Faecal Sources in the Avon River/Ōtakaro, Heathcote River/Ōpāwaho and the Estuary of the Heathcote & Avon Rivers/Ihutai 2015

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EXECUTIVE SUMMARY

In this study, six water samples were collected from nine sites between 16th April 2015 and 11th September 2015. There were three sites in the Avon River (The Antigua Boatsheds, Kerrs Reach and Owles Terrace), four sites in the Heathcote River (Ferniehurst Street, Bowenvale Avenue, Waltham Park and Catherine Street), and two sites in the Estuary of the Heathcote and Avon Rivers/Ihutai (Humphreys Drive, South New Brighton Domain). For each of these sites, three samples were collected during base flow conditions, and three were collected following rainfall events of >10mm rain in the previous 24 hours, with elevated flow conditions. Samples were taken after rainfall, as (a) many contaminants are entrained in stormwater (e.g. bird faeces from roads and roofs) which is then discharged into the rivers and (b) wastewater overflows often occur during wet weather, when the capacity of the sewer system is reduced by stormwater infiltration.

E. coli levels in the water samples were typically elevated, exceeding recreational water guideline values on a number of occasions during base flow, and after rainfall almost all samples exceeded recreational water guideline values.

Campylobacter were found in all but one of the river water samples taken, and at concentrations of up to 240 MPN (most probable number) per 100 ml during base flow and up to 460 MPN per 100 ml following rainfall. Speciation and genotyping of *Campylobacter* isolates suggested that base flow isolates were consistent with a wildfowl source. Following rainfall, wildfowl genotypes were still present, but supplemented by isolates more likely to come from ruminant or poultry sources. As *Campylobacter* isolates from ruminant and poultry sources are frequently found among human clinical cases, based just on *Campylobacter* genotyping, these isolates could also be from human sewage.

Additional faecal source tracking analysis was undertaken using molecular markers and faecal sterols. These supported wildfowl as the dominant faecal source during base flow with the highest levels observed at the Antigua boatsheds. At Kerrs Reach and Catherine Street, human sources were detected on occasion during base flow conditions.

Following rainfall, human sources were detected at much higher frequency, with the strongest human signals in the Waltham and Antigua sites after rainfall. Canine sources are also primarily detected following rainfall events. Ruminant sources were detected in the Heathcote River samples following rainfall, with both sheep and cow markers identified.

AC/TC faecal ageing ratio analysis suggested fresh sources of faeces present in almost all samples, and therefore, did not support aged pollution.

Analysis of sediment samples indicated relatively low concentrations of *E. coli* in most samples, and *Campylobacter* were detected only once.

Comparison of the faecal source tracking results with previous studies suggests that in the Avon River, the situation has now returned to a similar situation to that prior to the earthquakes with wildfowl the dominant source during base flow, and the input of canine and some sources during rainfall events.

Table 1 Summary of faecal source tracking

Area/River	Location	Base flow	Rainfall
Avon	Antigua Boatsheds	Wildfowl dominant source	Wildfowl, human, canine sources
	Kerrs Reach	Human and wildfowl sources	Human, wildfowl, canine sources
	Owles Tce	Wildfowl dominant sources	Wildfowl, human, canine sources
Heathcote	Ferniehurst St	Wildfowl, canine sources	Human, ruminant (both cow and sheep), wildfowl, and canine sources
	Bowenvale Ave	Wildfowl dominant sources	Human, ruminant (both cow and sheep), wildfowl, and canine sources
	Waltham Park	Wildfowl dominant sources	Human, ruminant (both cow and sheep), wildfowl, and canine sources
	Catherine St	Human and wildfowl sources	Human, ruminant (both cow and sheep), wildfowl, and canine sources
Estuary	South New Brighton Park	Wildfowl dominant sources	Wildfowl dominant sources
	Humphreys Drive	Wildfowl dominant sources	Human, wildfowl, canine sources

1. INTRODUCTION

The microbial water quality of the ocean, estuary and rivers in Christchurch is of high interest due to the potential health risk from diseases spread via the faecal oral route. In particular, areas frequently used for recreational activity have a heightened health risk, due to the possibility of accidental immersion and consumption of water.

Microbial quality is monitored by measuring levels of indicator bacteria such as *Escherichia coli*. The *New Zealand Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas* (MfE and MoH, 2003) specify in single samples, that levels of *E. coli* below 260 are acceptable, while single sample levels above 550 cfu/100 mL should result in warnings to the public, and actions to identify sources of contamination. Ongoing monitoring of the Avon River/Ōtakaro, Heathcote River/Ōpāwaho and the Estuary of the Heathcote & Avon Rivers/Ihutai has identified over a number of years, that many samples taken exceed this level (Margetts & Marshall, 2015).

Prior to and post the Canterbury Earthquakes (2009-present day) ESR has undertaken studies to identify the sources of faecal pollution in river water and sediment and to quantify the zoonotic pathogens present. These previous studies have concentrated on the Avon River and Estuary.

A brief summary of the findings of previous reports are included below. Each study varied slightly in terms of the sites examined and the organisms looked for. Different faecal source tracking (FST) tools were used in each study, depending on the issues at the time and the budget of the project.

1.1 STUDY 1: AVON RIVER JULY 2009

Previous monthly monitoring of two sites on the Avon River identified, that levels of the water quality indicator *Escherichia coli* regularly exceed the MfE/MoH (2003) 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* concentrations.

FST 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 boatsheds

on Antigua Street (12 samples), and from the boat ramp at Kerrs Reach (17 samples). No recognised sewage overflows occurred during this period**.**

The key findings of this study were:

- During low flow events, wildfowl appeared to be the source of *E. coli.*
- Rainfall resulted in a significant degradation of the microbial water quality of the Avon River, with the primary sources of this degradation related to wildfowl and possibly dog faecal material.
- Human markers were detected from high flow events at Kerrs Reach, but at relatively low levels compared to the number of *E. coli* detected. This suggested either a distant source of these human markers, or an aged source of these human markers.

1.2 STUDY 2: AVON RIVER AND ESTUARY DURING AND POST-EARTHQUAKE, FEBRUARY 2012

A study was undertaken at three sites along the Avon River (Antigua Boatsheds, Kerrs Reach, Owles Terrace), and at two sites in the Estuary (South New Brighton Park and Humphreys Drive), with collection of river water and sediment. The samples were evaluated for a range of pathogens, indicators and FST markers. At this time large volumes of wastewater was being discharged directly into the Avon River and the Estuary.

The key findings of this study were:

- After a major faecal contamination event, measurement of *E. coli* in the waterway is a suitable indicator for establishing a public health risk. In this study, *E. coli* levels in water above 550 cfu/100 mL were correlated with an increased likelihood of detection of potential pathogens including *Campylobacter*, *Giardia* and *Cryptosporidium*.
- Overall, levels of pathogens were lower than may have been expected due to the dilution of the sewage and microorganisms present by high levels of groundwater infiltration. There was no increase in community levels of infection, which together with the groundwater infiltration resulted in lower levels of pathogens in the sewage entering the Avon River. The health risks were, therefore, less than may have been expected.
- All the microorganisms tested for in this study could be recovered from sediments. The indicator bacteria *Clostridium* accumulated in the sediments, and there is evidence to support the low-level persistence of *Cryptosporidium, Giardia* and viruses in sediments after cessation of sewage discharges. Bacteriophage and *Campylobacter* did not appear to accumulate in Avon River sediments. Faecal sterols and FWAs did accumulate in the sediments.

- In the event of disturbances of the sediment, it is highly probable that there could be re-mobilisation of microorganisms, including pathogens, into the water column. Chemical contaminants in the sediment may also be re-mobilised. Re-suspension events, therefore, increase the potential risk to human health for those who participate in recreational and work-related activities in the river and estuary environment.

1.3 STUDY 3: AVON RIVER AND ESTUARY POST- EARTHQUAKE FEBRUARY 2013

A follow-up study was undertaken at the same sites as Study 2, again with collection of river water and sediment. The samples were evaluated for a range of pathogens, indicators and FST markers. At this time wastewater was not being discharged directly into the Avon River and the Estuary.

The key findings of this study were:

- The Antigua Boatshed's site had similar water quality to that seen during active sewage discharge into the Avon River. FST tools indicated the likely sources of faecal pollution at the boatsheds to be wildfowl, with intermittent human pollution.
- The water quality at Kerrs Reach was also polluted with the average *E. coli* concentrations close to those seen during active sewage discharge (2011). FST results indicated fresh human inputs as well as wildfowl faeces.
- Owles Terrace had the greatest improvement in water quality when the levels of *E. coli* were compared with Study 2 results. FST analysis suggested the source of *E. coli* as wildfowl.
- The results from this study showed a marked decrease in *Giardia* concentrations in sediments, which indicates the long term quality of the water is improving.
- All sites, except Kerrs Reach, had a decrease in average concentration of all microorganisms tested for in the sediment.
- The two estuarine sediment sites showed a significant decrease in indicator microbial concentrations compared with Study 2. This indicates that there is no significant health risk related to contact with the estuarine sediments based on these results.
- Recreational contact with the Avon River water and sediments, particularly at Kerrs Reach, may continue to pose health risks, and the public should continue to minimise ingestion of the water.

1.4 STUDY 4: THIS REPORT – AVON AND HEATHCOTE RIVERS, CHRISTCHURCH ESTUARY MAY-SEPT 2015

Since the 2013 study was carried out there has been no significant earthquakes in the Canterbury region. Therefore, there has been no unintentional or intentional large discharges of wastewater into the river network. There are, however, considerable ongoing repairs to the wastewater network, which may require activities such as over-pumping and dewatering to occur.

In this current study the microbial quality of the Avon and Heathcote Rivers as well as the Estuary were examined under base flow and also following heavy rainfall. The aim was to assess the suitability of the waterways for recreational activity and to identify the faecal pollution sources and where these are occurring.

The microbial indicators and pathogens studied were *E. coli*, enterococci, *Campylobacter* spp. as well as FST to determine the source of the faecal pollution. The FST tools employed were PCR Markers and Faecal Sterols. In addition to the enumeration of *Campylobacter* present in the samples, up to six isolates from each sample were genotyped to determine the likely source of the pathogen.

2. METHODOLOGY

2.1 SAMPLING LOCATIONS, CONDITIONS AND METHODOLOGY

For the Avon River the sampling sites were the same as in Studies 2 and 3: The Antigua Boatsheds, Kerrs Reach and Owles Terrace (Figure 1). Two Estuary sites were sampled in this study, one of which was used previously (Humphreys Drive), while the other site, South New Brighton Domain was new to the study. This new site is used as a regular monitoring site by Environment Canterbury.

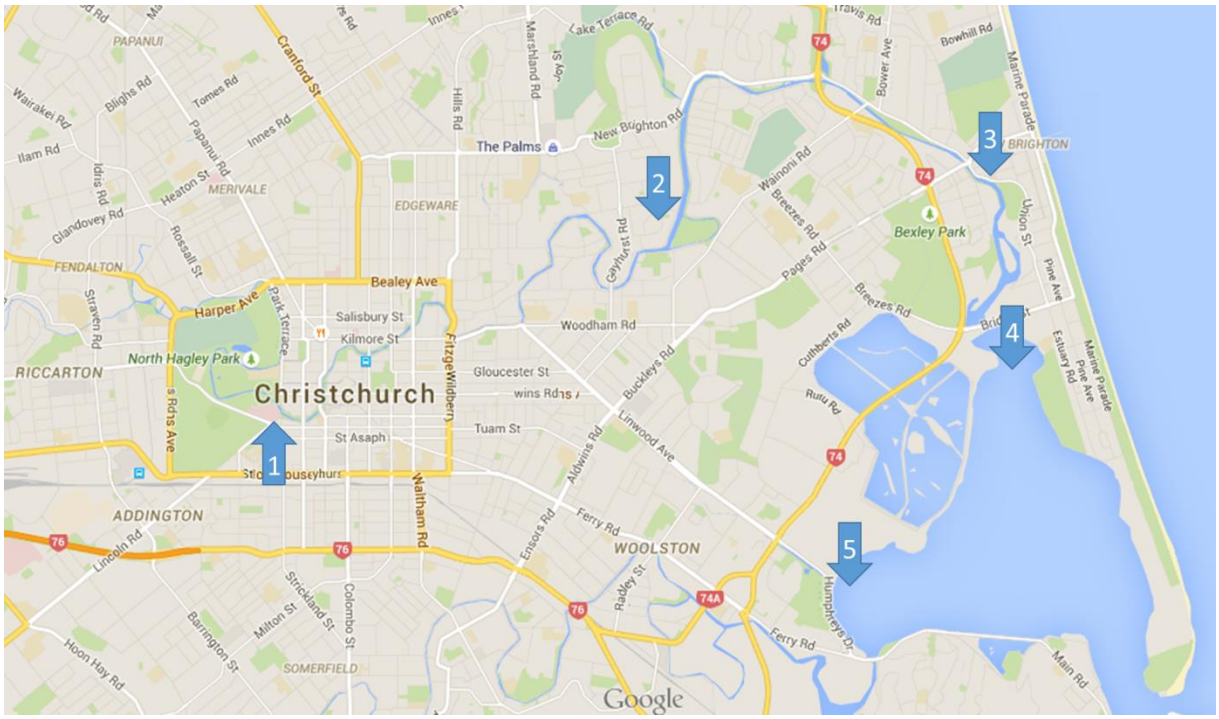


Figure 1. Sampling sites on the Avon River and in the Estuary. (1) The Antigua Boatsheds, (2) Kerrs Reach, (3) Owles Terrace, (4) Humphreys Drive, (5) South New Brighton Domain

For the first time, sites on the Heathcote River were also tested. These sites were Ferniehurst Street, Bowenvale Avenue, Waltham Park and Catherine Street (Figure 2).

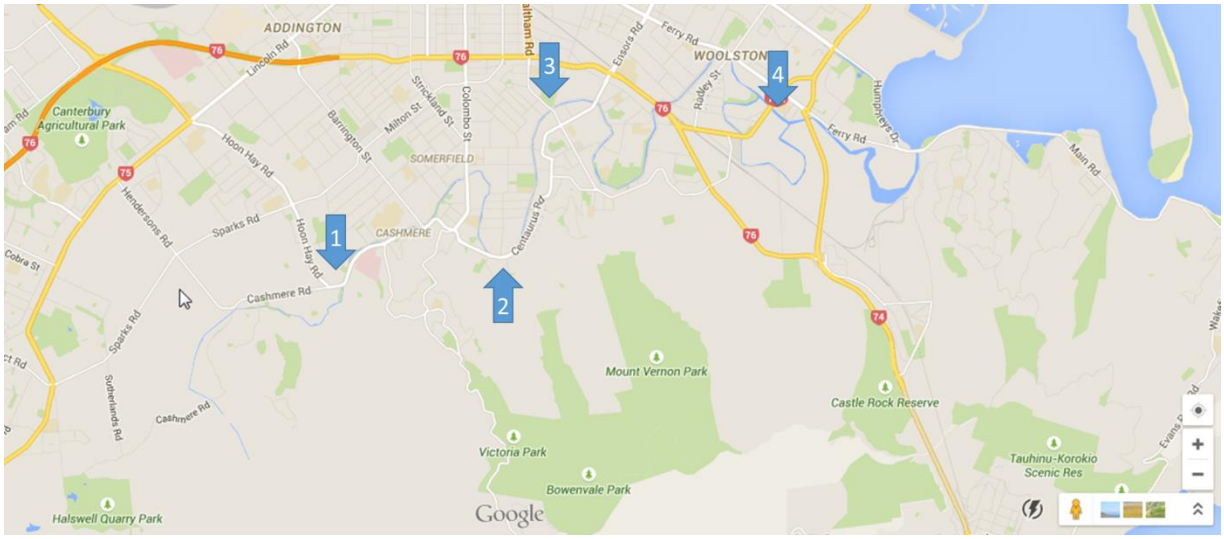


Figure 2 Sampling sites on the Heathcote River. (1) Ferniehurst Street, (2) Bowenvale Avenue, (3) Waltham Park and (4) Catherine Street.

