Technical Report

Investigations and Monitoring Group

Faecal source tracking in the Avon River, Christchurch March - May 2009

Report No. R09/67

Environment Canterbury

Your regional council

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Faecal Source Tracking in the Avon River, Christchurch March-May 2009

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Plain English Summary

Background: Water quality in the Avon River is monitored for the presence of the faecal indicator *Escherichia coli* (*E. coli*). *E. coli* are found in the faeces of humans, animals and birds. The detection of *E. coli* in water indicates the presence of faeces and therefore the potential presence of faecal-oral micro-organisms (such as *Campylobacter, E. coli* O157, and *Cryptosporidium*) that can cause disease in humans. MfE/MoH guidelines recommend that freshwater recreational areas should have less than 260 *E. coli*/100ml. At levels above 550 *E. coli*/100ml the guidelines recommend undertaking a sanitary survey, reporting on the sources of contamination, the erection of warning signs and informing the public through the media that a public health problem exists.

The problem: Water measurements in the Avon River regularly exceed 260 *E. coli*/100ml and in many cases 550 *E. coli*/100ml. For water managers to implement appropriate responses to elevated *E. coli* levels, information is required on where the faecal pollution comes from. This report specifically addresses the question of whether these *E. coli* are from a human, animal or wildfowl source.

What we did: Collected water samples from the Antigua Boatsheds and Kerrs Reach during low flow conditions, or during rainfall impacted flows. Twelve samples from each site were analysed using chemical and molecular tools capable of identifying faecal pollution and whether the pollution is from a human or animal/wildfowl source. No sewage overflows were reported during the sampling period.

What we found: In the absence of rainfall, *E. coli* levels of up to 540 *E. coli*/100ml were measured in the Avon River. The primary sources of these *E. coli* are wildfowl, with secondary contributions from dog faecal material. There was no indication of a human sewage contribution. During, and immediately following rainfall, *E. coli* counts in the Avon River increased up to 3,600 *E. coli*/100ml. The faecal source profile changed to be dominated by what appeared to be dog faeces, with secondary contributions from wildfowl. At the Antigua Boatshed there was no indication of a human sewage contribution. In contrast at Kerrs Reach following heavy rainfall faecal pollution with a human signature was detected.

What does it mean? Wildfowl and dogs are the primary contributors to degraded water quality in the Avon River. Measures to encourage dog owners to pick up dog faeces, especially along river banks, and to design and install stormwater systems to treat stormwater at source may result in lower levels of *E. coli* in the river. During the sampling period, a human sewage contribution was only detected during very heavy rainfall.

Executive Science Summary

Previous monthly monitoring of two sites on the Avon River has identified that levels of the water quality indicator *Escherichia coli* regularly exceed the MfE/MoH guidelines. The aim of this work was to identify if the application of faecal source tracking tools could improve understanding of the sources of the elevated *E. coli*. Faecal source tracking tools applied included faecal sterol analysis (faecal chemicals which differ between human and animal sources), fluorescent whitening agents (FWAs – washing powder agents that are usually associated with human faecal pollution), and DNA based molecular markers (assays indicative of human, wildfowl and canine sources).

Between March 17th and May 21st 2009, water samples were collected during high and low flow events from the Boat Sheds on Antigua Street (12 samples), and from the Boat ramp at Kerrs Reach (17 samples). No recognised sewage overflows occurred during this period.

Boat Shed, Antigua St

Six samples were collected during low flow conditions (river height 1.4 -1.5 m), of which five exceeded 260 *E. coli*/100ml (geometric mean 403, median 360). None exceeded the MfE/MoH action level of greater than 550 *E. coli*/100ml. During high flow conditions (>1.65 m), all samples collected exceed 550 *E. coli*/100ml (mean 1157, median 1150). Apart from FWAs detected in two of the low flow samples in March (which may be result of non-faecal associated cleaning products in stormwater), there is no evidence of a human source of pollution in the samples in either high or low flow at the Antigua Street Boatsheds. During low flow conditions the wildfowl PCR marker dominated, while during high flow, increased relative inputs were observed from the dog indicative marker.

Kerrs Reach

Eleven samples were collected during low flow conditions, of which only one (12th May, 380 *E. coli*/100ml) exceeded 260/100ml. The remaining samples ranged from 41 to 230 *E. coli*/100ml (mean 138, median 180). Five of the later samples were analysed for faecal source markers, despite four of them falling below 260 *E. coli*/100ml (mean 177, median 180). During high flow conditions all six samples collected exceeded 260 *E. coli*/100ml (mean 1561, median 2350). During low flow sampling not preceded by a high flow event, there was no evidence of human faecal pollution. The low levels of *E. coli* present appear to have a wildfowl source. High flow events separated into two groups. The first group did not have a human contribution to the high *E. coli* levels observed. In contrast human faecal markers (faecal sterols, FWAs, molecular markers) did contribute to the samples taken on the 11th and 20th May. Wildfowl and canine sources contributed to all the samples.

Conclusions and implications

Rainfall results in significant degradation of the microbial water quality of the Avon River. The primary sources of this degradation appear to be related to wildfowl and possibly dog faecal material. Human markers were detected from high flow events at Kerrs Reach, but at relatively low levels relative to the number of *E. coli* detected. This suggests either a distant source of these human markers, or an aged source of these human markers. The high levels of *E. coli* still appear to be primarily from wildfowl and animal sources.

The significant presence of the dog indicative molecular marker was somewhat surprising. To support the validity of this, a survey of the riverbanks for dog faecal material is suggested. Additional validation of the specificity of this marker with any other faecal or point source inputs is also recommended. If, however, this contribution of dog faecal material is confirmed, then significant improvements to microbial water quality may be achieved through better control of dog defecation and disposal, and the use of measures, such as low impact devices to reduce the volume of direct stormwater runoff and the treatment of stormwater at source. Reducing wildfowl inputs may be more difficult.

The health risk posed by this water is largely unknown. Wildfowl are known to carry a number of pathogens with the potential to cause disease in humans including *Campylobacter*, *Cryptosporidium*, *Giardia*, and *E. coli* O157. Dog faecal material may also contained pathogens. To better quantify the health risks related to this water two approaches are possible. The first would be to screen this water for a range of potential pathogens (*Campylobacter, Cryptosporidium, Giardia, E. coli* O157).

Pathogen testing is, however, expensive and would need to be fairly extensive to have statistical validity. A second approach would be to undertake an epidemiological study of the health impacts to water users from either the rowing clubs or the Antigua Boatsheds. The contribution of sediment re-suspension during high flows is an unknown factor which would benefit from further investigation.

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1 Project brief

E. coli monitoring undertaken during the summer months indicates that at recreational sites on the Avon River the *E. coli* levels frequently fail to meet the standards set by the MfE and MoH, even during dry periods. Potential sources of *E. coli* on the Avon River include sewage discharges during heavy rain events, stormwater with potential cross-connections, ducks, domestic animals and sediments within the river. To gain a better understanding of the sources of *E. coli*, this project applied faecal source tracking tools to answer the following questions:

- What are the sources of *E. coli* in the river during low flow conditions, which are when most people are using the river for recreation?
- What is the source(s) of raised levels of *E. coli* within the river during a rain event?

The two key monitoring sites were Kerrs Reach at the launch ramp and Antigua Street Boat Sheds as both sites are regularly used for recreational activity (Figures 1-3).

2 Experimental approach

2.1 Sample collection

Water samples were collected weekly between March 17th and May 21st at two key monitoring sites - Kerrs Reach at the rowing clubs, and Antigua Street Boat Sheds (Figures 1-3). Seventeen samples were collected from Kerrs Reach and 12 from the Antigua Boat Sheds. Water samples that contained greater than 260 *E. coli*/100 ml were then analysed using faecal source tracking tools. The aim was to collect:

- Five samples each from Boat Shed, Antigua St and Kerrs Reach during low water flow (1.4 - 1.5 m), with *E. coli* levels exceeding 260 *E. coli*/100ml.
- Five samples each from Boat Shed, Antigua St and Kerrs Reach during high water flow (over 1.65 m), with *E. coli* levels exceeding 260 *E. coli*/100ml.

If heavy rainfall and/or elevated river levels occurred outside of the routine weekly sampling, extra samples were collected and processed by CCC staff.

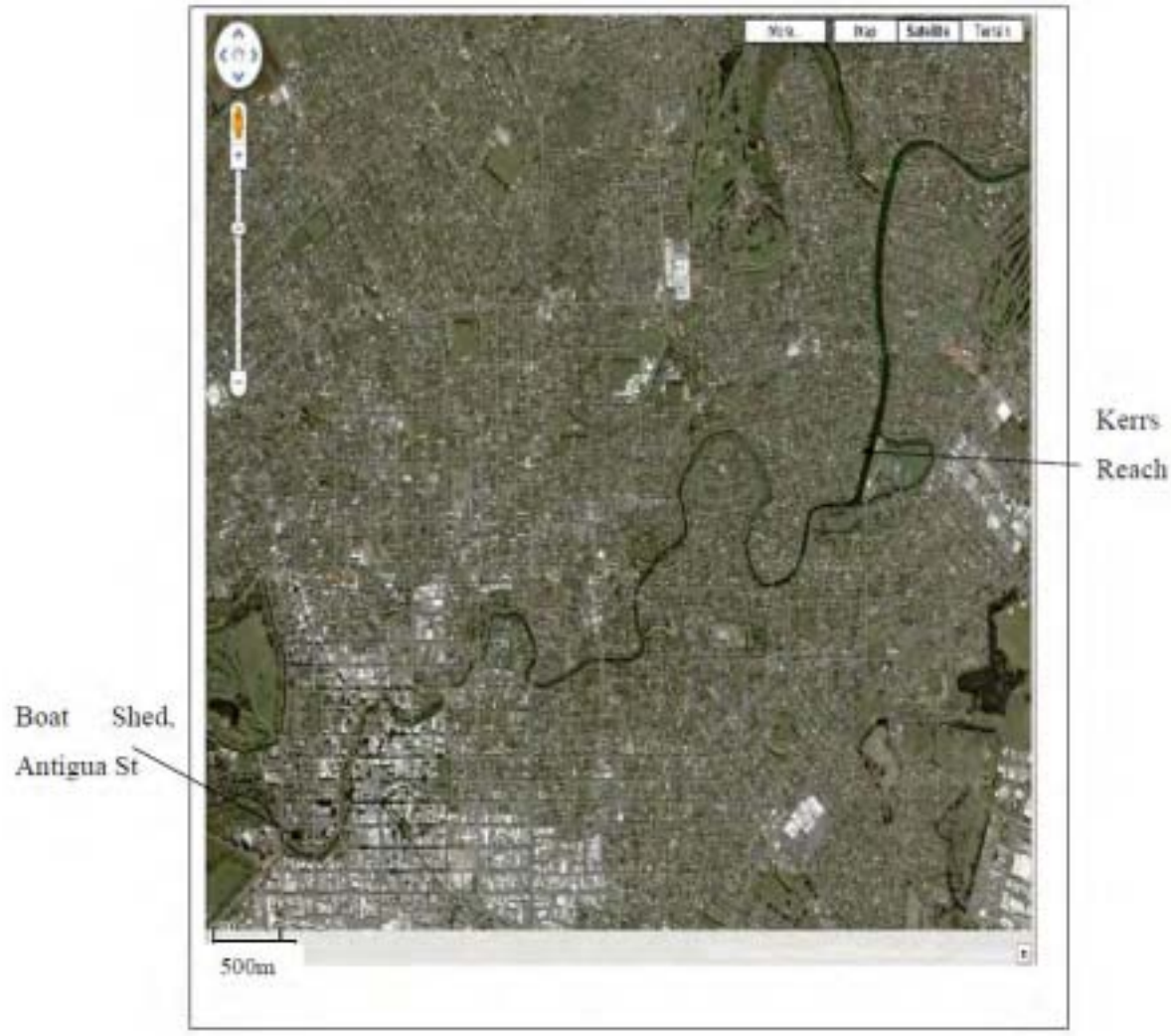

Figure 1 Aerial Map of Avon River, Christchurch with sampling locations marked

Figure 2 Sampling at the Boat Shed, Antigua St

Figure 3 Sampling at the Boat Ramp, Kerrs Reach

2.2 *E. coli* analysis

Water samples were analysed for *E. coli* by Christchurch City Council according to APHA 9213 D. *E. coli* results are expressed as colony forming units (cfu) per 100 ml of the stream water sample, with medians and geometric means calculated.

2.3 Rainfall and river flow data

Information pertaining to rainfall events and volume were sourced from NIWA National Climate Data Website (http://cliflo.niwa.co.nz/). The weather station at NIWA Eyre St, Riccarton, Christchurch was used for all data and values expressed as mm of rainfall per 24 hrs. Information pertaining to river flow was sourced from Environment Canterbury. The stage height for the Avon River is recorded every 15 minutes at the Gloucester St Bridge. Results are expressed in this report as maximum daily heights of the river as measured at this river stage in meters.

2.4 Faecal source tracking tools

There are an increasingly large number of methods available that can be used to identify the possible sources of faecal pollution. In this study molecular markers, fluorescent whitening agents (FWAs) and faecal sterols were examined in each of the water samples.

2.4.1 Molecular markers

There are a range of microorganisms other than faecal coliforms, *Escherichia coli* and enterococci present in the faeces, which are specific to animal hosts. Difficulties in culturing and identifying these organisms have 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 the polymerase chain reaction (PCR) for DNA from source-specific organisms. Five assays have been applied to the samples in this study. The first targets the *E. coli* bacteria (lacZ gene) and is not source specific. The second targets *Bacteroidales* bacteria which are indicative of faecal pollution. The third targets human-specific *Bacteroidales*, the fourth also targets *Bacteroidales*, but in this case indicates an animal specific, canine dominant source (referred to a dog marker). The final marker is wildfowl specific E2 marker and is referred to as duck marker. This marker is common in duck faeces, and has also been detected in geese, seagulls and swans (Devane *et al.* 2007).

2.4.2 Fluorescent Whitening Agents

Fluorescent whitening agents 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. Consequently, FWAs are usually associated with human faecal contamination in both septic tanks and community wastewater systems. The detection of FWAs therefore indicates the potential presence of human faecal pollution from a sewage system. In general levels of FWA greater than 0.2 ppb typically indicate recent or local source of human pollution, while lower levels indicate increasingly dilute or distant sources of human pollution. Levels below 0.1 typically are not associated with local source of human pollution.

2.4.3 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 24ethylepicoprostanol 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. **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.

Faecal sterols analysis was performed, by filtering 4 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 detected is expressed as parts per trillion (ppt).

Interpretation of the sterol is based on comparisons of ratios of key sterols. The ratios used in this study are include two indicators of faecal pollution, three indicators of human faecal source, two of herbivore source, and one suggesting plant decay (may be related to both natural plant breakdown in water, and plant consumption).

Table 1 Faecal sterol ratios

Ratio	Stanols/Sterols	Intepretation
Faecal 1	Coprostanol:cholestanol	>0.5 suggests faecal contamination, <0.3 in situ bacteria (sediments)
Faecal 2	24-ethylcoprostanol/24 ethylcholestanol	>0.5 suggests faecal contamination, <0.3 in situ bacteria (sediments)
Human 1	coprostanol/(coprostanol+chole stanol)	>0.7 suggests human, <0.7 herbivore
Human 2	%coprostanol/total sterols	>5-6% human, <5-6% herbivore
Human 3	Coprostanol:24 ethylcoprostanol	Human faecal pollution typically has a ratio greater than one
Herbivore 1	24-ethylcoprostanol/total sterols	> 5–6% herbivore
Herbivore 2	24-ethylcholesterol/24-ethylcoprostanol	<1.0 suggests animal
Plant Decay	24-ethylcholesterol/24-ethylcoprostanol	>4.0 suggests plant decay

In this report, to facilitate visualisation on graphs, all ratios have been converted to give a significance value of 1. Appendix 2 uses actual ratios on log scale.

3 Results

3.1 *E. coli* analysis

E. coli analysis was performed on 29 water samples, 12 from the Boat Sheds on Antigua Street, and 17 from the Boat ramp at Kerrs Reach (Figure 4). Six high flow (>1.65 m) samples were processed for each site with the remainder (6 Boat Shed and 11 Kerrs Reach) sampled during low flow (1.4 – 1.55 m). While the original aim was to process only samples that exceeded the MoH/MfE guidelines for recreational water quality (260 *E. coli*/100ml), it was necessary to relax this criterion in order to obtain sufficient samples from Kerrs Reach in low flow conditions.

