

WATER SERVICES ASSOCIATION
of Australia

Distinguishing Sources of
Faecal Pollution in Australian
Inland and Coastal Waters
using Sterol Biomarkers and
Microbial Faecal Indicators

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Pollution in Australian Inland
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Biomarkers and Microbial
Faecal Indicators**

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Executive Summary

The project aims were to: (i) estimate the numbers of faecal indicator microorganisms (bacteria and bacteriophages of *Bacteroides fragilis*) and concentrations of sterols present in the faeces of native and domestic animals and in sewage to validate whether profile differences measured in pilot studies could be used to quantify sources of faecal contamination, (ii) determine the relationship between, and relative persistence of, faecal sterols and indicator microorganisms during microcosm field experiments and (iii) develop a quantitative method which could distinguish how much faecal matter is present from various sources in a polluted receiving water and test the technique in the field.

The results for objective (i) indicate that the differences in faecal sterols and bacterial indicators between the animal groups were consistent over a range of environments. The concentrations of faecal sterols and bacterial indicators measured can be variable, but the ratios of the parameters, on the basis of populations, are consistent within animal groups and compared to reference samples. Reference samples were collected during field studies from sewer mains or overflows, livestock saleyards and bird resting sites adjacent to field sites and results from these samples provided corroborating *in situ* ratios to estimate faecal contributions to specific environments. The independent use of at least two and preferably four subgroups of bacterial indicators (thermotolerant coliforms, *Escherichia coli*, faecal streptococci and enterococci) as a common denominator of all faecal pollution, strengthens the degree of confidence in the estimates. Estimates from calculations performed for field studies, where the wider range of bacterial indicators was used, generally varied by less than 10%. The use of a range of ratio values based on the variance measured in the faecal and field samples enables a range of contributions to be expressed. These are summarised in the legend of Figure 4 in the report.

The bacteriophages to various strains of *B. fragilis* used to characterise human, pig or poultry, cattle and sheep faeces were often absent from the faeces of individual animals. Nonetheless, when bacteriophages were present in the faeces of cattle or pigs, they consistently contained only phages specific to their respective host strain, and human sewage always contained the bacteriophages to HSP40, the human-specific strain. As observed for the HSP40 strain in North America, the results indicated that only about 10% of individuals excreted their respective bacteriophages, and if faecal contamination from cattle, pigs or humans was present, then up to 1000 plaque forming units of their specific phages per millilitre of effluent could be expected. Hence, given the likely low numbers of phages in environmental waters, enrichments for the specific phages probably will be needed to confirm the presence of cattle, pig or human faecal contamination.

With the use of any single or multiple indicator system, the efficacy of the result or relevance of the interpretation is a function of time and / or distance from the source. Results for objective (ii) indicated that faecal sterols degrade at the same rate as each other and most bacterial indicators die-off more rapidly than faecal sterols degrade, with *C. perfringens* spores being the exception. However, significant divergence of the ratios between faecal sterols and bacterial indicators are unlikely to occur within 2-3 days. Under the environmental situations of field studies undertaken so far, this time constraint was generally not considered to have been exceeded. This was because the survey / catchment areas were not large and even low flow rates occurring in dry weather were estimated to transport faecal matter along the length of the catchments inside 2-3 days. During wet weather when faecal contamination is most prevalent, residence times are even shorter. A settling experiment was conducted to examine the particle size distribution for faecal sterols and bacterial indicators. The results indicated that only in the very fine particle range (equivalent settling velocity to 9 ϕ quartz particles) was there any significant increase in bacterial indicators. This means that only in standing waters would the ratio of bacterial indicators to faecal sterols increase.

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Results from field studies showed the technique conclusively distinguished episodes of human faecal contamination. Faecal contamination from humans and herbivores such as cattle, sheep, kangaroos, etc. could be distinguished from each other with a high degree of accuracy. Based on the findings of this study, faecal contamination from dogs might also be evident, *if present in significant amount*, because of a distinctive faecal profile of high *C. perfringens* spores (\approx equal to thermotolerant coliforms) and high cholesterol concentrations. However, in field studies performed during the period of this study, dogs were not considered to be a significant source of faecal contamination, hence the efficacy of the technique to distinguish faecal contamination from dogs remains untested. In the absence currently of a specific marker for bird faeces, the faecal profile for birds is high abundances of thermotolerant coliforms and faecal streptococci, low abundances of *C. perfringens* spores and high concentrations of Δ^5 -sterols with 5β -stanols absent or in environmentally insignificant trace amounts. Other possible sources of faecal contamination which share a similar faecal profile are invertebrates, reptiles and fish. Because there are presently no specific biomarkers for faeces from these species, faecal contamination from these sources are designated "birds, dogs and other diffuse sources". However, in several field studies, birds were proposed to be the dominant contributor to this grouping because they were ubiquitous and prolific.

In summary, the technique can, with a high degree of certainty, determine and distinguish human versus herbivore faecal contamination and these two sources versus other sources. The technique can then, with a diminished degree of precision, estimate the contribution from birds, dogs and other diffuse sources from total faecal contamination. The results from field studies show the technique is a great benefit to water managers for making effective management decisions.

Future Issues

The water industry has already responded positively to the concept of using additional indicators of faecal contamination to characterise and understand catchments. No one indicator or one approach could represent all the facets and issues associated with contamination of our waterways with faecal matter. The technique described herein has, as of publication, been used in over 20 different environments for local councils, environment protection agencies and water authorities with consistent results. This technique is only a first step toward devising a set of tools that water managers will have at their disposal to investigate the entry of faecal contamination and other organic matter inputs into aquatic systems.

In light of the results of this study, it is recommended that further research be conducted in several complementary areas. Firstly, other classes of lipid biomarkers (e.g. cholanic bile acids and bile pigments) show distinctive profiles between animals. Additional research to discover specific biomarkers for birds and/or dogs would be highly desirable and appears achievable. Secondly, research on bacterial phages could provide qualitative confirmations of faecal sources to complement biomarker assays and to promote a diversity of approaches to solve current problems. Finally, combined PCR (polymerase chain reaction) and biomarker analyses of environmental samples could also provide an advantageous and diversified approach to distinguishing faecal pollution.

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INTRODUCTION

In Australia, sewage contamination of waterways has been a problem since 1788 when the first settlers camped beside the Tank Stream in Sydney Cove (Beder, 1989). By 1826 the Tank Stream had to be abandoned as a water supply because of pollution from refuse and effluents (Beder, 1989). Since then, public interest and government concern over the issue of sewage contamination of our waters has waxed and waned depending to a large degree on public perceptions of the problem and the level of public outcry. Today, faecal contamination of Australia's waterways continues to be a chronic problem adding nutrients and degrading the water quality. In many regions, creeks and stormwater drains have been shown to be a significant source of faecal pollution and excess nutrients to waterways, with this observation recently highlighted by an investigation of nutrients entering Port Phillip Bay, Victoria, (O'Leary *et al.*, 1994).

In addition to human sewage pollution, birds, wildlife, domestic and agricultural animals also contribute to the faecal contamination of Australia's inland and coastal waterways to previously unrecognised, or at least unproven, levels. Faecal matter from virtually all animals except humans is deposited randomly over catchments to lie in varying states of decomposition until transport, usually by rain, into the nearest waterway. No wonder that in many countries, local authorities have been concerned for some time that domestic pets, livestock, birds and native animals may also be a large source of background bacterial contamination in urban creeks and rivers.

Thermotolerant coliform bacteria (faecal coliforms) and faecal streptococci, are commonly used to indicate faecal contamination. But faecal contamination by whom? The ratio between thermotolerant coliforms and faecal streptococci previously had been used to distinguish human and herbivore-derived faecal matter by Geldreich (1976). However, this method is no longer recommended (Howell *et al.*, 1995) and none of the currently used bacterial indicators on their own can confidently distinguish different sources of faecal matter (Cabelli, *et al.*, 1983), although genetic typing of *E. coli* shows some potential (Muhldorfer *et al.*, 1996). The virological methods can specifically identify human faecal presence, however, they are difficult and expensive procedures, and are not readily quantifiable. Other microbiological options include specific identification of phenotypes of *Bifidobacterium* spp. (Gavini *et al.*, 1991), *Bacteroides* spp. (Kreader, 1995), serotypes of F-specific RNA bacteriophages (Osawa *et al.*, 1981) or bacteriophages to *Bacteroides fragilis* (Puig *et al.*, 1997). Yet none of these techniques are suited to quantifying the proportion of human faecal contamination. Indeed, no one indicator or one approach is likely to represent all the facets and issues associated with contamination of our waterways with faecal matter.

To overcome these various drawbacks, Vivian (1986), in his review of sewage tracers, suggested that using more than one method of determining the degree of sewage pollution would be prudent and advantageous. The use of alternative indicators, in this case faecal sterols as biomarkers, in conjunction with existing microbiological indicators, offers a new way to distinguish sources of faecal contamination and monitor river health.

Aims

The aims of this project were to: (i) estimate the numbers of indicator microorganisms (bacteria and bacteriophages of *Bacteroides fragilis*) and concentrations of sterols present in sewage and in the faeces of native and domestic animals to validate whether profile differences measured in pilot studies can be used to quantify sources of faecal contamination; (ii) determine the relationship between, and relative persistence of, faecal sterols and indicator microorganisms during microcosm field experiments; and (iii) develop a quantitative method which could distinguish how much faecal matter is present from various sources in a polluted receiving water and test the technique in the field.

Faecal Sterols - a brief overview

The most commonly known faecal sterol, coprostanol (5 β -cholestan-3 β -ol), is produced in the digestive tract of humans by microbial hydrogenation of cholesterol (cholest-5-en-3 β -ol) (Rosenfield and Gallagher, 1964). The term "sterols" is both a collective name for all sterols and stanols (i.e. "faecal sterols") and a more specific term denoting a steroidal alcohol with some degree of unsaturation. Examples of sterols and stanols are illustrated in Figures 1 and 2. Sterols are based upon a standard framework of four cyclic rings (Figure 1); three six member rings and one five member ring. The ring system possesses several methyl groups attached to the rings, a hydroxyl group at carbon position 3 and a side chain (R) of eight or more carbon atoms attached to the five member ring (Figure 1).

The structural difference between cholesterol and coprostanol and related isomers, is the presence in cholesterol of a double bond at the C₅-C₆ position (Figure 1). Prior to 1980, numerous studies had determined the chemical structure, origin and transformation pathways of coprostanol and related sterols and these are detailed by Walker *et al.* (1982). Two pathways have been proposed for the biotransformation of cholesterol to coprostanol; (i) direct hydrogenation of the double bond and (ii) reduction through intermediates such as cholest-4-en-3-one and 5 β -cholestan-3-one (Rosenfield and Gallagher, 1964; Parmentier and Eyssen, 1974). In the reduced stanol, orientation of the hydrogen atom at the carbon 5 position can be either β -configuration, as for coprostanol, or α -configuration as for 5 α -cholestan-3 β -ol (5 α -cholestanol, Figure 2). The α -configured cholestanol is the most thermodynamically stable of the reduction products and is found ubiquitously in the environment. Unlike cholestanol, the reduction of cholesterol to β -configured coprostanol is mediated solely by obligate anaerobic microorganisms. Martin *et al.* (1973) suggested that Gram-negative anaerobes were the principal microbial mediators in the conversion, however, Parmentier and Eyssen (1974) isolated a Gram-positive anaerobe that could hydrogenate cholesterol to coprostanol. 5 β -Cholestan-3 α -ol (*epicoprostanol*; Figure 2) is also β -configured at the carbon 5 position, but differs from coprostanol by having the hydroxyl group at the carbon 3 position in the α -configuration. *Epicoprostanol* is found in trace amounts (relative to coprostanol) in human faeces, but increases in relative proportions in digested sewage sludges either through epimerisation of coprostanol to *epicoprostanol* or because of diversification of anaerobic microorganisms present in the sludge (McCalley *et al.*, 1981). *Epicoprostanol* has also been identified as a major sterol constituent in the faeces of blue and fin whales (Venkatesan and Santiago, 1989).

Coprostanol has been proposed as a measure of human faecal pollution by a large number of researchers since the late 1960's (e.g. Murtaugh and Bunch, 1967; Dukta *et al.*, 1974; Walker *et al.*, 1982). However, coprostanol has never really been embraced as a sanitary indicator for

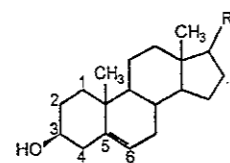
sewage pollution because its presence is not considered as indicative of a health risk. Previous investigations of the overall sterol composition of faeces from animals and invertebrates determined that coprostanol was not present in a range of species of molluscs, crustaceans, invertebrates and birds with the exception of chickens (Walker *et al.*, 1982). It is hypothesised that the modified diet of production chickens, which most often includes antibiotics, alters the microbial composition in the chicken's gut with the consequence that low concentrations of coprostanol, relative to other sterols, can be measured in chicken faeces. Monkeys, pigs, sheep, cows and rodents were also found to have some coprostanol (Walker *et al.*, 1982). But these studies focused on whether these animals produced coprostanol alone and if so, in what amounts relative to the weight of faeces, not relative to the composition of other sterols for diagnostic purposes.

The faecal sterol biomarker technique offers many diagnostic and quantitative advantages when used in conjunction with traditional techniques for detecting sewage pollution. In 1994, scientists from AWT-EnSight and CSIRO investigated differences in the neutral lipid composition (including sterols, hydrocarbons and long chain alcohols) and bacterial indicator profiles of faecal matter from a range of common animals (Leeming *et al.*, 1994 & 1996). Because of the breadth of the study, the number of replicate samples from each species was limited to 6 or 7. It confirmed that herbivores did have coprostanol in their faeces, but that the dominant stanol was the C₂₉ homologue of coprostanol, 24-ethylcoprostanol (24-ethyl-5 β (H)-cholestan-3 β -ol). It was thought that if consistent, these differences could be exploited to determine the contribution of faecal matter from these two sources relative to each other by calculating the ratio of coprostanol to 24-ethylcoprostanol in human and herbivore faeces.

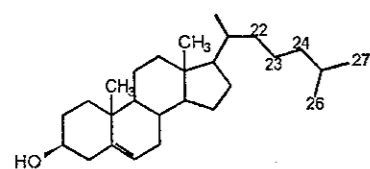
Furthermore, it was discovered that animals such as dogs and birds either do not have coprostanol in their faeces or it is present in trace and definable amounts relative to other sterols (Leeming *et al.*, 1996). Provided these findings were consistent, the faecal matter from birds and dogs could be distinguished from humans and herbivores by comparing the *measured* abundances of bacterial indicators (e.g. thermotolerant coliforms, faecal streptococci, enterococci) common to these animal groups with the abundance of bacterial indicators *predicted* from human and herbivore sources based on faecal sterol concentrations. Further differences in the relative abundances of faecal coliforms and *Clostridium perfringens* spores were also observed (Leeming, 1996a) and the possibility of exploiting those differences to distinguish contamination from birds and dogs was identified as needing further investigation.