Two distinct sample types were taken - base flow and wet weather. The base flow samples were taken in the absence of rainfall in the previous 72 hours and were collected 1 hour either side of low tide as measured in Lyttelton.

Wet weather sampling took place following 10 mm of rainfall as measured at Christchurch Airport. On the final sampling occasion only 7 mm of rainfall had occurred but it was deemed suitable due to the lack of rainfall in the preceding weeks. Samples were taken after rainfall, as (a) many contaminants are entrained in stormwater (e.g. bird faeces from roads and roofs) which is then discharged into the rivers and (b) wastewater overflows often occur during wet weather, when the capacity of the sewer system is reduced by stormwater infiltration.

During base flow, water and sediment samples were collected from the river sites. Following wet weather events only water samples were collected from the river. On all occasions only water samples were collected from the Estuary sites.

For water samples, a bucket was dropped into the waterway, rinsed once and then a water sample was collected and dispensed into appropriate containers. For sediment samples a Mighty Gripper was used. An empty plastic container was placed within the metal holder and dragged along the top 1 cm of the sediment. The jar was removed and excess water removed prior to transportation to the laboratory.

2.2 EVALUATION OF WATER QUALITY

There are a number of methods available that can be used to evaluate water quality and identify the possible sources of faecal pollution. In this study, water quality was evaluated by enumerating the levels of *E. coli*, enterococci and *Campylobacter*. To identify sources of faecal pollution, three main tools were used. PCR markers were used to evaluate whether the water contained human, herbivore, canine or wildfowl sources. Faecal sterol analysis was undertaken to identify human, herbivore, wildfowl or plant sources. Finally, in samples where *Campylobacter* were isolated, analysis of these was undertaken using MBiT analysis. All microbial methods with the exception of the *Campylobacter* typing method (MBiT) have been previously used in studies on the Avon River (Devane *et al*. 2014 and Devane *et al.* in preparation). A brief summary of methodology is included below with greater detail on the new MBiT analysis method.

2.2.1 Enumeration of microorganisms.

E. coli and enterococci were enumerated using Brilliance *E. coli* Coliform Selective Medium and Chromocult Enterococci Agar (Merck) respectively. Volumes of water between 1 ml and 0.001 ml were analysed for each sample in duplicate and incubated and enumerated according to the manufacturer's instructions.

Campylobacter were enumerated using a 4 x 3 most probable number (MPN) enrichment. Triplicate samples of volumes 100, 10, 1 and 0.1 ml were filtered using 0.45 µM filters with incubation in Exeter broth for 48 hours at 42°C. A loopful of each enrichment broth was then streaked onto Exeter Agar, and incubated for a further 48 hours. Colonies typical of *Campylobacter* were then streaked onto Blood Agar and incubated for a further 48 hours. Well isolated colonies were analysed by PCR to identify *C. jejuni, C. coli* or other thermotolerant *Campylobacter* (Cornelius *et al*. 2014*)*.

2.2.2 Faecal ageing ratio of atypical coliforms to total coliforms (AC/TC)

The atypical coliforms to total coliforms (AC/TC) ratio compares the background microflora found in the river (identified as atypical colonies (AC) on m-endo agar), with the high numbers of total coliforms (TC) identified in fresh faecal runoff/discharge into the river (Brion 2005). Fresh faecal material in the water, therefore, generates a low AC/TC ratio (Table 6). After discharge into water, the total coliforms progressively die-off and their numbers are lower in comparison to the river microflora suggesting an aged faecal event with a correspondingly higher AC/TC ratio.

Duplicate samples of appropriate volumes (1-100 mL) of Avon River water were filtered through 47 mm diameter, 0.45 µm cellulose ester membrane filters (Millipore) and placed onto modified (m-Endo agar (Fort Richard Laboratories). Ten-fold dilutions of each sample

were prepared in 0.1% peptone water as appropriate (n = 4). Plates were incubated at 35ºC (±1ºC) for 22 (± 2) hours. After incubation, atypical colonies (AC) were enumerated by counting pink/red colonies and total coliforms were enumerated by counting colonies with a green metallic sheen. The AC/TC ratio was calculated by dividing AC (Colony forming units (cfu)/100 mL) counts by TC (cfu/100 mL) counts.

2.2.3 Multiplex ligation-dependent probe amplification-binary typing (MBiT) subtyping of *Campylobacter jejuni* and *Campylobacter coli*.

Campylobacter species, notably *Campylobacter jejuni* and *Campylobacter coli*, are the most commonly reported bacterial causes of human gastroenteritis in New Zealand with over 6,000 notified cases each year resulting in a rate of >150 cases per 100,000 of population. *Campylobacter* are found in a range of animal reservoirs including poultry, cows, sheep, deer, and wildfowl and are readily recoverable from environmental water samples in New Zealand. Speciation of *Campylobacter* is important, as the isolation methods will recover not only *C. jejuni* and *C. coli*, but also other thermotolerant *Campylobacter* including *C. lari*. These other species have been reported to cause disease, but are not commonly reported among notified cases.

While speciation is important, so is subtyping as it is recognised that there is a large genetic diversity among *Campylobacter*. Methods such as multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) have been widely used, and are able to differentiate a large number of genotypes of *Campylobacter*. They are also used to attribute sources. Neither of these techniques is particularly rapid, with analysis times of several days at a minimum. They are also relatively expensive which limits their application to large numbers of isolates.

ESR has developed a multiplex ligation-dependent probe amplification-binary typing (MBiT) assay for subtyping *Campylobacter jejuni* and *Campylobacter coli*. This assay targets 18 pathogenicity- or survival-associated genes, and, using a multiplex ligation-dependent probe amplification (MLPA) format, allows analysis of an isolate in a single reaction (Cornelius *et al*. 2014). MBiT requires isolation of a colony of *Campylobacter*. A simple heat-lysis preparation can be used to release DNA from the bacterial cells, followed by MLPA detection of the gene targets via a process including hybridisation, ligation, and PCR. The result of the analysis is a profile for each isolate with the presence or absence of each gene target resulting in a six-digit number. This six digit nomenclature is then used to describe each pattern (Figure 3).

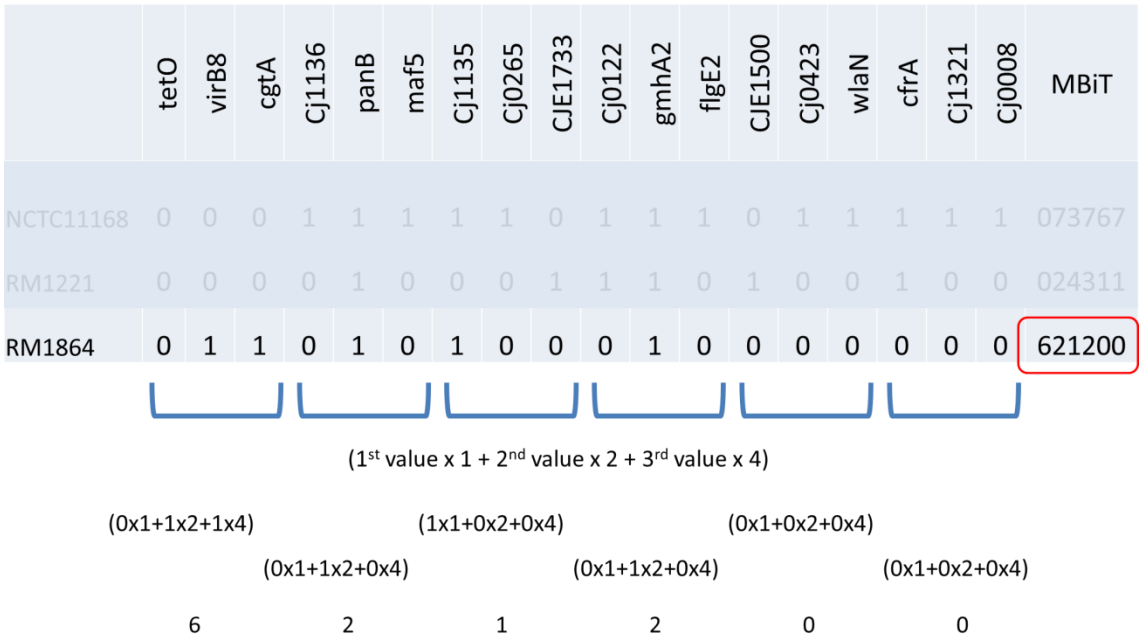


Figure 3. Example of MBiT pattern naming

Isolates with the same pattern of gene targets are described as indistinguishable. It is then possible to use the pattern of gene products to produce phylogenetic comparisons of isolates.

Source attribution is possible on the basis that *Campylobacter* from different faecal sources tend to cluster separately from one another. There is of course some overlap, and some genotypes may cluster separately from isolates from known sources. The effectiveness of the attribution depends on the size of the source library of known isolates, which should ideally have temporal and spatial overlap with isolates of interest.

Details of the MBiT methodology are described in greater detail in Cornelius *et al*. (2014). Briefly, isolates for MBiT analysis were purified to obtain single colonies and then one colony resuspended in 250 µl of 2% Chelex buffer. The tube was heated for 5 min at 98°C to denature the DNA, cooled and then the MLPA reaction was performed as described in Cornelius *et al.* (2014). Subsequently, the sample was diluted 1:10, a LIZ500 size standard added, and products separated by capillary electrophoresis on an ABI 3700. Analysis of electropherograms, subsequent band assignment, cluster analysis and burst diagram production was performed using BioNumerics v7.5 (Applied Maths).

In terms of peak detection, we used thresholds of 5% of the OD range and 5% of the curve range with correction for peak intensity profile. Filtering by relative peak height was also performed using a minimum relative height of 15% and a maximum distance of

30%. Bands were then assigned to 18 band classes, using a position tolerance of 0.75%. Manual adjustment of bands was made as necessary.

For cluster analysis, categorical value similarity matrix with UPGMA cluster analysis was applied. Burst diagrams were created using minimum spanning tree analysis for categorical data. The size of each circle in a burst diagram represents the number of isolates with that MBiT profile. Branches in burst diagrams are thick bold if only one locus was different, a thinner solid line represents two or three differences in loci, a dashed line is used, if there are four differences, and a dotted line if more than four differences were observed.

Up to six isolates from each water sample were analysed and assigned to a source cluster by comparison with isolates from known sources. The ability of the MBiT assays to determine the source of a pathogen depends on the quality of the source library which you compare isolates with, and intrinsic separation of isolates into different genotypes based on that source.

The analysis in this project was based on a source library of:

- 952 human isolates
- 377 poultry isolates
- 344 ruminant isolates (cows and sheep)
- 156 wildfowl
- 65 deer
- 20 pig

As shown in Figure 4, depending on the genotypes, separation of isolates according to source does occur, although there are overlaps in some genotypes. Notably, wildfowl isolates tend to separate from some ruminant genotypes and from some poultry isolates. The water isolates from this project (blue in the figure), do tend to cluster primarily with wildfowl.

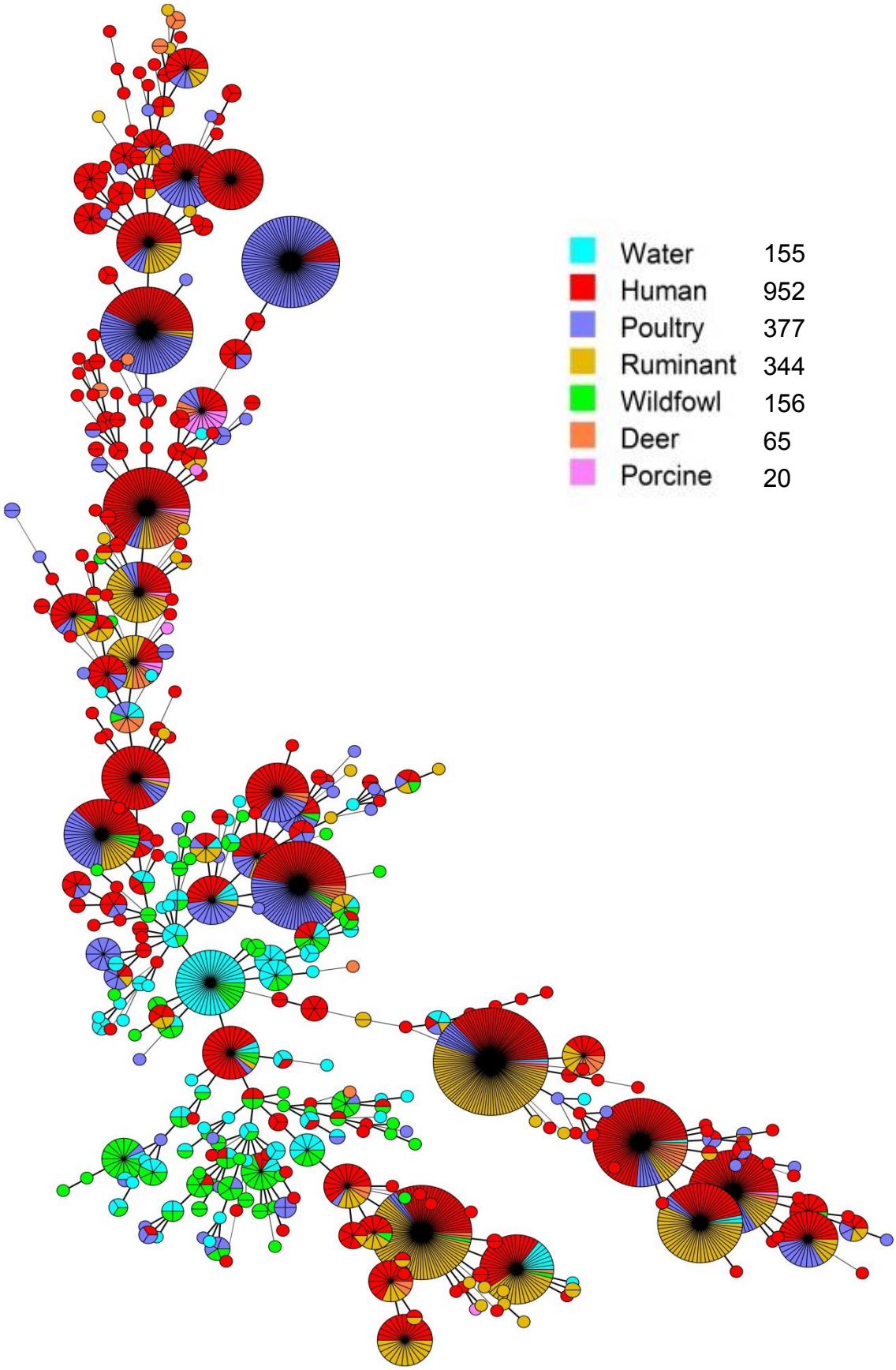


Figure 4 Minimum spanning tree of *Campylobacter* isolates analysed using MBiT

2.2.4 Faecal source tracking using polymerase chain reaction (PCR) markers

There are a range of microorganisms other than faecal coliforms, *E. coli* and enterococci present in faeces, some of 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. Microorganisms targeted by these assays and their specificities are listed in Tables 1 and 2.

