Faecal Source Discrimination Trial: Environment Southland

Prepared by Brent Gilpin

Institute of Environmental Science & Research Ltd

June 2006

Prepared with support from Envirolink 106

For further information please contact:

Brent J. Gilpin Ph.D.
Senior Scientist
Institute of Environmental Science & Research Limited
Christchurch Science Centre
27 Creyke Road
PO Box 29-181
Christchurch
NEW ZEALAND
Phone: +64 - 3 - 351 0044 (direct)
+ 64 - 3- 351 6019 (operator)
Fax: + 64 - 3 - 351 0010
email: brent.gilpin@esr.cri.nz
Web site: http://www.esr.cri.nz

Executive summary

This report discusses several tools available for faecal source discrimination, and explores the potential value of faecal sterol analysis to contribute to reducing faecal pollution of waterways in Southland.

Samples were collected from ten surface water sites, and one meat processing effluent. Faecal coliform and *E. coli* levels counts were determined in the ten surface water sites, and seven of the samples were analysed for presence and quantity of faecal sterols. Six of the sampling sites were also analysed using molecular assays.

The sheep effluent from Blue Sky Meats contained very high levels of sterols and was consistent with a herbivore origin. Stream sampling identified very high levels of *E. coli*, and sterols in the Blue Sky Meats downstream site. Sterol analysis indicates a profile consistent with a **human** source of the sterols. The upstream site in contrast had lower *E. coli* and sterol levels consistent with a herbivore source of the sterols. Further upstream at the beginning of the stream sterol analysis indicated **human** sources of the sterols. This was also the only site where human specific PCR marker was detected. The other samples contained herbivore specific markers. Further investigation is required to decipher these and previous microbial results. The presence of human indicative sterol profiles at the downstream result may be from an unrecognised human input between the upstream site and this site, or may be a “slug” of contamination from further upstream. The irregular nature of microbial counts upstream and downstream suggests little correlation between the sites at the times sampled. Upstream inputs before the Blue Sky Meats upstream site should be addressed before meaningful assessment of impact of Blue Sky Meats on microbial water quality can be made. The absence of human specific molecular marker in the downstream site, *may* indicate that there has been a significant travel distance between human input and sampling site. That is however purely a hypothesis as relative transport and survival studies are still in development to address this issue.

Sterols and molecular markers analysed from the duck pond confirmed that water containing faeces from ducks would not falsely be identified as being either of human or herbivore origin.

The remaining sites analysed all contained sterols consistent with a herbivore source of sterols.

Introduction

Potential faecal source tools

The public has become increasingly aware of the potential health hazards of faecally contaminated water. This heightened awareness is resulting in an increased frequency of water quality monitoring for the traditional microbial indicators, faecal coliforms, *E. coli* and enterococci. There is also an expectation that when these indicators are detected, corrective action will be taken to eliminate these faecal indicators - and by inference the faecal pollution - from the water. While these traditional indicators are usually a good indication of microbial quality, and therefore the health risk posed, they provide little guidance as to the source of the faecal pollution. Faecal coliforms and other traditional indicators are present in the faeces of humans, cows, sheep, dogs, ducks, seagulls and a wide range of other animals. Identifying the source of faecal pollution can be crucial for effective water management, particularly when for example, a single seagull can excrete per day as many as 3×10^8 faecal coliforms and 3×10^9 enterococci (Wood and Trust 1972).

Faecal source discrimination is a developing area of research worldwide. The summary below outlines some of the approaches ESR has explored in recent research.