A positive correlation was observed ($P < 0.05$) between rainfall and the level of the river (Figure 4). A positive correlation was also observed with higher concentrations of *E. coli* following heavy rainfall ($P < 0.05$) (Figure 4).

3.1.1 Boat shed, Antigua St

Six samples were collected during low flow conditions (1.4 -1.5 m), of which five exceeded 260 *E. coli*/100ml (geometric mean 403, median 360 Appendix 1). None exceeded the MfE /MoH action level of greater than 550 *E. coli*/100ml. One sample contained only 180 *E. coli*/100ml. During high flow conditions (>1.65 m), all samples collected exceed 550 *E. coli*/100ml (mean 1157, median 1150, Appendix 1).

3.1.2 Kerrs Reach

Eleven samples were collected during low flow conditions (1.4-1.5 m), of which only one (12th May, 380 *E. coli*/100ml) exceeded 260/100ml. The remaining samples ranged from 41 to 230 *E. coli*/100ml (mean 138, median 175, Appendix 1). Five of the later samples were analysed for faecal source markers, despite four of them falling below 260 *E. coli*/100ml (mean 177, median 180). During high flow conditions (>1.65 m), all six samples collected exceeded 260 *E. coli*/100ml (mean 1561, median 2350, Appendix 1).

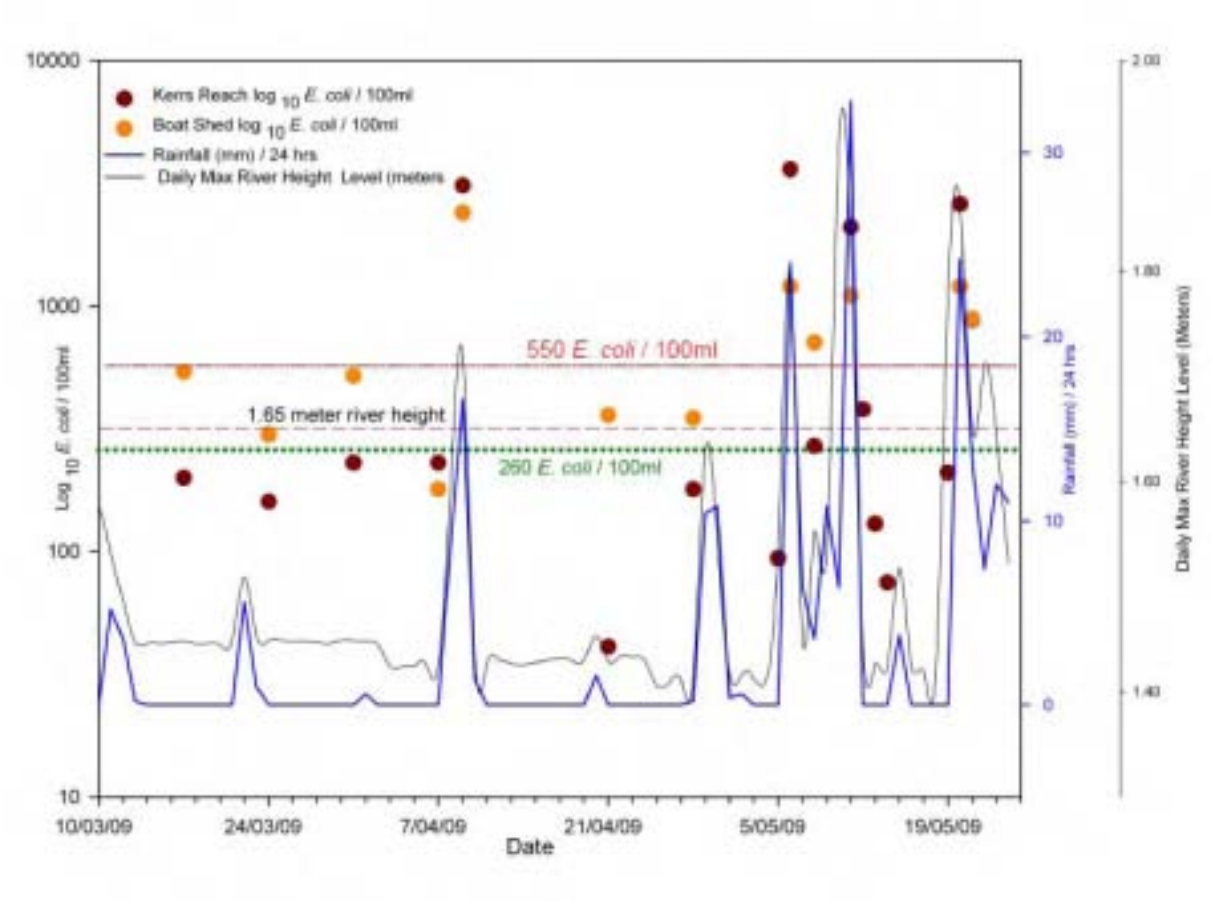

Figure 4 ***E. coli*** **concentrations at Kerrs Reach and Antigua Boat Shed with corresponding daily rainfall and height of the Avon River**

3.2 Faecal source tracking - Boat shed, Antigua Street

3.2.1 Faecal sterol analysis

During low flow total sterols ranged from 5,600 to 31,000 ppt (median 11,000), while during high flow total sterols ranged from 9,300 to 26,500 ppt (median 13,000). These levels are sufficient to allow further interpretation of all samples.

The ratio of 24-ethylcoprostanol/24-ethylcholestanol (Faecal 2 ratio) exceeded the threshold in all samples suggesting a faecal source of the sterols. This was supported in high flow samples by the ratio of coprostanol:cholestanol (Faecal 1 ratio), but in low flow samples this tended to be more indicative of sediment source (Figure 5). Examination of the three ratios indicative of human source of faecal pollution did not support a human source of these sterols (Figure 6). Herbivore marker 2 was negative in all samples, whilst the first two low flow samples exceeded the threshold for Herbivore marker 1 (Figure 7). All samples contained evidence of plant decay related sterols (Figure 7). Appendix 2 provides alternative representation of sterol analysis.

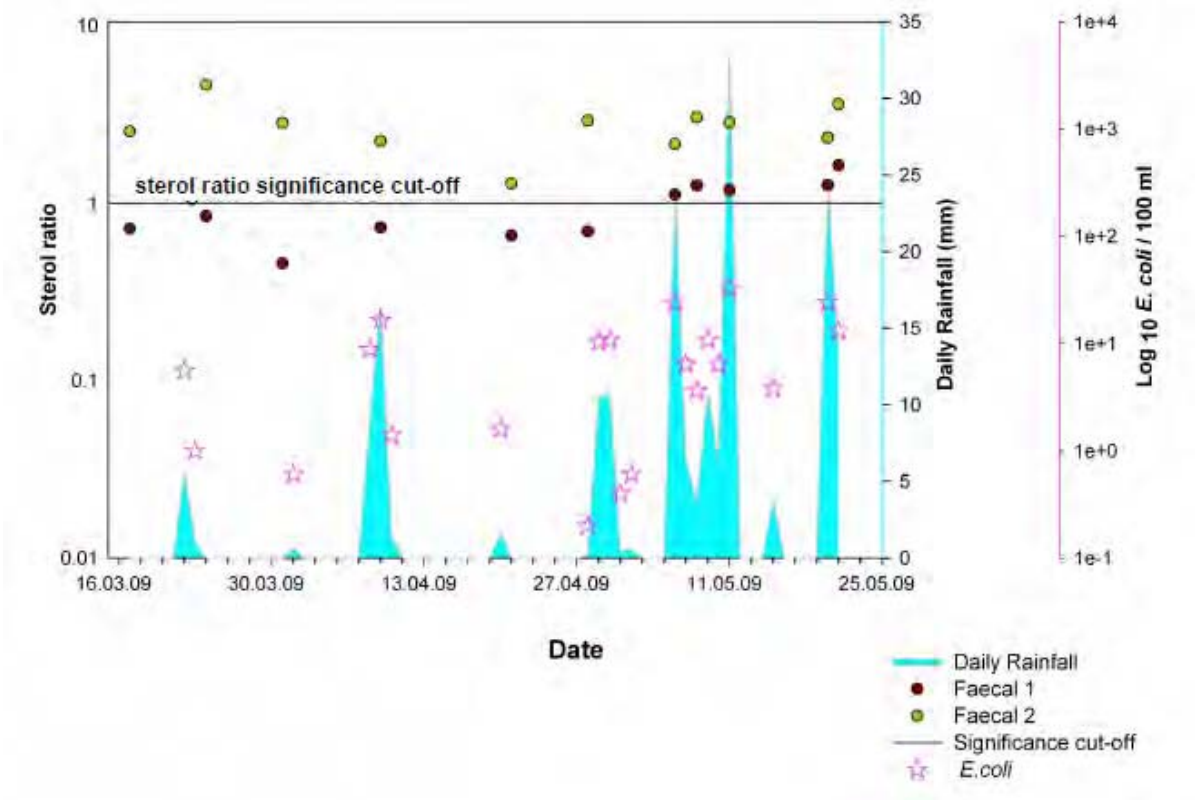

Figure 5 **Antigua Boat Sheds: Faecal sterol ratios greater than 1 are indicative of a faecal source of sterols**

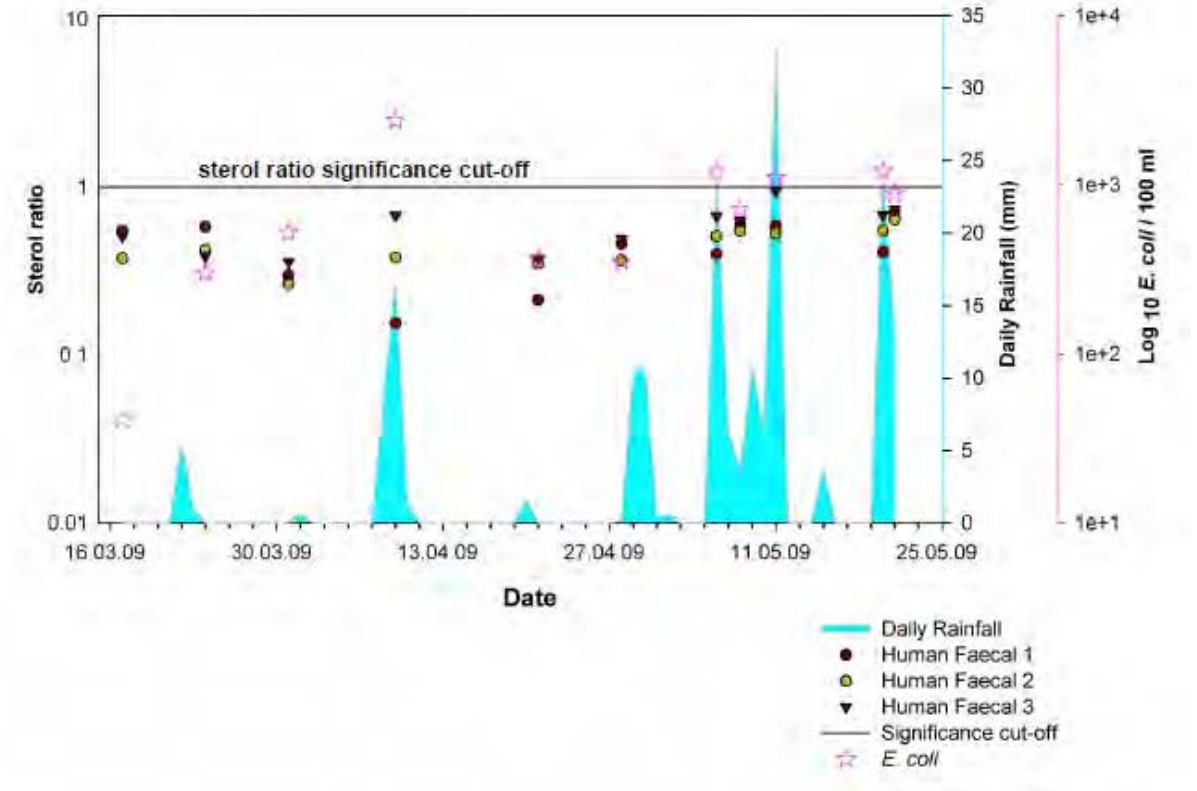

Figure 6 **Antigua Boat Sheds: Faecal sterol ratios indicative of a human source of sterols**

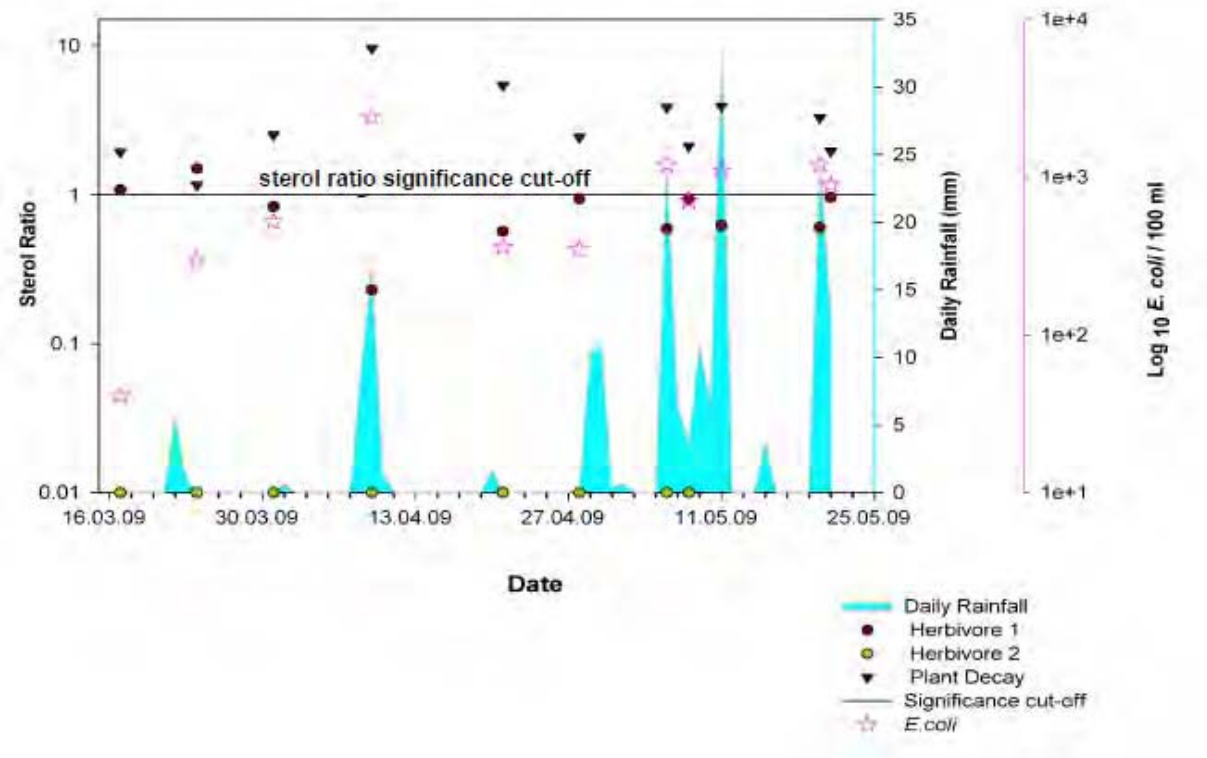

Figure 7 **Antigua Boat Sheds: Faecal sterol ratios indicative of a non-human sources of sterols**

3.2.2 Fluorescent Whitening Agents (FWAs)

Fluorescent Whitening Agents (FWAs) were below the detection limit (<0.01) in all the samples except for two of the low flow samples (Figure 8). One on the 24th March which contained 0.19 ppb, and one a week later (31st March) that contained 0.08 ppb.