Preliminary Field Trials

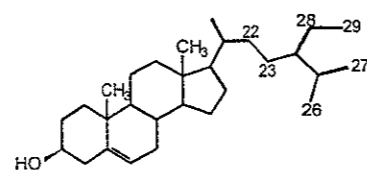
Subsequent field trials in Lake Tuggerah, NSW (Leeming and Nichols, 1995a&b) highlighted the potential to estimate the proportions of faecal pollution in water samples by calculating the ratios of bacterial indicators to faecal sterols. It was recognised that the ratios of faecal sterols to bacterial indicators for faecal matter from populations needed to be as accurate as possible (given the inherent variability likely to be encountered in environmental samples) and that a variety of processes that could potentially change the ratios during transport into receiving waters needed to be identified and understood. In response, the Water Services Association of Australia (WSAA) funded this study, which commenced in July 1996, to address these issues and to validate and refine the basis for distinguishing faecal pollution using faecal sterols and bacterial indicators.



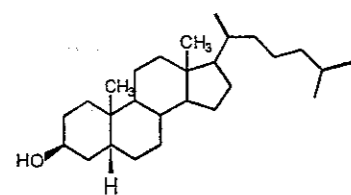
(i) Basic Δ^5 sterol structure



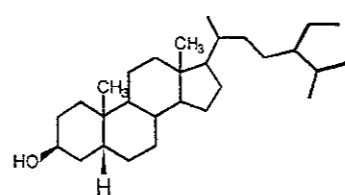
(ii) cholesterol (C₂₇ sterol)



(iii) 24-ethylcholesterol (C₂₉ sterol)

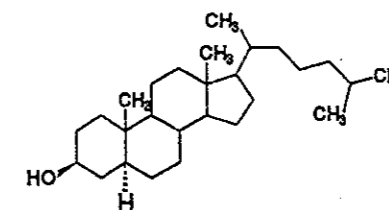


(iv) 5 β -coprostanol (C₂₇ stanol)

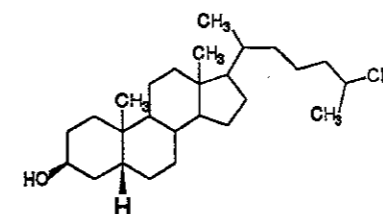


(v) 24-ethyl-5 β -coprostanol (C₂₉ stanol)

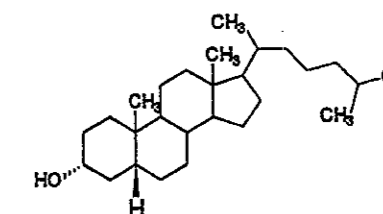
Figure 1: Illustration of the basic structure of a Δ^5 sterol and the structural differences between C₂₇ and C₂₉ sterols and stanols.



(i) cholestanol
(5 α -cholestan-3 β -ol)



(ii) coprostanol
(5 β -cholestan-3 β -ol)



(iii) epi-coprostanol
(5 β -cholestan-3 α -ol)

Figure 2: Stereochemical differences in the three C₂₇ saturated stanols, (coprostanol, *epicoprostanol* and cholestanol) occur at the hydrogen atom at the carbon 5 position and the hydroxyl group at the carbon 3 position.

In parallel to these investigations, detailed field studies in Lake Macquarie (funded by Hunter Water), the Yarra River and Rippleside stormwater drains in Geelong (funded by the Victorian Environment Protection Authority), the Murray / Serpentine Rivers (funded by the Peel Inlet Management Authority) and other smaller studies for local councils were undertaken. As noted in the original proposal, several of these field studies were earmarked to provide ground truthing of techniques developed and refined within the WSAA study. Furthermore, these studies have established a database of faecal pollution and its source(s) in a variety of environments and also tested ongoing improvements to the broader experimental protocols. The Lake Macquarie and Rippleside studies are summarised in this report to illustrate application of the technique and the benefits to water managers; other referenced reports are available from the CSIRO Marine Laboratory's library. The field studies were undertaken prior to the completion of this study which is why there may be some small differences in the ratios and / or percentages used compared with those proposed in this report. Similarly, important issues about potential changes to ratios and the source(s) of the "unidentified faecal contamination" were first discussed in the text of the reports, but without the benefit of the final results from this study. The primary aim of each field study was to determine whether faecal pollution was derived principally from human faecal matter. The supplementary aim was to identify and estimate contributions from non-human sources and provide feed back to this study.

METHODS

For faecal samples, only obviously fresh samples (generally 5-10 replicates for each animal type) were collected and transported to the laboratory in less than 8 hours. One aliquot was taken for dry weight analysis. A second 1-2 g aliquot was weighed into a sterile collection flask and 10 mL of Ringers solution was added. A slurry was made from this mixture whereupon 1 or 2 mL aliquots were taken for bacterial indicator assays and faecal sterol analysis. Water samples were collected in sterilised and pre-rinsed 5 L polyurethane containers; up to 9.5 L were used for sterol analyses and the remainder used for bacterial assays. Sterols are generally present in nanogram (ng) or microgram (μg) per litre concentrations in natural waters and are largely bound to particulate matter (Brown and Wade, 1984; Eganhouse *et al.*, 1988). Samples for sterol analysis were filtered (Whatman GF/F nominal pore size 0.5 μm) and stored frozen at -20°C until received at the CSIRO Marine Laboratories.

Two protocols were employed during degradation experiments (Leeming, 1996a). The first entailed batch cultures of effluent and a receiving water mixed at various ratios and placed in 500 mL glass Schott bottles (i.e. *in vitro*). The bottles had Teflon lined screw cap lids which were loosened during incubation to allow gas exchange with the atmosphere. A maximum of 12 bottles at a time were placed in a water bath which had a rotating base to stir the contents of the bottles. Bottles were rotated at 200 revolutions per minute (rpm) and the water bath was filled to half way up the Schott bottles. A refrigeration unit with immersible probe and a heater were used to keep the temperature of water in the bath at the set temperatures. Seawater and sewage mixtures were homogenised as much as possible and every effort was made to ensure that aliquots for the batch cultures were as similar as possible. Batch cultures (2-3) were analysed at the beginning of each experiment and periodically, Schott bottles were removed and the contents analysed for sterols and bacterial indicators.

The second type of degradation experiment entailed the same mixture of effluent and receiving water, but the mixture was placed in cellulose membrane dialysis tubing (Sigma, cat. no. D-9402) with the ends tied to form a bag. The tubing was prepared in accordance with the manufacturers instructions to remove glycerin added as a humectant and sulfur compounds present. Lengths of tubing ($\approx 30\text{ cm}$) were cut, tied at one end, filled with the effluent / water mix and sealed at the top with plastic ties. The dialysis bags were then placed inside a specially constructed cage and deployed at a depth of 0.5-1 m in a freshwater lagoon (Long Bay, NSW). The water temperature was $\approx 17^\circ\text{C}$ throughout the experiment. Replicate bags were sampled at time zero, then periodically (usually twice per day) for three weeks. The settling velocity experiment was conducted in a 1 L measuring cylinder at 23°C in the dark. Raw effluent (200 mL) was mixed with creek water from an urban stormwater drain (800 mL) and 50 mL aliquots were collected at time intervals (0, 22.04 min, 5 hr 55 min, 23 hr 28 min) equating to mineral particle sizes ($>4\phi$, 6ϕ , 8ϕ and 9ϕ respectively) according to Waddell's Law.

All filters and / or faecal slurries were thawed and extracted quantitatively by the one-phase CHCl_3 -MeOH- H_2O (chloroform / methanol / water) Bligh and Dyer (1959) method. Aliquots from degradation experiments were extracted using a liquid-liquid extraction technique where 100-300 mL of sample was mixed with 50 mL each of CHCl_3 and MeOH and shaken in a mechanical shaker for 20 minutes (Leeming, 1996a). After phase separation, the lipids were

recovered in the lower CHCl_3 layer (solvents were removed *in vacuo*) and were made up to a known volume and stored sealed under nitrogen at -20°C . The total sterol fraction was obtained following alkaline saponification of an aliquot of the total lipids. Sterols were converted to their corresponding OTMSi ethers by treatment with bis(trimethylsilyl)trifluoroacetamide (BSTFA, 100 μL , 60°C , 60 min). Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890 GC. A flame ionisation detector (FID) was used for the analysis of the sterol fraction with 5α -cholestane used as an internal standard. The GC was equipped with a 50 m x 0.32 mm i.d. cross-linked 5% phenyl-methyl silicone (HP5) fused-silica capillary column and hydrogen was used as the carrier gas. Peak identifications were based on comparison of retention time data with data obtained for authentic and laboratory standards. Peak areas were quantified using DAPA chromatography software and personal computers operating Windows 95. Concentrations of individual sterols in water samples detected below 10 ng L^{-1} were labelled trace (TR), those not detected labelled (ND); the detection limit for individual sterols was $2\text{--}5\text{ ng L}^{-1}$ for water samples and $1\text{--}2\text{ }\mu\text{g g}^{-1}$ (dry weight) for faecal slurries.

GC-MS analyses of samples for verification of identification were performed on a Fisons MD800 benchtop quadrupole mass spectrometer fitted with a direct capillary inlet. Data were acquired and processed on a Fisons data station operated in scan acquisition or selective ion monitoring mode. The nonpolar column (HP5) and operating conditions were similar to that described above for GC-FID analyses, but helium was used as the carrier gas.

Bacterial indicator bacteria were enumerated from faecal slurries (1 mL) diluted in Ringers then collected on cellulose acetate membrane filters (0.45 μm Millipore), whereas 100 mL of water samples were directly filtered prior to filters being placed on selective media for estimation of colony forming units (cfu). Standard methods (APHA, 1989) were used for the enumeration and confirmation of thermotolerant coliforms (on mThC agar) and faecal streptococci/enterococci (membrane Slanetz and Bartley agar). Estimated numbers of *Escherichia coli* were also made from a random selection of samples for each animal group, from the ThC agar plates, using standard methods. Spores of *C. perfringens* were enumerated on Perfringens Agar (Oxoid CM543, containing Oxoid supplements A [SR 76] and B [SR 77]) modified by the addition of 0.004% methyl umbelliferyl phosphate (MUP, Merck), following heat treatment (10 min at 75°C) as previously described (Davies *et al.*, 1995). Positive control cultures were used with each batch on media prepared (*E. coli* 080300, *Enterococcus fragilis* 054400, *C. perfringens* 082401, from the School of Microbiology and Immunology, UNSW).

Bacteriophages present in 1 mL of faecal slurries were enriched in BPRM (*Bacteroides* phage recovery medium) (Tartera *et al.*, 1992) containing the specific host (in log phase) and incubated at 37°C , anaerobically, overnight as described by Grabow *et al.* (1995). Strains of *B. fragilis* for various animal groups were: HSP40 (ATCC 51477) [human], RYC2056 [pigs and poultry], RYC3317 [pigs, cows and poultry], RYC3318 [pigs, sheep and poultry], supplied by Rosa Araujo [University of Barcelona]. The broths were treated with chloroform (0.3 to 1 mL of broth), centrifuged (7000 rpm 5min) and then spotted onto BPRM plates with a freshly spread lawn of host bacteria; once dry they were incubated anaerobically as described above. Plates were examined for plaques after 48 hr incubation. Faecal slurries shown to be positive in the enrichments had phages enumerated by the double-agar layer pour plate method (Tartera *et al.*, 1992).

RESULTS AND DISCUSSION

The CSIRO-AWT pilot study which preceded this research examined a wide range of neutral lipids in the faeces of various animals and compared these with the commonly used bacterial indicators (Leeming *et al.*, 1994 and 1996). Because of the breadth of the study, the number of replicate samples from each species was limited to 6 or 7. In contrast, the WSA study focused on validating with greater replication, the diagnostic differences which were identified in the pilot study. This meant determining the population mean concentrations and various ratios of two sterols, six stanols, thermotolerant coliforms, faecal streptococci, and *C. perfringens* spores. Confirmations of *E. coli* and enterococci were performed on a subset of samples.

The systematic and trivial names of sterols and stanols measured and a precis of their attributes in the context of this study are provided in Table 1. The mean concentrations of sterols, stanols, bacterial indicators and the pertinent ratios derived from human and animal faecal samples examined are shown in Tables 2-7. The important results to take note of in respect of these are; (i) the dominance of 5β -stanols compared to 5α -stanols in humans and herbivores, (ii) the different proportions of stanols compared to their precursor sterols, (iii) the higher relative proportions of C_{27} sterols and stanols compared to their C_{29} homologues in human versus herbivore faeces and vice versa, and (iv) the proportions of $5\beta(\text{H}),3\beta(\text{OH})$ -stanols like coprostanol compared to $5\beta(\text{H}),3\alpha(\text{OH})$ -stanols like epicoprostanol.

Table 1: Systematic and trivial names and description of sterols used in this study.

Systematic name	Trivial name	Description
C_{27} sterols		
5β -cholestan- 3β -ol	coprostanol	human faecal biomarker - high relative amounts indicate fresh human faecal contamination
5β -cholestan- 3α -ol	<i>epi</i> -coprostanol	present in sewage sludges - high relative amounts suggest older faecal contamination
cholest-5-en- 3β -ol	cholesterol	C_{27} precursor to 5α and 5β -stanols
5α -cholestan- 3β -ol	cholestanol	thermodynamically most stable isomer is ubiquitous - if the ratio of coprostanol / cholestanol is $< 0.3\text{--}0.5$, origin of 5β -stanols may not be faecal.
C_{29} sterols		
24-ethyl- 5β -cholestan- 3β -ol	24-ethylcoprostanol	herbivore faecal biomarker - high relative amounts indicate herbivore faecal contamination
24-ethyl- 5β -cholestan- 3α -ol	24-ethyl- <i>epi</i> -coprostanol	also present in some herbivore faeces
24-ethylcholest-5-en- 3β -ol	24-ethylcholesterol	C_{29} precursor to 5α and 5β -stanols
24-ethyl- 5α -cholestan- 3β -ol	24-ethylcholestanol	thermodynamically most stable isomer is ubiquitous

5β(H),3β(OH)-stanols: coprostanol and 24-ethylcoprostanol

Measurement of coprostanol alone is not an unambiguous measure of human faecal pollution. However, provided the suite of sterols and stanols shown in Table 1 are analysed, a clear and often conspicuous picture of the sources of these sterols can be ascertained. Tables 2-5 show that in addition to humans, animals such as sheep, cows, kangaroos, possums, pigs and rats all have sufficient coprostanol in their faeces to potentially confound interpretations for environmental samples. However, many other factors need to be considered. On a dry weight basis, humans have a higher concentration of 5β-stanols than the herbivores. While this is empirically true, the difference in terms of microbial activity to hydrogenate Δ⁵-sterols to 5β-stanols might actually be smaller because some of the weight of herbivore faecal matter is undigested plant material. However, it is not the absolute concentration of specific indicators, but their proportions and overall fingerprint which determines the source.

To determine whether sterols present in a sample are from a herbivore such as a cow or from a human origin, one must also consider the concentration of 24-ethylcoprostanol.