As illustrated in Table 2, each marker is strongly associated with, but not exclusive to the source tested for. They each have some degree of non-specificity. The detection limit of these methods is 1.00×10^3 copies/100 mL.

While a semi-quantitative method is used to evaluate PCR markers, the results are generally presented as presence/absence. Where a very high level of a marker is detected, this is reported as High Level, indicating a likely major or dominant source. Lower levels may still represent a dominant source, but due to variable levels of the marker in host source faeces and dilution effects upon entering the waterway, the marker may not be present at high levels.

For human sources of pollution at least two PCR markers are used, with human faeces supported when two markers are detected.

The BacR ruminant marker is consistently present in ruminant faeces at high levels relative to the general marker. Therefore results for BacR are reported using a percentage value based on levels of this marker relative to the general indicator in fresh ruminant faeces.

Samples reported as 50-100% ruminant are consistent with all of the general faecal marker having come from a ruminant source.

Lower levels reported (10-50%) may be a consequence of the presence of other sources of pollution, or in fact ruminant sources may still account for all the pollution, but this may include aged faecal material, where relative levels of the ruminant marker decline more rapidly than the general indicator.

Levels less than 10% ruminant suggest a very minor contribution from ruminant sources.

Table 2 Summary of PCR Markers, Sensitivity and Microbial Targets

Marker Assay	Sensitivity	Target
General GenBac3	High	*Bacteroides* 16S rRNA
Human BiADO	Medium - less sensitive than BacH	*Bifidobacterium adolocentis*
Human BacH	Medium - most sensitive human assay	*Bacteriodales* species
Human HumM3	Low – least sensitive human assay but indicative of fresh faeces	*Bacteriodales* species
Ruminant BacR	High	*Bacteriodales* species
CowM2	Low	Bovine faeces-specific genetic markers
Schill Sheep	Medium	Cytochrome *b*
Avian GFD	Medium	16S rRNA gene
Avian E2	Low	*Desulfovibrio* species

Table 3 Specificity of PCR Markers

Marker Assay	Detected in faeces from:	Negative in faeces from:
General GenBac3	Human, cow, sheep, deer, goat, pig, rabbit, possum, cat, dog, horse, duck, swan, seagull, geese, chicken	Can be low in seagull and geese faeces.
Human BiADO	Human, Seagulls	Cow, Sheep, Deer, Horse, Goat, Pig, Rabbit, Geese, Chicken, Cat. Very low levels in faeces from Possum, Dog, Duck, Swan.
Human BacH	Human, Cat, Dog, Rabbit, Possum, Chicken, Goat	Cow, Sheep, Deer, Pig, Duck, Swan, Geese, Chicken, Cat, Horse, Goat, Dog, Seagull.
Human HumM3	Human, Possum, Rabbit	Cow, Sheep, Deer, Horse, Duck. Very low levels in faeces from Swans, Geese, Seagulls, Pigs.
Ruminant BacR	Cow, Sheep, Deer, Goat	Human (individuals), Horse, Pig, Rabbit, Duck, Swan, Seagull, Chicken, Dog. Very low levels in faeces from cats, possum, geese.
CowM2	Cow	Sheep, Goat, Horse, Pig, Human (individuals), Ducks, Swan, Geese, Seagulls, Cat, Dog, Possum, Rabbit. Very low levels in faeces from deer.
Schill Sheep	Sheep	Cow, Deer, Human (individuals), Swan, Geese, Seagull, Chicken, Horse, Cat, Pig, Possum, Rabbit. Very low levels in faeces from Goat, Duck, Dog.
Avian GFD	Duck, Swan, Seagull, Geese, Chicken	Human, Cow, Sheep, Deer, Horse, Goat, Pig, Rabbit, Possum Cat, Dog.
Avian E2	Duck	Human, Cow, Sheep, Deer, Horse, Goat, Rabbit, Possum Cat, Dog. Very low levels in faeces from swan, Seagull, Geese, Chicken, Pig.

2.2.5 Faecal source tracking using 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, 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 humans, 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.

Initially, use of faecal sterols in FST utilised the presence of coprostanol - which is the principal human biomarker - as an indicator of human faecal pollution. High relative amounts can indicate fresh human faecal material. Coprostanol constitutes 60% of the total sterols found in human faeces, while dogs and birds typically have either no coprostanol or only trace amounts present in their faeces. However, herbivores and other animals can have considerable amounts of coprostanol in their faeces, although at lower levels than the amount of 24-ethylcoprostanol.

Therefore, the ratios of one sterol to another are a better approach to assigning sources of pollution. Table 3 lists the key ratios used by ESR, which are evaluated using a decision tree approach. Pure faeces is relatively simple to evaluate, but when faecal sources are mixed, and when plant sterols and other environmental sources are added, the interpretation can become more complex and requires expert analysis.

Faecal sterols analysis was performed, by filtering 1–4 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).

Table 4 Faecal sterol ratios indicative of faecal pollution

Ratio	Sterols	Interpretation
Ratios indicative of faecal pollution (either human or animal)		
F1	coprostanol/cholestanol	Ratios >0.5 indicative of mammalian faecal source of sterols
F2	24-ethylcoprostanol/ 24-ethylcholestanol	
Human indicative ratios (values exceeding threshold in red)		
H1	% coprostanol	Ratio >5-6% suggests human source
H2	coprostanol/ (coprostanol+cholestanol)	Ratio >0.7 suggests human source
H3	coprostanol/24-ethylcoprostanol	Ratio >1 suggests human source
H4	coprostanol/(coprostanol+ 24-ethylcoprostanol)	Ratio >0.75 suggests human source
Ruminant indicative ratios (values exceeding threshold in blue)		
R1	% 24-ethylcoprostanol	Ratio >5-6% suggests ruminant source
R2	coprostanol/(coprostanol +24-ethylcoprostanol)	Ratio <30% suggests ruminant source
R3	24-ethylcholesterol/ 24-ethylcoprostanol	Ratio <1 suggests ruminant source, ratio >4 suggests plant decay
Avian indicative ratios (values exceeding threshold in yellow)		
A1	24-ethylcholestanol/ (24-ethylcholestanol +24-ethylcoprostanol +24-ethylepicoprostanol)	A1 Ratio >0.4 suggests avian source AND A2 Ratio >0.5 suggests avian source
A2	cholestanol/(cholestanol +coprostanol+epicoprostanol)	

2.2.6 Analysis of sediment samples

Sediment samples were analysed using the same methodology as for water samples, with the following exceptions. Analytes in sediments are reported as gram wet weight (ww).

- The dilutions used were 10 fold lower than those used for water, as no undiluted sediment sample could be analysed. Instead, a 0.1 ml sample was analysed, as well as 0.01 and 0.001 ml samples for indicator bacteria.
- For *Campylobacter* analysis a 1 g sample was analysed in triplicate, followed by a tenfold dilution series down to 0.001 g.

2.3 PRESENTATION OF RESULTS

Tables 5, 6, 7 and 8 provide a key for interpretation of results, which can be used to assist with reviewing results provided for each sampling site in the following sections.

Table 5 Explanation of results for general data, microbial results and MBiT interpretation.

<table>
<tr><td>Site</td><td colspan="5">Site name</td></tr>
<tr><td>Conditions</td><td colspan="5">Base flow or rainfall impacted</td></tr>
<tr><td>Date sampled</td><td colspan="5">Date sampled</td></tr>
<tr><td>E. coli (cfu/100ml)
E. coli (cfu/g)</td><td colspan="5">Colony forming units/100 ml for water samples
Colony forming units/gram for sediment samples (wet weight)</td></tr>
<tr><td>Enterococci (cfu/100ml)
Enterococci (cfu/g)</td><td colspan="5">Colony forming units/100 ml for water samples
Colony forming units/gram for sediment samples (wet weight)</td></tr>
<tr><td>Campylobacter (MPN/100ml)</td><td colspan="5">Most probable number (MPN) count of Campylobacter/100 ml</td></tr>
<tr><td>Species</td><td colspan="5">Determined by PCR as either C. jejuni, C. coli or other thermotolerant Campylobacter</td></tr>
<tr><td rowspan="3">MBiT Typing</td><td colspan="5">MBiT patterns of analysed isolates.
Colours reflect source attribution. Campylobacter isolates attributed to ruminant or poultry sources may also be from human sewage source, as these genotypes commonly cause disease in humans. Evaluation in conjunction with other faecal source indicators and site inspections is required assist in determining Campylobacter source.</td></tr>
<tr><td colspan="5">BOLD number indicates C. coli, italics thermotolerant, and normal text C. jejuni.</td></tr>
<tr><td>Wildfowl</td><td>Ovine/ Bovine/Deer</td><td>Poultry</td><td>Poultry and/or ruminants</td><td>Unknown</td></tr>
</table>

Table 6 Indicative Faecal ageing ratios for AC/TC

Source	Event	Average AC/TC ratio
Fresh human raw sewage/faeces, cow and horse faeces	Day 1	<1.0
Human sewage*	Sewage discharge into river	1.5 - 3.9*
River water	Ongoing inputs, can be a mix of fresh and historical inputs from faecal sources e.g. avian	5.0 - 10.0
	Heavy rainfall	3.0
	Day 3 after heavy rainfall	10.0
	Day 7 after heavy rainfall	79.0
	River returning to a healthier environment, less likelihood of pathogen detection including viruses	>15.0

*Human sewage discharges can be a mixture of fresh and aged faecal material

Table 7 below outlines the decision criteria applied to the PCR marker data to interpret the faecal source results presented in Section 3. The colour code for faecal sources identified is as follows: blue = ruminant, red = human, yellow = wildfowl, brown = canine.

Table 7 Interpretation of PCR based markers

General Bacteria (GenBac)	Indicator of possible faecal pollution. Scale indicates level detected, with samples with Positive or greater levels generally valid for examination of other markers					
Abbreviation	VS positive	S positive	Positive	Low levels	ND	
Full name	Very strong positive	Strong positive	Positive	Low levels	Not Detected	
Ruminant	Percentage of herbivore faecal pollution relative to the GenBac marker					
Final result in the table	Up to 100%	Up to 50%	10-50%	Up to 10%	1%	ND
	The remaining markers are reported as present/absent, although when a very high level is detected it is shown as PRESENT in bold. This suggest a major source. Lower levels may also indicate a significant source.					
Cow	**PRESENT**		Present		ND	
Sheep	**PRESENT**		Present		ND	
Human-BacH	**PRESENT**		Present		ND	
Human -BAdo	**PRESENT**		Present		ND	
Wildfowl - GFD	**PRESENT**		Present		ND	
Wildfowl - E2	**PRESENT**		Present		ND	
Canine	**PRESENT**		Present		ND	

ND = not detected, VS = very strong, S = strong

Table 8 below presents all the decision criteria applied to the sterol ratio data to interpret the faecal source. Only the **final decision** on source is presented in the results tables of Section 3. The colour code for sterol sources identified is as follows: red = human; blue = ruminant; yellow = wildfowl; green = plant runoff. For example, if the source is human, it will be coloured red in the results table with Yes (3) or Yes (2) if all three or two ratios were indicating human pollution (respectively), whereas >1 indicates that only the H3 ratio was indicating human, and therefore, is not a reliable indicator of human pollution.

Table 8 Interpretation of faecal sterol results.

Total Sterols (ng/L)	Total sterols expressed at ng/L			
Coprostanol (ng/L)	Level of coprostanol expressed as ng/L			
Faecal	If ratio F1 (coprostanol/cholestanol) or ratio F2 (24-ethylcoprostanol/24-ethylcholestanol) are greater than 0.5 it suggests human or animal faecal material. F1 tends to dominate human faeces, F2 in herbivore faeces.			
	Result in brackets indicates that close to reaching threshold			
Final decision	F1 + F2	F1	F2	No
Human	Human sources of faecal contamination are indicated when: Ratio H1 (%coprostanol/total sterols) is > 5-6% Ratio H2 (5β/(5β+5α stanols)) is > 0.7 Ratio H3 (coprostanol/24-ethylcoprostanol) is ≥ 1.0			
	H1, H2 and H3 meet thresholds	2 of 3 ratios meet thresholds	H3 meets threshold	None meet threshold
Final decision	Yes (3)	Yes (2)	>1	No
Herbivore[≠]	Herbivore sources of faecal material are indicated when: Ratio R1 (24-ethylcoprostanol/total sterols) is >5-6% Ratio R2 (coprostanol/coprostanol+24-ethylcoprostanol) is <30% Ratio R3 (24-ethylcholesterol/24-ethylcoprostanol) is <1.0			
	R1, R2 and R3 meet thresholds	2 of 3 ratios meet thresholds	R2 meets threshold	None meet threshold
Final decision	Yes (3)	Yes (2)	<30	No
Wildfowl*	Wildfowl sources of faecal material are indicated when: %Coprostanol/total sterols is <4% 24-ethylcoprostanol/total sterols is <4% %of alpha stanols/cholestanol, 24-ethylcholestanol is >2% 24-ethylcholesterol/24-ethylcoprostanol is >7% 24-ethylcholestanol/(24-ethylcholestanol+24-ethylcoprostanol+24-ethylepicoprostanol) is >0.4 cholestanol/(cholestanol+coprostanol+epicoprostanol) is >0.5			
	Meets all criteria	Almost meets criteria		
Final decision	Yes	(Yes)	No	
Plant Sterols**	Plant sterols are indicated when: Ratio of 24-ethylcholesterol/24-ethylcoprostanol is			
	>20	>10	>4	<4
Final decision	**YES**	YES	Yes	No

[≠]Herbivore includes ruminant faecal pollution

*Avian sterol ratios are not valid when moderate levels of human /herbivore/ruminant pollution are identified ;**NB high levels of plant sterols can also be indicative of avian pollution sources

3. RESULTS

3.1 WATER SAMPLING RESULTS

E. coli concentrations in the water samples were typically elevated, exceeding recreational water guideline values on a number of occasions during base flow, and after rainfall almost all samples exceeded MfE and MoH (2003) guideline values (Table 9).