Sorbitol fermenting Bifidobacterium

Bifidobacteria comprise a high proportion of the microflora inhabiting the human intestine, with densities of 10^9-10^{10} per gram of faeces. Sorbitol fermenting bifidobacteria are reported to be almost exclusive to humans and include the species *Bifidobacterium adolescentis* and *B. breve*, which contribute between 58% and 90% of the *Bifidobacterium* isolates identified in humans. Sorbitol fermenting bifidobacteria have been isolated from pigs but no other animals species including feral animals. However, the pig isolates do not grow on the Human Bifid Sorbitol Agar (HBSA), which was developed to specifically enumerate the sorbitol fermenting bifidobacteria present in the human intestine (Mara and Oragui, 1983). ***The growth of Sorbitol fermenting bifidobacteria on HBSA indicates the presence of human effluent.***

Molecular Indicators

There is a range of other microorganisms present in the faeces, which are specific to animal hosts. Difficulties in culturing and identifying these organisms have however limited their useful application to faecal source identification. An alternative approach is to extract total DNA from a water sample and examine the sample using PCR for DNA from source specific organisms. Three assays have been applied to the samples in this study. The first targets *Bacteroidetes* bacteria which are indicative of faecal pollution. The second targets human specific *Bacteroidetes*, and the third herbivore specific *Bacteroidetes*.

Results are expressed as gene copy number. Faecal Bact indicates total faecal pollution. The human and herbivore markers target a subset of the *Bacteroidetes* specific for human and herbivore sources. These assays are very much in development. Data is still being collected to determine relative transport and survival of these molecular indicators and prevalence relationship between total faecal indicator and any specific faecal indicator.

Fluorescent Whitening Agents (FWAs)

Fluorescent whitening agents (FWA) are common constituents of washing powders that adsorb to fabric and brighten clothing. There is a range of FWAs, but only one (4,4’-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)-amino]stilbene-2,2’-disulfonate) is used in New Zealand. Most household plumbing mixes effluent from toilets with "grey water" from washing machines and, as a consequence FWAs are usually associated with human faecal contamination in both septic tanks and community wastewater systems. FWAs were extracted from 50 mL samples and analysed by High Pressure Liquid Chromotography (HPLC) as previously described (Gregor *et al.*, 2002). Results are expressed in parts per billion (ppb) equivalent to μg/litre. ***The presence of FWAs indicates human effluent.***

Faecal Sterols

Faecal sterols are a group of C27-, C28- and C29-cholestane-based sterols found mainly in animal faeces. The sterol profile of faeces depends on the interaction of three factors. Firstly, the animal's diet determines the relative quantities of sterol precursors (cholesterol, 24-ethylcholesterol, 24-methylcholesterol, and/or stigmasterol) entering the digestive system. Secondly, animals differ in their endogenous biosynthesis of sterols (for example, human beings on a low cholesterol diet synthesise cholesterol). Thirdly, and perhaps most importantly, is that the anaerobic bacteria in the animal gut biohydrogenate sterols to stanols of various isomeric configurations.

The sterol cholesterol can be hydrogenated to one or more of four possible stanols. In human beings, cholesterol is preferentially reduced to coprostanol, whereas in the environment cholesterol is predominately reduced to cholestanol. Similarly, plant-derived 24-ethylcholesterol is reduced to 24-ethylcoprostanol and 24-ethylepicoprostanol in the gut of herbivores, whereas in the environment it is primarily reduced to 24-ethylcholestanol. As a consequence, analysis of the sterol composition of animal faeces can generate a sterol fingerprint, which can be quite distinctive from one species to another.

Faecal sterols analysis was performed, by filtering ~24 litres of river water onto glass fibre filters. Filters were stored frozen until they were analysed using the extraction procedure described by Gregor et al. (2002). Each sterol and stanol result is expressed as parts per trillion (ppt). The key sterol values reported were the level of coprostanol, and two key ratios.

Coprostanol is the principal human biomarker. High relative amounts indicate fresh human faecal material. Coprostanol constitutes 60% of the total sterols found in human faeces, while dogs and birds have either no coprostanol or only trace amounts, present in their faeces.

Coprostanol:24-ethylcoprostanol: These stanols are present in both human and herbivore faeces, but in significantly different amounts. Therefore, the relative contributions of each stanol can be determined by examining the ratio of one stanol to the other. Human faecal pollution typically has a ratio greater than one.

Coprostanol:cholestanol: The ratio of coprostanol:cholestanol can indicate whether the coprostanol present is of faecal origin. A ratio greater than 0.5, suggests faecal contamination (preferential reduction from sterol by gut microbiota), whereas a ratio less than 0.3 may suggest environmental reduction by, for example, anaerobic bacteria in sediments.