24-Ethylcoprostanol is the C₂₉ homologue of coprostanol and is present in higher relative proportions than coprostanol in herbivore faecal matter. Table 2 shows that the percentage of coprostanol compared to the sum of coprostanol and 24-ethylcoprostanol in human faeces is 75% ± 1.8. The same ratio in herbivores show that cows, sheep, kangaroos and possums have between 26 to 28% ± 2 coprostanol and conversely between 72 to 74% ± 2 24-ethylcoprostanol (Tables 4 and 5). This ratio is diagnostic of human versus herbivore faecal contamination and had the least variability of all the parameters measured in this study.

Compared to the obligate herbivores, other animals such as rats (42% coprostanol, 58 % 24-ethylcoprostanol) and pigs (56% coprostanol, 44% 24-ethylcoprostanol) have increased percentages of coprostanol which probably reflects their more omnivorous diet (Tables 5 and 6). Dogs had no measurable 5β-stanols (Table 6). Coprostanol and other 5β-stanols were generally absent in bird faeces, but a few individuals had trace amounts (< 20 μg g⁻¹ dry wt., Table 7). Chickens have previously been reported as having coprostanol in their faeces and indeed all the faecal samples tested had small amounts. However, the concentrations were similarly low as in other birds when it was present (i.e. < 24 μg g⁻¹ dry wt., Table 7). The reasons chickens probably have a higher incidence of coprostanol in their faeces may be due to their manipulated diet and / or changes in gut microbiota caused by antibiotics they receive with their feed. However, the occurrence of trace amounts of 5β-stanols from birds is inconsequential because the proportion of 5β-stanols compared to 5α-stanols is as low or lower than that found in uncontaminated sediments (see below). Since the hydrogenation of Δ⁵-sterols to 5β-stanols is preferentially mediated by gut microbes (Parmentier and Eyssen, 1974), this finding suggests dogs and birds do not have the necessary intestinal biota to preferentially accomplish this transformation. The distribution of sterols found in faeces, and hence their source-specificity, is therefore caused by a combination of diet, the animal's ability to synthesise its own sterols and the intestinal microbiota in the digestive tract. The combination of these factors determines "the sterol fingerprint".

Table 2: Concentrations and ratios of sterols and bacterial indicators of diagnostic relevance in human faeces.

Variable	Human faeces from this project ^{1,2}		Human faeces from pilot study ^{1,3}	
	mean	% std err	mean	% std err
coprostanol (cop)	12740	17%	3770	13%
epicoprostanol	bd	-	bd	-
cholesterol	5080	31%	293	9%
5α-cholestanol	678	14%	76	17%
24-ethylcoprostanol (ethylcop)	3680	12%	1330	18%
24-ethylcholesterol	1720	30%	114	12%
24-ethylcholestanol	254	11%	59	7%
thermotolerant coliforms (ThC)	3.9E+8	28%	3.6E+7	56%
faecal streptococci (FS)	8.2E+7	63%	1.4E+8	70%
<i>C. perfringens</i> spores (CP)	⁵ 1.7E+5	66%	⁶ 2.3E+4	39%
⁴ ThC / coprostanol	19 (30)	34%	13 (9.5)	65%
⁴ FS / coprostanol	2.6 (6.4)	91%	11 (37)	77%
% cop / (cop + ethylcop)	75%	1.8	74%	5
% ethylcop / (cop + ethylcop)	25%	1.8	26%	14
cop / cholesterol	19	12%	53	15%
ethylcop / 24-ethylcholestanol	16	13%	22	14%

bd: below detection;

¹ Sterols (μg g⁻¹) & bacterial indicators (cfu g⁻¹) dry weight;

² Sterol & ratio data based on n=13, bacterial indicators based on n=31;

³ Data based on n=6;

⁴ Bacterial indicator to sterol ratios based on cfu g⁻¹ divided by ng g⁻¹ and calculated as means of individual ratios; ratios of mean concentrations are given in parentheses.

⁵ Only 10 out of 31 samples had measurable *C. perfringens* spores;

⁶ Only 3 out of 6 samples had measurable *C. perfringens* spores - detection limit for *C. perfringens* spores was ≈ 6.4+E2 cfu g⁻¹ dry wt.

Table 3: Concentrations and ratios of sterols and bacterial indicators of diagnostic relevance in cow and sheep faeces.

Variable	Cows ^{1,2}		Sheep ^{1,2}	
	mean	% std err	mean	% std err
coprostanol (cop)	553	17%	394	13%
epicoprostanol	167	16%	87	20%
cholesterol	426	16%	324	9%
5 α -cholestanol	300	14%	198	12%
24-ethylcoprostanol (ethylcop)	1478	15%	1031	12%
24-ethylepicoprostanol	2162	15%	613	8%
24-ethylcholesterol	587	13%	272	8%
24-ethylcholestanol	736	12%	615	12%
thermotolerant coliforms (ThC)	2.3E+7	33%	1.3E+7	61%
faecal streptococci (FS)	7.4E+6	74%	1.1E+7	55%
<i>C. perfringens</i> spores (CP)	< 1.2E+3	-	⁴ < 7.0E+2	-
³ ThC / 24-ethylcoprostanol	9.8 (15)	24%	7.1 (12)	30%
³ FS / 24-ethylcoprostanol	3.7 (5.0)	52%	4.3 (10)	45%
% cop / (cop + ethylcop)	27%	1.4	28%	1.5
% ethylcop / (cop + ethylcop)	73%	1.4	72%	1.5
cop / cholesterol	1.9	7%	2.2	9%
ethylcop / 24-ethylcholestanol	1.9	7%	1.8	9%

¹ Sterols ($\mu\text{g g}^{-1}$) & bacterial indicators (cfu g^{-1}) dry weight;

² Sterol & ratio data based on n=20, bacterial indicators based on n=30;

³ Bacterial indicator to sterol ratios based on cfu g^{-1} divided by ng g^{-1} and calculated as means of individual ratios; ratios of mean concentrations are given in parentheses.

⁴ Only 6 out of 30 samples had measurable *C. perfringens* spores.

Table 4: Concentrations and ratios of sterols and bacterial indicators of diagnostic relevance in kangaroo and possum faeces.

Variable	Kangaroos ^{1,2}		Possums ^{1,3}	
	mean	% std err	mean	% std err
coprostanol (cop)	127	9%	310	20%
epicoprostanol	0.6	32%	25	51%
cholesterol	142	9%	303	20%
5 α -cholestanol	56	10%	108	23%
24-ethylcoprostanol (ethylcop)	356	10%	981	17%
24-ethylepicoprostanol	48	14%	51	37%
24-ethylcholesterol	206	9%	758	19%
24-ethylcholestanol	79	10%	108	21%
thermotolerant coliforms (ThC)	9.2E+6	87%	6.3E+7	80%
faecal streptococci (FS)	1.1E+7	70%	1.4E+6	79%
<i>C. perfringens</i> spores (CP)	⁵ < 7.0E+2	-	< 1.0E+3	-
⁴ ThC / 24-ethylcoprostanol	4.1 (25)	46%	14 (64)	38%
⁴ FS / 24-ethylcoprostanol	8.6 (30)	25%	2.8 (1.5)	89%
% cop / (cop + ethylcop)	27%	0.9	26%	2.6
% ethylcop / (cop + ethylcop)	73%	0.9	74%	2.6
cop / cholesterol	2.4	5%	9.3	30%
ethylcop / 24-ethylcholestanol	5.5	15%	22	44%

¹ Sterols ($\mu\text{g g}^{-1}$) & bacterial indicators (cfu g^{-1}) dry weight;

² Sterol & ratio data based on n=20, bacterial indicators based on n=32;

³ Sterol & ratio data based on n=9, bacterial indicators based on n=17;

⁴ Bacterial indicator to sterol ratios based on cfu g^{-1} divided by ng g^{-1} and calculated as means of individual ratios; ratios of mean concentrations are given in parentheses.

⁵ Only 6 out of 32 samples had measurable *C. perfringens* spores.

Table 5: Concentrations and ratios of sterols and bacterial indicators of diagnostic relevance in rat faeces and all herbivore faeces.

Variable	Rats ^{1,2}		All herbivores sampled ^{1,3}	
	mean	% std err	mean	% std err
coprostanol (cop)	603	17%	410	9%
epicoprostanol	66	41%	74	15%
cholesterol	764	11%	403	8%
5 α -cholestanol	223	18%	187	9%
24-ethylcoprostanol (ethylcop)	825	16%	953	8%
24-ethylepicoprostanol	bd	-	941	13%
24-ethylcholesterol	949	13%	555	9%
24-ethylcholestanol	235	14%	377	10%
thermotolerant coliforms (ThC)	2.3E+6	62%	1.8E+7	38%
faecal streptococci (FS)	7.2E+6	39%	8.3E+6	30%
<i>C. perfringens</i> spores (CP)	⁵ 8.3E+3	-	⁶ < 1.0E+3	-
⁴ ThC / 24-ethylcoprostanol	3.3 (2.7)	36%	7.3 (18)	5%
⁴ FS / 24-ethylcoprostanol	6.7 (8.7)	29%	5.6 (8.7)	17%
% cop / (cop + ethylcop)	42%	1.4	30%	0.7
% ethylcop / (cop + ethylcop)	58%	1.4	70%	0.7
cop / cholestanol	2.8	8%	5.7	43%
ethylcop / 24-ethylcholestanol	4.4	22%	7.9	30%

bd: below detection;

¹ Sterols ($\mu\text{g g}^{-1}$) & bacterial indicators (cfu g^{-1}) dry weight;

² Sterol & ratio data based on n=23, bacterial indicators based on n=28;

³ Sterol & ratio data based on n=92, bacterial indicators based on n=137;

⁴ Bacterial indicator to sterol ratios based on cfu g^{-1} divided by ng g^{-1} and calculated as means of individual ratios; ratios of mean concentrations are given in parentheses.

⁵ Only 13 out of 28 samples had measurable *C. perfringens* spores;

⁶ Only 25 out of 137 samples had measurable *C. perfringens* spores - detection limit below 1.0E+3.

Table 6: Concentrations and ratios of sterols and bacterial indicators of diagnostic relevance in pig and dog faeces.

Variable	Pigs ^{1,2}		Dogs ^{1,3}	
	mean	% std err	mean	% std err
coprostanol (cop)	5496	7%	bd	-
epicoprostanol	182	16%	bd	-
cholesterol	1338	9%	1482	29%
5 α -cholestanol	1027	8%	98	37%
24-ethylcoprostanol (ethylcop)	4301	6%	bd	-
24-ethylepicoprostanol	275	6%	bd	-
24-ethylcholesterol	1132	14%	518	39%
24-ethylcholestanol	1294	7%	55	33%
thermotolerant coliforms (ThC)	7.5E+7	41%	3.5E+8	25%
faecal streptococci (FS)	7.1E+7	49%	5.7E+7	76%
<i>C. perfringens</i> spores (CP)	⁴ 4.8E+5	80%	9.2E+8	38%
³ ThC / 24-ethylcoprostanol	14 (17)	44%		
³ FS / 24-ethylcoprostanol	20 (16)	63%		
% cop / (cop + ethylcop)	56%	1.6		
% ethylcop / (cop + ethylcop)	44%	1.6		
cop / cholestanol	5.5	5%		
ethylcop / 24-ethylcholestanol	3.4	3%		

bd: below detection;

¹ Sterols ($\mu\text{g g}^{-1}$) & bacterial indicators (cfu g^{-1}) dry weight;

² Sterol data on n=20, bacterial indicators based on n=31;

³ Sterol data based on n=5, bacterial indicators based on n=32;

⁴ 21 out of 31 samples had measurable *C. perfringens* spores.

Table 7: Concentrations and ratios of sterols and bacterial indicators of diagnostic relevance in chicken and coastal bird faeces.

Variable	Chickens		Coastal Birds	
	mean	% std err	mean	% std err
coprostanol (cop)	9.7	20%	2.5	49%
epicoprostanol	bd	-	bd	-
cholesterol	1093	15%	2209	23%
5 α -cholestanol	99.8	11%	39.9	18%
24-ethylcoprostanol (ethylcop)	bd	-	bd	-
24-ethylepicoprostanol	bd	-	bd	-
24-ethylcholesterol	1641	11%	409	26%
24-ethylcholestanol	96.2	13%	29.6	39%
thermotolerant coliforms (ThC)	9.7E+8	55%	7.2E+7	53%
faecal streptococci (FS)	5.2E+7	35%	1.6E+8	77%
<i>C. perfringens</i> spores (CP)	< 5.0E+2	-	⁴ < 1.0E+3	-

bd: below detection;

¹ Sterols ($\mu\text{g g}^{-1}$) & bacterial indicators (cfu g^{-1}) dry weight;

² Sterol data based on n=10, bacterial indicators based on n=30;

³ Sterol data based on n=19, bacterial indicators based on n=80;

⁴ Only 10 out of 80 samples had measurable *C. perfringens* spores.

5 β (H),3 α (OH)-stanols: epicoprostanol and 24-ethyl-epi-coprostanol

In this study, epicoprostanol was below detectable levels in human faeces (Table 2), but it has previously been reported as a minor constituent (Ferezou *et al.*, 1978). However, McCalley *et al.* (1981) showed that epicoprostanol while not prevalent in primary sewage effluent, could indicate the presence of treated or aged faecal matter because of its increasing presence as sewage sludge aged. This suggests that in humans, gut microbes preferentially hydrogenate cholesterol to the 5 β (H),3 β (OH)-coprostanol and little if any of the 5 β (H),3 α (OH)-epicoprostanol or 5 α (H),3 β (OH)-cholestanol is produced *in vivo*. Nonetheless, once transferred to a different environment, epicoprostanol can be produced either through epimerisation of coprostanol to epicoprostanol, or the direct hydrogenation of epicoprostanol from cholesterol mediated by a different group of anaerobic microbes.

The C₂₉ homologue of epicoprostanol is 24-ethylepicoprostanol (24-ethyl-5 β -cholestan-3 α -ol). This compound was present in often equal quantities as 24-ethylcoprostanol in sheep and cow faeces (600-2000 $\mu\text{g g}^{-1}$); epicoprostanol was also present albeit in smaller quantities (Table 3). 24-Ethyl-epi-coprostanol was also measured in kangaroo and possum faeces, but in much lower proportions to 24-ethylcoprostanol (Table 4). Rat faeces contained a low amount of epicoprostanol (66 $\mu\text{g g}^{-1}$) but no measurable 24-ethyl-epi-coprostanol. Epi-coprostanol has been reported by Venkatesan and Santiago (1989) as the dominant faecal sterol in the faeces of blue and fin whales from Antarctica. These data suggest that microbial mediation is a more likely mechanism for the hydrogenation of 5 β (H),3 α (OH)-stanols than epimerisation of existing 5 β (H),3 β (OH)-stanols however, the latter transformation pathway cannot be ruled out.