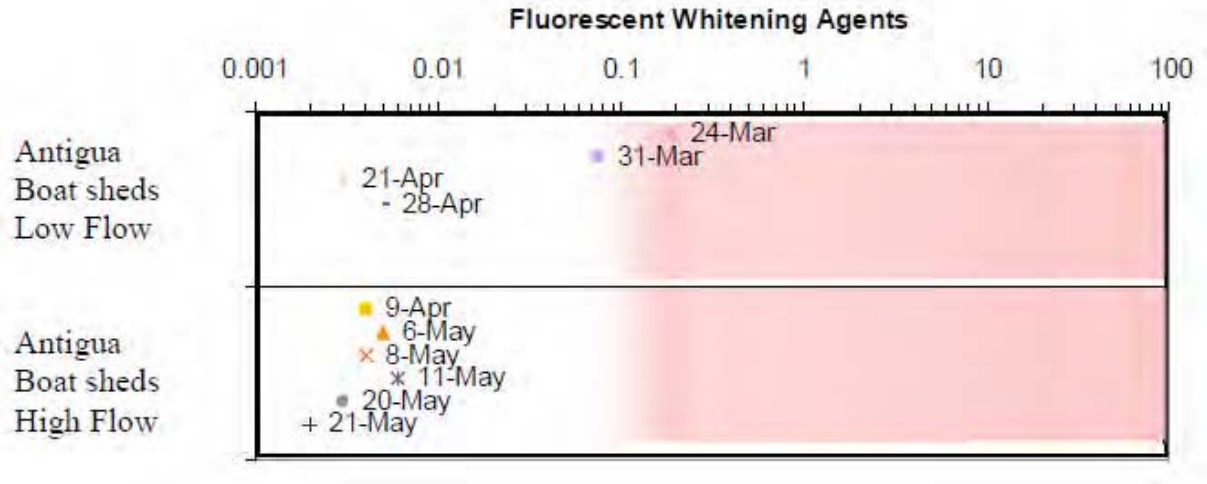

Figure 8 **Measured levels of FWAs at Antigua Boat Sheds**

3.2.3 Molecular markers

The lacZ marker indicative of *E. coli* was detected in all samples analysed (the first four samples were not analysed) (Figure 9, 11, 12). The human indicative bacteroidales marker was not detected in any of the samples (Figure 10-12). In contrast the wildfowl marker (Duck E2) and dog indicative marker were detected in almost all the other samples (Figure 10-12). In the low flow samples the wildfowl marker dominated, while in the high flow samples the dog marker dominated.

3.2.4 Conclusion

Apart from the FWAs detected in two of the low flow samples in March, there is no evidence of a human source of pollution in the samples in either high or low flow at the Antigua Street Boatsheds. During low flow conditions the wildfowl PCR dominated, while during high flow there were increased relative inputs from the dog dominated marker.

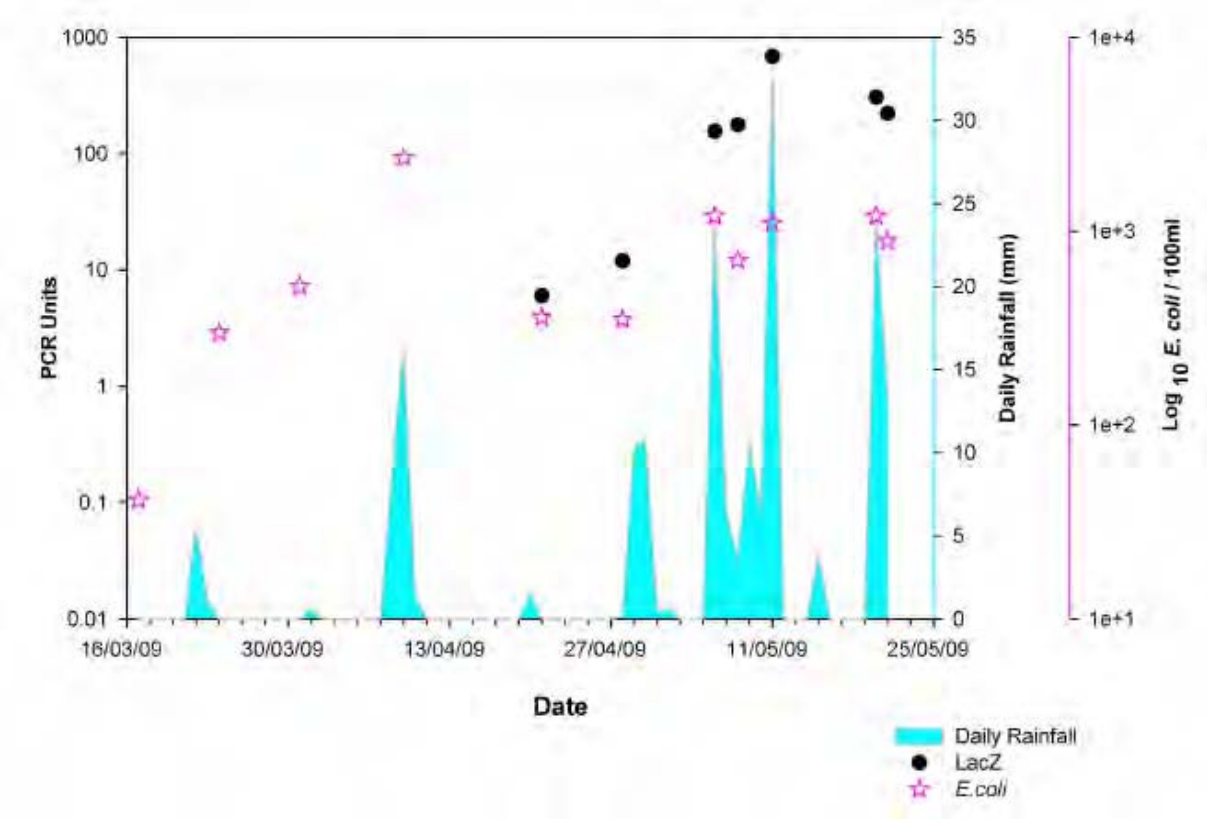

Figure 9 Antigua Boat Sheds: Detection of LacZ *E. coli* indicative PCR marker

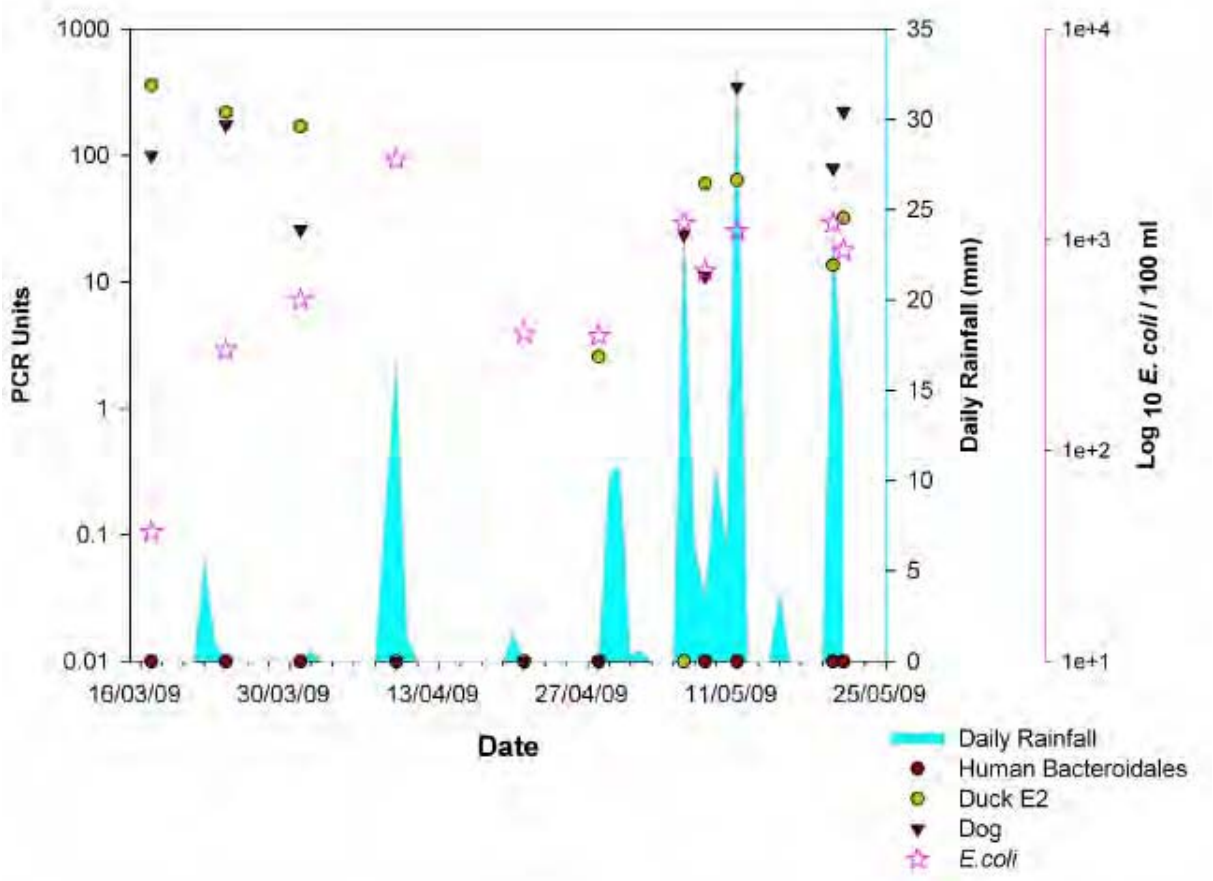

Figure 10 Antigua Boat Sheds: Detection of human, duck and dog indicative PCR markers

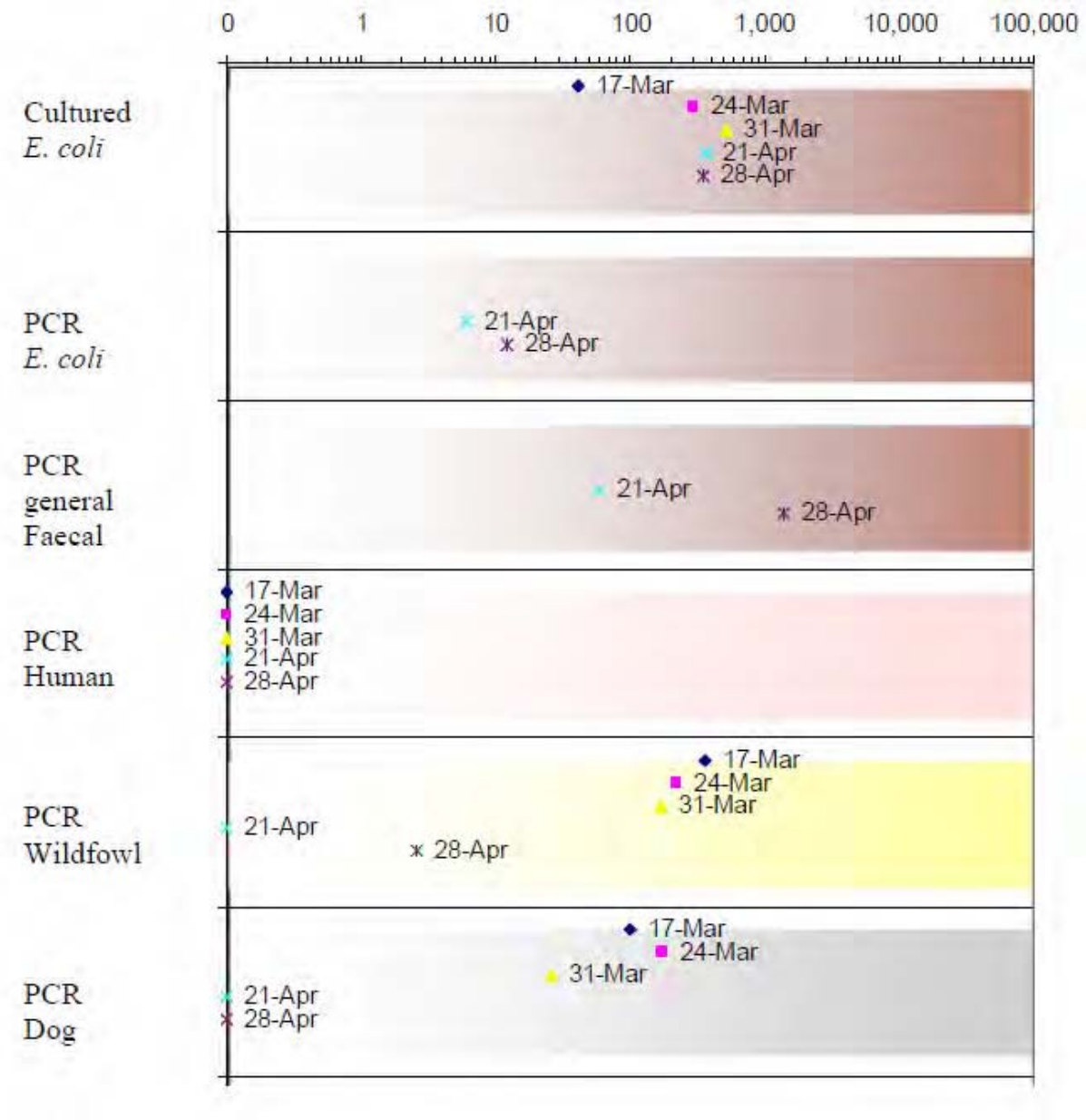

Figure 11 **Levels of cultured *E. coli* (CFU.100ml) and PCR based markers (copy number) detected at Antigua Boatsheds under low flow conditions. The further to the right that point is detected, the higher the level of marker. Note: log scale used for samples. The *E. coli* and General faecal PCR assays were only performed on two samples.**

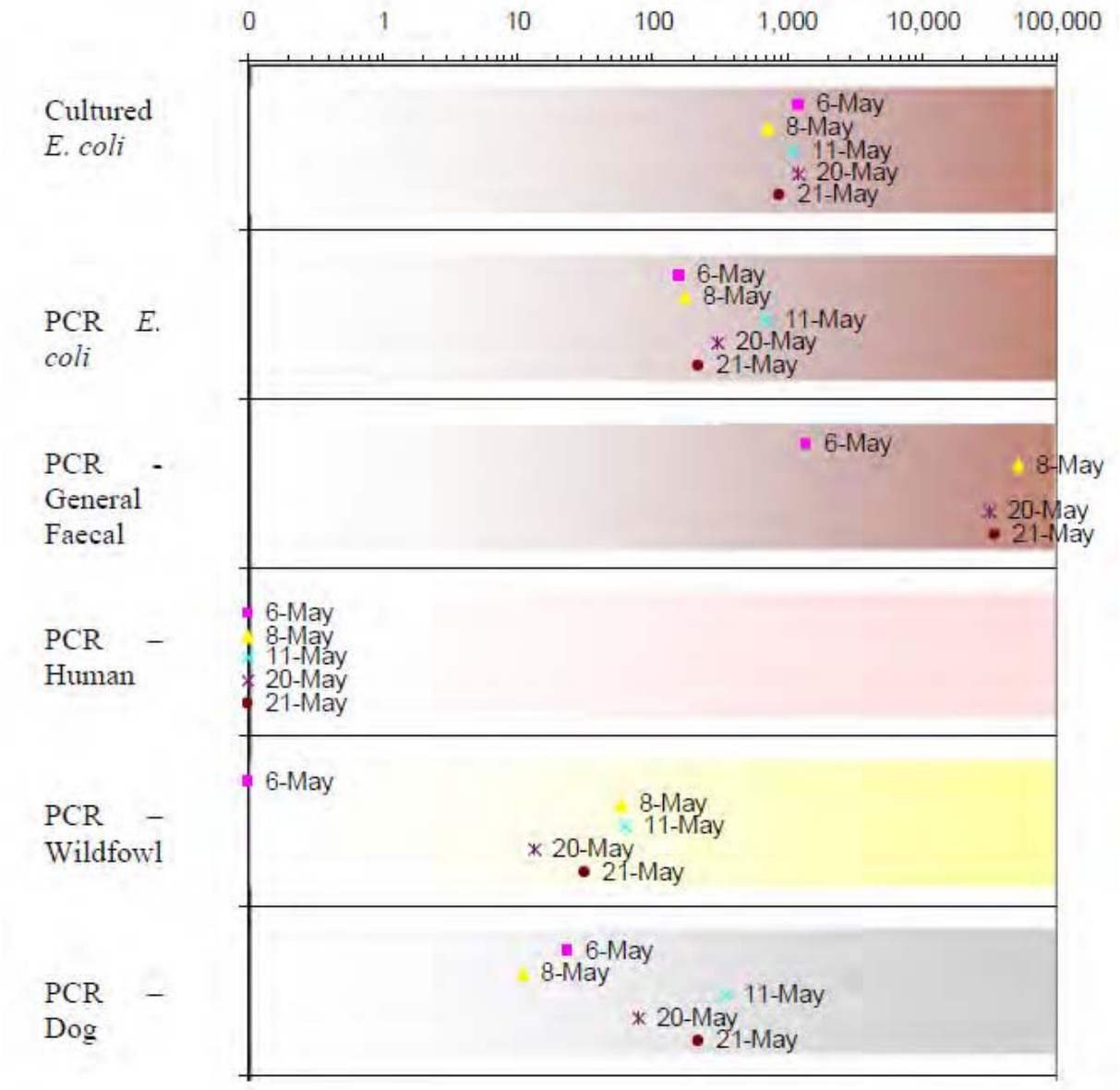

Figure 12 **Levels of cultured *E. coli* (CFU/100ml) and PCR based markers (copy number) detected at Antigua Boatsheds under high flow conditions. The further to the right that point is detected, the higher the level of marker. Note: log scale used for samples.**

3.3 Faecal source tracking - Kerrs Reach

3.3.1 Faecal sterol analysis

During low flow total sterols ranged from 3,500 to 5,700 ppt (median 5,400), while during high flow total sterols ranged from 8,500 to 16,000 ppt (median 9,900). These levels are sufficient to allow further interpretation of all samples. The ratios of both faecal indicative ratios exceeded the threshold in almost all of the samples suggesting a faecal source of the sterols (Figure 13). The three ratios indicative of human source of faecal pollution all exceeded the thresholds on the high flow 11th May sampling, and this signature was also detected the following day under low flow conditions (Figure 14). One of these ratios (Human faecal 3), was also detected following the final high flow event on the 20th and 21st May. The two herbivore markers were negative in almost all the samples (Figure 15). All samples contained evidence of plant decay related sterols (Figure 15). Appendix 2 provides alternative representation of sterol analysis.