Table 9 Average *E. coli* concentrations in water samples (cfu/100 ml)

Area	Site	Base flow	Rainfall
Avon	Antigua Boatsheds	533	2933
	Kerrs Reach	300	2283
	Owles Tce	466	466
Heathcote	Ferniehurst St	650	4450
	Bowenvale Ave	1150	3567
	Waltham Park	300	9684
	Catherine St	1050	3700
Estuary	South New Brighton Park	52	202
	Humphreys Drive	300	2025

3.1.1 Antigua Boatsheds, Avon River

Table 10 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Antigua Boatsheds. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 10 Water sampling results for Antigua Boatsheds, Avon River

Sample Type		Water					
Site		Antigua Boatsheds					
Conditions		Base flow			Rainfall Impacted		
Date Sampled		16/04/15	14/05/15	28/05/15	28/04/15	4/06/15	11/09/15
Bacteria	*E. coli*(cfu/100ml)	300	600	700	3750	4700	350
	Enterococci (cfu/100ml)	200	250	300	7800	5050	1000
	Campylobacter (MPN/100ml)	4.3	0.4	<0.3	9.3	15	9
	- Species	*C. jejuni*	*C. jejuni*	ND	*C. jejuni*	*C. jejuni*	*C. jejuni*
	- MBiT Typing	040210	440202		406200 006040 002060	064121	033767 42000
	AC/TC Ratio	2.7	3.5	1.1	1.3	0.0	#N/A
PCR Markers	General Bacteria	VS positive	VS positive	VS positive	VS positive	VS positive	VS positive
	Human - BacH	present	ND	present	present	PRESENT	present
	Human - BAdo	ND	ND	ND	present	present	ND
	Human- HumM3	ND	ND	ND	ND	present	ND
	Ruminant	ND	ND	ND	up to 10%	1 - 10%	up to 1%
	Cow	NA	NA	NA	NA	NA	ND
	Sheep	NA	NA	NA	present	ND	ND
	Wildfowl - GFD	present	present	present	present	present	present
	Wildfowl - E2	PRESENT	PRESENT	PRESENT	present	present	present
	Canine	ND	ND	ND	present	present	present
Faecal Sterols	Total Sterols (ng/L)	5,308	7,250	11,246	14,921	21,956	3,846
	Coprostanol (ng/L)	132	286	363	229	536	33
	Faecal	F2	F1+F2	F1+F2	No	F1+F2	(F1)+F2
	Human	No	No	No	No	No	No
	Herbivore	No	No	Yes (2)	No	No	No
	Wildfowl	(Yes)	No	No	Yes	No	Yes
	Plant	Yes	Yes	Yes	Yes	Yes	Yes
Summary		Wildfowl dominant source			Wildfowl, human, canine sources		

NA = not applicable, ND = not detected, VS = very strong, present = positive for that source
Colour code: blue = ruminant; yellow = wildfowl; red = human; brown = canine and green = plant

Interpretation for Antigua Boatsheds

Base flow conditions

Under base flow conditions numbers of *E. coli* exceeded 550 *E. coli*/100ml on two of the three sampling occasions. The enterococci levels are lower than the *E. coli* as you would expect in base flow events, where typically diffuse faecal pollution from fresh sources is the source of the indicator bacteria i.e. bird defecations. C*ampylobacter* was present in low concentrations on two of the three occasions and was found by MBiT subtyping to be

sourced from wildfowl. The AC/TC ratio reflects a fresh pollution source in the river with all ratios below 3.9.

PCR markers provide a consistent indication of wildfowl pollution, with high levels of the duck indicative E2 markers in all three samples. Human BacH marker was detected on two occasions, but was not supported by either of the other two human indicative markers (nor sterol analysis).

Faecal sterol analysis was dominated by plant sterols, with none of the three base flow samples having a clear source of pollution based on sterols. However, high levels of plant sterols can support an avian source of faecal pollution as indicated by PCR markers.

Rainfall impacted conditions

Under rainfall impacted conditions the microbial loading of the river increased markedly (10 fold on one occasion), with *E. coli* concentrations well above 1000 cfu/100 ml on two occasions.

Increased concentrations of *Campylobacter* were observed under rainfall conditions, with a maximum of 15 MPN per 100 ml on the second sampling occasion. The first event was dominated by *Campylobacter* from avian sources, while for the second event the MBiT pattern is indicative of a herbivore source. As humans are readily infected with *Campylobacter* from herbivore sources, the presence of this *Campylobacter* subtype could indicate human and/or herbivore sources. The presence of human PCR markers (see section below), would support *Campylobacter* being from a human sewage source. The AC/TC ratio reflects fresh faecal pollution being dominant during the rainfall events.

Wildfowl PCR markers were again detected in all three samples, but at lower relative levels than under base flow. Canine faecal source was also consistently detected, and in two of the three rainfall impacted samples a human signature was evident. The second sampling event had all three human indicative PCR markers present providing very strong evidence of human source. When the HumM3 PCR marker is detected it also suggests a very recent faecal input.

Surprisingly, low levels of ruminant faeces were detected in all three rainfall impacted sampling occasions, with a sheep indicative marker supporting the first event. The ruminant marker is highly specific, with only cow, sheep, deer, and goat giving positive results. The low levels, and lack of support from sterols suggests a distant upstream source, or a relatively minor, more local source.

Faecal sterols did support wildfowl sources of pollution, but not the human inputs. The level of coprostanol was elevated suggesting human source, but perhaps swamped by plant based sources as indicated by high concentrations of plant sterols. Plant sterols could be attributed to avian sources, but in the case of rainfall events is more likely to be related to overland flow of plant sourced material. The human faecal input may also have high levels of kitchen or other plant waste (e.g. from waste disposal discharge into the sewer system), which may mask the signal.

Overall Conclusion for Antigua Boatsheds

Overall base flow pollution is characterised by wildfowl faecal pollution, while sampling after rainfall identifies, that in addition to wildfowl sources, canine, human faecal pollution.

3.1.2 Kerrs Reach, Avon River

Table 10 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Kerrs Reach. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 - Presentation of Results.

Table 11 Water sampling results for Kerrs Reach, Avon River

Sample Type		Water					
Site		Kerrs Reach					
Conditions		Base flow			Rainfall Impacted		
Date Sampled		16/04/15	14/05/15	28/05/15	28/04/15	4/06/15	11/09/15
Bacteria	***E. coli***(cfu/100ml)	450	400	50	4350	2050	450
	Enterococci (cfu/100ml)	350	350	<10	12800	4450	2500
	Campylobacter (MPN/100ml)	2.3	1.5	4.3	93	24	0.4
	- Species	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*
	- MBiT Typing	066000	040200 441302	040102 040220 044102	440260 166000 002000	460127	444010
	AC/TC Ratio	1.3	0.8	1.6	1.4	0.0	0.0
PCR Markers	**General Bacteria**	VS positive	VS positive	VS positive	VS positive	VS positive	VS positive
	Human - BacH	present	present	present	present	present	present
	Human - BAdo	present	present	present	present	present	present
	Human- HumM3	ND	ND	ND	ND	ND	present
	Ruminant	ND	ND	ND	1 - 10%	1 - 10%	up to 1%
	Cow	NA	NA	NA	NA	NA	ND
	Sheep	NA	NA	NA	ND	ND	ND
	Wildfowl - GFD	present	present	present	present	present	present
	Wildfowl - E2	present	present	**PRESENT**	present	present	present
	Canine	ND	ND	ND	present	present	present
Faecal Sterols	**Total Sterols** (ng/L)	1135	1003	1194	2624	4377	2305
	Coprostanol (ng/L)	68	97	30	35	63	35
	Faecal	F1+F2	F1+F2	F1+F2	F1+F2	F1+F2	F1+F2
	Human	Yes	Yes	No	No	No	No
	Herbivore	No	No	No	No	No	No
	Wildfowl	No	No	No	Yes	Yes	No
	Plant	YES	Yes	Yes	**YES**	**YES**	**YES**
Summary		Human and wildfowl sources			Human, wildfowl, canine sources		

NA = not applicable, ND = not detected, VS = very strong, present = positive for that source
Colour code: blue = ruminant; yellow = wildfowl; orange = non-wildfowl; purple = poultry; red = human; brown = canine, and green = plant

Interpretation for Kerrs Reach

Base flow conditions

Under base flow conditions, numbers of *E. coli* exceeded the acceptable value of 260 cfu per 100 ml on two occasions. C*ampylobacter* was present in low concentrations and was found by MBiT subtyping to be sourced from wildfowl on two occasions, and from non-wildfowl source on the other. The AC/TC ratio reflects a fresh faecal source in the river with all ratios below 1.6.

PCR markers provide a consistent indication of human faecal pollution in the absence of HumM3 in all three samples suggesting an aged or dilute human faecal input.

Faecal sterol analysis was dominated by plant sterols, but human sources were detected on two of three occasions.

Rainfall impacted conditions

Under rainfall impacted conditions the microbial loading of the river increased markedly, with two samples exceeding 2000 cfu *E. coli* per 100 ml. The enterococci concentrations exceed the *E. coli* values in the river on all occasions at this site following rainfall.

Increased concentrations of *Campylobacter* were observed under rainfall conditions, with a maximum of 93 MPN per 100 ml on the first sampling occasion. The first event is dominated by *Campylobacter* from avian sources, while for the second and third events the MBiT pattern is indicative of poultry genotypes, which may indicate a human source of these *Campylobacter*, as poultry related genotypes are common in human sewage. The AC/TC ratio reflects fresh pollution being dominant during the rainfall events.

Human PCR markers were again detected in all three samples, with HumM3 detected in one sample. When the HumM3 PCR marker is detected it also suggests a very recent faecal input. A canine faecal source was also consistently detected, and in all rainfall impacted samples a wildfowl signature was evident.

Very low levels of ruminant faecal markers were detected on all three rainfall impacted sampling occasions. The ruminant marker is highly specific, with only cow, sheep, deer, and goat giving positive results. The low levels and lack of support from sterols suggests a distant upstream source, or a relatively minor more local source.

Faecal sterols did support wildfowl sources of pollution, but not the human inputs. This may be due to the high levels of plant sterols swamping the human signal.

Overall Conclusion for Kerrs Reach

Overall base flow pollution is characterised by human and wildfowl sources. In contrast, sampling after rainfall identified in addition to human and wildfowl, that canine sources and plant runoff into water is evident.

3.1.3 Owles Terrace, Avon River

Table 12 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Owles Terrace. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 12 Water sampling results for Owles Terrace, Avon River

Sample Type		Water					
Site		Owles Terrace					
Conditions		Base flow			Rainfall Impacted		
Date Sampled		16/04/15	14/05/15	28/05/15	28/04/15	4/06/15	11/09/15
Bacteria	*E. coli*(cfu/100ml)	1150	150	100	1000	300	100
	Enterococci (cfu/100ml)	1250	200	<10	4000	600	700
	Campylobacter (MPN/100ml)	4.3	4.3	21	24	24	9.3
	- Species	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni, C. coli*	*C. jejuni*
	- MBiT Typing	*002000* 405230	*440260* 404230	*401200* 425121 002000 002020	*441302*	**024021** 002000 401200 040200	407000 040220 002040 003202
	AC/TC Ratio	4.7	1.0	2.1	0.0	0.0	0.0
PCR Markers	General Bacteria	VS positive	VS positive	VS positive	VS positive	VS positive	VS positive
	Human - BacH	present	ND	present	present	present	present
	Human - BAdo	ND	ND	ND	present	present	present
	Human- HumM3	ND	ND	ND	ND	ND	ND
	Ruminant	ND	ND	ND	1 - 10%	ND	up to 1%
	Cow	NA	NA	NA	NA	NA	ND
	Sheep	NA	NA	NA	ND	NA	ND
	Wildfowl - GFD	present	present	present	present	present	present
	Wildfowl - E2	present	present	present	present	present	present
	Canine	ND	ND	ND	present	present	ND
Faecal Sterols	Total Sterols (ng/L)	1691	914	1182	1529	1254	1114
	Coprostanol (ng/L)	62	25	26	28	32	23
	Faecal	F1+F2	F1+F2	F1+F2	F2	F1+F2	F1+F2
	Human	>1	>1	No	No	No	No
	Herbivore	No	No	No	No	No	No
	Wildfowl	Yes	Yes	Yes	Yes	No	No
	Plant	YES	YES	YES	YES	YES	Yes
Summary		Wildfowl dominant sources			Wildfowl, human, canine sources		

NA = not applicable, ND = not detected, VS = very strong, present = positive for that source
Colour code: blue = ruminant; yellow = wildfowl; orange = non-wildfowl; purple = poultry; red = human; brown = canine, and green = plant

Interpretation for Owles Terrace

Base flow conditions

Under base flow conditions the *E. coli* concentration exceeded the acceptable value of 260 cfu per 100, with 1150 cfu *E. coli* per 100 ml on one occasion. Enterococci numbers are at a similar level compared to *E. coli*. *Campylobacter* was present in low concentrations on all

occasions and was found by MBiT subtyping to be sourced from wildfowl, although one genotype was unable to be assigned to a source. The AC/TC ratio, in general, reflects recent faecal pollution sources in the river with all ratios below 4.7.

PCR markers indicate a consistent wildfowl source, with only the BacH human PCR marker detected on occasions. As it was detected in the absence of the other human markers, the presence of human faecal pollution is not supported.

Faecal sterol analysis was dominated by plant sterol and wildfowl signatures.

Rainfall impacted conditions

Under rainfall impacted conditions the microbial loading of the river did not increase markedly, with only one value above 550 cfu *E. coli* per 100 ml. The enterococci population exceeded *E. coli* values in the river on all occasions at this site following rainfall.

Increased concentrations of *Campylobacter* were observed under rainfall conditions, with a maximum of 24 MPN per 100 ml on the first two sampling occasions. The genotypes of *Campylobacter* were consistent with wildfowl sources, although one genotype couldn't be assigned to a source. The AC/TC ratio reflected fresh faecal pollution.

Human PCR markers were again detected in all three samples, but as HumM3 was not detected in any sample, the faecal pollution may not have been recent, or may have been diluted.

Low levels of ruminant markers were detected in two of the rainfall impacted sampling occasions. The ruminant marker is highly specific, with only cow, sheep, deer, and goat giving positive results. The low levels and lack of support from sterols suggests a distant upstream source, or a relatively minor more local source.

Faecal sterol analysis suggested wildfowl sources on one occasion, with plant sterols dominating.

Overall Conclusion for Owles Terrace

Overall, base flow pollution is characterised by wildfowl faecal pollution, while sampling after rainfall identified that human and canine faecal sources may also be present.