5 α (H),3 β (OH)-stanols: 5 α -cholestanol and 24-ethyl-5 α -cholestanol

The naturally occurring C₂₇ stanol in pristine environments is 5 α -cholestanol because it is the most thermodynamically stable isomer (Nishimura, 1982). 5 β -Stanols such as coprostanol do not occur naturally in fresh or marine waters or in aerobic sediments because only anaerobic bacteria appear capable of hydrogenating Δ^5 -sterols to the 5 β -stanols. Increasing amounts of 5 α -cholestanol relative to cholesterol in sediments usually indicates increasing anaerobic conditions. Under these circumstances, small relative amounts of 5 β -stanols can also be found in anaerobic sediments not contaminated by faecal pollution (Nishimura, 1982). If some of these sediments were remobilised into the water column, the presence of coprostanol could be misinterpreted as coming from human faecal contamination.

A useful measure for assessing whether coprostanol found in organic-rich and partially anoxic sediments is of faecal origin (i.e. the *in-situ* background level) is by comparison of the ratio of coprostanol to 5 α -cholestanol (see Table 8). For the animal faeces examined, the ratio of C₂₇ and C₂₉ 5 β -stanols divided by their corresponding 5 α -stanols were generally above 2 and for humans were greater than 10. Those values were for undiluted faecal samples. In lake or river samples the faecal sterol fingerprint is superimposed by the sterol fingerprint of a broad range of other organic matter including allochthonous detritus and autochthonous algal and higher plants. In addition, the sterol profile may also reflect changes due to chemical digenesis or microbial processes. These processes can dilute and lower the 5 β /5 α stanol ratio, but not completely eliminate the elevating effect caused by preponderance of 5 β -stanols if human or herbivore faeces is present.

Table 8: Concentration of coprostanol, total organic matter and ratio of coprostanol to 5 α -cholestanol in sediment from a range of pristine sites in eastern Australia. Data from sites adjacent to Sydney's Malabar ocean outfall (Leeming and Nichols, 1998) are included as a comparison to other sites considered uncontaminated by sewage pollution.

Location & site no.	Ratio $\frac{5\beta\text{-coprostanol}}{5\alpha\text{-cholestanol}}$	Coprostanol (ng g ⁻¹)	Total organic matter (mg g ⁻¹)	Depth (m)	Distance from point source (km)	Latitude (south)	Longitude (east)
Derwent Estuary¹							
Z2	0.15	9	14	-	-	-	-
D9	0.49	357	186	25	-	-	-
G4	0.80	779	166	23	-	-	-
H1	2.12	1812	160	22	-	-	-
U5	0.51	707	188	9	-	-	-
Jervis Bay²							
12	0.18	18	21.7	51	-	35° 14.23	150° 37.76
13	0.2	48	52.6	68	-	35° 14.70	150° 39.16
14	0.19	40	38.4	93	-	35° 15.16	150° 40.31
15	0.19	58	49.9	107	-	35° 15.59	150° 41.46
17	0.24	19	39.1	136	-	35° 18.07	150° 48.19
18	0.16	19	39.5	353	-	35° 19.82	150° 53.95
Great Keppel Island³							
s1	0.16	7.5	-	-	-	-	-
s2	0.16	7.4	-	-	-	-	-
r1	0.19	22	-	-	-	-	-
r2	0.14	15	-	-	-	-	-
Sydney⁴							
36	0.13	13	109.9	1031	48	33° 58.83	151° 49.26
35	0.17	12	56.3	169	24	33° 58.18	151° 33.74
34	0.17	26	52.2	141	17	33° 57.90	151° 27.88
33	0.29	38	24.9	95	6.2	33° 58.03	151° 22.02
31	3.4	1255	56.1	88	2.4	33° 58.31	151° 19.50
27	2.1	106	11.6	49	1.7	33° 58.27	151° 16.61
28	2.8	560	15.3	70	1.2	33° 58.14	151° 17.25
29	6.8	1846	26.7	78	0.4	33° 58.00	151° 18.13
30	9.3	2869	42.8	78	0.4	33° 50.07	151° 18.19

¹ Leeming and Nichols, 1998, ² previously unpublished data related to Nichols et al., 1993, ³ Leeming and Nichols, 1992, ⁴ Nichols et al., 1993 plus additional unpublished data.

In pristine sediments, the ratio of coprostanol / 5 α -cholestanol is generally below 0.3 (see Great Keppel Is. and Jervis Bay, Table 8). Changes in the ratio caused by proximity to a point source are illustrated by comparing sites adjacent to Sydney's sewage outfalls. Sites near the outfall had a ratio greater than 5 as opposed to the remote sites where the ratio decreased to background values of 0.1-0.2 (Table 8). Likewise, the background value in Storm Bay south of Hobart was 0.15 but ratios were greater than 2 nearer the sewage outfalls adjacent to the city. From these and other similar results (Leeming and Nichols, 1998) it is proposed that 0.3 to 0.4 represent the highest ratio values likely to be measured in uncontaminated environments.

Δ^5 -sterols: cholesterol and 24-ethylcholesterol

The proportions of stanols compared to their precursor sterols provide supporting information about the environment from which the sample was taken and can help confirm interpretations about faecal sources. As noted above, anaerobic sediments often contain equal or higher proportions of 5 α -stanols compared to Δ^5 -sterols (Nishimura, 1982). In contrast, it was 5 β -stanols that were 2-2.4 times more abundant than Δ^5 -sterols in humans, cows and sheep and 0.8-1.4 times more abundant in rats, kangaroos and possums. However, in birds and dogs, Δ^5 -sterols were the dominant compounds with the respective 5 α -stanols usually less than 10% of the sterols and 5 β -stanols less than 10% of the 5 α -stanols or absent altogether (Tables 6 and 7).

In the case of dogs and coastal birds (pelican, seagull, ibis) the dominant sterol was cholesterol reflecting a diet of meat, fish and small organisms with a low plant intake. In the Lake Macquarie field trial, the percentage of Δ^5 -sterols (cholesterol and 24-ethylcholesterol) was often more than 50% of the total sterols (total includes algal derived sterols) in water samples from Lake Macquarie. This was at sites where coastal birdlife was extremely prolific and the percentage of faecal contamination from birds, dogs and other diffuse sources was calculated at greater than 90%. Cholesterol is ubiquitous in the environment and is a major component of the sterol profile of water samples. However, it rarely dominates the profile unless a single source rich in cholesterol is present. Because the faecal sterol signature's of seagulls and pelicans are overwhelmingly dominated by Δ^5 -sterols (> 80%), faecal matter from these birds may have noticeably elevated the proportion of Δ^5 -sterols in the Lake Macquarie samples. While such a result cannot unambiguously identify a source like pelican or seagull faeces, it does provide supporting evidence.

Bacterial Indicators & *B. fragilis* Bacteriophages

Estimated numbers of bacterial indicators determined in this study were generally not significantly different from the same species sampled in the pilot study. Furthermore, additional animal species were examined for the first time in this study.

In most animal species, the percentage of thermotolerant coliforms confirmed as *E. coli* was 80-90%. In humans, however, the percentage was found to be more variable, ranging from 2 to 60%, rather than being some 80% as determined in the pilot study. Nonetheless, it is important to remember, that as human faeces "matures" in the sewer, the proportion of *E. coli* in the population of coliforms decreases from some 80% to 25% (Bitton, 1994). Hence, as an indicator of sewage input, thermotolerant coliforms may be simpler and preferred for the determination of ratios with faecal sterols. However, there are some environments when thermotolerant coliforms can be misleading, such as waterways receiving pulp and paper

wastewaters, due to the presence of *Klebsiella* spp. (Niemi *et al.*, 1987) or some warm waters, due to the growth of *Enterococci* spp., *Klebsiella* and sometimes *E. coli* (Niemi *et al.*, 1987; Ashbolt *et al.*, 1996). In a similar vein, faecal streptococci are simpler and quicker to confirm than enterococci, and given the problems with faecal streptococci / enterococci ratios for sourcing faecal pollution, there is little advantage with using enterococci. Nonetheless, with the advent of defined substrate media for direct enumeration of *E. coli* and enterococci (e.g. Colilert™ and Enterolert™, IDEXX, USA), it is interesting that for the animal species tested (Tables 2, 6 & 7), it makes little difference to the interpretation of the bacterial indicator / faecal sterol ratio results (given in the next section). Furthermore, the independent use of four subgroups of bacterial indicators as a common denominator of all faecal pollution, could only smooth the analytical and environmental variability and strengthen the degree of confidence in estimates of faecal sources.

Spores from the bacterial indicator *Clostridium perfringens* were only detected in about 30% of human, 20-40% of herbivores, but in over 60% of pig and in 95% of dog faeces. The average estimate of spores in human faeces was 1.7×10^5 cfu g⁻¹ dry wt., and the mean number estimated from Sydney's sewage is 1.3×10^5 cfu 100 ml⁻¹ (95% CI 99,000-160,000) (Ashbolt unpublished). In contrast, dogs had an average of 9.2×10^8 cfu g⁻¹ dry wt., whereas pigs were similar to humans (4.8×10^5 cfu g⁻¹ dry wt., Tables 2 and 6). Hence, while dogs have a similar number of thermotolerant coliforms and faecal streptococci to that found in humans, their relatively higher numbers of *C. perfringens* spores may be a useful indicator when fresh faecal contamination is being investigated. It is important to note that spores of *C. perfringens* have half-lives orders of magnitude longer than the other bacterial indicators (Davies *et al.*, 1995).

The bacteriophages to *Bacteroides fragilis* were investigated for the first time in Australia, to see if the various host strains of *B. fragilis* could discriminate between human, pig, cattle, sheep or chicken faeces. The results in general were disappointing in that the phages were generally not detected, even when enrichment cultures were employed. Storage of faecal material at -80° C may have reduced the numbers of phages, but it is the recommended method for storage (Araujo pers. comm.). Nonetheless, phages to the human-specific strain *B. fragilis* HSP40 were detected from sewage at concentrations of up to 140 plaque forming units [pfu] mL⁻¹. Less than 5% of humans excrete this phage (<10% reported for North America, Prof. Mark Sobsey, *per. comm.*). Three of the host strains were described as being sensitive to infection by pig *B. fragilis* phages (Dr Araujo, University of Barcelona, *pers. comm.*), but only one host strain, RYC2056 produced plaques (4/30 pig faecal samples). The estimated numbers from pig faeces varied from 780-820,000 pfu/g⁻¹ dry wt. Fourteen of the thirty seagull and 2/30 chicken samples were also positive for this poultry-pig strain, RYC 2056. On the other hand, one Ibis faecal sample was positive to the poultry-sheep-pigs strain RYC 3317. Unexpectedly, one dog faecal sample was positive to the pig-sheep-poultry strain RYC 3318, the strain that was also positive for 2/30 sheep samples.

Ratios of Bacterial Indicators to Faecal Sterols

As long as the relative concentrations of other stanol isomers and Δ^5 -sterols are also considered, the presence of 5 β -stanols can provide a very clear indication of human and / or herbivore faecal contamination and distinguish those two sources quantitatively against the total faecal contamination. Because bird and dog faecal matter does not contain consequential amounts of 5 β -stanols, contributions from these animals is obtained by difference; i.e. the

difference between predicted bacterial indicators (*based on faecal biomarkers*) and measured bacterial indicators. To provide the maximum information to water managers, the concentrations of both faecal sterols and the commonly used bacterial indicators need to be equated.

The mean ratios for bacterial indicators divided by coprostanol in Table 2 are based on bacterial indicator (cfu L⁻¹ or cfu g⁻¹) divided by coprostanol (ng L⁻¹ or ng g⁻¹) and are means of individual ratios. The ratios of mean concentrations are given in parentheses. Taking into account the measured variability for individuals, the ratios from the human faecal samples are consistent with the ratios elucidated from the pilot study (Table 2), previously published data from Australia and overseas (Leeming and Nichols, 1996) and sewer reference samples collected during two separate field studies (Table 9). For example, the ratio of thermotolerant coliforms divided by coprostanol was calculated to be 19 [this study, Table 2], 30 [this study using the ratio of means], 13 [pilot study, Table 2], 25 [Leeming and Nichols, 1996], and 18, 38 and 41 in field studies [see Table 9]. While there is considerable variation in the ratios between individuals, variation diminishes across populations large enough to cause measurable faecal pollution under high dilution conditions in receiving waters.

Using the measured variance from the faecal samples and the data available from reference samples, a margin for error can be incorporated into estimates of faecal contributions. A mid point ratio of 25 ± 15 for the thermotolerant coliforms / coprostanol ratio is proposed to be a reasonable approximate of the true mean ratio whilst accommodating a range of values within the 90% confidence limits (see legend in Figure 4 for a summary of proposed ratios). The data set for confirmed *E. coli* was more limited, but assuming the percentage of thermotolerant coliforms confirmed as *E. coli* in human faecal matter is $\approx 80\%$ (Bitten, 1994), the proposed mid point ratio and range for *E. coli* is 20 ± 12 . As is shown in the accompanying field studies, such an apparently wide range of values for the bacterial indicator to faecal sterol ratio rarely culminate in widely divergent interpretations. Rather a range of estimates is provided within which the real contribution from a faecal source is almost certain to lie.

The results for the faecal streptococci / coprostanol ratio followed a similar pattern to the thermotolerant coliform / coprostanol ratio. Since enterococci are a subgroup of faecal streptococci it was not surprising that on average enterococci comprised 83% of faecal streptococci and that ratios using confirmed enterococci instead of faecal streptococci were slightly lower. The ratio of faecal streptococci divided by coprostanol was calculated to be 2.6 [this study, Table 2], 6.4 [this study using the ratio of means], 11 [pilot study, Table 2] and 2.9, 4.9 and 5.9 in field studies [see Table 9]. Using a similar procedure as above, a mid point ratio of 6.5 ± 4 is proposed to be a reasonable approximate of the true mean ratio whilst accommodating a range of values between 2.5-10.5. The proposed mid point ratio for enterococci is 5.5 ± 4 .

Table 9: Concentrations and ratios of sterols and bacterial indicators of diagnostic relevance in reference sewer samples collected during field studies.

Variable	¹ Winding Ck	¹ Rippleside sewers 1 & 2	
coprostanol (cop)	262	2650	3400
cholesterol	106	1260	1410
5 α -cholestanol	16	bd	bd
24-ethylcoprostanol (ethylcop)	49	525	639
24-ethylcholesterol	25	244	229
24-ethylcholestanol	7	45	44
thermotolerant coliforms (ThC)	4.8E+5	1.0E+7	1.4E+7
<i>E. coli</i> (EC)	na	1.0E+7	1.4E+7
faecal streptococci (FS)	7.6E+4	1.3E+6	2.0E+6
enterococci (Ent)	na	1.3E+6	1.5E+6
<i>C. perfringens</i> spores (CP)	2.6E+4	4.0E+4	1.0E+3
² ThC / coprostanol	18	38	41
² EC / coprostanol	nq	38	41
² FS / coprostanol	2.9	4.9	5.9
² Ent / coprostanol	nq	4.9	4.4
% cop / (cop + ethylcop)	84%	83%	84%
% ethylcop / (cop + ethylcop)	16%	17%	16%
cop / cholesterol	16	nq	nq
ethylcop / 24-ethylcholestanol	6.9	12	15

na: not analysed; nq: not-quantifiable; bd: below detection (see methods);

¹ Sterols ($\mu\text{g L}^{-1}$) & bacterial indicators (cfu 100 ml⁻¹);

² Bacterial indicator to sterol ratios based on cfu L⁻¹ divided by ng L⁻¹.