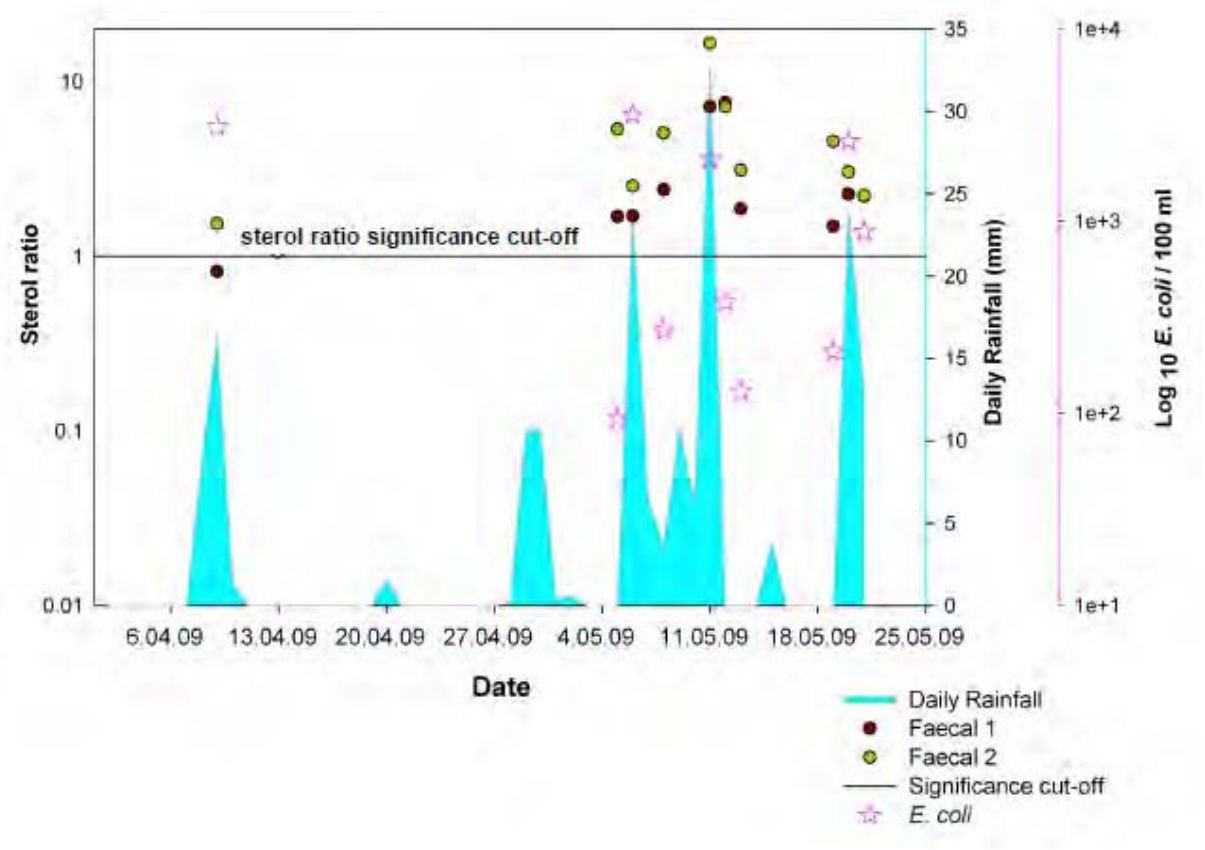

Figure 13 Kerrs Reach: Faecal sterol ratios indicative of faecal source of sterols

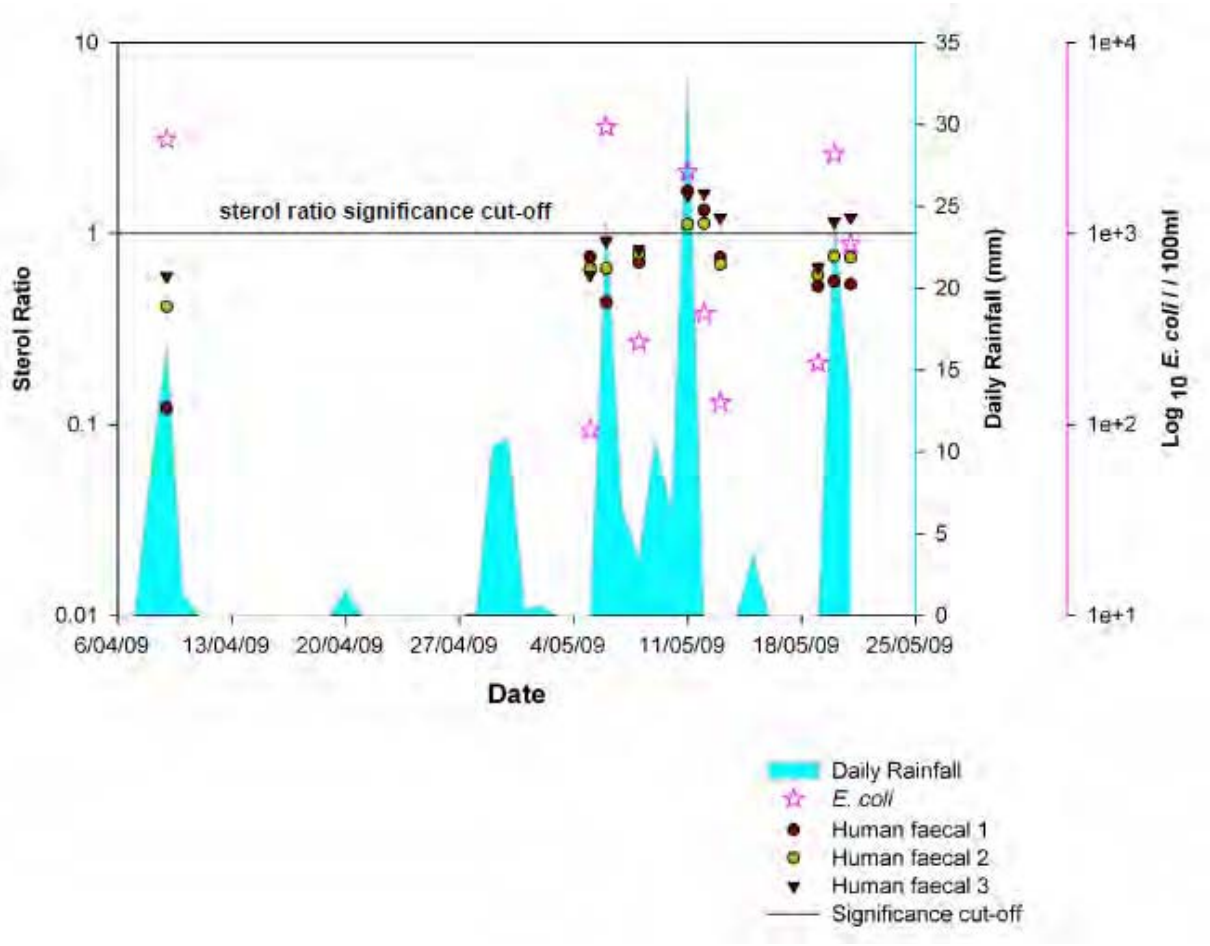

Figure 14 Kerrs Reach: Faecal sterol ratios indicative of a human source of sterols

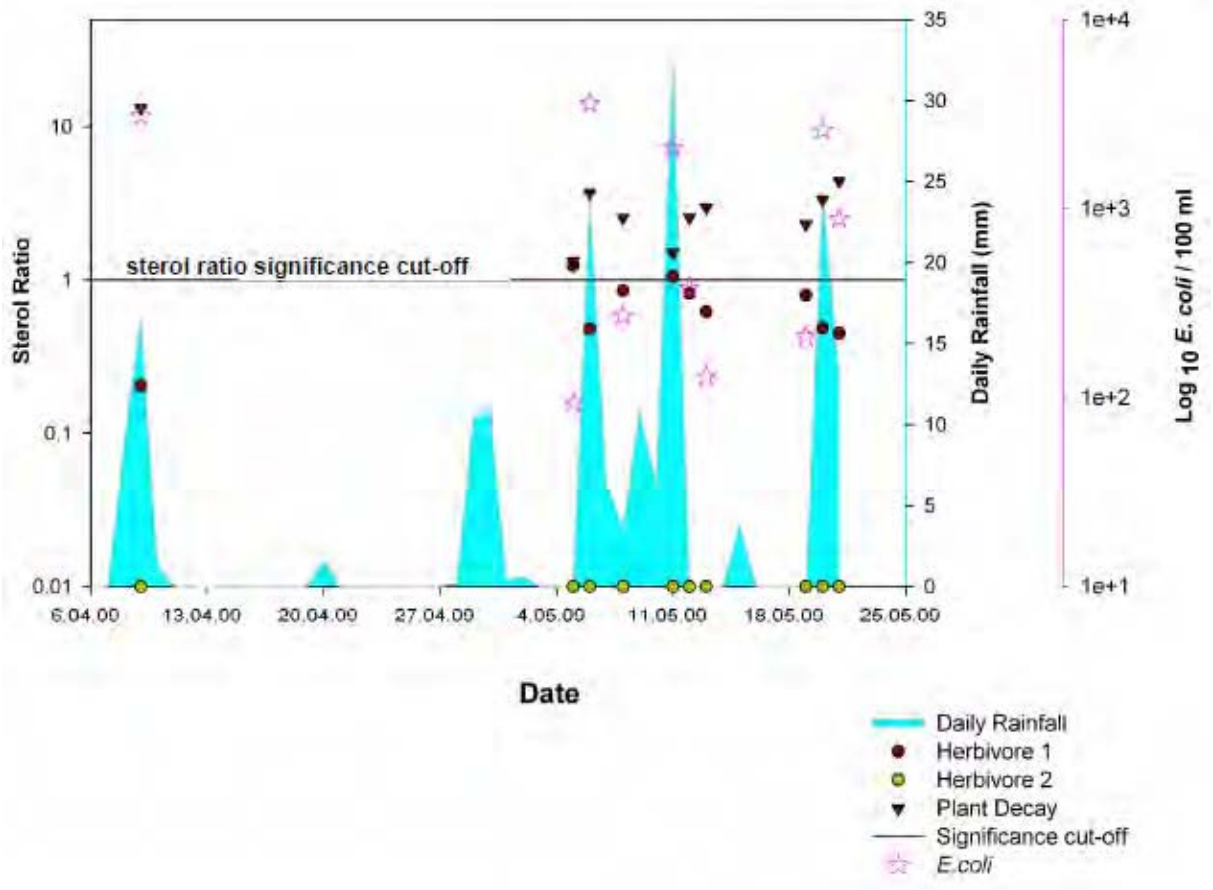

Figure 15 Kerrs Reach: Faecal sterol ratios indicative of a non-human sources of sterols

3.3.2 Fluorescent Whitening Agents (FWAs)

Fluorescent Whitening Agents (FWAs) were below the detection limit (<0.01) in all the samples except for the samples collected under high flow on the 11th May (0.01 ppb) and the following day on 12th May (0.02 ppb) (Figure 16).

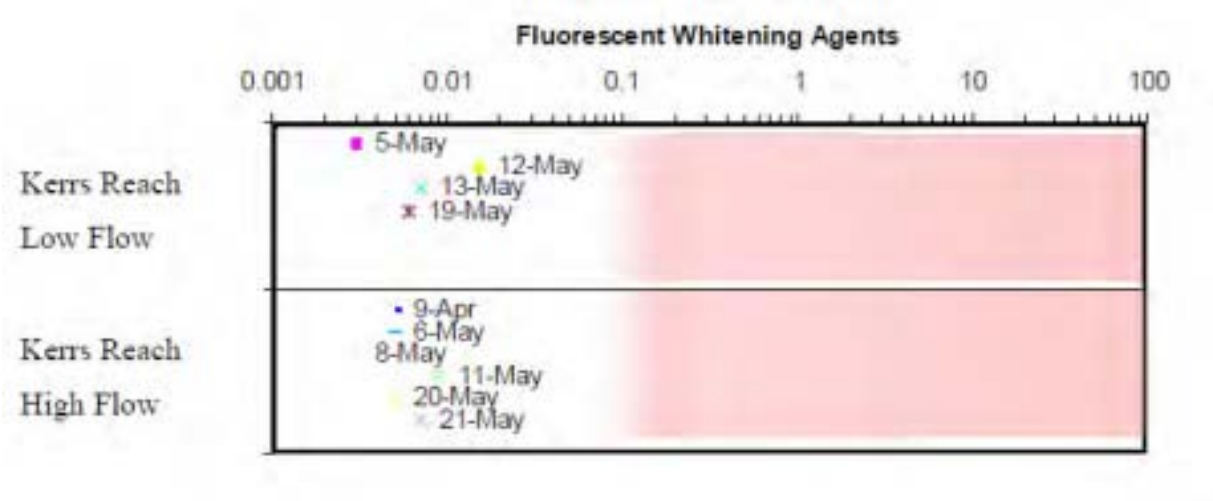

Figure 16 Measured levels of FWAs at Kerrs Reach

3.3.3 Molecular markers

The lacZ marker indicative of *E. coli* was detected in all samples analysed (the first four samples were not analysed) (Figure 17, 19, 20). The human indicative bacteroidales marker was detected at low levels in samples taken on the 11and 12May and again on the 20th May (Figure 18-20). Both of these periods were high flow events. In contrast the wildfowl marker (Duck E2) and dog indicative marker were detected in all the samples, and at levels higher than the human indicative marker (Figure 18-20). In the high flow samples the dog marker tended to predominate.

3.3.4 Conclusion

During low flow sampling not preceded by a high flow event, there was no evidence of human faecal pollution. The low levels of *E. coli* present appear to have wildfowl source. High flow events separated into two groups. The first group did not have a human contribution to the high *E. coli* levels observed. In contrast human faecal markers did contribute to the samples taken on the 11and 20 May. Wildfowl and canine sources contributed to all the samples.