Synchronous scanning spectrofluorophotometry

Fluorescent compounds absorb light at a lower wavelength (higher energy) and re-emit light at a higher wavelength (lower energy). Each fluorescent compound has a signature fluorescent spectrum, which is dependent on its excitation and emission wavelengths. For example, the Fluorescent Whitening Agents (FWAs), which are used as indicators of human pollution, absorb light at 350 nm (the excitation wavelength) and re-emit the light as fluorescence at a higher wavelength (emission wavelength) in the range 430 nm (Poiger et al., 1993).

A Synchronous Scanning Spectrofluorophotometer (SSS) is programmed to scan through a defined wavelength range for both the excitation and emission wavelengths of a fluorescent compound. This analysis produces a plot of fluorescence intensity versus emission wavelength and is very useful for identifying a mixture of fluorescent molecules in an unknown sample. Fluorescence sensitivity is up to a thousand times better than absorbance methods and allows analysis to nanogram and picogram levels. SSS is a rapid and inexpensive method, which is able to provide preliminary and discriminatory information on the types of contaminants present in a sample, e.g. the presence of FWAs, fuels, polyaromatic hydrocarbons and pesticides in an aquatic environment.

Project Brief

To explore the potential value of faecal source tools to contribute to reducing faecal pollution of waterways in Southland.

Experimental approach

Sampling and analysis of microbial faecal indicators
Water samples were collected on Friday 24 March 2006 between 8.55 am and 12:10 pm. Samples were analysed locally at Envirolab, 142 Esk Street, Invercargill for faecal coliforms and E. coli using membrane filtration based methods APHA 9222 D and G respectively. Samples were tested within 6 hours of collection. Four-litre volumes of water from each sample (except for sample 5 which only 5 ml was processed) were filtered in Southland through 142mm G/F filters, and the filter subsequently analysed at ESR Christchurch for faecal sterols.

Initial microbial results.

Microbial results are shown below. Based on these results, samples 6, 9 and 11 were not analysed further (budgetary constraints).

Site	Description	Time sampled	Faecal coliforms	*E. coli*
4	Upstream of Spray site	9:45 am	1200	1030
2	Spray site upstream Blue Sky Meats	9:18 am	2800	2800
3	Sheep effluent before pond	9.30 am	nt	nt
1	Spray site downstream Blue Sky Meats	8.55 am	130000	16300
5	Duck pond Pinknees Property	10:14 am	6500	5400
6	Spurhead Creek at Dacre Morton Mains	10:35 am	500	390
7	Waihopai River at Woodlands Street	11:05 am	1500	1100
8	Waihopai River at Woodlands Bridge	11:12 am	1400	930
9	Waihopai River at Waihopai Dam	11:40 am	480	340
10	Waihopi River at Queens Drive	11:55 am	530	470
11	Waihopi River at Victoria Ave	12:10 pm	670	470
12	tap water blank		nt	nt

All results cfu/100ml.
Nt: not tested

Molecular markers

Results are expressed as gene copy number. Faecal Bact indicates total faecal pollution. The human and herbivore markers target a subset of the *Bacteroidetes* specific for human and herbivore sources. These assays are very much in development. Data is still being collected to determine relative transport and survival of these molecular indicators and prevalence relationship between total faecal indicator and any specific faecal indicator.

Site	Description	Faecal Bact	Herbivore Bact	Human Bact
4	Upstream of Spray site	10^5	0	10^2
2	Spray site upstream Blue Sky Meats	10^5	10^2	0
3	Sheep effluent before pond	10^8	10^6	0
1	Spray site downstream Blue Sky Meats	10^7	10^4	0
5	Duck pond Pinknees Property	10^5	0	0
6	Spurhead Creek at Dacre Morton Mains	10^5	10^3	0

Interpretation

All sites sampled contained DNA which indicated the presence of bacteria derived from a faecal source. A human specific marker was only detected in site 4, while herbivore specific markers were detected at the remaining sites except for the duck pond.

Faecal sterol quality control.