The mean ratios for herbivore faecal contributions (Tables 3-5) were based on bacterial indicators (cfu L⁻¹ or cfu g⁻¹) divided by 24-ethylcoprostanol (ng L⁻¹ or ng g⁻¹) and again are means of individual ratios. The ratios of mean concentrations are given in parentheses. While the ratio data for human inputs could come from a variety of sources thereby corroborating each other, the data for herbivore ratios comes solely from faecal samples. All together 92 samples were collected from herbivores (see footnotes Tables 3-5 for details) to provide a representative estimate of the ratios. Since the ratios among herbivores were generally similar (Tables 3-5), the same procedure used to determine a mid point ratio plus or minus a range of values for human inputs was used for herbivores. The proposed mid point ratio for thermotolerant coliforms / 24-ethylcoprostanol in herbivores is 8 ± 5 which is a close approximate of the mean ratio for all herbivores (Table 5) and gives a range of values between 3-13. For faecal streptococci / 24-ethylcoprostanol the proposed mid point ratio is 4.5 ± 1.5 giving a range of values between 3-6. *E. coli* and enterococci were not confirmed for all samples. As noted above, in most animal species the percentage of these confirmed sub-groups was 80-90%. The ratios for these indicators divided by 24-ethylcoprostanol are therefore extrapolated from the available data. Hence the ratio for *E. coli* would be 7 ± 4 and for enterococci 4 ± 1.5 .

Faecal contamination from pigs is most likely to be from a point source such as a piggery. Because the proportions of coprostanol and 24-ethylcoprostanol are close to midway between humans and herbivores (Table 6), this could potentially confuse the calculation of contributions if all 3 sources were present in significant amounts in the one sample. However, recent results indicate that pig faeces can be distinguished from human and herbivore faeces using cholanic bile acids (*pers. comm.* R. Evershed). Despite the possibility for confusion, in a case study of the Murray and Serpentine Rivers (Leeming, 1996b) the faecal sterol profile of sediments satisfactorily distinguished where faecal contamination from pigs and humans was predominant. On the basis of data in Table 6, the proposed mid point ratio for thermotolerant coliforms / 24-ethylcoprostanol in pigs is 14 ± 6 and for faecal streptococci / 24-ethylcoprostanol the proposed mid point ratio is 20 ± 12 .

To illustrate how well defined the various groupings are, Figure 3 shows a principal components analysis of faecal samples, reference samples and water samples from a stormwater drain in Rippleside, Geelong. The samples from the wet survey were well correlated with reference samples of human faeces. In contrast, dry weather samples were grouped in the same hemisphere as birds, dogs and other diffuse sources, but were grouped to the left of these animals because of differences in the absolute abundance of bacterial indicators (Fig. 3).

Empirical Calculations for % Faecal Source

The next step is to establish a procedure using the above results to empirically calculate the proportions of faecal contamination in water samples. A flow diagram is shown in Figure 4 to simplify the steps. It is emphasised that the estimation of contributions from a faecal source is not based solely on the empirical calculations, rather these are used as a starting point. Furthermore, the level of accuracy that can be expected from the estimates diminishes, not only with time and distance from a point of entry, but also as one proceeds through steps in the protocol. To put this in context, we can with a high degree of certainty, determine human versus other sources of faecal pollution. We can also, with a high degree of certainty but slightly less precision, determine and distinguish human versus herbivore faecal contamination

and these two sources versus other sources. We can then, with a much diminished degree of precision, estimate faecal contamination from birds versus domestic pets provided these constitute a significant proportion to the overall faecal contamination. Despite the implied imprecision of the technique, particularly with the latter animals, results from the various field studies have proved to be logical in the context of anecdotal evidence and consistent within data sets. This has enabled water managers to make effective decisions with regard to remediation of identified inputs. Further advances in applying the various strains of *B. fragilis* to identify birds and pigs will strengthen the approach.

Human and Herbivore Contributions: Using the proportions of coprostanol versus 24-ethylcoprostanol (Tables 2-4) it is possible to estimate the relative contributions of faecal pollution of herbivorous animals and humans where faecal matter from both sources are present. The percentage of coprostanol relative to the sum of coprostanol and 24-ethylcoprostanol in humans was $75\% \pm 2$ and for all herbivores $30\% \pm 1$. Where both sources of faecal pollution are present in the environment, the proportions of each source can be calculated between the above percentages relative to each other using the following calculations. It is stressed that the following calculations provide an empirical basis for determining proportions of faecal pollution and are not used in isolation from other quantitative, qualitative and anecdotal data.

$$\text{If } \left(\frac{5\beta\text{-C}_{27}\Delta^0}{5\beta\text{-C}_{27}\Delta^0 + 5\beta\text{-C}_{29}\Delta^0} \right) \times 100 > 75$$

where $5\beta\text{-C}_{27}\Delta^0$ is coprostanol and $5\beta\text{-C}_{29}\Delta^0$ is 24-ethylcoprostanol, then faecal contamination originates solely from humans.

$$\text{If } \left(\frac{5\beta\text{-C}_{27}\Delta^0}{5\beta\text{-C}_{27}\Delta^0 + 5\beta\text{-C}_{29}\Delta^0} \right) \times 100 < 30$$

then faecal contamination originates solely from herbivores.

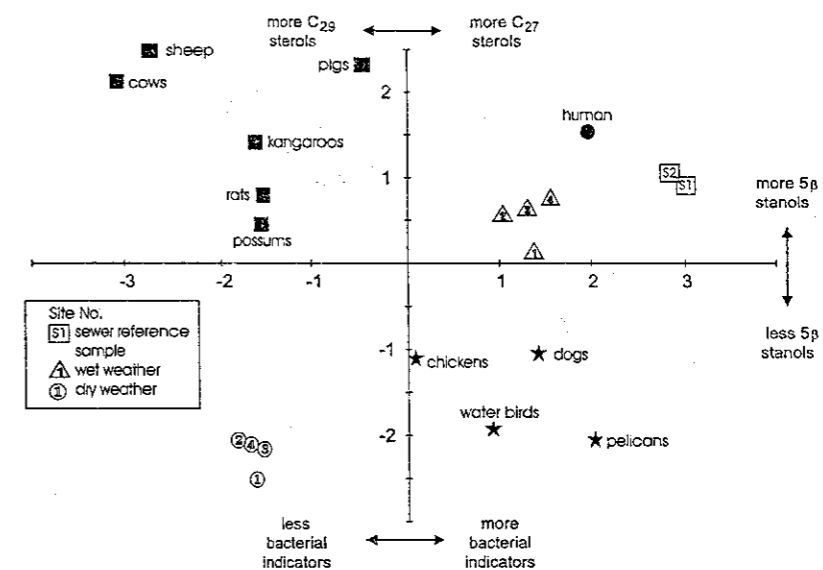


Figure 3: Plot of principal components analysis of human, herbivore, bird, and dog faeces, sewer reference samples and samples from the Rippleside stormwater drain. Components were separated on the basis of the faecal sterols listed in Table 1 (except $5\beta(\text{H}),3\alpha(\text{OH})\text{-epi-stanols}$) and data for *E. coli*, faecal streptococci and *C. perfringens* spores; faecal samples were means with $n > 10$. The main differences which separated the components were the abundance of 5β -stanols compared to $\Delta 5$ -sterols, the carbon number of the sterol precursors ($\text{C}_{27} - \text{C}_{29}$), and the abundance of bacterial indicators.

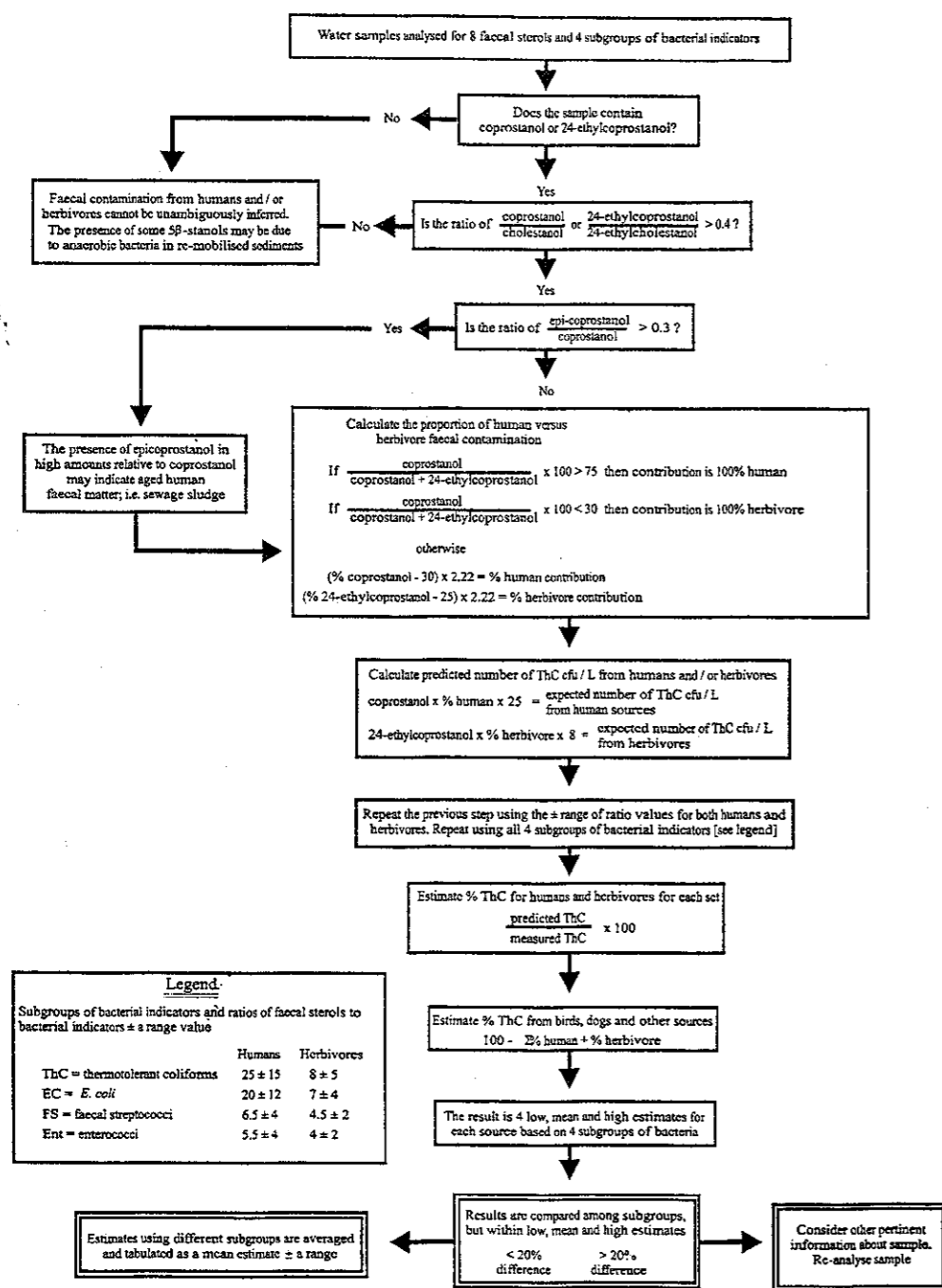


Figure 4: Flow diagram of steps used to distinguish sources of faecal contamination.

In the case of a water sample containing both human and herbivore faecal contamination, the difference between 100% human input and 100% herbivore input is 45% points; i.e. 75% coprostanol compared to 30% coprostanol (respectively) as a proportion of the sum of coprostanol plus 24-ethylcoprostanol = 45. The 45% points are divided into 100% to give a factor of 2.2. For every one percent less of coprostanol below 75%, the proportion of human faecal contamination *relative only to faecal biomarkers* is 2.2% less. Therefore if a sample had 45% coprostanol and 55% 24-ethylcoprostanol then;

$$[\% \text{coprostanol} - 30] \times 2.22 = \% \text{ human contribution}$$

$$[\% 24\text{-ethylcoprostanol} - 25] \times 2.22 = \% \text{ herbivore contribution}$$

Other Sources of Faecal Contamination: Taking these proportions into account, it is possible to estimate the number of bacterial indicators predicted for given concentrations of coprostanol and 24-ethylcoprostanol using the ratios proposed above and summarised in Figure 4. The bacterial indicator abundances are used as common denominators taking into account the percentage of faecal sterols attributed to either human or herbivore sources.

For example, a sample with 80 ng L⁻¹ of coprostanol x 25 (ratio of 25:1) x 0.75 (75% human derived coprostanol) would be *predicted* to have a thermotolerant coliform abundance of 1,500 cfu / L or 150 cfu / 100 ml. This is compared to the *measured* abundance of thermotolerant coliforms and normalised to 0-100% if necessary. The same formula is then applied using *E. coli*, faecal streptococci and enterococci respectively and also applied to 24-ethylcoprostanol and the various bacterial indicators to arrive at estimated herbivore contributions. Where % human and % herbivore are < 100%, the difference is the % birds, dogs and other diffuse sources of faecal contamination. The estimates from each of the calculations (using the different bacterial indicators) are then compared. Those estimates that vary by less than 20% are averaged to provide a final estimate of the contribution from a faecal source. Samples where estimates vary by more than 20% are flagged for further investigation and/or reported as such in the text.

The component of faecal contamination termed "birds, dogs and other diffuse sources", refers to bacterial indicators which represent faecal matter, but come from sources that do not convert Δ⁵-sterols to 5β-stanols. Possible sources of "diffuse faecal contamination" include invertebrates, reptiles and fish (Walker *et al.*, 1982). However, one of the most likely sources of this faecal contamination is birds. This is because they are ubiquitous and abundant in the environment. Dogs are also a large potential source of faecal contamination. But while some dog faeces undoubtedly are carried into waterways, a large proportion also decomposes in grass covered areas such as backyards. It was previously identified that dogs appeared to have uncommonly high abundances of *C. perfringens* spores (Leeming *et al.*, 1996). The present study confirms that dog's faeces had a mean concentration of 9.2E+8 cfu g⁻¹ dry wt. of *C. perfringens* spores (Table 6). In all other animals including humans, but excepting pigs, *C. perfringens* spores could only be measured in less than a third of samples with a detection limit of ≈ 1.0E+3 cfu g⁻¹ (Tables 2-7). The one third of samples where the abundance of *C. perfringens* spores was above this detection limit suggest that the likely population mean abundance for human faecal contamination would be in the order of 1.0E+4 cfu g⁻¹ and for other animals probably less. This indicates that dogs have at least 3-4 orders of magnitude higher *C. perfringens* spores than any other animal. On this basis, unexpectedly high *C. perfringens* spores in comparison to either faecal sterols or other bacterial indicators might

indicate that dog faeces are a significant contributor to a particular sample. However, this concept would only be valid in situations where faecal contamination was fresh and residence times were very short. This is because *C. perfringens* spores are considerably more resistant to die-off in the environment than other bacterial indicators and increase relative to other indicators over time. Until another independent measure for dog faecal contamination can be established (such as a bile acid marker or unique bacteriophage), it is not recommended to unequivocally quantify dog faecal pollution in the above manner. What the above premise suggests is that a background level of non-human faecal contamination is always prevalent. The results from the field studies support this notion. A proportion of low level faecal pollution present during dry weather is probably caused by faecal matter from animals deposited in or adjacent to waterways. During rain events, much larger quantities of faecal matter in varying states of decay can be swept into waterways from catchments; human faecal contamination is just a further burden on water quality albeit often a very significant one.