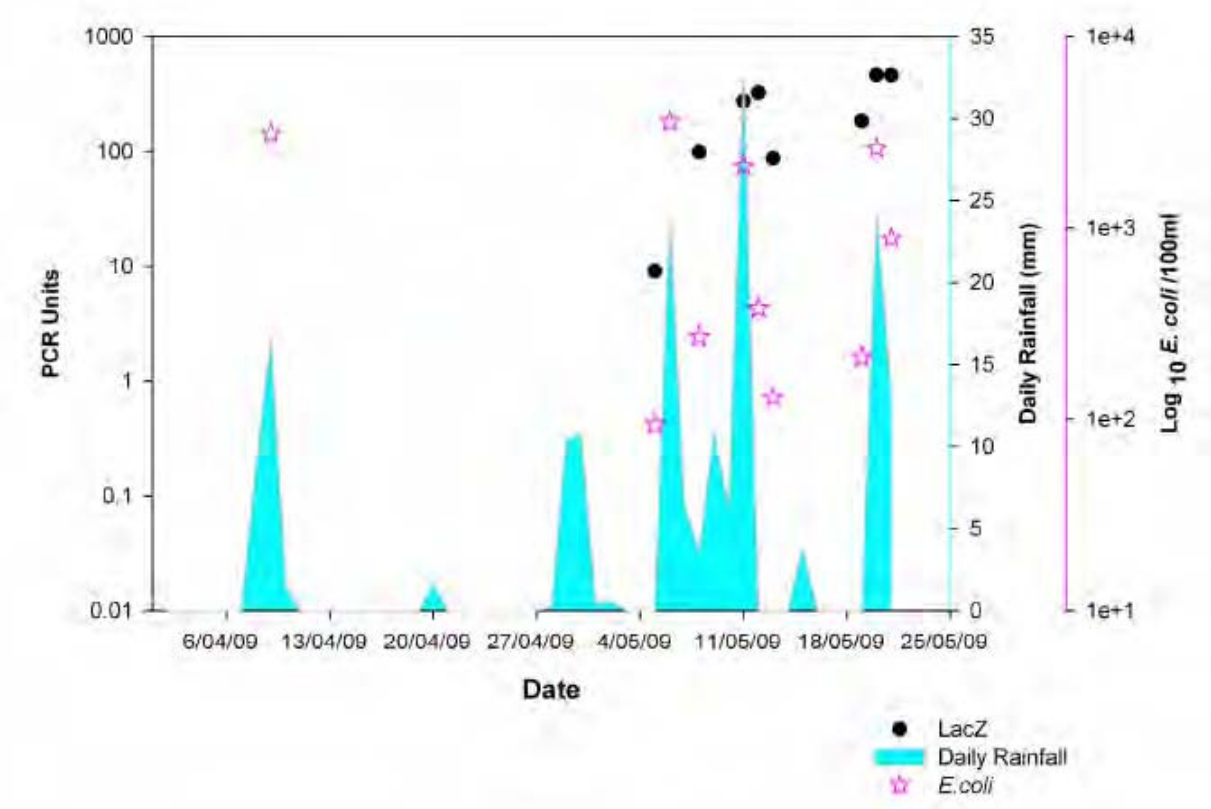

Figure 17 Kerrs Reach: Detection of LacZ *E. coli* indicative PCR marker

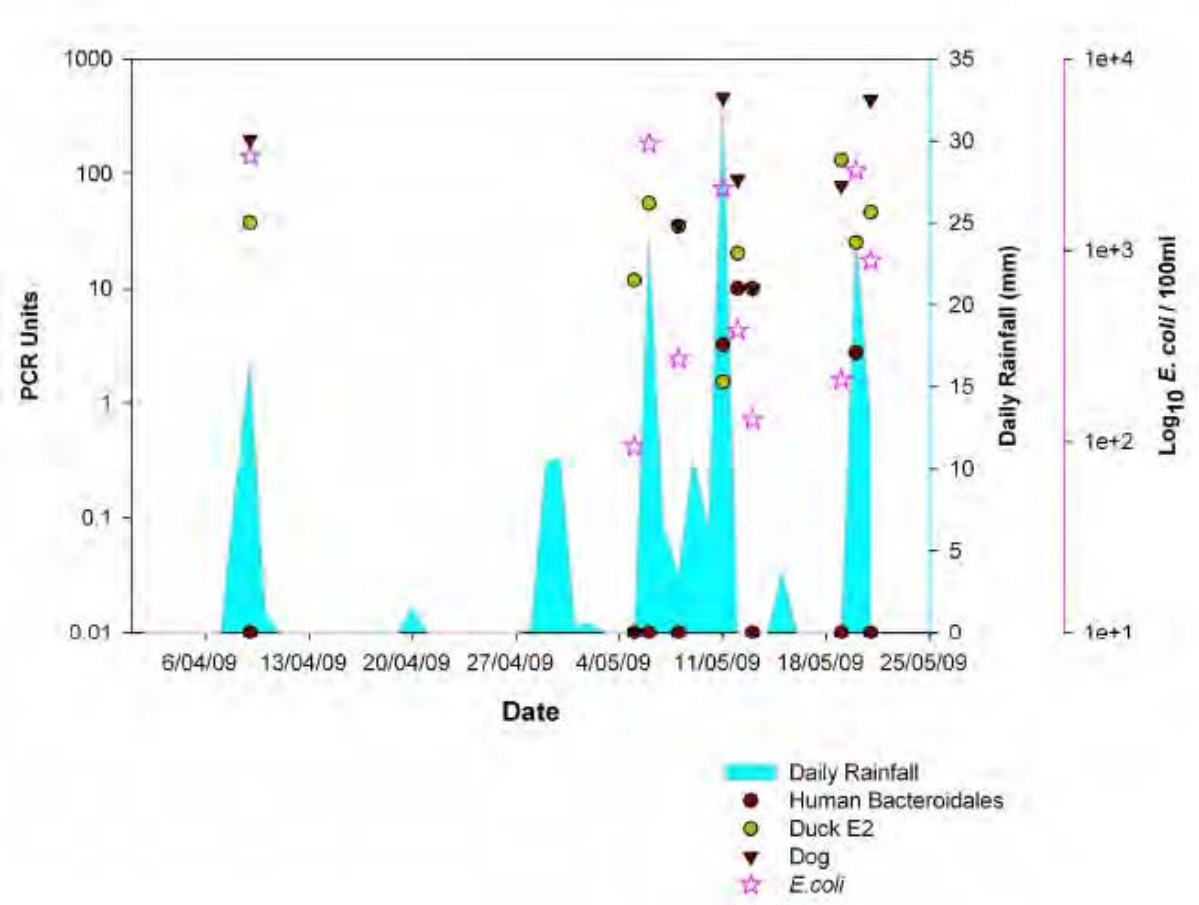

Figure 18 Kerrs Reach: Detection of human, duck and dog indicative PCR markers

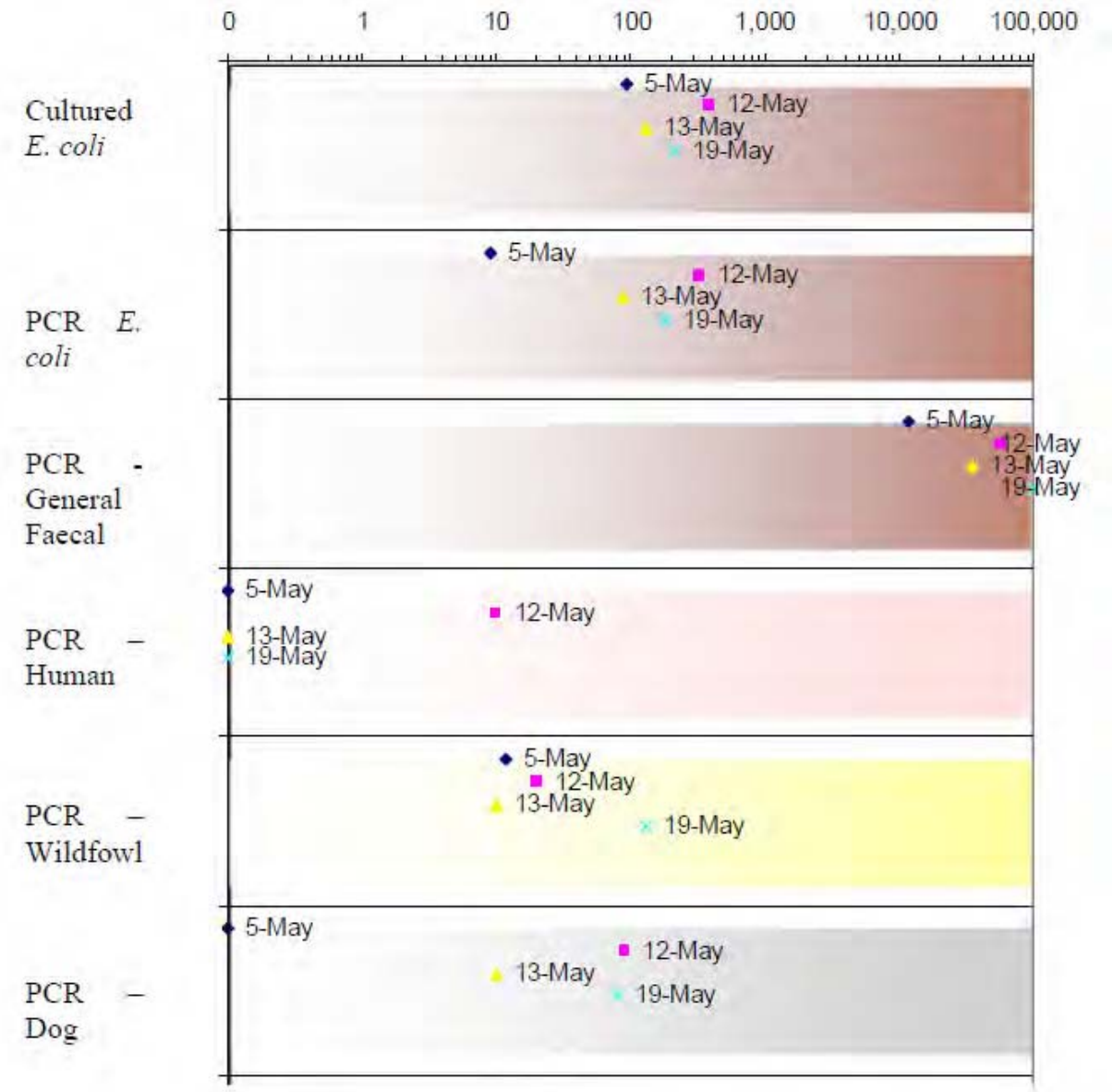

Figure 19 **Levels of cultured *E. coli* (CFU/100ml) and PCR based markers (copy number) detected at Kerrs Reach under low flow conditions. The further to the right that point is detected, the higher the level of marker. Note: log scale used for samples.**

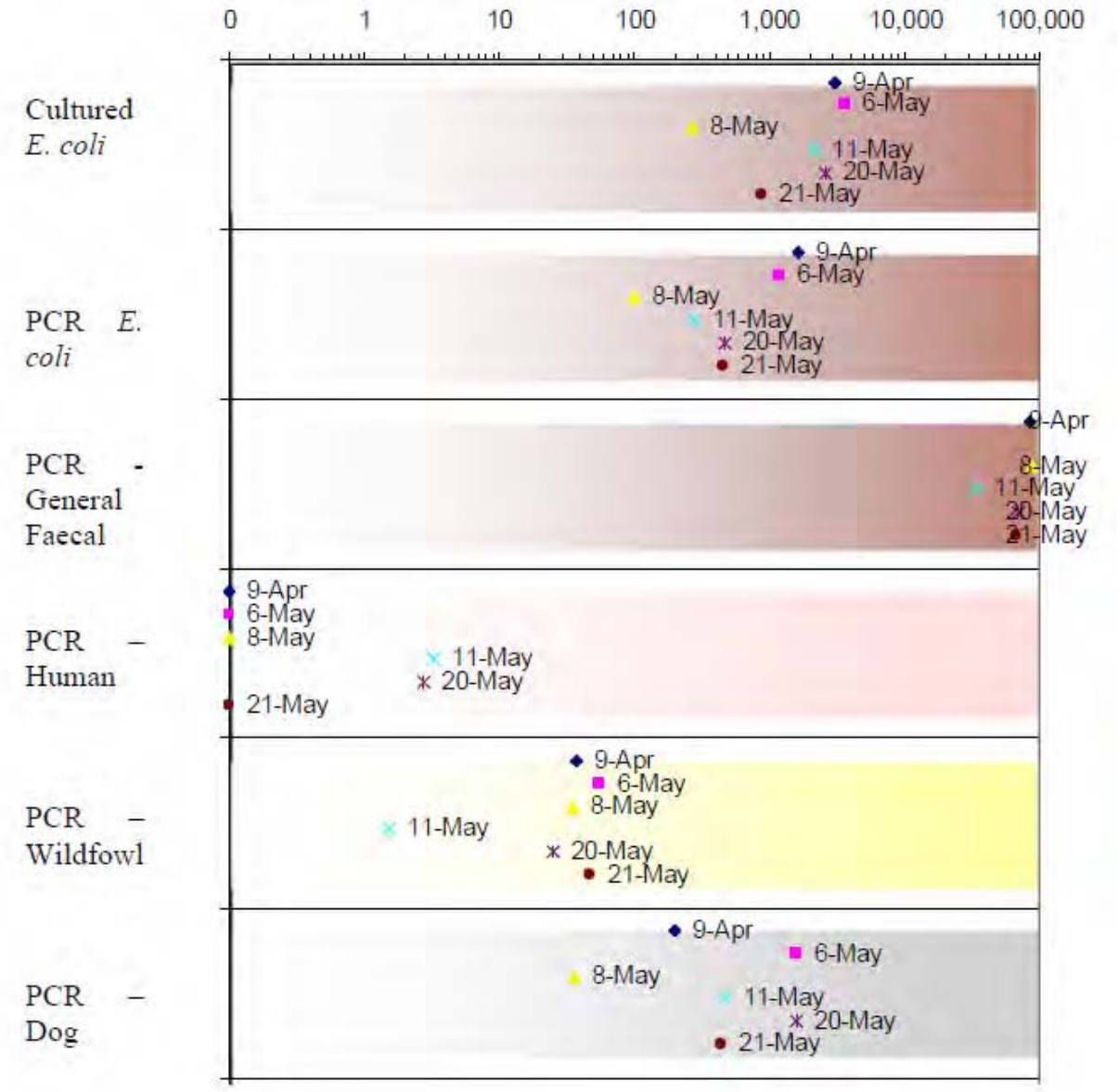

Figure 20 **Levels of cultured *E. coli* (CFU/100ml) and PCR based markers (copy number) detected at Kerrs Reach under high flow conditions. The further to the right that point is detected, the higher the level of marker. Note: log scale used for samples.**

4 Discussion

The public has become increasingly aware of the potential 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, *Escherichia 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 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.

Previous monthly monitoring of two sites on the Avon River has identified that levels of the water quality indicator *Escherichia coli* regularly exceed the MfE/MoH recreational contact guidelines

(MfE/MoH, 2003). The aim of this work was to explore the application of faecal source tracking tools to better understanding the sources of the elevated *E. coli* in the Avon River. Faecal source tracking tools applied included faecal sterol analysis (faecal chemicals which differ between human and animal sources), fluorescent whitening agents (FWAs – washing powder agents that are usually associated with human faecal pollution), and DNA based molecular markers (assays indicative of human, wildfowl and canine sources).

One aim of this study was to understand water quality during high and low flow events. A number of the samples were sampled close to one another, so samples are not independent of one another, and indeed influences from high flow events appear to have carried over to subsequent low flow sampling in several occasions.

Rainfall results in significant degradation of the microbial water quality of the Avon River. During high flow events median *E. coli* levels at both sites exceeded 1,000 *E. coli*/100ml. Measured levels of microbes at each site will represent not just local inputs, but also contributions from upstream sources. The increases observed following rainfall events may be due to a combination of inputs from overland flow, stormwater discharges, and resuspension of bacteria from the stream sediments. The primary sources of this degradation appear to be related to wildfowl and dog faecal material. During low flow conditions the wildfowl PCR marker dominated, while during high flow, increased relative inputs were observed from the dog indicative marker.

Human markers were detected from high flow events at Kerrs Reach, but at relatively low levels relative to the number of *E. coli* detected. This suggests either a distant source of these human markers, or an aged source of these human markers. The high levels of *E. coli* still appear to be primarily from wildfowl and animal sources.

The FWAs detected in two of the low flow samples in March at the Boatshed were not supported by the other indicators. This suggests a non-faecal association of FWAs from for example cleaning products in stormwater or runoff.

The significant presence of the dog indicative molecular marker was somewhat surprising. To support the validity of this, a survey of the riverbanks for dog faecal material is suggested. Additional validation of the specificity of this marker with any other faecal or point source inputs is also recommended. If, however, this contribution of dog faecal material is confirmed, then significant improvements to microbial water quality may be achieved through better control of dog defecation and disposal, and the use of measures, such as low impact devices to reduce the volume of direct stormwater runoff and to treat the stormwater at source. Reducing wildfowl inputs may be more difficult.

4.1 Additional investigations

This small study has looked at only two sites on the Avon River between March and May 2009. How representative these sites are of other areas of the river, and other time periods remains to be determined. Investigations could be assisted by sampling of the sediments to determine what type and what concentrations of micro-organisms are present. Faecal source tools can be applied to sediment analysis. Stormwater discharges could also be tested to determine the levels of microbial indicators and then the sources of these indicators. Whether all stormwater discharges contribute equally, or whether particular stormwater discharges are proportionally responsible for more of the pollution would inform any management solutions.