3.1.4 Humphreys Drive, Estuary

Table 13 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the estuarine sampling site of Humphreys Drive. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 13 Water sampling results for Humphreys Drive, Estuary

Sample Type		Humphreys Drive					
Site		Estuary					
Conditions		Base flow			Rainfall Impacted		
Date Sampled		16/04/15	14/05/15	28/05/15	28/04/15	4/06/15	11/09/15
Bacteria	***E. coli***(cfu/100ml)	250	450	200	1600	2450	No Sample Taken
	Enterococci (cfu/100ml)	150	600	50	6300	6250	
	Campylobacter (MPN/100ml)	<0.3	<0.3	<0.3	9.3	4.3	
	- Species	-	-	-	*C. jejuni*	*C. jejuni*	
	- MBiT Typing				046240 046342	064103	
	AC/TC Ratio	0.8	1.6	1.8	1.6	0.0	
PCR Markers	**General Bacteria**	S positive	VS positive	VS positive	VS positive	VS positive	
	Human - BacH	present	present	ND	present	present	
	Human - BAdo	ND	ND	ND	present	present	
	Human- HumM3	ND	ND	ND	ND	ND	
	Ruminant	up to 10%	ND	ND	ND	1 - 10%	
	Cow	ND	ND	ND	ND	ND	
	Sheep	ND	ND	ND	ND	present	
	Wildfowl - GFD	present	ND	present	present	present	
	Wildfowl - E2	present	present	present	present	present	
	Canine	ND	ND	ND	present	present	
Faecal Sterols	**Total Sterols** (ng/L)	1587	1335	1424	2178	4747	
	Coprostanol (ng/L)	36	45	35	82	249	
	Faecal	F2	F1+F2	F2	F1+F2	F1+F2	
	Human	No	>1	>1	>1	Yes (2)	
	Herbivore	No	No	No	No	No	
	Wildfowl	Yes	Yes	Yes	Yes	No	
	Plant	YES	YES	YES	YES	YES	
Summary		Wildfowl dominant sources			Human, wildfowl, canine sources		

NA = not applicable, ND = not detected, VS = very strong, present = positive for that source
Colour code: blue = ruminant; yellow = wildfowl; orange = non-wildfowl; purple = poultry; red = human; brown = canine, and green = plant

Interpretation for Humphreys Drive

Base flow conditions

The indicator bacteria used under saline conditions are enterococci as they survive better in this environment and are, therefore, a more conservative indicator of human health risk. Under base flow conditions the enterococci concentration exceeded the action value of 280

enterococci per 100 ml with 600 enterococci per 100 ml on one occasion. C*ampylobacter* was absent on all occasions at this site under base flow. The AC/TC ratio reflects a fresh faecal source with all ratios below 1.9.

PCR markers point to a consistent wildfowl source of faecal pollution, with only the BacH human PCR marker detected on two occasions. As it was detected in the absence of the other human markers, the presence of human faecal pollution is not supported. Low level ruminant sources were detected in the first sampling.

Faecal sterol analysis was dominated by plant sterol and wildfowl signatures.

Rainfall impacted conditions

Only two rainfall impacted samplings took place. On the third rainfall event the tide was out too far for sampling to take place. Under rainfall impacted conditions the microbial loading of the estuary did increase markedly, with the enterococci concentration exceeding the red alert value on all occasions at this site. The *E. coli* concentrations also increased, indicating fresh faecal inputs to the site. This assumption is supported by low AC/TC ratios indicating fresh faecal sources.

Low levels of *Campylobacter* were observed under rainfall conditions, with a maximum of 9.3 MPN per 100 ml on the first sampling occasion. Avian sources of *Campylobacter* were detected on the first rainfall impacted sampling, while herbivore sources were determined on the second sampling. This may also reflect a human source of *Campylobacter*.

Human PCR markers were again detected in both rainfall impacted samples, but as HumM3 was not detected in any sample, the faecal source may not have been recent or it was a low level of human pollution.

Low levels of ruminant faeces were detected on one of the rainfall impacted sampling occasions. The ruminant marker is highly specific, with only cow, sheep, deer, and goat giving positive results. The low levels, and lack of support from sterols suggests a distant upstream source, or a relatively minor more local source. Analysis suggested a contribution from sheep source to the ruminant signal. A canine faecal source was detected on both rainfall occasions.

Faecal sterol analysis suggested a human source on the second rainfall sampling and a wildfowl source of sterols during the first rainfall sampling.

Overall Conclusion for Humphreys Drive

Overall, base flow pollution is characterised by wildfowl faecal pollution, while sampling after rainfall identified, that in addition to wildfowl, human and canine faecal pollution was also present.

3.1.5 South New Brighton Park, Estuary

Table 14 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the estuarine sampling site of South New Brighton Park. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 14 Water sampling results for South New Brighton Park, Estuary

Sample Type		Estuary					
Site		South New Brighton Park					
Conditions		Base flow			Rainfall Impacted		
Date Sampled		16/04/15	14/05/15	28/05/15	28/04/15	4/06/15	11/09/15
Bacteria	***E. coli***(cfu/100ml)	100	<10	50	<10	550	50
	Enterococci (cfu/100ml)	50	<10	<10	150	850	2300
	Campylobacter (MPN/100ml)	<0.3	4.3	<0.3	<0.03	0.9	24
	- Species	ND	*C. jejuni*	ND	ND	*C. jejuni*	*C. jejuni*
	- MBiT Typing		144200 146202			002000	040210
	AC/TC Ratio	0.6	1.5	2.1	0.0	1.2	0.0
PCR Markers	**General Bacteria**	VS positive	S positive	VS positive	VS positive	VS positive	VS positive
	Human - BacH	present	ND	present	present	ND	present
	Human - BAdo	ND	ND	ND	present	ND	ND
	Human- HumM3	ND	ND	ND	ND	ND	ND
	Ruminant	1 - 10%	ND	ND	up to 1%	ND	1 - 10%
	Cow	ND	ND	ND	ND	ND	ND
	Sheep	ND	ND	ND	ND	ND	ND
	Wildfowl - GFD	ND	present	present	present	present	present
	Wildfowl - E2	present	present	present	present	present	present
	Canine	ND	ND	ND	ND	ND	present
Faecal Sterols	**Total Sterols** (ng/L)	1362	1347	1109	1365	1326	1388
	Coprostanol (ng/L)	32	16	28	24	23	44
	Faecal	No	F2	F2	(F1)+F2	F2	F1+F2
	Human	No	No	No	No	No	>1
	Herbivore	No	No	No	No	No	No
	Wildfowl	Yes	Yes	Yes	Yes	Yes	Yes
	Plant	YES	YES	YES	YES	YES	Yes
Summary		**Wildfowl dominant sources**			**Wildfowl dominant sources**		

NA = not applicable, ND = not detected, VS = very strong, present = positive for that source
Colour code: blue = ruminant; yellow = wildfowl; orange = non-wildfowl; purple = poultry; red = human; brown = canine, and green = plant

Interpretation for South New Brighton Park

Base flow conditions

Under base flow conditions the enterococci concentration were below the action level of 280 enterococci per 100 ml on all occasions, and the *E. coli* concentrations were also very low. C*ampylobacter* (MPN 4.3 per 100 ml) was present on one occasion at this site under base flow, and was found by MBiT subtyping to be sourced from a mixture of wildfowl and poultry types. The AC/TC ratio reflects a fresh faecal pollution source with all ratios below 2.1.

PCR markers point to a consistent wildfowl source of faecal pollution, with only the BacH human PCR marker detected on two occasions. As this marker was detected in the absence of the other human markers, the presence of human faecal pollution is not supported.

Faecal sterol analysis was dominated by plant sterol and wildfowl signatures.

Rainfall impacted conditions

Under rainfall impacted conditions the microbial loading of the estuary did increase markedly, particularly on the final sampling, with enterococci levels above 2000 cfu per 100ml at this site. *E. coli* levels were elevated on one occasion only, while the remaining times revealed concentrations of 50 *E. coli* per 100 ml and lower (<10).

Low levels of *Campylobacter* were observed under rainfall conditions on two occasions, with a maximum of 24 MPN per 100 ml. MBiT analysis revealed wildfowl sources of *Campylobacter* on both sampling occasions. The AC/TC ratio reflected fresh faecal pollution.

Human PCR markers were again detected in rainfall impacted samples, but as HumM3 was not detected in any sample, the faecal source may not have been recent.

Very low levels of ruminant markers were detected in two of the rainfall impacted sampling occasions. The low levels, and lack of support from sterols suggests a distant upstream source, or a relatively minor more local source. A canine faecal source was detected on one occasion only.

Faecal sterols supported the wildfowl sources of faecal pollution with a consistent plant sterol ratio.

Overall Conclusion for South New Brighton Park

Overall, base flow faecal pollution is characterised by wildfowl faecal pollution, while sampling after rainfall identifies that in addition to wildfowl, there may be canine and human faecal pollution present on occasion.

3.1.6 Ferniehurst Street, Heathcote River

Table 15 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Ferniehurst Street in the Heathcote River. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 - Presentation of Results.

Table 15 Water sampling for Ferniehurst Street, Heathcote River

River		Heathcote					
Site		Ferniehurst Street					
Conditions		Base flow			Rainfall Impacted		
Date Sampled		16/04/15	14/05/15	28/05/15	28/04/15	4/06/15	11/09/15
Bacteria	***E. coli***(cfu/100ml)	1000	650	300	7700	4850	800
	Enterococci (cfu/100ml)	600	900	150	36000	8500	3050
	Campylobacter (MPN/100ml)	110	43	240	460	93	21
	- Species	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*
	- MBiT Typing	401200 007200 002020 002000	407202 473363	002000	002000 046000 421001	460127	401200 002000 002202 044000
	AC/TC Ratio	2.6	1.9	1.1	0.7	0.0	0
PCR Markers	**General Bacteria**	VS positive	VS positive	VS positive	VS positive	VS positive	VS positive
	Human - BacH	ND	ND	present	present	present	present
	Human - BAdo	present	ND	ND	ND	present	present
	Human - HumM3	ND	ND	ND	ND	ND	present
	Ruminant	1 - 10%	up to 10%	1 - 10%	50 - 100%	up to 100%	up to 50%
	Cow	ND	ND	ND	present	present	present
	Sheep	ND	ND	ND	present	ND	present
	Wildfowl - GFD	present	present	present	present	present	present
	Wildfowl - E2	**PRESENT**	present	**PRESENT**	present	present	present
	Canine	present	ND	present	present	present	present
Faecal Sterols	**Total Sterols** (ng/L)	1990	1418	1608	6098	19386	4319
	Coprostanol (ng/L)	28	25	26	57	178	42
	Faecal	F2	F1+F2	F2	F2	F2	F1+F2
	Human	No	No	No	No	No	No
	Herbivore	No	No	No	<30	<30	Yes (R1+R2)
	Wildfowl	Yes	(Yes)	Yes	No	(Yes)	No
	Plant	Yes	YES	YES	YES	YES	Yes
Summary		**Wildfowl, canine sources**			**Human, ruminant (both cow and sheep), wildfowl, and canine sources**		

NA = not applicable, ND = not detected, VS = very strong, present = positive for that source
Colour code: blue = ruminant; yellow = wildfowl; orange = non-wildfowl; purple = poultry; red = human; brown = canine, and green = plant

Interpretation for Ferniehurst Street

Base flow conditions

Under base flow conditions the numbers of *E. coli* exceeded the acceptable value of 260 cfu per 100 ml on two occasions. *Campylobacter* were present in relatively high concentrations on all occasions and were found by MBiT subtyping to be sourced mainly from wildfowl, with ruminant or undetermined sources present on one occasion. The AC/TC ratio reflects a fresh faecal pollution source.

PCR markers provide a consistent indication of wildfowl faecal pollution, with high levels of the duck indicative E2 markers also detected in two samples. A low level of ruminant faecal pollution was evident in all samples. Intermittent human PCR markers were detected but not sufficiently to assign human faecal pollution. Canine faecal pollution was also detected twice during base flow sampling at this site.

Faecal sterol analysis was dominated by plant sterols, with wildfowl faecal pollution also detected.

Rainfall impacted conditions

Under rainfall impacted conditions the microbial loading of the river increased markedly. The enterococci population exceeded the *E. coli* values at the site following rainfall.

Campylobacter were observed under rainfall conditions on all occasions, with a maximum of 460 MPN per 100 ml on the first sampling event. This was the highest value of all sampling occasions. The *Campylobacter* were determined to be of a mixture of wildfowl, herbivore and poultry sources by MBiT pattern analysis. As humans are readily infected with *Campylobacter* from herbivore and poultry sources, the presence of these sources of *Campylobacter* could indicate human or herbivore/avian sources.

The AC/TC ratio reflected fresh faecal pollution.

Human PCR markers were again detected in all three samples. The HumM3 PCR marker was detected on the third sampling occasion, suggesting a recent faecal input. BacH was the only human marker detected in the first rainfall impacted sampling; this suggests there is not a human source. . A ruminant source was detected on all three occasions, constituting up to 100 % of the faecal pollution. A bovine marker was detected on all three occasions

with sheep present in two. The wildfowl marker was detected on each occasion and canine pollution was also found in all rainfall sampling events.

The sterols were consistent with plant sources on all occasions, with herbivore and wildfowl sources detected on one occasion each. Detection of herbivore sources by sterols reduces the ability to concurrently detect wildfowl faecal pollution by sterol analysis.

Overall Conclusion for Ferniehurst Street

Overall, base flow faecal pollution is characterised by wildfowl and canine faecal inputs, while sampling after rainfall, identifies that high levels of ruminant faecal pollution, along with human faecal pollution are also present.

3.1.7 Bowenvale Avenue, Heathcote River

Table 16 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Bowenvale Avenue. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 16 Water sampling for Bowenvale Avenue, Heathcote River

River		Heathcote					
Site		Bowenvale Ave					
Conditions		Base flow			Rainfall Impacted		
Date Sampled		16/04/15	14/05/15	28/05/15	28/04/15	4/06/15	11/09/15
Bacteria	***E. coli***(cfu/100ml)	550	2650	250	5400	4450	850
	Enterococci (cfu/100ml)	850	1450	50	25000	7400	3450
	Campylobacter (MPN/100ml)	110	24	150	93	43	43
	- Species	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. coli*	*C. jejuni, C. coli*
	- MBiT Typing	407202 002000 002040 042200 002200	002020 402202	002000 401200 002000	423001 421001	073563	24121 400002 002202 401200 000220
	AC/TC Ratio	11.6	0.7	3.2	0.7	0.0	0.0
PCR Markers	**General Bacteria**	VS positive	VS positive	VS positive	VS positive	VS positive	VS positive
	Human - BacH	ND	ND	present	present	present	present
	Human - BAdo	ND	ND	ND	ND	present	present
	Human - HumM3	ND	ND	ND	ND	present	ND
	Ruminant	1 - 10%	1 - 10%	1 - 10%	50 - 100%	up to 50%	up to 50%
	Cow	NA	NA	NA	present	present	present
	Sheep	ND	ND	ND	present	ND	present
	Wildfowl - GFD	present	present	present	present	present	present
	Wildfowl - E2	**PRESENT**	present	present	present	present	present
	Canine	present	ND	ND	present	present	present
Faecal Sterols	**Total Sterols** (ng/L)	1622	1017	1425	12065	17742	4399
	Coprostanol (ng/L)	22	20	22	53	374	59
	Faecal	F2	F1+F2	F2	F2	F1+F2	F1+F2
	Human	No	No	No	No	No	No
	Herbivore	No	No	No	<30	No	Yes (R1 and R2)
	Wildfowl	Yes	(Yes)	Yes	No	(Yes)	No
	Plant	YES	YES	YES	YES	YES	Yes
Summary		**Wildfowl dominant sources**			**Human, ruminant (both cow and sheep), wildfowl, and canine sources**		

NA = not applicable, ND = not detected, VS = very strong, present = positive for that source
Colour code: blue = ruminant; yellow = wildfowl; orange = non-wildfowl; purple = poultry; red = human; brown = canine, and green = plant

Interpretation for Bowenvale Avenue

Base flow conditions

Under base flow conditions the numbers of *E. coli* exceeded the acceptable value of 260 cfu *E. coli* per 100 ml on two occasions, with more than 2600 cfu/100 ml on the second sampling. C*ampylobacter* was present in relatively high concentrations on all occasions and was found by MBiT subtyping to be sourced from wildfowl. The AC/TC ratio reflects fresh faecal pollution on two of the three sampling events.