Field Studies

To validate and develop the methodology devised during the course of this investigation, a series of companion field projects were performed working in close association with local councils, water authorities and state environment agencies. A listing of such studies is included in Appendix 1 and the references. Summaries of the Rippleside study undertaken in Geelong, Victoria and the Lake Macquarie study undertaken in Newcastle, NSW follow.

Field Application #1 - Rippleside Stormwater Drain

The Rippleside stormwater project was designed to provide preliminary information to the Victorian Environmental Protection Authority about the input of faecal matter to stormwater drains in the Rippleside area of Geelong, Victoria. The principal aim of this study was to determine whether faecal pollution, measured by traditional bacterial indicators (i.e. thermotolerant coliforms) in the stormwater, was principally derived from human faecal matter. The full report is available from the CSIRO Division of Marine Research library (Leeming, 1996c).

The results showed that during wet weather, all the sites sampled except for the Golf Course site, were affected by human faecal contamination (see Fig. 5 for site locations). Coprostanol concentrations of $\approx 500 \mu\text{g L}^{-1}$ were recorded at all sites from Ballarat Rd. downstream except for Rippleside Beach ($169 \mu\text{g L}^{-1}$); such concentrations are generally found in treated albeit undiluted sewage effluent. Clearly the sewerage system was compromised above Ballarat Rd. (and perhaps below as well) by an overflow of sewage into the stormwater drain, either by design or by accident, and human faecal matter could be identified right down to Rippleside Beach. Four other samples had coprostanol concentrations greater than 400 ng L^{-1} that were of human origin; Weddle Rd. south during the first dry weather survey and first wet weather survey, Ballarat Rd. during the second wet weather survey and Railway Bridge [below Weddle Rd. south] during the first dry weather survey. The second wet weather survey was undertaken a little over a week after the first wet weather survey and much lower levels of both bacterial indicators and faecal biomarkers were observed; i.e from $\approx 10^6$ down to $10^2 E. coli$ cfu 100 ml^{-1} . This may reflect that either the second rain event was much smaller than the first or that the catchment and stormwater drain had been flushed out by the first rain event. Despite this it was clear that human faecal contamination had again entered the stormwater drain above Ballarat Rd as a consequence of wet weather

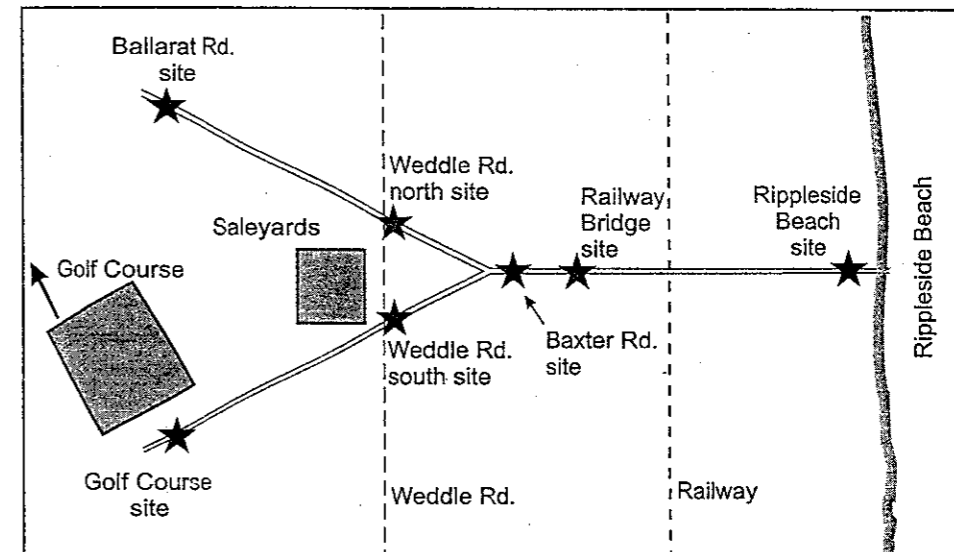


Figure 5: Schematic layout of Rippleside stormwater drains and location of sampling sites.

Underlying the acute episodes of human faecal contamination, there were also high levels of faecal contamination, based on bacterial indicators ($\approx 10^3$ *E. coli* cfu 100 ml⁻¹), from birds, dogs and other diffuse sources. During dry weather, this faecal contamination constituted the majority of the faecal pollution and was still a significant component during wet weather as yet more diffuse faecal matter from the catchment was swept into the drains. A low level of chronic human faecal contamination was also present in both the dry weather surveys. There was no indication of faecal contamination from herbivores at the saleyards entering the southern stormwater drain; while this might seem surprising, it is consistent with information that prior to this study a sump was constructed at the saleyards to capture runoff during rain events. A distinctive faecal biomarker profile for herbivores was identified at the Golf Course site during the first wet weather survey.

Field Application #2 - Lake Macquarie

The sources of faecal pollution to Lake Macquarie and its influent creeks and drains were investigated by examining and comparing concentrations of faecal sterols and bacterial indicators in water samples collected during high water flows (rain events) and low water flows. The full report is available from the CSIRO Division of Marine Research library (Leeming, 1997a). Overall, very high concentrations of bacterial indicators were measured at most sites ($\approx 10^3$ - 10^4 thermotolerant coliforms and/or faecal streptococci cfu 100 ml⁻¹). Faecal contamination from human waste was, on a relative basis, generally only a small proportion (< 3%) of the total faecal pollution measured. However, the absolute amount of human faecal contamination would still have been high enough for nine samples from six of the sites to have exceeded the primary contact limit of 150 cfu / 100 mL of thermotolerant coliforms. Furthermore, some human faecal contamination was measured at every site on one occasion or more. Of greatest concern were the results from Winding Creek which contained the human faecal biomarker coprostanol at concentrations greater than 1,500 ng L⁻¹ which is indicative of considerable leakage of raw sewage. Elevated concentrations of coprostanol attributed to human sources (> 60 ng L⁻¹) were also measured in Cockle Creek, North Creek, South Creek, Warners Bay, Rathmines and Dora Creek. Faecal matter from herbivores was also identified as a small component of the overall faecal pollution, particularly in South Creek and North Creek.

Although some human and herbivore faecal contamination was often present, the concentrations of faecal biomarkers were not high enough to account for the high concentrations of both thermotolerant coliforms and faecal streptococci that were measured during these surveys. This component was designated "other sources" and refers to birds, dogs and other diffuse sources which do not convert $\Delta 5$ -sterols to 5β -stanols. Several issues which might affect or explain differences in the concentration of bacterial indicators compared to faecal biomarkers used are discussed below in more detail. Even allowing for a reasonable degree of error in the estimates, we propose from this data that the estimates of human faecal contributions reflect the known and likely inputs to Lake Macquarie.

Issues for Consideration

We have explained the theoretical basis for distinguishing faecal contamination and presented data to support the protocol proposed. We have also detailed the procedures which enable estimates of the proportion of faecal contamination to be made and demonstrated their application in the environment (see Leeming, 1996a-f, 1997a-f in the reference section and Appendix 1). This section deals with several issues which might affect or explain differences

in the concentration of bacterial indicators compared to faecal biomarkers which are not due to the amount of faecal contamination. These include; (i) degradation of faecal biomarkers and / or bacterial indicators thus changing the faecal biomarker to bacterial indicator ratios, (ii) the affect of adhesion of faecal sterols and bacterial indicators to different particles and their potentially different settling rates, (iii) regrowth of bacterial indicators and (iv) inadvertent measurement of other species of bacteria.

These issues need to be examined since; (i) no biomarker(s) have been identified to date that are specific to birds and / or dogs and, (ii) the efficacy of the procedures developed in this study depends on a thorough understanding of the potential of these issues to influence any interpretation. These are particularly important issues as seen in the field studies (Leeming, 1996c & 1997a) where in some samples bacterial indicator abundances were high, but faecal sterols were low. These instances occur more often during dry weather probably because "emergency relief structures" or sewer overflows only operate during wet weather, and similarly, leaks from broken sewer pipes have a much higher likelihood of impacting waterways during rain events. Whenever sewer overflows could be shown to have occurred and obviously constituted the majority of the faecal contamination, the ratios between faecal sterols and bacterial indicators were shown to be accurate and robust (Leeming, 1997a-f).

Relative Persistence of Faecal Sterols and Bacterial Indicators

Previous experiments have shown that the half-lives of faecal sterols in marine waters are about 4-10 days depending on the water temperature (Leeming, 1996a). These findings are supported by earlier studies with coprostanol (Switzer-Howse and Dukta, 1978). An additional degradation experiment in freshwater was undertaken during this study to complement previous experiments conducted in marine waters. The similarity in the relative persistence of sterols and stanols in all the experiments is illustrated in Figure 6 which shows sterol and stanol degradation at 12°C. The exception in this experiment was 24-ethylcholesterol which degraded at the same rate as other sterols, but was also synthesised by phytoplankton during the experiment. This was confirmed by the co-production of the algal sterol isofucosterol (24-ethylcholesta-5,24(28)Z-dien-3 β -ol) which along with 24-ethylcholesterol were the principal constituents of algae measured in the seawater stock used in the experiments. The same phenomenon was observed by Marty *et al.* (1996) during degradation experiments of sterols and fatty acids in seawater.

The relative persistence of bacterial indicators and faecal sterols during degradation experiments at 12°C in marine waters and at 17°C in freshwater are shown in Figures 7 and 8 respectively. Removal (or die-off) of both faecal sterols and bacterial indicators could be explained with 1st order decay rates. Preliminary experiments conducted by Leeming (1996a) in seawater indicated that if high concentrations of sewage effluent were used or if populations of sterol degrading bacteria were very low in the batch culture to start with, an induction period of \approx 24-36 hr was observed during which faecal sterol degradation was minimal. This phenomenon was not observed during the experiments illustrated in Figures 7 and 8 probably due to the comparatively high (and realistic) dilution of the original water /sewage effluent mixture and the fact that sterol degrading microbes were in sufficient numbers in the original water or effluent.

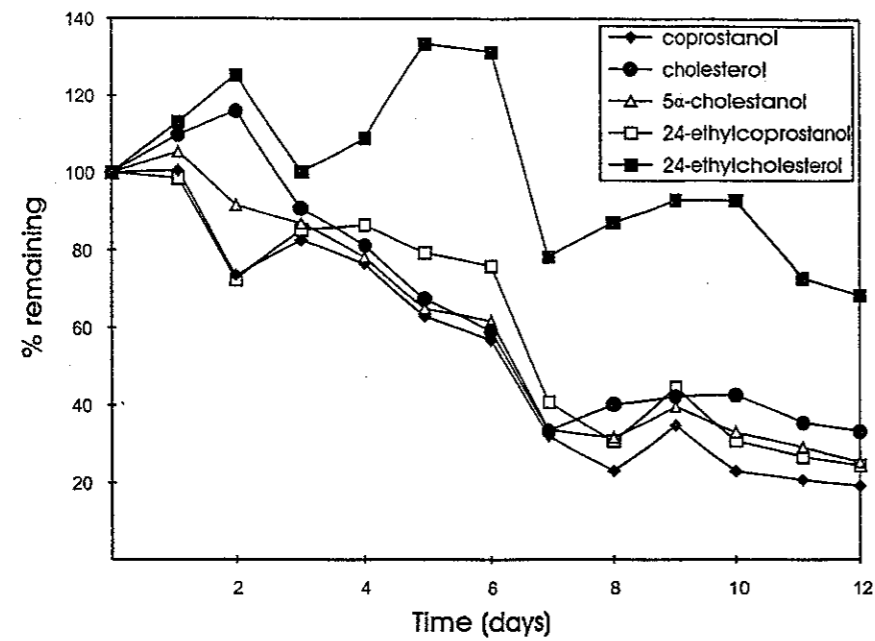


Figure 6: Percent removal of sterols and stanols from seawater in batch culture bottles (*in vitro*) inoculated with sewage effluent and maintained at 12° C

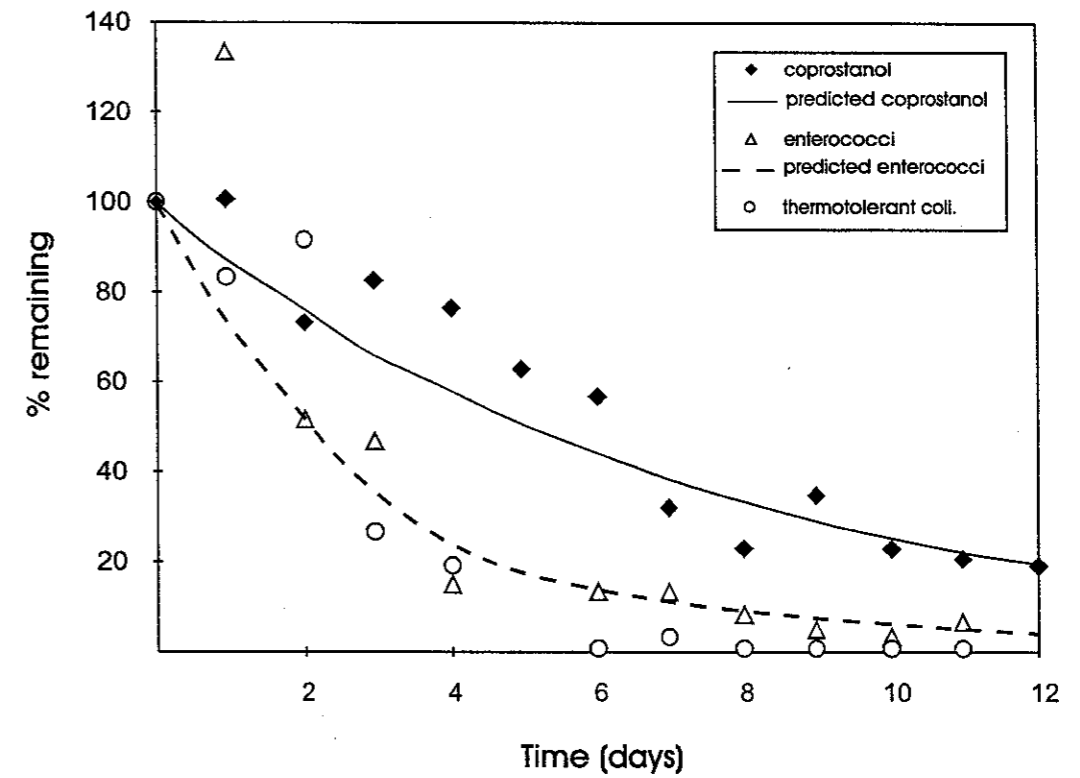


Figure 7: Observed and predicted percent removal of coprostanol and percent removal of thermotolerant coliforms and enterococci from batch culture bottles (*in vitro*) and maintained at 12° C. Batch cultures were comprised of seawater inoculated with sewage effluent at a dilution rate of 10:1. Equations for predicted relationships are shown on page