4.2 Sewer overflows

In the original project brief it was intended to sample the river following an overflow event along the Avon. During the period of the study no overflow events were reported, therefore all samples which were analysed during the high flow/heavy rainfall were not impacted by sewage overflows. To better understand the impact of sewer overflows, a targeted sampling of the sewer overflow may be a better strategy. The first question is what is the microbial quality of the overflow. Overflows are likely to contain significant infiltration of stormwater. Collection and analysis of actual sewer overflow during overflow events would provide information on the number of bacteria in the overflow, and how this changes during the discharge. The issue of what happens to that overflow, how far downstream it will impact and how long the overflow contamination will persist are then primarily hydrological issues

which could be addressed through combination of modelling supported by experimental sampling. Faecal source tracking tools may be required to differentiate sewer overflow from other sources.

4.3 Health risks of Avon River water

The health risk posed by this water is largely unknown. Indicator bacteria such as *E. coli* are used to detect the presence of faecal material, and therefore potentially pathogenic organisms transmitted by the faecal-oral route. A number of factors influence whether any pathogens will actually be present including the source of the faeces, time since excretion and the influence of various attenuation factors including sunlight, predation, and sedimentation.

Waterfowl are significant excreters of faecal material both directly into water, and in the vicinity of waterways, particularly on riverbanks. The daily faecal outputs of a variety of waterfowl have been measured. Seagulls were found to excrete on average 50 g wet weight a day (Wood and Trust 1972), black swans an average of 418 g (Mitchell and Wass 1995), and ring billed gulls an average of 0.48 g (Alderisio and DeLuca 1999). A survey has recently been completed by ESR on the microbial loading of bacterial indicators (*E. coli* and enterococci) and pathogens (*Campylobacter* and *Salmonella* spp.) present in the fresh faeces of Canada Geese (n=80), Gulls (n=80), Ducks (n=80) and Black Swans(n=80) from various locations in NZ. *Campylobacter* were detected in 30% of duck, 58% of gull, 40% Canada Geese and 46% of Black Swan scats. No *Salmonella* were detected. Mean concentrations per gram of *Campylobacter* ranged up to 4,840 CFU/gram. High levels of *E. coli* were measured in duck faeces – a mean of 9.5 x 107 per gram. Dog faecal material may also contain microorganisms pathogenic to humans (Cook, 1989, Robertson and Thompson, 2002, Palmer *et al.* 2008).

To better quantify the health risks related to this water two approaches are possible. The first would be to screen this water for a range of potential pathogens (*Campylobacter, Cryptosporidium, Giardia, E. coli* O157). Pathogen testings is, however, expensive and would need to be fairly extensive to have statistical validity. Unlike microbial indicators such as *E. coli*, which is almost always present in faecal material, pathogens are often only intermittently present in faeces, and therefore intermittently present in faecally contaminated water. Infective doses of some pathogens can be as low as a single organism, so testing of water needs the analysis of large volumes of water.

A second approach would be to undertake an epidemiological study of the health impacts to water users from either the rowing clubs or the Antigua Boatsheds. In the case of the rowing clubs, a partnership with users could be established to follow-up any gastrointestinal illness events following recreational water contact. This would need to be a one to two year study. Water users could also be enlisted to keep a diary of frequency and type of water contact. At the Boatsheds an epidemiological study may be more difficult, but could involve giving everyone who hires a boat, a card with an 0800 number to ring if they have any gastrointestinal illness in the seven days following boating activity. Partnership with the Boatsheds would be required.

4.4 Recreational water standards

The appropriateness of contact recreation standards to water impacted primarily by non-human sources is a topic which requires more investigation. Environment Southland have proposed using a standard of 1,000 faecal coliforms/100ml for the assessment of microbial water quality in lowland streams used for either stock drinking water or secondary contact recreation. Sinton and Weaver (2008) recently reviewed the scientific justification for this standard, and review of that document by readers is highly recommended. *E. coli* are a subset of faecal coliforms, and in fresh faeces 90% or more of faecal coliforms will be *E. coli*. Therefore, water with median levels of *E. coli* of 1,000 or more, will contain at least that many faecal coliforms. Using this threshold, both sites on the Avon River exceed this secondary contact recreation level during high flow.

4.5 Conclusion

Rainfall results in significant degradation of the microbial water quality of the Avon River. The primary sources of this degradation appear to be related to wildfowl and possibly dog faecal material. Human markers were detected from high flow events at Kerrs Reach, but at low levels relative to the number

of *E. coli* detected. This suggests either a distant source of these human markers, an aged source of these human markers, or a small (possibly isolated) spill. The high levels of *E. coli* still appear to be primarily from wildfowl and animal sources.

5 Acknowledgements

Environment Canterbury commissioned this project, as part of its 2008-2009 contribution towards implementing the Ihutai Management Plan 2004. Many thanks to the Avon-Heathcote Estuary Ihutai Trust for prioritising that this project be undertaken and their interest and involvement.

Many thanks to Lyndsey Husband, formerly of Christchurch City Council, for the initial request and project design. We are most grateful to Nicholas Ohs, Christchurch City Council, for sample collection (in all sorts of weather) and enumeration of *E. coli* in water samples.

We would also like to acknowledge the staff at Christchurch City Council and Environment Canterbury for their assistance and information provided during the project.

6 References

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Appendix 1 Summary of results

Table 2. Results summary for Boat Shed, Antigua St

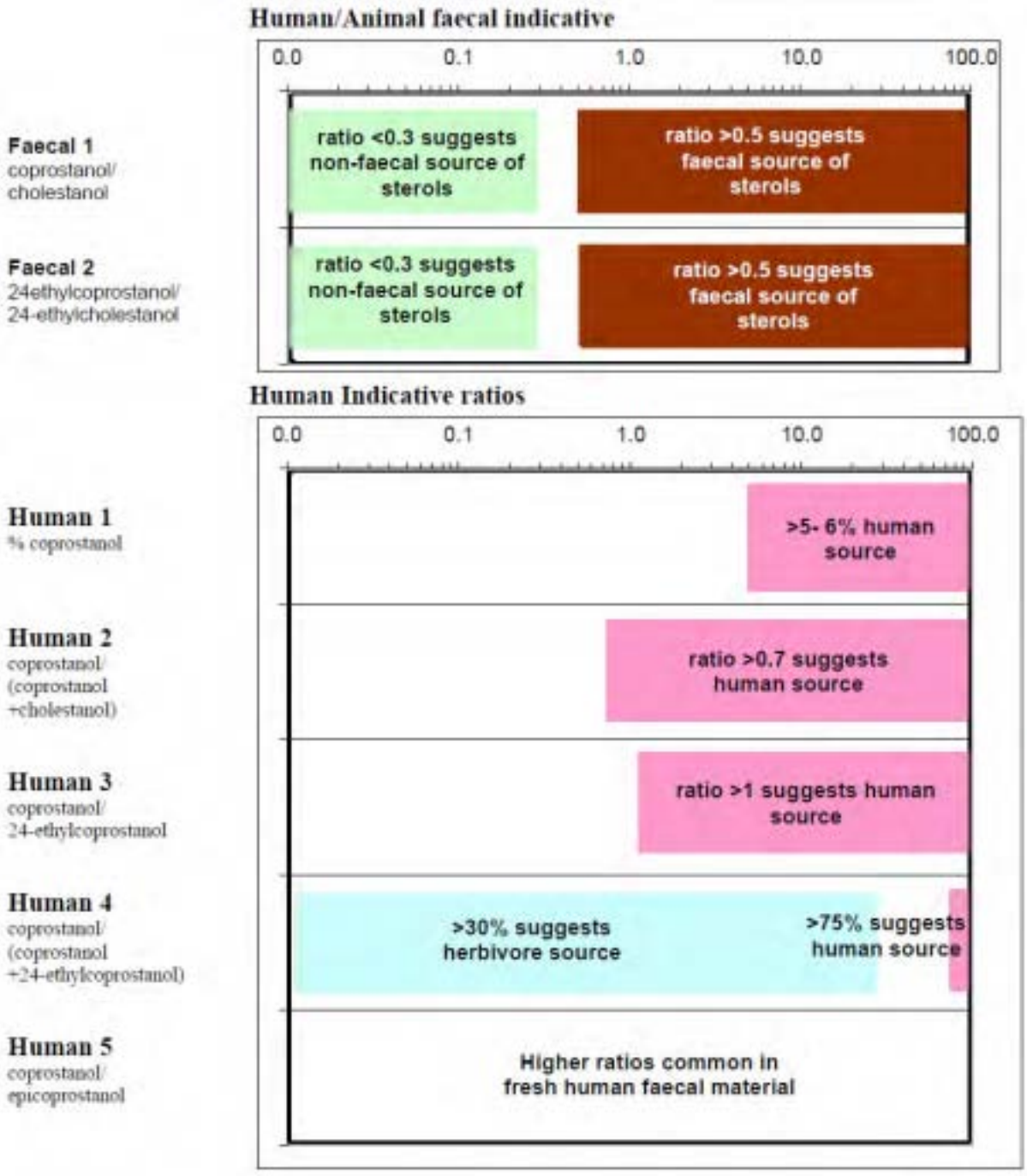
Collection Date	Time	River Flow	Stage Level (mm)	Rainfall (24hrs) / mm	*E. coli* /100ml	FWAs	Sterols			PCR			Conclusions	
							Hum	Herb	Plant	Hum	Duck	Dog		
17/3/2009	10:50	**Low**	**1448**	**0**	**540**	×	×	✓	✓	×	✓	✓	Not human	Dog, Duck
24/3/2009	10:40	**Low**	**1449**	**0**	**300**	✓	×	✓	✓	×	✓	✓	Not human	Dog, Duck
31/3/2009	10:52	**Low**	**1449**	**0**	**520**	✓	×	×	✓	×	✓	✓	Not human	Dog, Duck
7/4/2009	10:30	Low	1425	0	180									
9/04/09	11:08	**High**	**1721**	**16.6**	**2400**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
21/4/2009	13:32	**Low**	**1429**	**0**	**360**	×	×	×	✓	×	×	×	Not human	
28/4/2009	11:12	**Low**	**1415**	**0.2**	**350**	×	×	×	✓	×	✓	×	Not human	Duck
6/05/09	14:21	**High**	**1801**	**24.0**	**1200**	×	×	×	✓	×	×	✓	Not human	Dog.
8/05/09	11:51	High	1553	3.6	710	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
11/05/09	12:27	**High**	**1826**	**32.8**	**1100**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
20/05/09	11:08	**High**	**1862**	**24.2**	**1200**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
21/05/09	14:34	**High**	**1650**	**13.2**	**890**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck

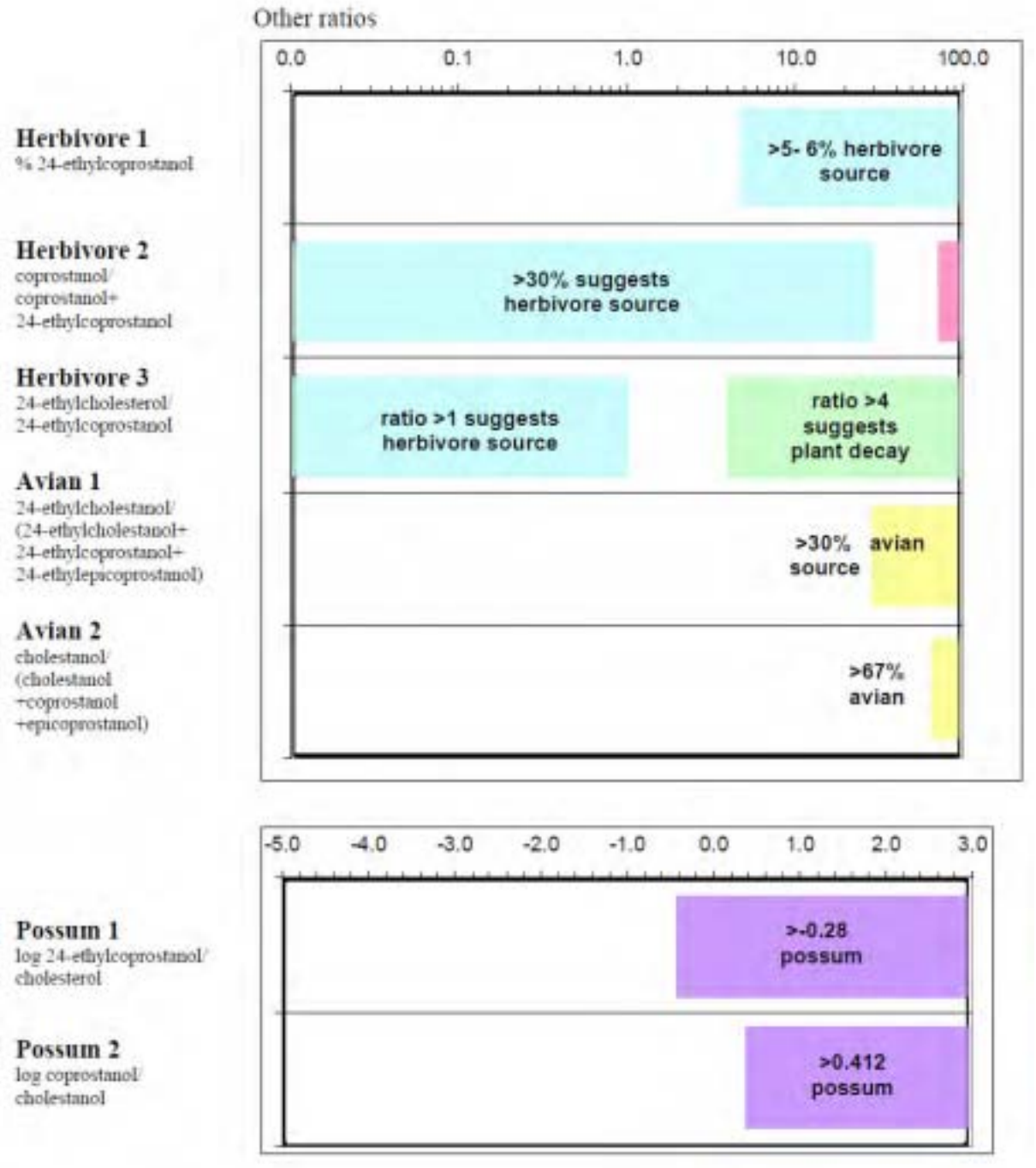
Table 3. Results summary for at Kerrs Reach

Collection Date	Time	River Flow	Stage Level (mm)	Rainfall (24hrs) / mm	*E. coli* /100ml	FWAs	Sterols			*PCR*			Conclusions	
							Hum	*Herb*	*Plant*	*Hum*	*Duck*	*Dog*		
17/03/09	10:15	low	1448	0	200									
24/03/2009	10:15	low	1449	0	160									
31/03/2009	10:22	low	1449	0	230									
7/04/2009	9:55	low	1425	0	230									
9/04/2009	10:34	**high**	**1721**	**16.6**	**3100**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
21/04/2009	13:01	low	1429	0	41									
28/04/2009	10:50	**low**	**1415**	**0.2**	**180**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
5/05/2009	11:27	**low**	**1539**	**0**	**94**	×	×	×	✓	×	✓	×	Not human	
6/05/2009	14:00	**high**	**1801**	**24.0**	**3600**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
8/05/2009	11:17	**high**	**1553**	**3.6**	**270**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
11/05/2009	12:01	**high**	**1826**	**32.8**	**2100**	×	✓	×	✓	✓	✓	✓	Human	Dog, Duck
12/05/2009	11:00	**low**	**1444**	**0**	**380**	×	✓	×	✓	✓	✓	✓	Human	Dog, Duck
13/05/2009	9:15	**low**	**1427**	**0**	**130**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
14/05/2009	10:34	low	1422	0	75									
19/05/2009	10:43	**low**	**1461***	**0**	**210**	×	×	×	✓	×	✓	✓	Not human	Dog, Duck
20/05/2009	10:44	**high**	**1862**	**24.2**	**2600**	×	(✓)	×	✓	✓	✓	✓	Some human	Dog, Duck
21/05/2009	14:10	**high**	**1649**	**13.2**	**880**	×	(✓)	×	✓	×	✓	✓	Some human	Dog, Duck