Sampling was performed at Environment Southland using single filtering apparatus which was washed with tap water between samples. To ensure that minimal cross contamination occurred between samples, 4 L of tap water (from Environment Southland) was filtered and this analysed. Faecal sterol analysis

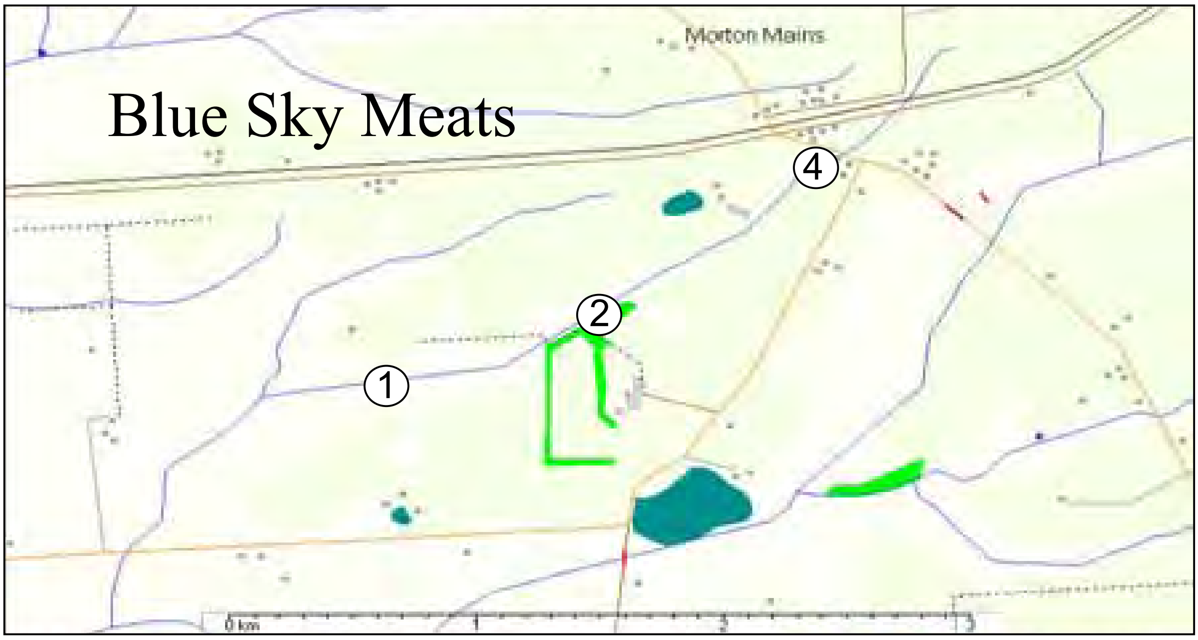
Name	LOQ	Blank	QA	Exp QA	CFC0610296
					Tap water
coprostanol	60	ND	975	951	*3*
24-ethylcoprostanol	10	ND	255	213	*2*
epicoprostanol	60	ND	1058	995	*6*
cholesterol	60	50	1183	1096	*110*
cholestanol	60	ND	1188	1049	*20*
24-methylcholesterol	80	ND	1484	1301	*8*
24-ethylepicoprostanol	100	ND	1869	1562	*1*
stigmasterol	70	ND	1406	1157	*8*
24-ethylcholesterol	60	91	1318	1001	*87*
24-ethylcholestanol	80	ND	1766	1322	*12*
Results in PPT					

LOQ = Limit of Quantitation (based on lowest standard)
Bold = Significantly over top of calibration**.**
Italics = Below Lowest standard

Interpretation

Quality control all very good. No significant cross-contamination as indicated by tap water analysis.

Area 1. Series of samples around Blue Sky meats.



Sampling site 3. Blue Sky Meats sheep effluent (site 3).

Sample was collected from the solids separator of factory effluent before it enters the storage pond.