PCR markers provide a consistent indication of wildfowl faecal pollution, with high levels of the duck indicative E2 markers also detected. The human BacH marker was detected on one occasion but was not supported by either of the other two human indicative markers (nor sterol analysis). The canine marker was detected on one occasion. A low level of ruminant faecal pollution was detected in all samples.

Faecal sterol analysis was dominated by plant sterols, with wildfowl faecal pollution also detected.

Rainfall impacted conditions

Under rainfall impacted conditions the microbial concentrations at the site increased markedly. *E. coli* concentrations exceeded 550 cfu per 100 ml on all occasions. The enterococci population exceeded the *E. coli* values in the river following rainfall.

Campylobacter were observed under rainfall conditions on all occasions, with a maximum of 93 MPN per 100 ml on the first sampling occasion. The *Campylobacter* were determined to be of ruminant and wildfowl sources by MBiT pattern analysis. As humans are readily infected with *Campylobacter* from herbivore sources, the presence of herbivore sourced *Campylobacter* could indicate human or herbivore sources.

The AC/TC ratio reflected fresh faecal pollution.

Human PCR markers were detected in all three rainfall associated samples. HumM3 PCR marker was detected on the second sampling occasion suggesting recent faecal input. Only BacH was detected in the first rainfall impacted sampling, this suggests it is not a human source. A ruminant source was detected on all three occasions constituting up to 100 % of the faecal pollution. The bovine marker was detected on all three occasions with sheep present in two. Wildfowl and canine faecal pollution were detected on each occasion.

The sterols were consistent with a plant source on all occasions, with herbivore and wildfowl sources detected on one occasion each. Detection of herbivore by sterols reduces the ability to concurrently detect wildfowl pollution by sterol analysis. There was elevated coprostanol in the sample where the three human PCR markers were detected, but the high levels of plant sterol runoff precluded a positive identification of human faecal pollution by sterol analysis.

Overall Conclusion for Bowenvale Avenue

Base flow sources were characterised by wildfowl faecal inputs, while in addition to wildfowl faecal pollution, sampling after rainfall identified increased incidence of ruminant (sheep and cow), canine and human faecal pollution.

3.1.8 Waltham Park, Heathcote River

Table 17 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Waltham Park. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 17 Water sampling for Waltham Park, Heathcote River

River		Heathcote					
Site		Waltham Park					
Conditions		Base flow			Rainfall Impacted		
Date Sampled		16/04/15	14/05/15	28/05/15	28/04/15	4/06/15	11/09/15
Bacteria	***E. coli***(cfu/100ml)	550	200	150	7200	21000	850
	Enterococci (cfu/100ml)	550	150	150	14550	11450	5950
	Campylobacter (MPN/100ml)	46	9.3	43	150	93	15
	- Species	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni, C. coli*
	- MBiT Typing	002040 407200 002000	003200 447000	002060 002000 002040	073767 421001 406060 401200 407240 402200 002060	464327	044000 007200 024121 405200 002202 003000
	AC/TC Ratio	4.3	0.9	2.6	0.7	0.0	0
PCR Markers	**General Bacteria**	VS positive	VS positive	VS positive	VS positive	VS positive	VS positive
	Human - BacH	present	ND	ND	present	**PRESENT**	present
	Human - BAdo	ND	ND	ND	present	**PRESENT**	present
	Human - HumM3	ND	ND	ND	ND	present	ND
	Ruminant	1 - 10%	1 - 10%	1 - 10%	up to 100%	up to 10%	up to 50%
	Cow	NA	NA	NA	present	ND	present
	Sheep	ND	ND	ND	ND	present	present
	Wildfowl - GFD	ND	present	present	present	ND	present
	Wildfowl - E2	present	present	present	present	present	present
	Canine	present	ND	ND	present	present	present
Faecal Sterols	**Total Sterols** (ng/L)	1294	923	2706	5443	16417	4638
	Coprostanol (ng/L)	16	21	20	43	4406	65
	Faecal	F2	F1+F2	No	F2	F1+F2	F1+F2
	Human	No	>1	No	No	Yes (3)	No
	Herbivore	<30	No	No	<30	No	Yes (R1+2)
	Wildfowl	Yes	Yes	Yes	No	No	No
	Plant	YES	YES	**YES**	YES	No	Yes
Summary		**Wildfowl sources**			**Human, ruminant (both cow and sheep), wildfowl, and canine sources**		

NA = not applicable, ND = not detected, VS = very strong, present = positive for that source
Colour code: blue = ruminant; yellow = wildfowl; orange = non-wildfowl; purple = poultry; red = human; brown = canine, and green = plant

Interpretation for Waltham Park

Base flow conditions

Under base flow conditions the numbers of *E. coli* exceeded the acceptable value of 260 cfu per 100 ml on one occasion. C*ampylobacter* was present on all occasions and was found by MBiT subtyping to be sourced from wildfowl with one undetermined source isolate. The AC/TC ratio reflects fresh faecal pollution.

The PCR markers provided an indication of wildfowl faecal pollution, with the duck indicative E2 markers present in all samples. A low level of ruminant faecal pollution was detected in all samples. Canine faecal pollution was detected once during base flow sampling at this site.

Faecal sterol analysis was dominated by plant sterols, with wildfowl faecal pollution also detected.

Rainfall impacted conditions

Under rainfall impacted conditions the microbial concentrations at the site increased markedly. The *E. coli* concentrations exceeded 550 cfu per 100 ml on all occasions. The enterococci concentrations exceeded the *E. coli* values following rainfall on two occasions. This may be due to re-suspension of enterococci from the sediment and inputs from the environment or from sources yet to be characterised such as dog faeces.

Campylobacter were observed under rainfall conditions on all occasions, with a maximum of 150 MPN per 100 ml on the second sampling event. The *Campylobacter* were determined by MBiT pattern analysis to originate from a mix of wildfowl and ruminant sources, with one isolate from a poultry source. As humans are readily infected with *Campylobacter* from herbivore/poultry sources, the presence of these *Campylobacter* MBiT profiles could indicate human or herbivore/poultry sources.

The AC/TC ratio reflected fresh faecal pollution .

Human PCR markers were detected in all three samples. The HumM3 PCR marker was detected on the second sampling occasion, suggesting a recent faecal input. A ruminant source was detected on all three occasions constituting up to 100 % of the faecal pollution. A bovine marker was detected on two occasions, with the sheep marker also identified on two occasions. Wildfowl and canine faecal pollution were detected on each occasion.

The sterols did not provide consistent source identification, with plant, herbivore, and human sources detected on occasion.

Overall Conclusion for Waltham Park

Overall, base flow pollution is characterised by wildfowl faecal inputs. Following rainfall wildfowl and human faecal pollution are present as well as ruminant and canine inputs.

3.1.9 Catherine Street, Heathcote River

Table 18 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Catherine Street. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 - Presentation of Results.

Table 18 Water sampling for Catherine Street, Heathcote River

River		Heathcote					
Site		Catherine Street					
Conditions		Base flow			Rainfall Impacted		
Date Sampled		16/04/15	14/05/15	28/05/15	28/04/15	4/06/15	11/09/15
Bacteria	***E. coli*** (cfu/100ml)	550	900	1700	4500	5850	750
	Enterococci (cfu/100ml)	850	450	450	14050	8400	8150
	Campylobacter (MPN/100ml)	4.3	0.9	2.3	46	240	9.3
	- Species	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*
	- MBiT Typing	002000 400002 002040	406000 440270	002040 104200	002020 406200 446012 446202 407202 402202	460127	044000 401200 042000 002000 040600
	AC/TC Ratio	2.5	0.6	0.6	1.2	0.0	0.0
PCR Markers	**General Bacteria**	VS positive	VS positive	VS positive	VS positive	VS positive	VS positive
	Human - BacH	present	present	present	present	present	present
	Human - BAdo	present	present	present	ND	present	present
	Human - HumM3	ND	ND	present	ND	ND	present
	Ruminant	1 - 10%	ND	up to 10%	up to 50%	10 - 50%	10 - 50%
	Cow	NA	NA	NA	present	ND	ND
	Sheep	ND	NA	ND	present	ND	ND
	Wildfowl - GFD	present	present	present	present	present	present
	Wildfowl - E2	present	present	present	present	present	present
	Canine	ND	ND	present	present	present	present
Faecal Sterols	**Total Sterols** (ng/L)	1975	1753	2305	2602	16711	5496
	Coprostanol (ng/L)	50	59	166	57	394	90
	Faecal	F1+F2	F1+F2	F1+F2	F1+F2	F1+F2	F1+F2
	Human	No	<1	Yes	No	No	No
	Herbivore	No	No	No	No	No	No
	Wildfowl	Yes	Yes	No	No	No	No
	Plant	YES	YES	Yes	YES	YES	YES
Summary		**Human and wildfowl sources**			**Human, ruminant (both cow and sheep), wildfowl, and canine sources**		

NA = not applicable, ND = not detected, VS = very strong, present = positive for that source
Colour code: blue = ruminant; yellow = wildfowl; orange = non-wildfowl; purple = poultry; red = human; brown = canine, and green = plant

Interpretation for Catherine Street

Base flow conditions

Under base flow conditions the numbers of *E. coli* exceeded the acceptable value of 260 cfu per 100 ml on all occasions. C*ampylobacter* was present in low concentrations on all occasions and was found by MBiT subtyping to be sourced from wildfowl, where a source was attributable. Two isolates were from unknown sources. The AC/TC ratio reflected fresh faecal pollution.

PCR markers provided a consistent indication of human faecal pollution, with BacH and BAdo detected in all samples and Hum-M3 on one occasion. Wildfowl PCR markers were detected in all samples, with canine markers present in the final sample. Faecal sterol analysis was dominated by plant sterols, with wildfowl faecal pollution also detected on two occasions and human once, which correlated with PCR markers. Please note, that wildfowl sterol ratios may not be identified if a dominant human or ruminant source is present, because the human or ruminant sterols tend to swamp wildfowl sterols.

Rainfall impacted conditions

Under rainfall impacted conditions the microbial concentrations at the site increased markedly. The *E. coli* concentrations exceeded 550 *E. coli* per 100ml on all occasions. The enterococci concentrations exceeded *E. coli* values in the river on all occasions following rainfall.

Campylobacter were observed under rainfall conditions on all occasions, with a maximum of 240 MPN per 100 ml on the second sampling event. The *Campylobacter* were determined by MBiT pattern analysis to be a mixture of wildfowl and poultry (therefore, a possible human source), with one isolate from an undetermined source. The second rainfall sampling corresponded with a sewage overflow event (M. Bourke, pers.com), hence the strong human signal and elevated *Campylobacter* numbers.

The AC/TC ratio reflected fresh faecal pollution.

Human PCR markers were present in all three samples. The HumM3 PCR marker was detected on the third sampling occasion, suggesting recent faecal input. Only BacH was detected in the first rainfall impacted sampling, which suggest this is not from a human source A ruminant source was apparent on all three occasions, constituting up to 50 % of the faecal pollution. The cow and sheep PCR markers were detected on the second sampling occasion. Wildfowl and canine markers were detected on each occasion.

The sterols were typical of plant sources on all occasions, and had a significant increase in level on two occasions.

Overall Conclusion for Catherine Street

Overall, base flow pollution was characterised by wildfowl and human faecal inputs. Sampling after rainfall identified in addition to wildfowl faecal pollution, an increased incidence of canine and ruminant (both sheep and cow) and also human faecal pollution.

3.2 SEDIMENT SAMPLING RESULTS

3.2.1 Antigua Boatsheds, Avon river

Table 19 below summarises all water sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Antigua Boatsheds. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 19 Sediment sampling results for Antigua Boatsheds, Avon River.

Type		**Sediments**		
Site		**Antigua Boatsheds**		
Conditions		**Base flow**		
Date Sampled		16/04/15	14/05/15	28/05/15
Bacteria	***E. coli*** *cfu/g ww*	110	110	105
	Enterococci cfu/g ww	120	130	40
	Campylobacter MPN/g ww	<0.3	<0.3	<0.3
	AC/TC Ratio	1.7	2.7	2.3
PCR Markers	**General Bacteria**	positive	strong positive	strong positive
	Human - BacH	ND	ND	ND
	Human - BAdo	present	present	present
	Human - HumM3	ND	ND	ND
	Ruminant	ND	ND	ND
	Cow	NA	NA	NA
	Sheep	NA	NA	NA
	Wildfowl - GFD	ND	present	present
	Wildfowl - E2	present	present	present
	Canine	ND	ND	ND
Faecal Sterols	**Total Sterols** (ng/L)	24930	13258	14587
	Coprostanol (ng/L)	180	110	208
	Faecal	F2	No	No
	Human	No	No	No
	Herbivore	<30	No	No
	Wildfowl	Yes	Yes	Yes
	Plant	YES	**YES**	**YES**
Summary		**Wildfowl sources**		

NA = not applicable, ND = not detected, present = positive for that source
Colour code: red = human; blue = ruminant; yellow = wildfowl; brown = canine, and green = plant

Interpretation for Antigua Boatsheds sediments

Sediment analysis only took place under base flow conditions. There are currently no microbial guidelines for acceptable levels of microbes present in sediments at recreational freshwater sites.

The *E. coli* concentrations present in the sediment were stable over the sampling period. Little change was also noted in enterococci concentrations. *Campylobacter* was absent on all occasions and the AC/TC ratio reflects fresh faecal pollution of the sediment.

PCR markers provide an indication of wildfowl faecal pollution, with the duck indicative E2 markers present in all samples. BAdo, one of the human markers was detected on each occasion, but in isolation we would determine that human pollution was not present. This may suggest a non-faecal reservoir of BAdo in sediment. Faecal sterol analysis was dominated by plant and wildfowl sterol ratios.