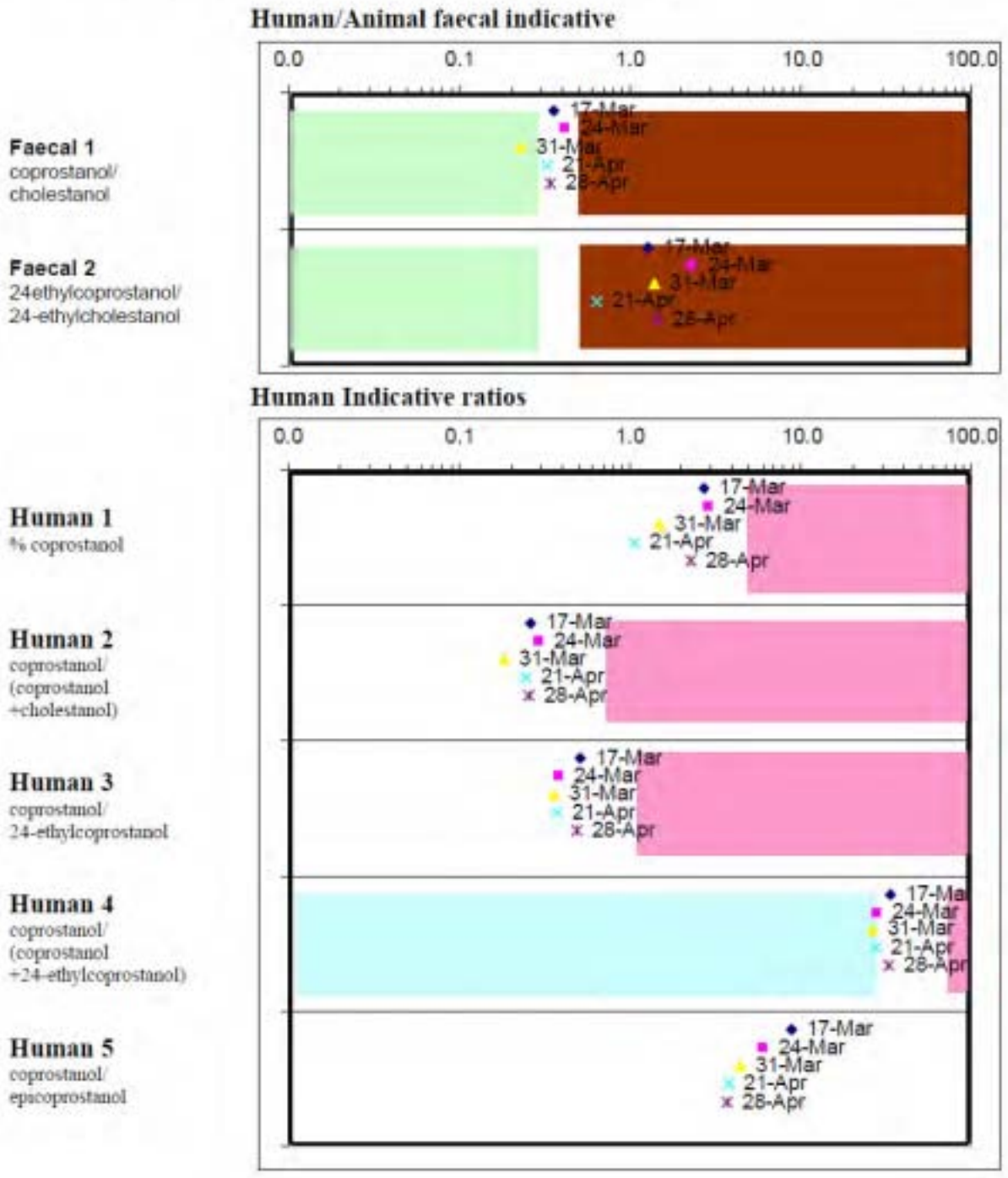
Appendix 2 Faecal sterol analysis

Sterols analysis guidelines

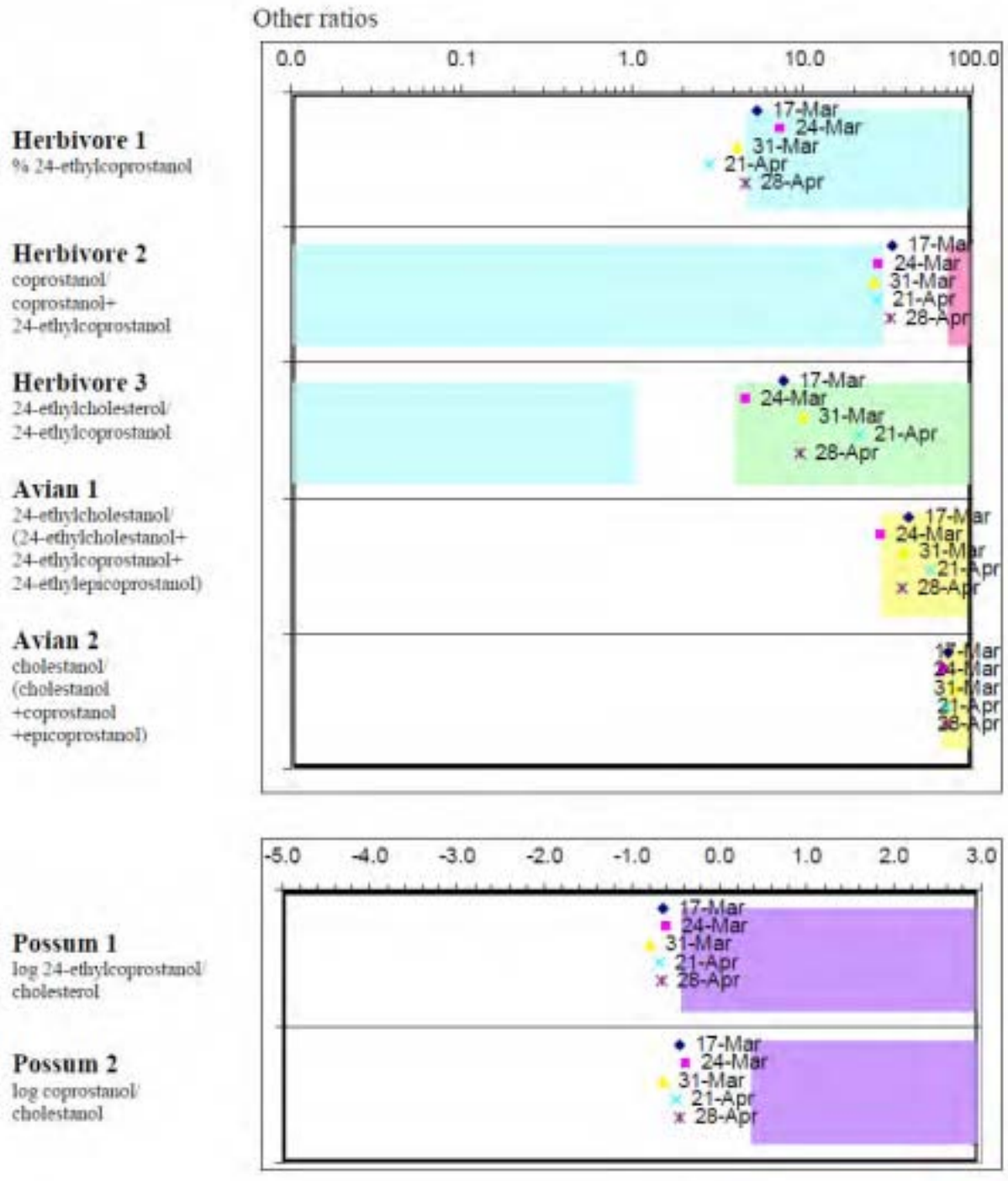



Other ratios
0.0
0.1
1.0
10.0
100.0
Herbivore 1
% 24-ethylcoprostanol
>5- 6% herbivore source
Herbivore 2
coprostanol/
coprostanol+
24-ethylcoprostanol
>30% suggests herbivore source
Herbivore 3
24-ethylcholesterol/
24-ethylcoprostanol
ratio >1 suggests herbivore source
ratio >4 suggests plant decay
Avian 1
24-ethylcholestanol/
(24-ethylcholestanol+
24-ethylcoprostanol+
24-ethylepicoprostanol)
>30% avian source
Avian 2
cholestanol/
(cholestanol
+coprostanol
+epicoprostanol)
>67% avian
-5.0
-4.0
-3.0
-2.0
-1.0
0.0
1.0
2.0
3.0
Possum 1
log 24-ethylcoprostanol/
cholesterol
>-0.28 possum
Possum 2
log coprostanol/
cholestanol
>0.412 possum

Sterols – Antigua Low flow

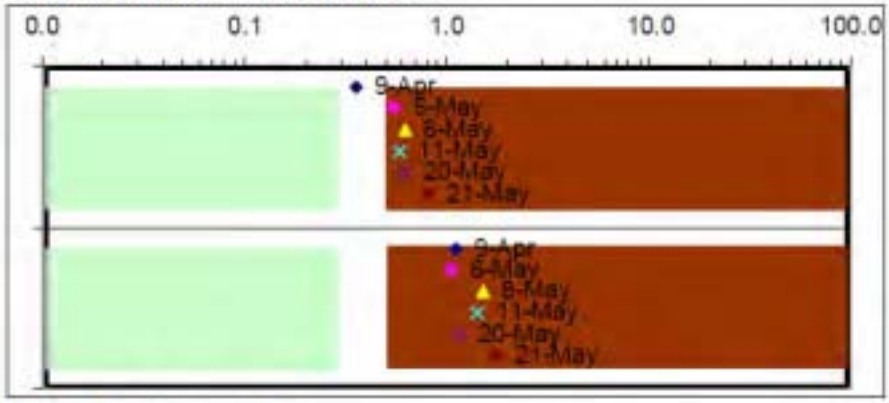


Sterols – Antigua Low flow



Sterols – Antigua High flow

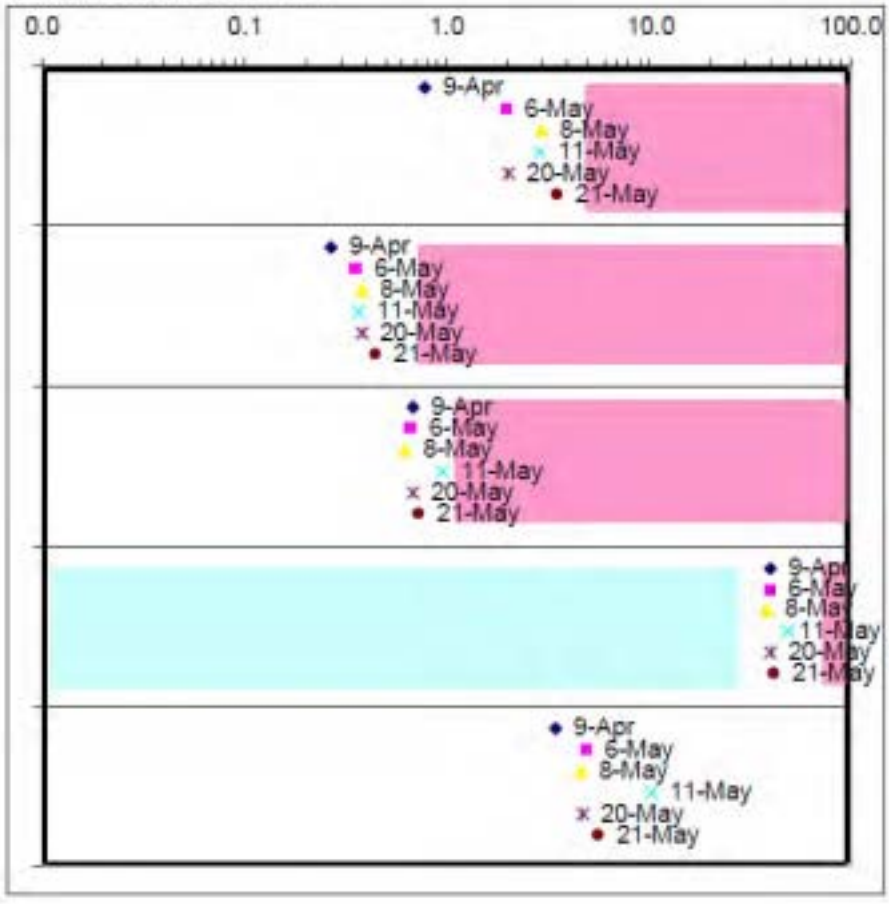
Human/Animal faecal indicative



Faecal 1
coprostanol/
cholestanol

Faecal 2
24ethylcoprostanol/
24-ethylcholestanol

Human Indicative ratios



Human 1
% coprostanol

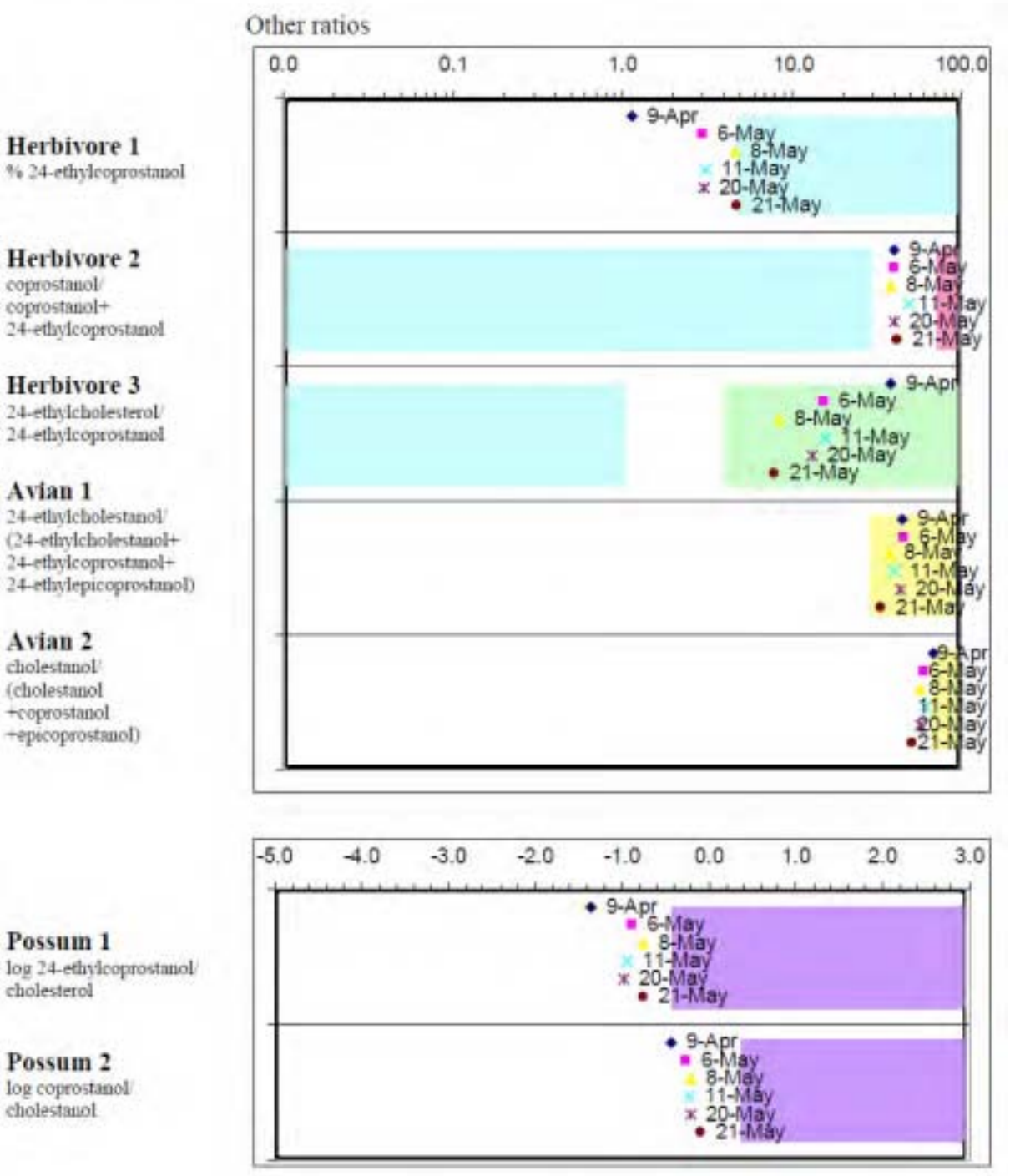
Human 2
coprostanol/
(coprostanol
+cholestanol)