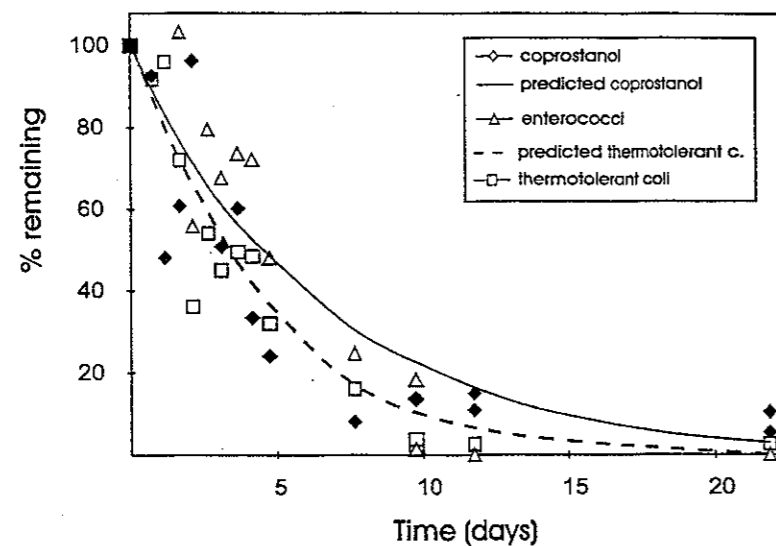


Figure 8: Observed and predicted percent removal of coprostanol and percent removal of thermotolerant coliforms and enterococci from cellulose membrane bags immersed in a freshwater lagoon at a depth of 0.5-1 m and at $\approx 17^\circ\text{C}$. Batch cultures were lagoon water inoculated with sewage effluent at a dilution rate of 50:1. Equations for predicted relationships are shown on page 35.

For coprostanol, % removal (Y) could be explained by the equation*;

$$Y = \text{Exp}[\text{Days} \times 0.137] \times 100 \quad (\text{at } 12^\circ\text{C}, R^2 = 0.91)$$

$$Y = \text{Exp}[\text{Days} \times 0.153] \times 100 \quad (\text{at } 17^\circ\text{C}, R^2 = 0.66)$$

For thermotolerant coliforms, % removal (Y) could be explained by the equation*;

$$Y = \text{Exp}[\text{Days} \times 0.510] \times 100 \quad (\text{at } 12^\circ\text{C}, R^2 = 0.88)$$

$$Y = \text{Exp}[\text{Days} \times 0.221] \times 100 \quad (\text{at } 17^\circ\text{C}, R^2 = 0.75)$$

For enterococci, % removal (Y) could be explained by the equation*;

$$Y = \text{Exp}[\text{Days} \times 0.344] \times 100 \quad (\text{at } 12^\circ\text{C}, R^2 = 0.89)$$

$$Y = \text{Exp}[\text{Days} \times 0.415] \times 100 \quad (\text{at } 17^\circ\text{C}, R^2 = 0.81)$$

* See Figures 7 and 8. Exp is the inverse of LN, the natural logarithm of number.

Thermotolerant coliforms and enterococci both had slightly higher die-off rates than faecal sterols over periods of up to 22 days. Inevitably these different removal rates must affect the ratios between faecal sterols and bacterial indicators. However, based on the measured removal rates above, the change in the ratios between faecal sterols and thermotolerant coliforms and enterococci would still be within the range of proposed values for these ratios 2-3 days after discharge or entry to a waterway (see legend in Fig. 4). Under the environmental conditions of the Rippleside and Lake Macquarie field studies (Leeming, 1996c & 1997a), this time constraint was not considered to have been exceeded (see Field Studies section). Catchment areas were not large and even dry weather flow rates would disperse fresh faecal contamination in the catchments within 2-3 days. During wet weather, when faecal contamination was most prevalent, residence times were almost certainly shorter. A settling experiment was conducted to compare the particle sizes upon which faecal sterols and bacterial indicators adhered. Figure 9 shows that the proportion of faecal sterols versus bacterial indicators remains fairly constant until only particles with a settling velocity of less than 0.14 mm min^{-1} remain. This settling velocity equates to quartz particle sizes of $\approx 9 \phi$ or less. Given this finding, only in standing waters would any differentiation occur that would be in favour of higher bacterial indicators compared to faecal sterols.

Mackay and Ridley (1983) and Ashbolt *et al.* (1996) have previously reported artificially high counts of thermotolerant coliforms caused by regrowth. A possible source of additional thermotolerant coliforms from non-human sources might be the inadvertent measurement of other species of coliforms of non-faecal origin such as those in the genus *Klebsiella*, common in pulp mill effluents and decaying wood (Cabelli *et al.*, 1983; Niemi *et al.*, 1987). However, contributions from *Klebsiella* would still not explain high and well correlated concentrations of faecal streptococci or enterococci. Indeed, measurement of faecal streptococci and / or enterococci provides a check for faecal presence versus regrowth of coliforms. For example, an initial increase of 10-20% in the density of thermotolerant coliforms (after 48 h) followed by a steady decline was observed by Leeming (1996a) during previous degradation experiments. Neither faecal streptococci nor enterococci showed any trend for regrowth in the same experiments (Leeming, 1996a) nor to our knowledge have they been reported to do so. In all of the degradation experiments there were just 3 observations for enterococci which were higher than the original concentrations (Figures 6 and 7) and given all the other evidence, these observations are suggested to be outlying values.

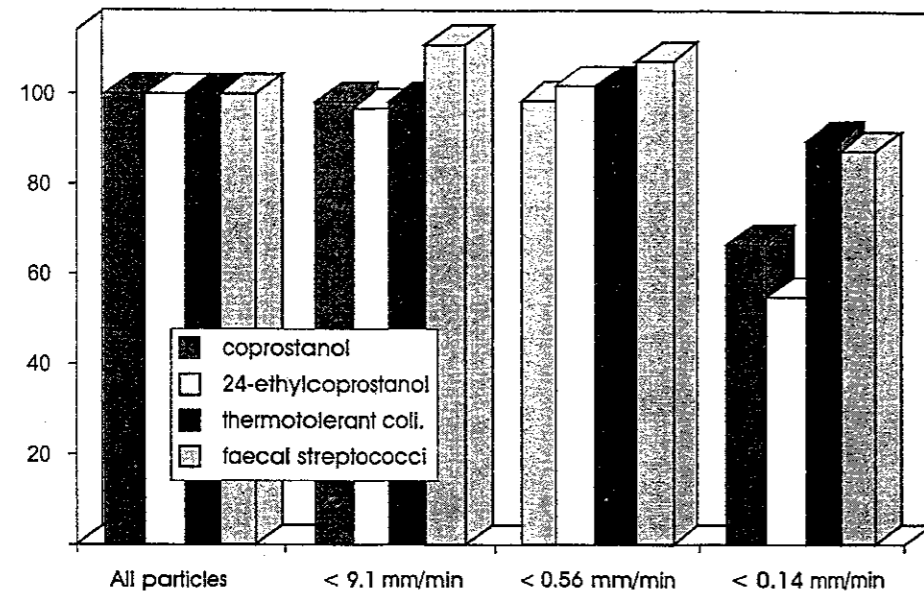


Figure 9: Results for settling experiment showing percent remaining of coprostanol, 24-ethylcoprostanol, thermotolerant coliforms and faecal streptococci associated with various particle size velocities. Velocities equate to quartz particle sizes of $>4\phi$, 6ϕ , 8ϕ and 9ϕ respectively.

CONCLUSIONS

This study has performed a more thorough examination of the theoretical basis of the new technique to distinguish faecal pollution than the preliminary experiments from which it evolved. The variables of diagnostic value now have a considerably more solid base to stand on. The efficacy of this technique to distinguish faecal contamination is, as with any indicator, a function of time and distance from the possible source. It is acknowledged that the ratios of bacterial indicators to faecal biomarkers may change over time and distance and that such changes would be more likely during dry weather when flows are low or have ceased altogether. The affects of degradation and die-off have been shown to be fairly similar although over time the divergence in relative concentrations inevitably increases, particularly for spores of *C. perfringens*. The possibility that faecal indicators (i.e. both bacterial indicators and biomarkers) adhere to different particles and consequently settle at different rates during low flows was shown to be only a potential issue for standing waters.

It is clear that a simplistic approach to the measurement of coprostanol or other indicators alone is inadequate to distinguish faecal contamination. However, in the companion field studies we were able to exploit the combined faecal sterols and bacterial indicator approach to clearly identify the trouble spots of human faecal contamination and thus confirm the utility of the biomarker approach. In many of the samples analysed during field studies, the source of the bacterial indicators was not of either human or herbivore origin. Bird life, which is prolific around Lake Macquarie, and coastal regions like Rippleside was proposed as the most likely source in those situations. Domestic pets were also potential sources in certain locations, but the low concentrations of *C. perfringens* spores relative to other bacterial indicators in most instances suggested faecal matter from dogs generally was not a major component of the faecal contamination.

The conclusion from the phage work is that human sewage contamination may be identified by the presence of bacteriophages to *B. fragilis* HSP40, but 1-20L samples may be necessary for enumeration of these relatively low density viruses. Furthermore, the presence of pig and certain bird faeces (but not water fowl), which currently can not be identified by the faecal sterol biomarkers, may also be indicated by phages to *B. fragilis* strain RYC 2056. An alternative to the less numerous *B. fragilis* bacteriophages is to serotype the more numerous F-RNA bacteriophages, where serogroup II is largely human while other serogroups represent other animals (Osawa *et al.*, 1981; Inokuchi *et al.*, 1982). Direct molecular probing of these F-RNA bacteriophages is now possible for rapid animal sourcing work (Beekwilder *et al.*, 1996; Sobsey *pers. comm.*).

In light of the results of this study, it is recommended that further research should be conducted in several complementary areas. Firstly, other classes of lipid biomarkers (e.g. cholanic bile acids, bile pigments and carbon isotope ratios of selected biomarkers) show distinctive profiles between animals. Additional research to discover specific biomarkers for birds would be highly desirable and appears achievable. Secondly, research on bacterial phages could provide qualitative confirmations of faecal sources to complement biomarker assays and to promote a diversity of approaches to solve current problems. Finally, combined PCR (polymerase chain reaction) and biomarker analyses of environmental samples could also provide an advantageous and diversified approach to distinguishing faecal pollution.

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REFERENCES

- APHA (1989) Standard Methods for the Examination of Water and Wastewater. 17th ed. American Public Health Association, Washington D.C.
- Ashbolt, N. J., Dorsch, M. R., Cox, P. T. and Banens, B. (1996) Blooming *E. coli*, what do they mean? In: *E. coli and Coliforms, Problems and Solutions*. (Ed.: Kay, D.) The Royal Society of Chemistry, Cambridge.
- Beder, S. (1989) Toxic fish and sewer surfing. Allen and Unwin publishers, Australia
- Beekwilder, J., Nieuwenhuizen, R. Havelaar, A. H. and van Duin, J. (1996) An oligonucleotide hybridization assay for the identification and enumeration of F-specific RNA phages in surface water. *J. Appl. Bacteriol.* **80**, 179-186.
- Bitton, G. (1994) Wastewater Microbiology. Wiley-Liss, John Wiley & Sons, Inc, New York.
- Bligh, E. G. and Dyer, W. M. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. and Physiol.* **35**, 911-917.
- Brown, R. C. and Wade, T. L. (1984) Sedimentary coprostanol and hydrocarbon distribution adjacent to a sewage outfall. *Water Res.* **18**, 621-632.
- Cabelli, V. J., Dufour, A. P., McCabe, L.J. and Levin, M. A. (1983) A marine recreational water quality criterion consistent with indicator concepts and risk analysis. *Res. J. Water Pollut. Control Fed.* **55**, 259-265.
- Davies, C. M., Long, J. A., Donald, M. and Ashbolt, N. J. (1995). Survival of fecal microorganisms in aquatic sediments of Sydney, Australia. *Appl. Environ. Microbiol.* **61**, 1888-1896.
- Dutka, B. J., Chau, A. S. Y. and Coburn, J. (1974) Relationship between bacterial indicators of water pollution and faecal steroids. *Water Res.* **18**, 1047-1055.
- Eganhouse, R. P., Olaguer, D. P., Gould, B. R. and Phinny, C. S. (1988) Use of molecular markers for the detection of municipal sewage sludge at sea. *Mar. Env. Res.* **25**, 7-22.
- Gavini, F., Poucher, A.M., Neut, C., Monget, D., Romond, C., Oge, C. and Izard, D. (1991) Phenotypic differentiation of bifidobacteria of human and animal origin. *Int. J. System. Bacteriol.* **41**, 548-557.
- Geldreich, E. E. (1976) Faecal coliform and faecal streptococcus density relationships in waste discharges and receiving waters. *Crit. Rev. Environ. Control*, **6**, 349-369.
- Grabow, W.O.K., Neubrech, T. E., Holtzhausen, C.S. and Jofre, J. (1995) *Bacteroides fragilis* and *Escherichia coli* Bacteriophages: Excretion by Humans and Animals. *Wat. Sci. Tech.* **31**(5-6), 223 - 230.