3.2.2 Kerrs Reach, Avon River

Table 20 below summarises all sediment sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Kerrs Reach. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 20 Sediment sampling results for Kerrs Reach, Avon River

Type		Sediments		
Site		Kerrs Reach		
Conditions		Base flow		
Date Sampled		16/04/15	14/05/15	28/05/15
Bacteria	***E. coli*** *cfu/g ww*	30	65	25
	Enterococci cfu/g ww	<10	<10	<10
	Campylobacter MPN/g ww	<0.3	<0.3	<0.3
	AC/TC Ratio	66.5	3.8	65.0
PCR Markers	**General Bacteria**	ND	S Positive	VS Positive
	Human - BacH	ND	ND	ND
	Human - BAdo	ND	ND	ND
	Human - HumM3	ND	ND	ND
	Ruminant	ND	ND	ND
	Cow	NA	NA	NA
	Sheep	NA	NA	NA
	Wildfowl - GFD	ND	ND	present
	Wildfowl - E2	ND	present	present
	Canine	ND	ND	ND
Faecal Sterols	**Total Sterols** (ng/L)	27736	27628	22798
	Coprostanol (ng/L)	1704	1564	547
	Faecal	F1+F2	F1+F2	No
	Human	Yes	Yes	No
	Herbivore	No	No	No
	Wildfowl	No	Yes	Yes
	Plant	Yes	Yes	YES
Summary		Wildfowl Sources		

NA = not applicable, ND = not detected, S = strong positive; VS = very strong positive; present = positive for that source
Colour code: red = human; blue = ruminant; yellow = wildfowl; brown = canine, and green = plant

Interpretation for Kerrs Reach sediments

The *E. coli* concentrations were low in the sediment over the sampling period, while the enterococci concentrations were below the limit of detection (<10/g) on all occasions. *Campylobacter* was absent on all occasions and the AC/TC ratio reflects an aged pollution source in the river sediment on two occasions and fresher faecal pollution on the middle sampling.

No faecal pollution was detected on the first sampling occasion using PCR. On the two later sampling occasions, PCR markers provide an indication of wildfowl faecal pollution with the duck indicative E2 markers present in both samples. Faecal sterol levels were very high,

which when analysed, suggested a human sterol profile in the first two samples. In the absence of PCR markers and elevated *E. coli* levels this would suggest a historical signal. All samples contained plant sterols and two samples wildfowl indicative sterol profiles.

3.2.3 Owles Terrace, Avon River

Table 21 below summarises all sedimentsampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Owles Terrace. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 21 Sediment sampling for Owles Terrace, Avon River

Type		Sediments		
Site		Owles Tce		
Conditions		Base flow		
Date Sampled		16/04/15	14/05/15	28/05/15
Bacteria	***E. coli*** *cfu/g ww*	85	15	<10
	Enterococci cfu/g ww	65	110	10
	Campylobacter MPN/g ww	<0.3	<0.3	<0.3
	AC/TC Ratio	2.8	2.3	11.5
PCR Markers	**General Bacteria**	positive	positive	weak positive
	Human - BacH	ND	ND	ND
	Human - BAdo	ND	ND	ND
	Human- HumM3	ND	ND	ND
	Ruminant	ND	ND	ND
	Cow	NA	NA	NA
	Sheep	NA	NA	NA
	Wildfowl - GFD	present	ND	ND
	Wildfowl - E2	present	present	ND
	Canine	ND	present	ND
Faecal Sterols	**Total Sterols** (ng/L)	13176	3679	17887
	Coprostanol (ng/L)	350	160	522
	Faecal	No	F1	F1+F2
	Human	No	(Yes)	>1
	Herbivore	No	No	No
	Wildfowl	Yes	Yes	Yes
	Plant	YES	YES	YES
Summary		Wildfowl dominant		

NA = not applicable, ND = not detected, present = positive for that source
Colour code: red = human; blue = ruminant; yellow = wildfowl; brown = canine, and green = plant

Interpretation for Owles Terrace sediments

The *E. coli* concentrations present in the sediment decreased over the sampling period to below the limit of detection (<10 /g). The enterococci concentrations were all low, with the highest recorded 110 cfu per g. *Campylobacter* was absent on all occasions and the AC/TC ratio reflects fresh faecal pollution in the sediment on two occasions and slightly older only on the final sampling.

No faecal pollution was detected on the final sampling occasion using PCR. On the two earlier sampling occasions, PCR markers provided an indication of wildfowl faecal pollution with the duck indicative E2 markers present in both samples. Canine faecal pollution was detected in the sediment on one occasion. Human faecal pollution was not present in the sediment samples using PCR markers, but was detected by faecal sterols on one occasion. This may reflect the ability of sterols to persist longer in the environment compared with DNA which degrades. Faecal sterol analysis was dominated by plant and wildfowl sterols.

3.2.4 Ferniehurst Street, Heathcote River

Table 22 below summarises all sediment sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Ferniehurst Street. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 22 Sediment sampling for Ferniehurst Street, Heathcote River

Type		**Sediments**		
Site		**Ferniehurst Street**		
Conditions		**Base flow**		
Date Sampled		16/04/15	14/05/15	28/05/15
Bacteria	***E. coli*** *cfu/g ww*	125	170	65
	Enterococci cfu/g ww	135	120	40
	Campylobacter MPN/g ww	<0.3	0.4	1.5
	- Species	ND	*C.jejuni*	*C.jejuni*
	- MBiT Typing		002200	002000 002020
	AC/TC Ratio	13.3	4.6	2.3
PCR Markers	**General Bacteria**	VS positive	VS positive	VS positive
	Human - BacH	ND	ND	ND
	Human - BAdo	present	ND	present
	Human- HumM3	ND	ND	ND
	Ruminant	ND	ND	ND
	Cow	NA	NA	NA
	Sheep	NA	NA	NA
	Wildfowl - GFD	present	present	present
	Wildfowl - E2	present	present	present
	Canine	ND	ND	ND
Faecal Sterols	**Total Sterols** (ng/L)	19503	17718	15290
	Coprostanol (ng/L)	78	82	99
	Faecal	No	No	No
	Human	No	No	No
	Herbivore	<30	<30	No
	Wildfowl	Yes	Yes	Yes
	Plant	**YES**	**YES**	**YES**
Summary		**Wildfowl sources**		

NA = not applicable, ND = not detected, VS = very strong positive; present = positive for that source
Colour code: red = human; blue = ruminant; yellow = wildfowl; brown = canine, and green = plant

Interpretation for Ferniehurst Street sediments

The *E. coli* and enterococci concentrations present in the sediment were at similar concentrations over the sampling period. *Campylobacter* was absent in two samples, with 0.4 cfu *Campylobacter* per g detected in the final sample. MBiT analysis of the *Campylobacter* pattern determined it to be from wildfowl. The AC/TC ratio reflects a mixture of a relatively fresh faecal pollution to an aged source in the sediment.

PCR markers provide an indication of wildfowl faecal pollution with both duck indicative markers E2 and GFD detected in all samples. Human specific BAdo was detected in two samples, which in the absence of other human specific markers does not indicate human faecal pollution. Faecal sterol analysis was dominated by plant and wildfowl sterol ratios.

3.2.5 Bowenvale Avenue, Heathcote River

Table 23 below summarises all sediment sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Bowenvale Avenue. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 23 Sediment sampling results for Bowenvale Avenue, Heathcote River

Type		**Sediments**		
Site		**Bowenvale Ave**		
Conditions		**Base flow**		
Date Sampled		16/04/15	14/05/15	28/05/15
Bacteria	***E. coli*** *cfu/g ww*	165	215	20
	Enterococci cfu/g ww	110	85	10
	Campylobacter MPN/g ww	<0.3	<0.3	<0.3
	AC/TC Ratio	5.9	6.0	3.2
PCR Markers	**General Bacteria**	S Positive	VS Positive	Positive
	Human - BacH	ND	ND	ND
	Human - BAdo	present	ND	ND
	Human- HumM3	ND	ND	ND
	Ruminant	ND	ND	ND
	Cow	NA	NA	ND
	Sheep	NA	NA	ND
	Wildfowl - GFD	present	ND	ND
	Wildfowl - E2	ND	present	ND
	Canine	ND	ND	ND
Faecal Sterols	**Total Sterols** (ng/L)	9375	11784	1218
	Coprostanol (ng/L)	85	82	19
	Faecal	No	No	No
	Human	No	No	No
	Herbivore	<30	No	No
	Wildfowl	Yes	Yes	Yes
	Plant	**YES**	**YES**	YES
Summary		**Wildfowl sources**		

NA = not applicable, ND = not detected, S = strong positive; VS = very strong positive; present = positive for that source
Colour code: red = human; blue = ruminant; yellow = wildfowl; brown = canine, and green = plant

Interpretation for Bowenvale Avenue sediments

The *E. coli* concentrations present in the sediment were relatively low over the sampling period. The enterococci concentrations were all lower than their respective *E. coli* concentrations, with the highest recorded 110 cfu per g. *Campylobacter* was absent on all occasions and the AC/TC ratio, in general, reflected a mixture of fresh and aged faecal sources in the sediment.

PCR markers provide an indication of wildfowl faecal pollution with the duck indicative E2 marker present in one sample and GFD in another. Ruminant faecal pollution was detected in none of the samples. Human specific BAdo was detected in one sample, which in the absence of other human specific markers does not indicate human faecal pollution, and may instead be the result non-specific detection. Faecal sterol analysis was dominated by plant and wildfowl sterols.

3.2.6 Waltham Park, Heathcote River

Table 24 below summarises all sediment sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Waltham Park. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 (Presentation of Results).

Table 24 Sediment sampling for Waltham Park, Heathcote River

Type		**Sediments**		
Site		**Waltham Park**		
Conditions		**Base flow**		
Date Sampled		16/04/15	14/05/15	28/05/15
Bacteria	***E. coli*** *cfu/g ww*	420	1150	155
	Enterococci cfu/g ww	160	250	20
	Campylobacter MPN/g ww	<0.3	<0.3	<0.3
	AC/TC Ratio	2.5	0.5	3.3
PCR Markers	**General Bacteria**	VS positive	VS positive	positive
	Human - BacH	present	present	ND
	Human - BAdo	present	present	present
	Human- HumM3	ND	ND	ND
	Ruminant	ND	ND	ND
	Cow	NA	NA	NA
	Sheep	NA	NA	NA
	Wildfowl - GFD	present	ND	present
	Wildfowl - E2	present	ND	ND
	Canine	ND	ND	ND
Faecal Sterols	**Total Sterols** (ng/L)	13489	9031	8858
	Coprostanol (ng/L)	276	278	192
	Faecal	F1	F1+F2	F1
	Human	>1	>1	>1
	Herbivore	No	No	No
	Wildfowl	Yes	No	Yes
	Plant	**YES**	**YES**	**YES**
Summary		**Wildfowl and human sources**		

NA = not applicable, ND = not detected, S = strong positive; VS = very strong positive; present = positive for that source
Colour code: red = human; blue = ruminant; yellow = wildfowl; brown = canine, and green = plant

Interpretation of Waltham Park sediments

The *E. coli* and enterococci concentrations present in the sediment at this site were the highest seen over all sediment samplings. The enterococci concentrations were lower than their respective *E. coli* levels on all occasions. *Campylobacter* was absent on all occasions and the AC/TC ratio reflects very fresh to relatively fresh faecal pollution in the sediment.

PCR markers provide an indication of wildfowl faecal pollution with the duck indicative markers E2 present in one sample and GFD in two samples. Human specific BAdo was detected in all samples, while BacH was detected twice. In the samples containing both BAdo and BacH a human pollution source is likely and supported by elevated *E. coli* and low AC/TC ratios. Faecal sterol analysis was dominated by plant and wildfowl sterol ratios.

3.2.7 Catherine Street, Heathcote River

Table 25 below summarises all sediment sampling results regarding bacterial load, PCR marker and faecal sterols from the sampling site Catherine Street. For further interpretation of criteria used for decision making on source detection refer to Section 2.3 Presentation of Results.

Table 25 Sediment sampling for Catherine Street, Heathcote River

Type		**Sediments**		
Site		**Catherine St**		
Conditions		**Base flow**		
Date Sampled		16/04/15	14/05/15	28/05/15
Bacteria	***E. coli*** *cfu/g ww*	50	180	30
	Enterococci cfu/g ww	135	80	25
	Campylobacter MPN/g ww	<0.3	<0.3	<0.3
	AC/TC Ratio	13.7	1.3	7.3
PCR Markers	**General Bacteria**	S Positive	VS Positive	S Positive
	Human - BacH	ND	ND	ND
	Human - BAdo	present	ND	present
	Human- HumM3	ND	ND	ND
	Ruminant	ND	ND	ND
	Cow	NA	NA	NA
	Sheep	NA	NA	NA
	Wildfowl - GFD	present	ND	ND
	Wildfowl - E2	present	present	present
	Canine	ND	ND	ND
Faecal Sterols	**Total Sterols** (ng/L)	15812	6132	17997
	Coprostanol (ng/L)	49	162	144
	Faecal	F1	F1+F2	F2
	Human	>1	>1	No
	Herbivore	No	No	No
	Wildfowl	(Yes)	Yes	Yes
	Plant	**YES**	**YES**	**YES**
Summary		**Wildfowl sources**		

NA = not applicable, ND = not detected, S = strong positive; VS = very strong positive; present = positive for that source
Colour code: red = human; blue = ruminant; yellow = wildfowl; brown = canine, and green = plant

Interpretation for Catherine Street sediments

The *E. coli* concentrations present in the sediment were relatively low over the sampling period. The enterococci concentrations were lower than their respective *E. coli* levels on two occasions. *Campylobacter* was absent on all occasions and the AC/TC ratio reflects a mixture of relatively aged and fresh faecal pollution in the sediment.

PCR markers provide an indication of wildfowl faecal pollution with the duck indicative E2 markers present in all samples and GFD in the first only. Human specific BAdo was detected in two samples, which in the absence of other human specific markers does not indicate human faecal pollution. Faecal sterol analysis was dominated by plant and wildfowl sterol ratios.

3.3 QUANTITATIVE COMPARISON OF PCR MARKERS IN WATER

In the previous sections PCR marker results are largely presented as presence/absence, despite the underlying assay being based on the number of copies of each DNA target present in a sample. Each marker has different prevalence and different levels of sensitivity, so presenting quantitative data can be somewhat misleading when comparing between markers.

However within an assay, a comparison of copies of each marker is more appropriate. The following figures present the number of copies of each PCR marker/100 mL.

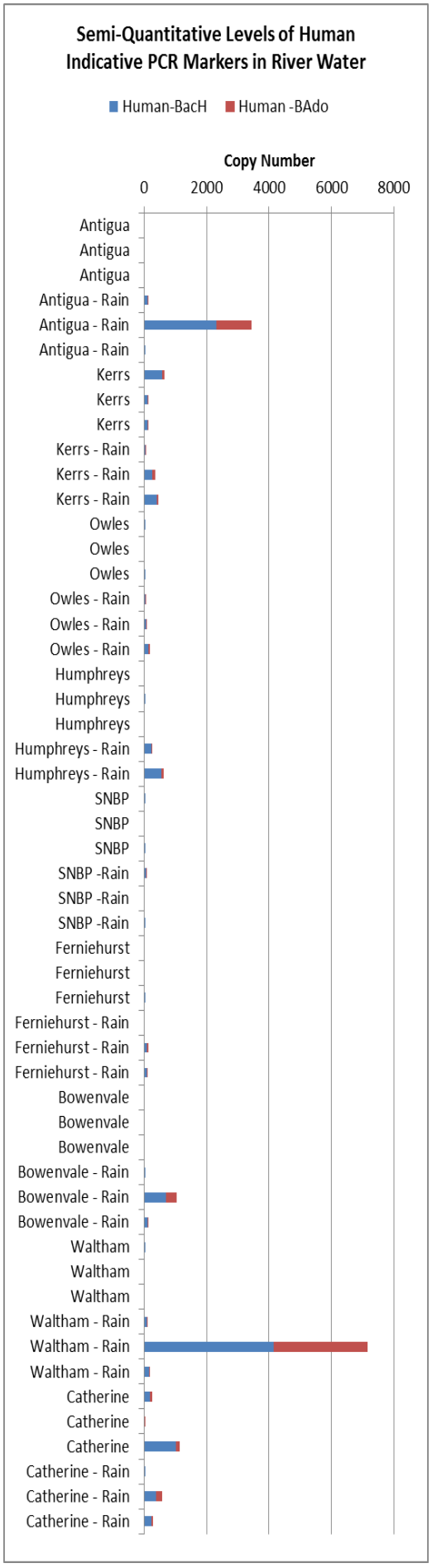
For each site the graphs present copy numbers of:

- Human indicative BacH and BAdo assays
- Human indicative Hum-M3 assay
- Wildfowl E2 and GFD assays
- Canine assay
- BacR ruminant assay
- Cow and Sheep assays (subset of ruminant assay).