Human 3
coprostanol/
24-ethylcoprostanol

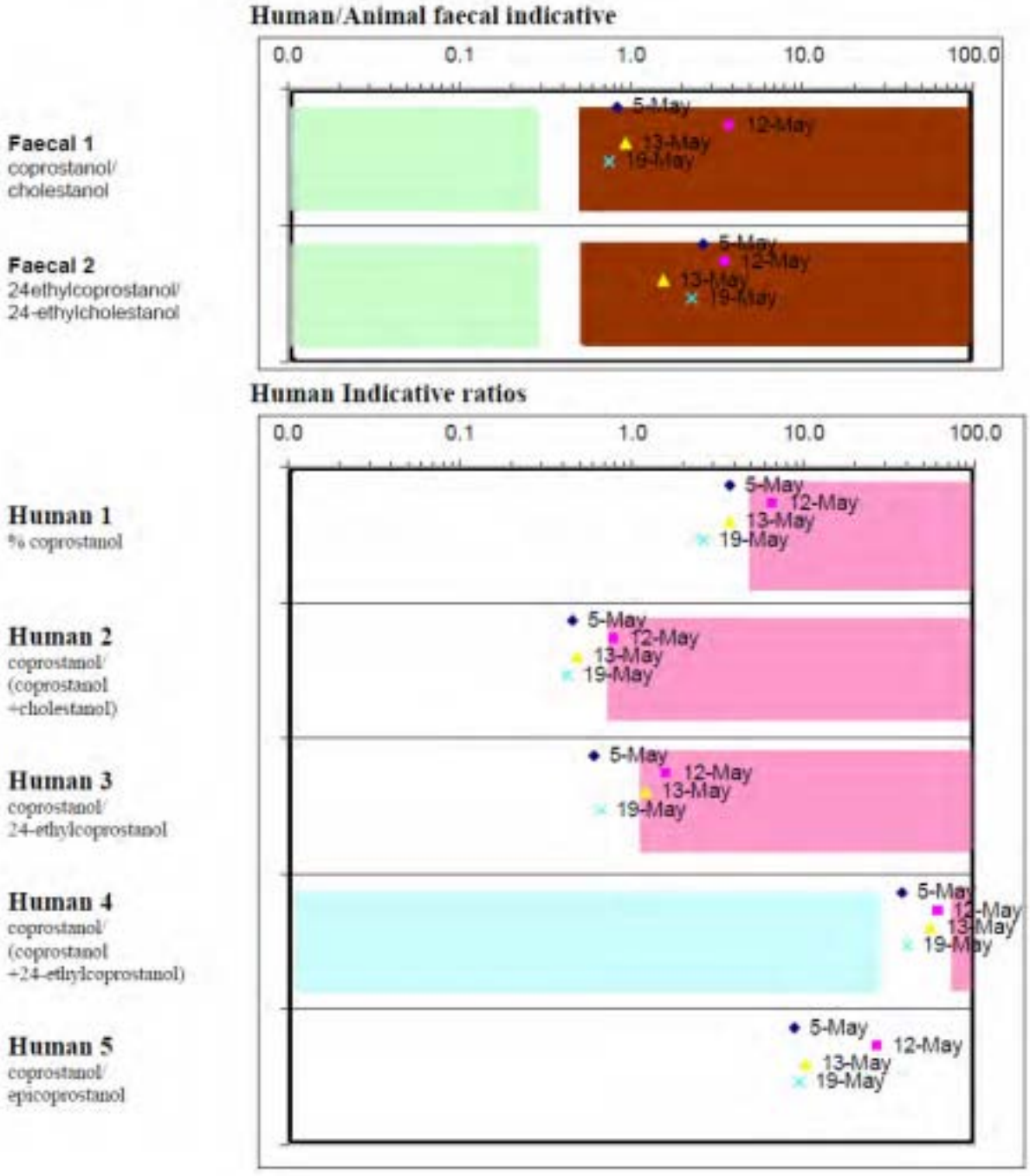
Human 4
coprostanol/
(coprostanol
+24-ethylcoprostanol)

Human 5
coprostanol/
epicoprostanol

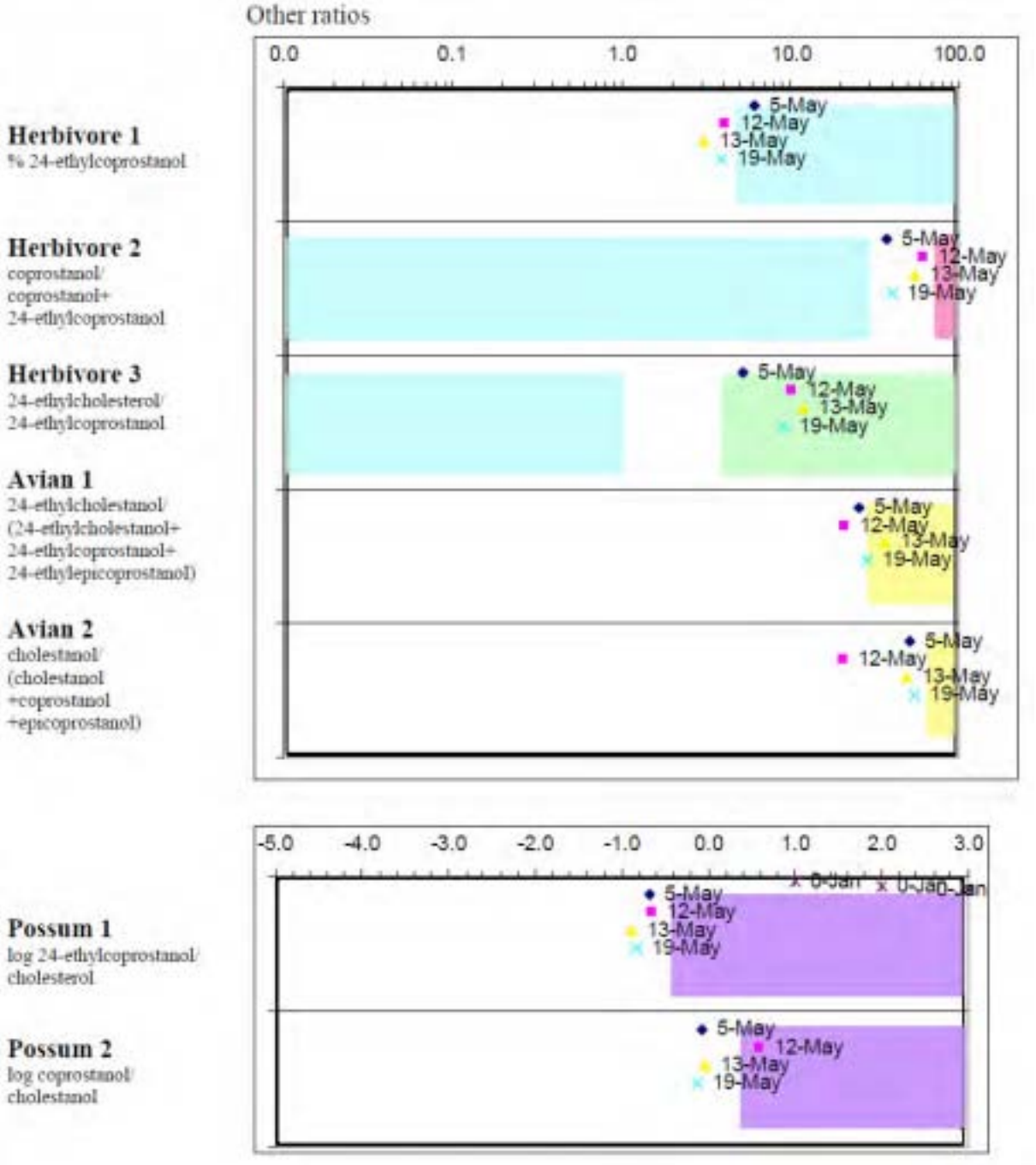
Sterols – Antigua High flow



Sterols – Kerrs Reach Low flow

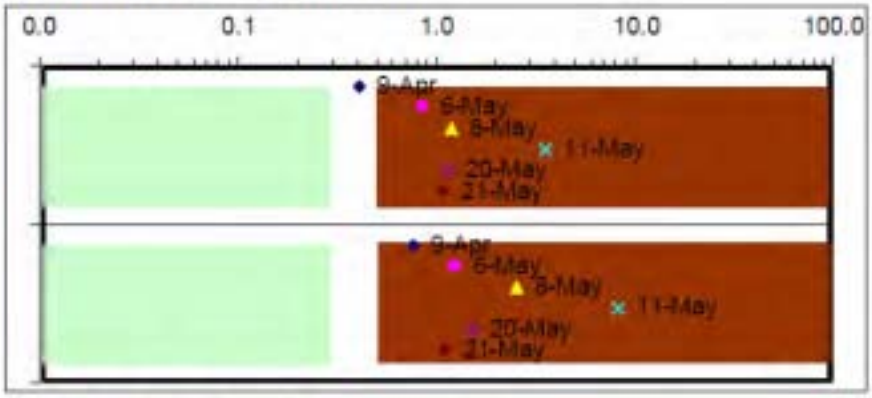


Sterols – Kerrs Reach Low flow



Sterols –Kerrs Reach High flow

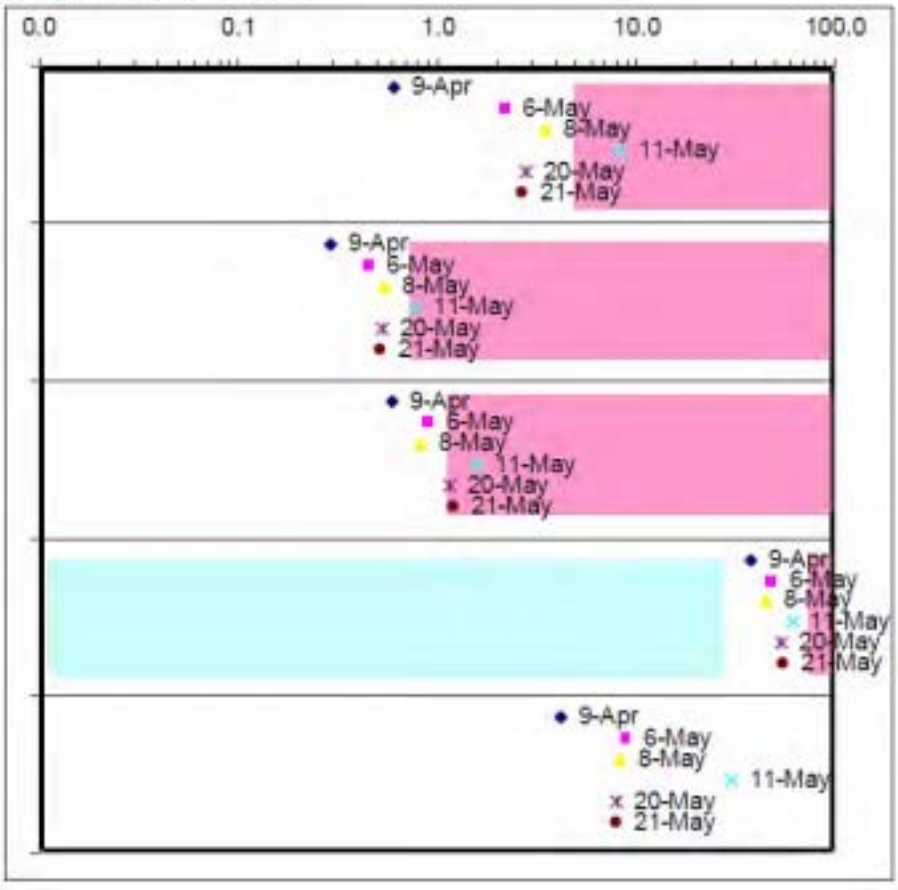
Human/Animal faecal indicative



Faecal 1
coprostanol/
cholestanol

Faecal 2
24ethylcoprostanol/
24-ethylcholestanol

Human Indicative ratios



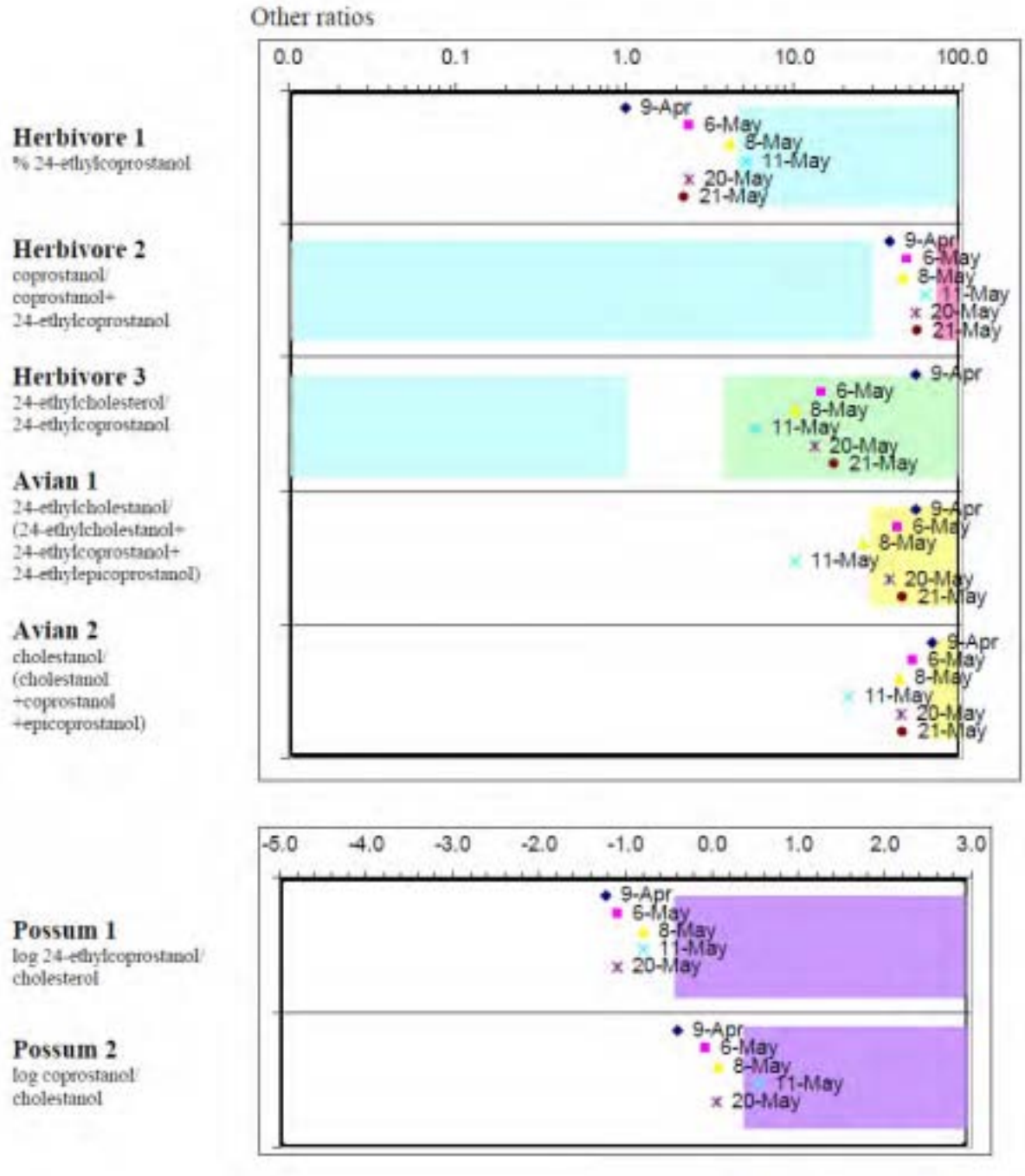
Human 1
% coprostanol

Human 2
coprostanol/
(coprostanol
+cholestanol)

Human 3
coprostanol/
24-ethylcoprostanol

Human 4
coprostanol/
(coprostanol
+24-ethylcoprostanol)

Human 5
coprostanol/
epicoprostanol


Other ratios
0.0
0.1
1.0
10.0
100.0
Herbivore 1
% 24-ethylcoprostanol
Herbivore 2
coprostanol/
coprostanol+
24-ethylcoprostanol
Herbivore 3
24-ethylcholesterol/
24-ethylcoprostanol
Avian 1
24-ethylcholestanol/
(24-ethylcholestanol+
24-ethylcoprostanol+
24-ethylepicoprostanol)
Avian 2
cholestanol/
(cholestanol
+coprostanol
+epicoprostanol)
9-Apr
6-May
8-May
11-May
20-May
21-May
-5.0
-4.0
-3.0
-2.0
-1.0
0.0
1.0
2.0
3.0
Possum 1
log 24-ethylcoprostanol/
cholesterol
Possum 2
log coprostanol/
cholestanol

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