Results

Sampling time	9.30am
Faecal coliforms	not tested
E. coli	not tested

Sterols	ppt
coprostanol	1165600
24-ethylcoprostanol	**2359600**
epicoprostanol	150800
cholesterol	**19164800**
cholestanol	2148000
24-methylcholesterol	570000
24-ethylepicoprostanol	498800
stigmasterol	152400
24-ethylcholesterol	880800
24-ethylcholestanol	3191200

Indicative Ratios		
coprostanol/cholestanol	0.54	>0.5 faecal
24-ethylcholesterol/24-ethylcoprostanol	0.37	>0.5 faecal
%Coprostanol/total sterols	3.8%	>5 to 6% suggests human source
5β/(5β+5α stanols)	0.35	> 0.7 suggests human source
coprostanol/24-ethylcoprostanol	0.49	>1.0 human
coprostanol/coprostanol+24-ethylcoprostanol	33.1%	
coprostanol:epicoprostanol	7.73	>2.0 fresh/untreated
24-ethylcoprostanol/24-ethylcholestanol	0.74	<1 faeces, >4 plant
Estimate of % Human sterols	**FNH**	

Interpretation

Very high levels of sterols were present in the sample – the bold numbers exceeding highest standard. All the ratios except 24-ethylcholesterol/24-ethylcoprostanol support the following interpretations:

- sterols of faecal origin are present,
- these sterols do not have a human faecal origin
- these sterols are consistent with herbivore origin.

Site 2 Upstream sampling site of Blue Sky Meats

Recent microbial sampling.

Date	Total Coliforms MPN/100mL	*E. coli* MPN/100mL
14-Oct-03	4,100	2,300
20-Nov-03	4,100	690
23-Dec-03	>240000	200,000
8-Jan-04	2,400,000	980,000
4-Mar-04	>240000	>240000
28-Apr-04	3,700	770
19-May-04	3,300	430
8-Jul-04	13,000	2,100
15-Sep-04	1,100	400
28-Sep-04	640	400
21-Oct-04	1,100	640
18-Nov-04	4,900	3,400
19-Jan-05		1,000
2-Mar-05	13,000	470
31-Mar-05	13,000	7,300
27-Apr-05	12,000	2,600
8-Jun-05	3,300	780
20-Sep-05		2,500
3-Nov-05		10,000
11-Jan-06		2,300

Microbial sampling results 24th March 2006

Site	Time sampled	FC	E. coli
2	9:18am	2800	2800

Faecal sterol analysis

Name	**CFC0610290**		
coprostanol	362		
24-ethylcoprostanol	1425		
epicoprostanol	63		
cholesterol	1914		
cholestanol	296		
24-methylcholesterol	562		
24-ethylepicoprostanol	424		
stigmasterol	627		
24-ethylcholesterol	1712		
24-ethylcholestanol	1095		
Results in PPT			
Ratios			
coprostanol/cholestanol	1.22	>0.5	faecal
24-ethylcoprostanol/24-ethylcholestanol	1.30	>0.5	faecal
24-ethylcholesterol/24-ethylcoprostanol	1.20	<1 faeces, >4 plant*	
%Coprostanol/total sterols	4.3%	>5 to 6% suggests human source	
5β/(5β+5α stanols)	0.55	> 0.7 suggests human source	
coprostanol/24-ethylcoprostanol	0.25	>1.0	human
coprostanol/coprostanol+24-ethylcoprostanol	20.3%		
coprostanol:epicoprostanol	5.75		
Estimate of % Human sterols	0		
	FNH		

* values between 1 and 4 do not allow an interpretation to be made.

Interpretation

Useful and detectable level of sterols were present in the. All the ratios support the following interpretations:

- sterols of faecal origin are present,
- these sterols do not have a human faecal origin
- these sterols are consistent with herbivore origin.

Site 1

Downstream sampling site for Blue Sky Meats.

Recent microbial sampling.