- Howell J. M., Coyne M. S. and Cornelius P. L. (1995) Faecal bacteria in agricultural waters of the bluegrass region of Kentucky. *J. Environ. Qual.* **24**, 411-419.
- Inokuchi, Y., Hirashima, A. and Watanabe, I. (1982) Comparison of the nucleotide sequences at the 3'(prml)-terminal region of RNAs from RNA coliphages. *J. Mol. Biol.* **158**, 711-730.
- Kreader, C.A. (1995) Design and evaluation of *Bacteroides* DNA probes for the specific detection of human fecal pollution. *Appl. Environ. Microbiol.* **61**, 1171-1179.
- Leeming, R. (1996a) Coprostanol and related sterols as tracers for faecal contamination in Australian aquatic environments.(Australia). PhD Thesis, Canberra University, Canberra, Australia.
- Leeming, R. (1996b) Examination of faecal sterols in sediments of the serpentine and Murray Rivers, WA. CSIRO Report 96-PIMA1. Report prepared for the Peel Inlet Management Authority.
- Leeming, R. (1996c) Use of faecal sterols and bacterial indicators to discriminate sources of faecal pollution entering Lake Macquarie, Newcastle, NSW. CSIRO Report 96-HWC1. Report prepared for the Hunter Water Corporation.
- Leeming, R. (1997a) Use of faecal sterols and bacterial indicators to discriminate sources of faecal pollution entering Rippleside Stormwater Drain, Geelong, Victoria. CSIRO Report 97-VEPA1. Report prepared for the Environment Protection Authority Victoria.
- Leeming, R. and Nichols, P. D. (1992) Lipid, sterol, fatty acid and hydrocarbon content of sediments from Great Keppel Island: A preliminary assessment of possible natural and pollutant carbon sources. CSIRO Report 92-UCQ-1. Report prepared in association with Q. Espey, University of Central Queensland.
- Leeming, R. and Nichols, P. D. (1995a) Use of faecal sterols to discriminate sources of faecal pollution in urban creeks, lakes and stormwater drains of Wyong, NSW. CSIRO Report 95-AWT-1. Report prepared in association with A. Ball, AWT-Science and Environment.
- Leeming, R. and Nichols, P. D. (1995b) Use of faecal sterols and bacterial indicators to discriminate sources of faecal pollution in urban creeks and lakes of Wyong, NSW. CSIRO Report 95-WYG-1.
- Leeming, R. and Nichols, P. D. (1996) Concentrations of coprostanol that correspond to existing bacterial indicator guideline limits. *Water Res.* **30**, 2997-3006.
- Leeming, R. and Nichols, P. D. (1998) Determination of the sources and distribution of sewage and pulp-fibre derived pollution in the Derwent Estuary using sterol biomarkers. *Marine and Freshwater Res.* **49/1**, 7-17.

- Leeming, R., Nichols, P. D. and Nespolo, S. (1994) Distinguishing human and non-human sources of sewage using faecal sterols. I. A survey of animal faeces from the Sydney catchment. CSIRO Report 94-AWT-1. Report prepared for AWT-Science and Environment.
- Leeming, R., Ball, A., Ashbolt, N., and Nichols, P. D. (1996) Using Faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. *Water Res.* **30**, 2893-2900.
- McCalley, D. V., Cooke, M. and Nickless, G. (1981) Effect of sewage treatment on faecal sterols. *Water Res.* **15**, 1019-1025.
- Mackay, S. J. and Ridley, J. P. (1983) Survival and regrowth of *Escherichia coli* in Lake Burragorang. In *Australian Water and Wastewater Association. 10th Federal Convention, Sydney, NSW*, pp:46-1 to 46-12.
- Martin, W. J., Ravi Subbiah, M. T., Kottke, B. A., Birk C. C. and Taylor, M. C. (1973) Nature of faecal sterols and intestinal bacterial flora. *Lipids*, **8**, 208-215.
- Marty, Y., Quemeneur, M., Aminot, A. and Le Corre, P. (1996) Laboratory study on degradation of fatty acids and sterols from urban wastes in seawater. *Water Res.* **30**, 1127-1136.
- Muhldorfer, I., Blum, G., Donohue-Rolfe, A., *et al.* (1996) Characterization of *Escherichia coli* strains isolated from environmental water habitats and from stool samples of healthy volunteers. *Res. Microbiol.* **147**, 625-635.
- Murtaugh, J. and Bunch, R. L. (1967) Sterols as a measure of faecal pollution. *J. Water. Pollut. Contr. Fed.* **39**, 404-409.
- Niemi, R.M., Niemelä, S. Mentu, J. and Siitonen, A. (1987) Growth of *Escherichia coli* in a pulp and cardboard mill. *Can. J. Microbiol.* **33**, 541-545.
- Nishimura, M. (1982) 5 β -Isomers of stanols and stanones as potential markers of sedimentary organic quality and depositional paleoenvironments. *Geochim. Cosmochim. Acta* **46**, 423-432.
- O'Leary, T., Leeming, R., Nichols, P. D. and Volkman, J. K. (1994) The distribution and concentrations of biomarkers for sewage, primary producers and bacteria in the major sediment types of Port Phillip Bay; Technical Report No. 17. CSIRO Institute of Natural Resources and Environment, Port Phillip Bay Environmental Study, October 1994.
- Osawa, S., Furuse, K. and Watanabe, I. (1981) Distribution of ribonucleic acid coliphages in animals. *Appl. Environ. Microbiol.* **41**, 164-168.
- Parmentier, G. and Eyssen, H. (1974) Mechanism of biohydrogenation of cholesterol to coprostanol by *Eubacterium* ATCC 21408. *Biochim. Biophys. Acta* **348**, 279-284.

- Puig, A., Jofre, J. and Araujo, R. (1997) Bacteriophages infecting various *Bacteroides fragilis* strains differ in their capacity to distinguish human from animal faecal pollution. *Wat. Sci. Tech.* 35(11-12), 359-362.
- Rosenfield, R. S. and Gallagher, T. F. (1964) Further studies of the biotransformation of cholesterol to coprostanol. *Steroids* 4, 515-520.
- Switzer-Howse K. D. and Dukta B. J. (1978) Fecal sterol studies: Sample processing and microbial degradation. *Sc. Ser.* 89, 1.
- Tartera, C. Araujo, R. Michel, T. and Jofre, J. (1992) Culture and decontaminating methods affecting enumeration of phages infecting *Bacteroides fragilis* in sewage. *Appl. Environ. Microbiol.* 58, 2670-2673.
- Venkatesan, I. M. and Santiago, C. A. (1989) Sterols in ocean sediments: novel tracers to examine habitats of cetaceans, pinnipeds, penguins and humans. *Marine Biology* 102, 431-437.
- Vivian, C. M. G. (1986) Tracers of sewage sludge in the marine environment: A review. *Science of the Total Environment.* 53, 5-40.
- Walker, R. W., Wun, C. and Litsky, W. (1982) Coprostanol as an indicator of faecal pollution. *CRC Crit. Rev. Environ. Control.* 10, 91-112.

Appendix 1: List of papers and reports not cited, but related to tracing faecal contamination in Australia with faecal sterols.

Research Publications

- Leeming, R. and Nichols, P. D. (1992) An integrated scheme to analyse naturally occurring and pollutant organic constituents in urban sewage. In *Proceedings of a Bioaccumulation Workshop: Assessment of the Distribution, Impacts and Bioaccumulation of Contaminants in Aquatic Environment*, (Ed. A. G. Miskiewicz), 153-161. Water Board and Australian Marine Science Association Inc., Sydney.
- Nichols, P. D., Leeming, R. and Cresswell, G. (1992a) Sydney deep ocean outfall studies. *Chemistry in Australia* 59, 389.
- Nichols, P. D., Leeming, R. and Cresswell, G. (1992b) Survey of Sydney's coastal environment. *Australian Wastewater Association Water Journal* 19, 32-33.
- Nichols, P. D., Leeming, R., Rayner, M. S., Latham, V., Ashbolt, N. J. and Turner, C. (1993) Comparison of the abundance of the fecal sterol coprostanol and fecal bacterial groups in inner-shelf waters and sediments near Sydney, Australia. *J. Chromatography* 643, 189-195.
- Leeming, R., Ball, A., Ashbolt, N., Jones, G. and Nichols, P. D. (1994) Distinguishing between human and animal sources of faecal pollution. *Chemistry in Australia.* 61, 434-435.
- Nichols, P. D., Leeming, R., Rayner, M., Latham, V. and Peterson, J. (1995) Sydney's inner-shelf environment after commissioning of the deep ocean outfalls. In: *Recent Advances in Marine Science and Technology '94. Proceedings PACON 1994 Conference* (Eds. O. Bellwood, H. Choat and N. Saxena) pp 289-298, Townsville, July 1994, James Cook University of North Queensland.
- Leeming, R. Latham, V., Rayner, M. and Nichols, P. (1997) Detecting and distinguishing sources of sewage pollution in Australian inland and coastal waters and sediments. In: *Application of molecular Markers in environmental geochemistry. American Chemical Society National Meeting, Division of Environmental Chemistry, Inc., American Chemical Society*, (Ed. R. P. Eganhouse) Orlando, FL, pp 306-319, ACS Washington, DC.
- Leeming, R. (1997) Sources of faecal pollution in Australian inland and coastal waters. *Chemistry in Australia* 64, No. 7, 10-11..
- Leeming, R., Bate, N., Hewlett, And Nichols, P. D. Discriminating faecal pollution: A case study of stormwater entering Port Phillip Bay, Australia. *Wat. Sci Tech.* (submitted)
- Wennberg, E., Sundin, A., Leeming, R. and Torstensson. A new method for the determination of faecal sterols in urine using non-chlorinated solvents. *J. Chromatography* (submitted)

Sundin, A., Leeming, R. and Stenström. Degradation of faecal sterols on source-separated human urine and urine sediment. (in preparation)

Research Reports

Leeming, R., Rayner, M., Latham, V. and Nichols, P. D. (1991a) Characterisation of urban sewage effluent in Sydney's coastal waters and sediments using specific organic markers. I. Malabar Precommissioning. CSIRO Report 91-SPCC1. Report prepared for the NSW State Pollution Control Commission.

Leeming, R., Rayner, M., Latham, V. and Nichols, P. D. (1991b) Characterisation of urban sewage effluent in Sydney's coastal waters and sediments using specific organic markers. II. North Head and Bondi Precommissioning. CSIRO Report 91-SPCC2. Report prepared for the NSW State Pollution Control Commission.

Leeming, R. and Nichols, P. D. (1992a) Quantitative analysis of coprostanol in sewerage effluent from the malabar treatment works and from sites adjacent to the malabar deep ocean outfall. I. CSIRO Report 92-SPCC1. Report prepared for the NSW State Pollution Control Commission.

Leeming, R. and Nichols, P. D. (1992b) Quantitative analysis of coprostanol in sewerage effluent from the malabar treatment works and from sites adjacent to the malabar deep ocean outfall. II. CSIRO Report 92-EPA-1. Report prepared for the NSW Environmental Protection Authority.

Leeming, R. and Nichols, P. D. (1992c) Lipid, sterol, fatty acid and hydrocarbon content of sediments from Great Keppel Island: A preliminary assessment of possible natural and pollutant carbon sources. CSIRO Report 92-UCQ-1. Report prepared in association with Q. Espey, University of Central Queensland.

Leeming, R., Latham, V., Rayner, M. and Nichols, P. D. (1992) Characterisation of beach grease from the Sydney region: Investigation of sterol and hydrocarbon inputs under winter conditions. CSIRO Report 92-EPA4. Report prepared for the NSW Environmental Protection Authority.

Nichols P. D. and Leeming R. (1992) Characterization of beach grease from the Sydney region: A pilot investigation using specific organic markers. II. CSIRO Report 92-EPA-2. Report prepared for the NSW Environmental Protection Authority

Nichols, P., Leeming, R. and Watson, M (1992) Quantitative analysis of coprostanol in sewerage effluent from the Malabar treatment works and particulate matter samples from the effluent plume emitted from the Malabar deep ocean outfall. III. CSIRO Report 92-EPA3. Report prepared for the NSW Environmental Protection Authority.

Nichols, P., Leeming, R., Rayner, M., Green, G. and Nespolo, S. (1993) Characterisation of stormwater and related samples from the Sydney coastal region using specific organic markers. CSIRO Report 93-WBS2. Report prepared for the Australian Water Technologies-Science and Environment Water Board, Sydney.

Leeming, R., Nichols, P. D. and Nespolo, S. (1994) Distinguishing human and non-human sources of sewage using faecal sterols. I. A survey of animal faeces from the Sydney catchment. CSIRO Report 94-AWT-1. Report prepared for AWT-Science and Environment.

Nichols, P. D., Leeming, R. and Jones, G. (1994) The use of the faecal sterol, coprostanol, as an indicator of sewage pollution. Report 94-EMIAA. Report prepared for Environment Management Industry Association of Australia.

O'Leary, T., Leeming, R., Nichols, P. D. and Volkman, J. K. (1994) The distribution of biomarkers for sewage, primary producers and bacteria in the major sediment types of Port Phillip Bay (Progress Report). CSIRO Report PPB94-1. Project undertaken for the Port Phillip Bay Environmental Study.

O'Leary, T., Leeming, R., Nichols, P. D. and Volkman, J. K. (1994) The distribution of biomarkers for sewage, primary producers and bacteria in the major sediment types of Port Phillip Bay (Final Report). CSIRO Report PPB94-F. Project undertaken for the Port Phillip Bay Environmental Study.

Leeming, R. and Nichols, P. D. (1995a) Use of faecal sterols to discriminate sources of faecal pollution in urban creeks, lakes and stormwater drains of Wyong, NSW. CSIRO Report 95-AWT-1. Report prepared in association with A. Ball, AWT-Science and Environment.

Leeming, R. and Nichols, P. D. (1995b) Use of faecal sterols and bacterial indicators to discriminate sources of faecal pollution in urban creeks and lakes of Wyong, NSW. CSIRO Report 95-WYG-1.

Volkman, J. K., Leeming, R., Revill, A. T. and Holdsworth, D. G. (1996) Changes in lipid biomarker distributions in a sediment core from Port Phillip Bay. CSIRO Report 96-PPB1. Project undertaken for the Port Phillip Bay Environmental Study.

Leeming, R. (1996d) Use of faecal sterols and bacterial indicators to discriminate sources of faecal pollution in stormwater entering the Yarra River, Melbourne. CSIRO Report 96-VEPA1. Report prepared for the Environment Protection Authority Victoria.

Leeming, R. (1996e) Use of faecal sterols and bacterial indicators to discriminate sources of faecal pollution in Kangaroo River, Kangaroo Valley, NSW. CSIRO Report 96-KV1. Report prepared for the Shoalhaven Shire Council.

Leeming, R. (1996f) Use of faecal sterols and bacterial indicators to discriminate sources of faecal pollution in Cabbage Tree Bay, Wyong, NSW. CSIRO Report 96-WYG1. Report prepared for the Wyong Shire Council.

Leeming, R. (1997b) Use of faecal sterols and bacterial indicators to discriminate sources of faecal pollution in Kangaroo River, Kangaroo Valley, NSW. CSIRO Report 97-KV1. Report prepared for the Shoalhaven Shire Council.

Leeming, R. (1997c) Faecal sterols and bacterial indicators in the Clarence River, NSW CSIRO Report 97-GFT1. Report prepared for the Grafton Shire Council.

Leeming, R. (1997d) Faecal sterols and bacterial indicators in estuarine lake waters around Bega, NSW. CSIRO Report 97-BE1. Report prepared for the Bega Shire Council.

Leeming, R. (1997e) Examination of faecal sterols in sediments of Merri Creek, Vic. CSIRO Report 97-WES1. Report prepared for Water Ecoscience.

Leeming, R. (1997f) Faecal sterols and bacterial indicators to discriminate sources of faecal pollution entering Lake Macquarie, Newcastle, NSW. CSIRO Report 97-HWC1. Report prepared for the Hunter Water Corporation.

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