catchment a different picture emerged. This was that much of the faecal contamination probably originated from a 1 km reach on the main catchment drainage line (Roser *et al.,* 2003). The rationale for this was that between reconnaissance sampling stations R3 and R4 there was a *ca* 7 fold increase in *E. coli,* Enterococci and the beta stanols and the ratios of the source sterols at R4 and the key monitoring site were much more similar to one another than R5 (Figure 2, Table 4).

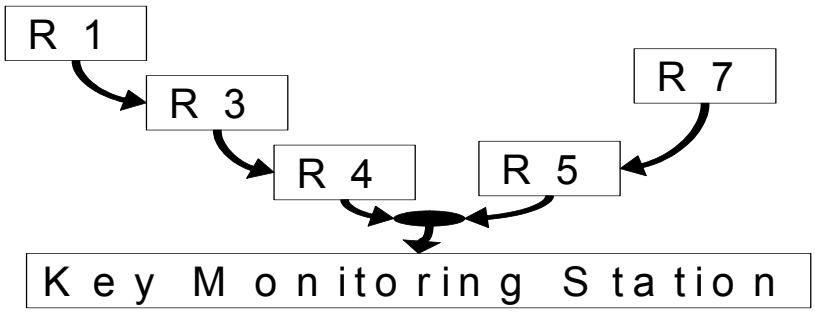

Figure 2. Relative Locations of Sampling Stations within Agricultural Catchment Drainage Lines

Table 4. Analyte Concentrations at the Agricultural Catchment Key Site and Reconnaissance Sampling Points

Analyte/Ratio	Sampling Station					
	Main Stream			Key Monitor-ing Station[1]	Tributary	
	R1. catchment head	R3 inter-mediate	R4 above key Site		R5 above key site	R7 catchment head
E. coli mpn.100 mL^{-1}	39,000	7300	55,000	28000	36,000	910
Enterococci mpn.100 mL^{-1}	21,000	10,000	12,000	8500	9,000	1700
Clostridium perfringens cfu.100 mL^{-1}	200	190	80	136	290	50
Coprostanol $ng.L^{-1}$	2060	120	970	700	682	16
24-ethylcoprostanol $ng.L^{-1}$	5500	540	4400	3600	3000	55
5α Cholestanol $ng.L^{-1}$	820	88	530	450	340	14
24-ethyl-α-cholestanol $ng.L^{-1}$	2800	420	2500	1900	2600	29
Cholesterol $ng.L^{-1}$	4800	1400	3900	2900	2200	128
24-ethylcholesterol $ng.L^{-1}$	5600	2700	5400	4900	3000	210
coprostanol:24-ethyl coprostanol	0.37	0.22	**0.22**[2]	**0.19**	0.22	0.29
coprostanol:cholestanol	2.51	1.36	**1.82**	**1.57**	2.01	1.11
coprostanol:cholesterol	0.43	0.09	**0.25**	**0.24**	0.31	0.12
24ethylcoprostanol:sitostanol	1.96	1.26	**1.75**	**1.91**	1.17	1.87
24ethylcoprostanol:sitosterol	0.97	0.20	**0.81**	**0.74**	1.02	0.26

Notes:

1. Flow weighted average means.
2. Bolded ratios show the key monitoring station and the most similar reconnaissance station.

Discussion

Load Estimation

The management of microbiological contamination has traditionally focused not on loads but on concentration measurements. This is evidenced even in the USEPA (2001) advice on TMDL case studies which still focused on receiving water quality (e.g. De George *et al.*, 2002; case studies reported with USEPA, 2001). The reasons for this conservatism are probably that clean water guidelines and legislation are focused on concentration based objectives, and the labile nature of microbial indicators. The results presented here show, however, that normal loading-style calculations can yield a range of data of use to management of both human and animal faecal matter.

Source tracking

The catchment analysis process undertaken highlighted a number of issues generally relevant to source tracking technology application. Source tracking data that focuses primarily on water quality, but includes no systematic consideration of the state of the environment at the point of sampling is potentially uninterpretable if there are confounding factors such as mixed sources or highly variable flow regimes. Yet in the recent reviews of source tracking technology (Scott *et al.*, 2002; Seurinck *et al* ., 2005) the approach seems to be to treat the environment as a black box incapable of providing any supporting information.

Collection of source tracking analytical data is clearly only the first step. To be useful for management it should then be analysed along with other information pertinent to

faecal contamination so as to assess the scale of contamination which will likely determine whether remediation is warranted. Clearly from our data contamination may range from relatively minor to very serious at the same locality.

The key issue of interest to water managers is not source tracking analytes but faecal contamination and tracking technology application needs to better recognise this. Put another way it is essential to be able to relate faecal contamination measures back to the faecal matter itself. The work undertaken here shows this is possible with faecal sterols and probably also some of the bacterial indicators. Indeed it may be in some instances be unnecessary to undertake exotic source tracking analyses where simply good sampling design programs allow sufficient assessment of whether land areas are emitting contamination or not as is needed to trigger active management. Such pragmatism could avoid investing the large up-front costs required in developing library-based faecal source tracking approaches.

Non-Point Source Pollution Characterisation
Both case studies shown consider examples of NPS. Far from being difficult to localize, it appears that once multiple analytes are measured concurrently effective characterisation of faecal NPS pollution was possible. The data here also highlights how NPS emissions are heavily dependent on environmental conditions and hence the need to understand hazardous event behaviour in a catchment before analysing any samples. Conversely if the trouble is taken to understand a catchment's structure and function then a sampling program should be able to provide a clear picture of faecal contamination patterns.

Use of Data for Management Decision Support
Collection of source tracking data in support of management brings a new additional criterion to the way source tracking assays should be compared and judged. Conventionally, technologies tend to be judged on the basis of test precision, accuracy, convenience and cost. In the present paper two other criteria are proposed as essential for consideration. Firstly a technology should allow estimation of faecal matter load and hence contamination severity. Secondly the data must be able to support environmental management and remediation.

Cost of Assessments
One final concern for Scott *et al.* (2002) was the cost of analyses, with sterols being identified specifically. Analysis cost is an important logistic issue especially where many water analyses are planned. However, other considerations should also be used in technology selection. Faecal sterol analyses at *ca* $500 per analysis are capable of providing several pieces of data as shown above, so the cost per numerical value is better than the single analysis cost indicates. The cost of many of the techniques proposed in often underestimated in that good quality sample collection and transport is itself costly and the cost of supporting expert staff is often not included in such assessments. For library-based approaches, literally thousands of strains are required in a database and even then may not be generally applicable (Moore *et al.*, 2005; Wiggins *et al.*, 2005). Finally there is the issue of cost benefit. Management schemes such as sewer line construction and active riparian zone management are extremely expensive. In such circumstances analysis costs may be trivial and the avoidance of good study design or high quality measurements as being 'too expensive' may be false economy in the extreme.

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