Date	Total Coliform MPN/100mL	*E. coli* MPN/100mL
14-Oct-03	2,200	1,000
20-Nov-03	24,000	11,000
23-Dec-03	16,000	8,300
8-Jan-04	98,000	44,000
4-Mar-04	12,000	1,700
28-Apr-04	4,100	520
19-May-04	20,000	14,000
30-Aug-04	2,600	1,500
15-Sep-04	>24000	17,000
21-Oct-04	>24000	24,000
18-Nov-04		>24000
19-Jan-05		11,000
2-Mar-05	>24000	>24000
31-Mar-05	3,900	280
27-Apr-05	>2400	>2400
8-Jun-05	4,000	3,000
20-Sep-05		240,000
3-Nov-05		16,000
11-Jan-06		2,100

Microbial indicators

Site		Faecal coliforms	*E. coli*
1	8.55am	130000	16300

Faecal sterols

Name	CFC0610289	Interpretation
	1	
coprostanol	25374	
24-ethylcoprostanol	13340	
epicoprostanol	1963	
cholesterol	69831	
cholestanol	8433	
24-methylcholesterol	4148	
24-ethylepicoprostanol	3858	
stigmasterol	7094	
24-ethylcholesterol	8906	
24-ethylcholestanol	15402	
Results in PPT		
Ratios		
coprostanol/cholestanol	3.01	>0.5 faecal
24-ethylcoprostanol/24-ethylcholestanol	0.87	>0.5 faecal
24-ethylcholesterol/24-ethylcoprostanol	0.67	<1 faeces, >4 plant
%Coprostanol/total sterols	16.0%	>5 to 6% suggests human source
5β/(5β+5α stanols)	0.75	> 0.7 suggests human source
coprostanol/24-ethylcoprostanol	1.90	>1.0 human
coprostanol/coprostanol+24-ethylcoprostanol	65.5%	
coprostanol:epicoprostanol	12.93	>2.0 fresh/untreated
Estimate of % Human sterols	78.69%	
	Human	

Interpretation

Elevated levels of sterols in this sample. Ratio of coprostanol/cholestanol is greater than 0.5 (actually 3.01), and ratio of 24-ethylcholesterol/24-ethylcoprostanol is less than 0.1 (actually 0.67), both of which indicate sterols are of faecal origin. The ratio of coprostanol/24-ethylcoprostanol is >1.0 (actually 1.90) which suggests a human source for the sterols. The proportion of coprostanol:total sterols is also elevated which suggests human source of sterols. Calculation of proportionality based on work of Leeming, estimates that human source comprises almost 80% of the total sterols.

The very high levels of sterols suggest a very concentrated source of human faecal material which would be consistent with direct septic tank input.

The contrast with the upstream site is marked and clearly warrants further investigation.

Upstream site 4

Sample was collected from beginning of this stream, downstream of housing.
Stream had no clearly visible flow.

Microbial Indicators

Site		Faecal coliforms	*E. coli*
4	9:45am	1200	1030

Faecal sterol analysis

Name	CFC061029 1	
	ppt	
Coprostanol	4243	
24-ethylcoprostanol	2410	
Epicoprostanol	295	
Cholesterol	6672	
Cholestanol	952	
24-methylcholesterol	2820	
24-ethylepicoprostanol	486	
Stigmasterol	2680	
24-ethylcholesterol	6036	
24-ethylcholestanol	1268	
Results in PPT		
Ratios		
coprostanol/cholestanol	4.46	>0.5 faecal
24-ethylcoprostanol/24-ethylcholestanol	1.90	>0.5 faecal
24-ethylcholesterol/24-ethylcoprostanol	2.50	<1 faeces, >4 plant
%Coprostanol/total sterols	15.2%	>5 to 6% suggests human source
5β/(5β+5α stanols)	0.82	> 0.7 suggests human source
coprostanol/24-ethylcoprostanol	1.76	>1.0 human
coprostanol/coprostanol+24-ethylcoprostanol	63.8%	
coprostanol:epicoprostanol	14.38	>2.0 fresh/untreated
Estimate of % Human sterols	73.65%	

Interpretation

Elevated levels of sterols in this sample. Ratio of coprostanol/cholestanol is greater than 0.5 (actually 4.46), which indicates sterols are of faecal origin. The ratio of 24-ethylcholesterol/24-ethylcoprostanol falls between 1 and 4 which does not enable an interpretation using this ratio to be made.

An interpretation that these sterols are of human origin is supported by all three ratios calculated including the ratio of coprostanol/24-ethylcoprostanol being >1.0 (actually 1.76), the proportion of coprostanol:total sterols being high (15%). Calculation of proportionality based on work of Leeming, estimates that human source comprises almost 75% of the total sterols.

The very high levels of sterols suggest a very concentrated source of human faecal material which would be consistent with direct septic tank input.

Duck Pond (site 5)

To contrast with the other sites, an isolated duck pond on the Pinkneys farm was sampled. This pond was fenced off from stock, and selected because it was believed unlikely that significant stock or human faecal inputs.

Microbial sampling

Site	Time sampled	Faecal coliforms	*E. coli*
5	10:14am	6500	5400

Faecal sterols

			FC	E. coli
4	Upstream of Spray site	9:45am	1200	1030
2	Spray site upstream Blue Sky Meats	9:18am	2800	2800
3	Sheep effluent before pond	9.30am	nt	nt
1	Spray site downstream Blue Sky Meats	8.55am	130000	16300
5	Duck pond Pinknees Property	10:14am	6500	5400

Name	CFC0610292	
	5	
coprostanol	561	
24-ethylcoprostanol	961	
epicoprostanol	349	
cholesterol	11795	
cholestanol	5143	
24-methylcholesterol	4422	
24-ethylepicoprostanol	246	
stigmasterol	4631	
24-ethylcholesterol	14397	
24-ethylcholestanol	1106	
Results in PPT		
Ratios		
coprostanol/cholestanol	0.11	>0.5 faecal
24-ethylcoprostanol/24-ethylcholestanol	0.87	>0.5 faecal
24-ethylcholesterol/24-ethylcoprostanol	14.98	<1 faeces, >4 plant
%Coprostanol/total sterols	1.3%	>5 to 6% suggests human source
5β/(5β+5α stanols)	0.10	> 0.7 suggests human source
coprostanol/24-ethylcoprostanol	0.58	>1.0 human
coprostanol/coprostanol+24-ethylcoprostanol	37%	
coprostanol:epicoprostanol	1.61	>2.0 fresh/untreated

While sterols are clearly detected in this sample, the ratios calculated do not support either a human or farmed herbivore source of the sterols.

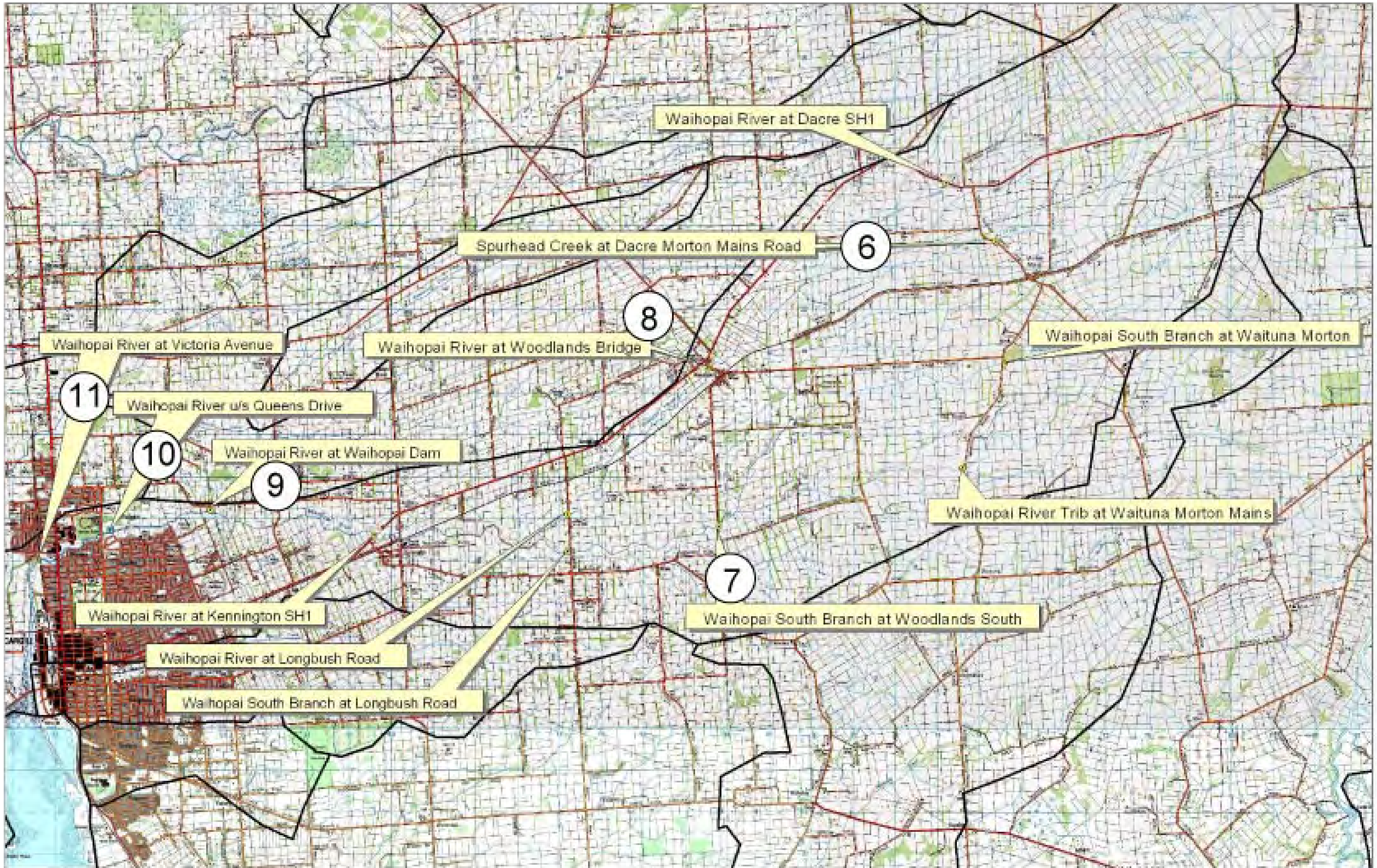
Sterols present would appear consistent with some wildfowl input, and plant sources of sterols. Low relative levels of coprostanol and 24-ethylcoprostanol relative to the other sterols.

Sites 7, 8 and 10

Site 7 Waihopai River at Woodlands Street

Site 8 Waihopai River at Woodlands Bridge

Site 10 Waihopai River at Queens Drive



environment SOUTHLAND

Waihopai Catchment WQ Sites

Date: 10/03/2006

N

1 : 82000

			Total coliforms	E. coli
7	Waihopai River at Woodlands Street	11:05am	1500	1100
8	Waihopai River at Woodlands Bridge	11:12am	1400	930
10	Waihopai River at Queens Drive	11:55am	530	470

Sterol	CFC0610 293	CFC0610 294	CFC0610 295	
	7	8	10	
coprostanol	1601	193	420	
24-ethylcoprostanol	6346	796	1547	
epicoprostanol	276	97	84	
cholesterol	5241	2213	2849	
cholestanol	975	272	313	
24-methylcholesterol	1383	710	502	
24-ethylepicoprostanol	2959	262	487	
stigmasterol	1118	663	476	
24-ethylcholesterol	3416	1510	1098	
24-ethylcholestanol	5408	498	1028	
Results in PPT				
Ratios				
coprostanol/cholestanol	1.64	0.71	1.34	>0.5 faecal
24-ethylcoprostanol/24-ethylcholestanol	1.17	1.60	1.50	>0.5 faecal
%Coprostanol/total sterols	5.6%	2.7%	4.8%	>6% suggests human source
5β/(5β+5α stanols)	0.62	0.42	0.57	> 0.7 suggests human source
coprostanol/24-ethylcoprostanol	0.25	0.24	0.27	>1.0 human
coprostanol/coprostanol+24-ethylcoprostanol	20.1%	19.5%	21.4%	
coprostanol:epicoprostanol	5.80	1.99	5.00	>2.0 fresh/untreated
24-ethylcholesterol/24-ethylcoprostanol	0.54	1.90	0.71	<1 faeces, >4 plant

Interpretation

Useful and detectable level of sterols were present in the. All the ratios support the following interpretations:

- sterols of faecal origin are present,
- these sterols do not have a human faecal origin
- these sterols are consistent with herbivore origin.