Please note the different scales in graphs.

It is appropriate to compare results within a graph.

Comparing quantitative levels between graphs may not be appropriate.


Semi-Quantitative Levels of Human Indicative PCR Markers in River Water
Human-BacH
Human -BAdo
Copy Number
0
2000
4000
6000
8000
Antigua
Antigua
Antigua
Antigua - Rain
Antigua - Rain
Antigua - Rain
Kerrs
Kerrs
Kerrs
Kerrs - Rain
Kerrs - Rain
Kerrs - Rain
Owles
Owles
Owles
Owles - Rain
Owles - Rain
Owles - Rain
Humphreys
Humphreys
Humphreys
Humphreys - Rain
Humphreys - Rain
SNBP
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SNBP -Rain
SNBP -Rain
SNBP -Rain
Ferniehurst
Ferniehurst
Ferniehurst
Ferniehurst - Rain
Ferniehurst - Rain
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Bowenvale
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Bowenvale - Rain
Bowenvale - Rain
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Waltham
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Waltham - Rain
Catherine
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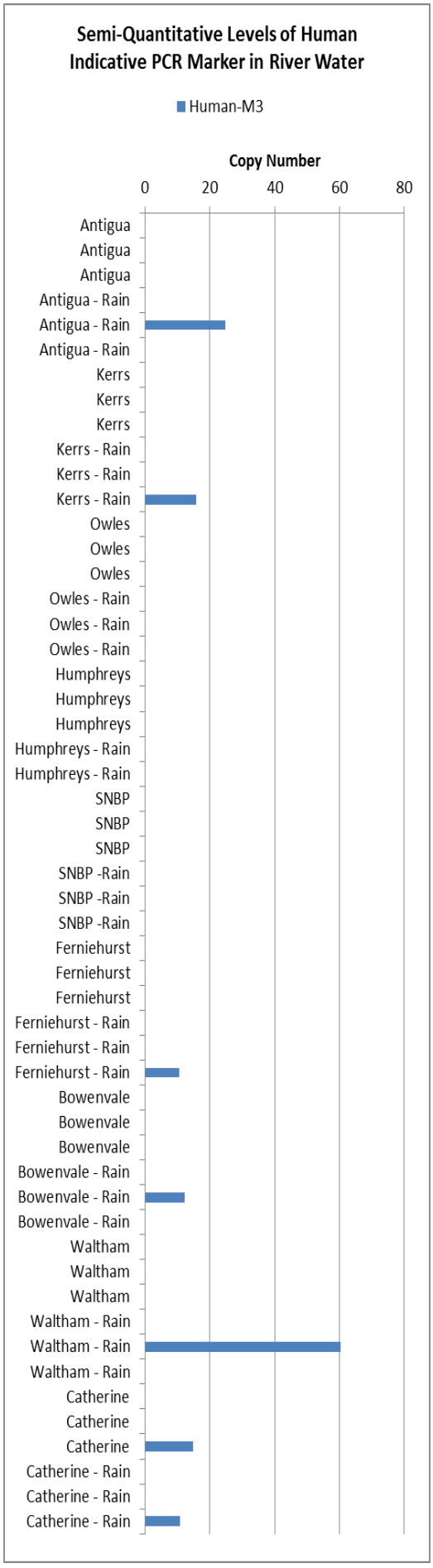
Catherine - Rain
Catherine - Rain
Catherine - Rain


Semi-Quantitative Levels of Human Indicative PCR Marker in River Water
Human-M3
Copy Number
0
20
40
60
80
Antigua
Antigua
Antigua
Antigua - Rain
Antigua - Rain
Antigua - Rain
Kerrs
Kerrs
Kerrs
Kerrs - Rain
Kerrs - Rain
Kerrs - Rain
Owles
Owles
Owles
Owles - Rain
Owles - Rain
Owles - Rain
Humphreys
Humphreys
Humphreys
Humphreys - Rain
Humphreys - Rain
SNBP
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SNBP
SNBP -Rain
SNBP -Rain
SNBP -Rain
Ferniehurst
Ferniehurst
Ferniehurst
Ferniehurst - Rain
Ferniehurst - Rain
Ferniehurst - Rain
Bowenvale
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Bowenvale - Rain
Bowenvale - Rain
Bowenvale - Rain
Waltham
Waltham
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Waltham - Rain
Waltham - Rain
Catherine
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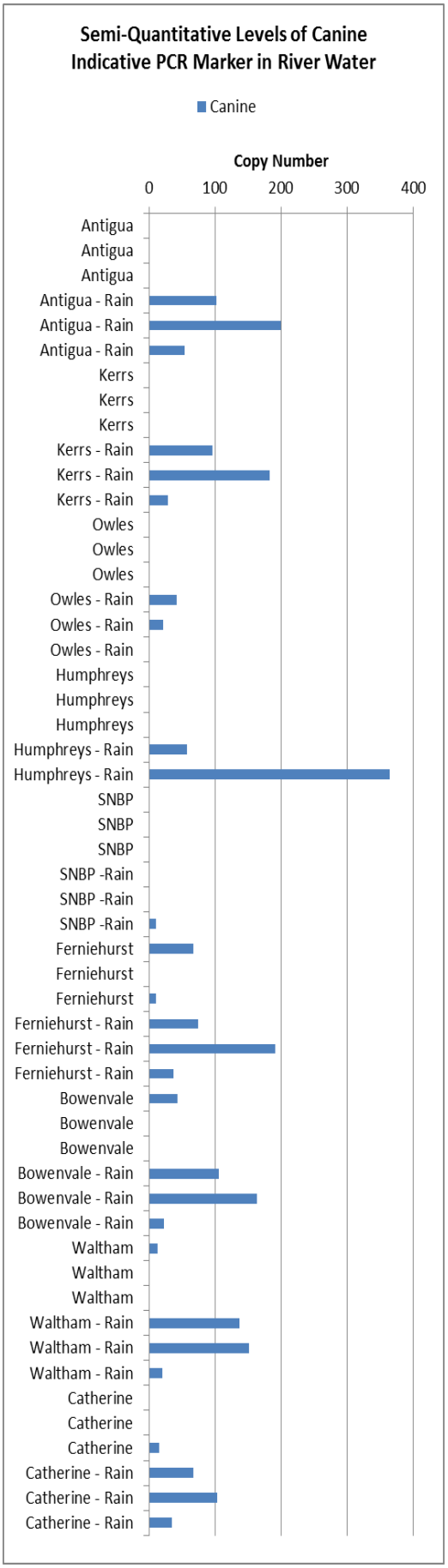
Catherine - Rain
Catherine - Rain
Catherine - Rain


Semi-Quantitative Levels of Canine Indicative PCR Marker in River Water
Canine
Copy Number
0
100
200
300
400
Antigua
Antigua
Antigua
Antigua - Rain
Antigua - Rain
Antigua - Rain
Kerrs
Kerrs
Kerrs
Kerrs - Rain
Kerrs - Rain
Kerrs - Rain
Owles
Owles
Owles
Owles - Rain
Owles - Rain
Owles - Rain
Humphreys
Humphreys
Humphreys
Humphreys - Rain
Humphreys - Rain
SNBP
SNBP
SNBP
SNBP -Rain
SNBP -Rain
SNBP -Rain
Ferniehurst
Ferniehurst
Ferniehurst
Ferniehurst - Rain
Ferniehurst - Rain
Ferniehurst - Rain
Bowenvale
Bowenvale
Bowenvale
Bowenvale - Rain
Bowenvale - Rain
Bowenvale - Rain
Waltham
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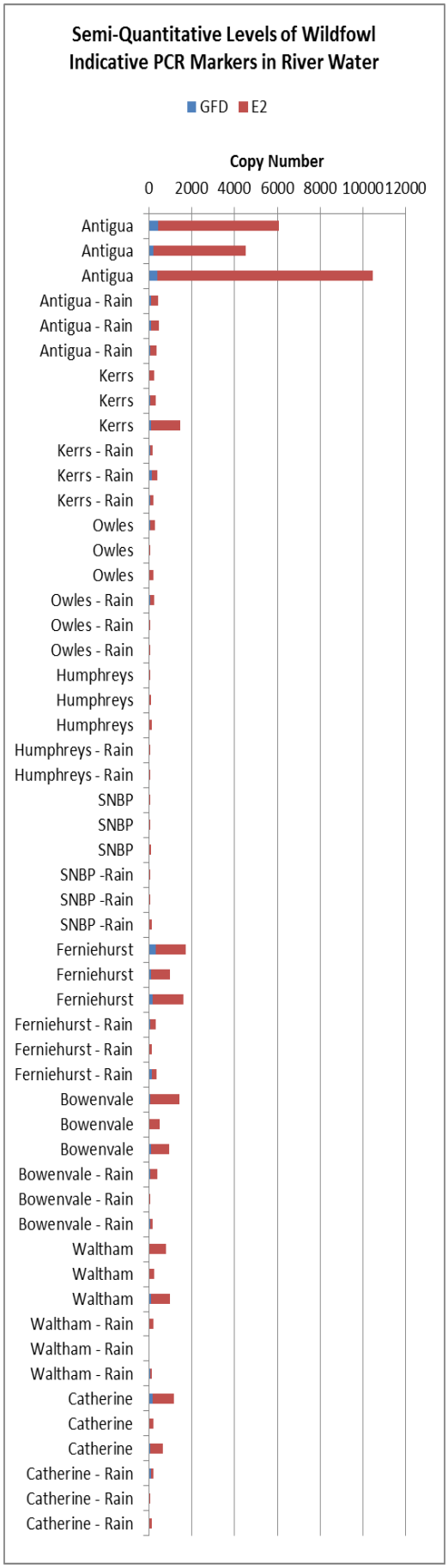
Catherine - Rain


Semi-Quantitative Levels of Wildfowl Indicative PCR Markers in River Water
GFD
E2
Copy Number
0
2000
4000
6000
8000
10000
12000
Antigua
Antigua
Antigua
Antigua - Rain
Antigua - Rain
Antigua - Rain
Kerrs
Kerrs
Kerrs
Kerrs - Rain
Kerrs - Rain
Kerrs - Rain
Owles
Owles
Owles
Owles - Rain
Owles - Rain
Owles - Rain
Humphreys
Humphreys
Humphreys
Humphreys - Rain
Humphreys - Rain
SNBP
SNBP
SNBP
SNBP -Rain
SNBP -Rain
SNBP -Rain
Ferniehurst
Ferniehurst
Ferniehurst
Ferniehurst - Rain
Ferniehurst - Rain
Ferniehurst - Rain
Bowenvale
Bowenvale
Bowenvale
Bowenvale - Rain
Bowenvale - Rain
Bowenvale - Rain
Waltham
Waltham
Waltham
Waltham - Rain
Waltham - Rain
Waltham - Rain
Catherine
Catherine
Catherine
Catherine - Rain
Catherine - Rain
Catherine - Rain


Semi-Quantitative Levels of Ruminant Indicative PCR Markers in River Water
Ruminant
Copy Number
0 1000 2000 3000 4000 5000 6000
Antigua
Antigua
Antigua
Antigua - Rain
Antigua - Rain
Antigua - Rain
Kerrs
Kerrs
Kerrs
Kerrs - Rain
Kerrs - Rain
Kerrs - Rain
Owles
Owles
Owles
Owles - Rain
Owles - Rain
Owles - Rain
Humphreys
Humphreys
Humphreys
Humphreys - Rain
Humphreys - Rain
SNBP
SNBP
SNBP
SNBP -Rain
SNBP -Rain
SNBP -Rain
Ferniehurst
Ferniehurst
Ferniehurst
Ferniehurst - Rain
Ferniehurst - Rain
Ferniehurst - Rain
Bowenvale
Bowenvale
Bowenvale
Bowenvale - Rain
Bowenvale - Rain
Bowenvale - Rain
Waltham
Waltham
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Catherine
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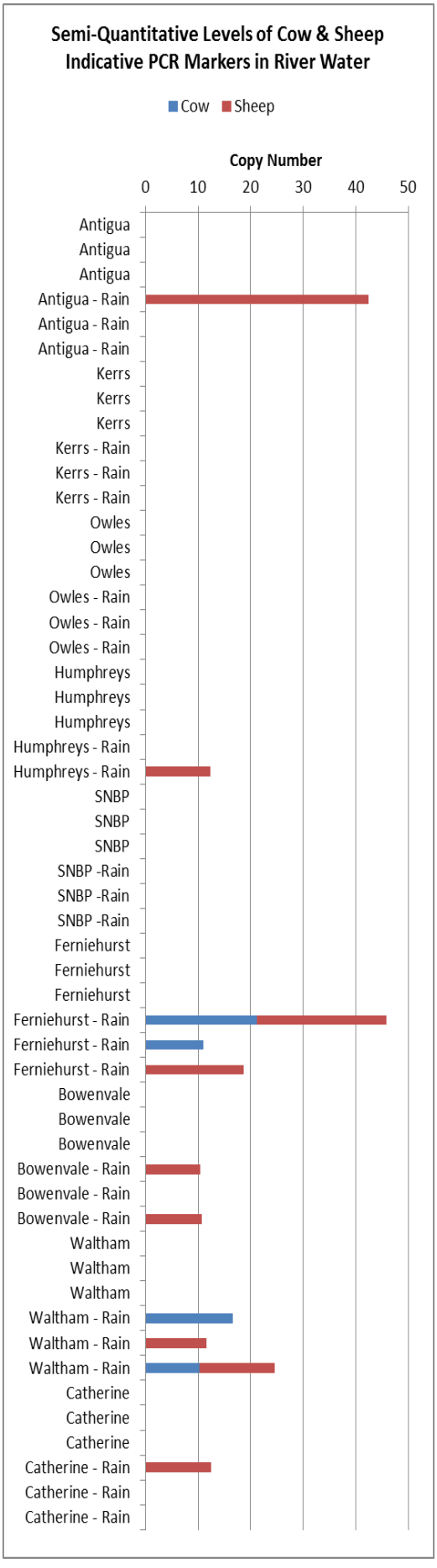
Semi-Quantitative Levels of Cow & Sheep Indicative PCR Markers in River Water
Cow
Sheep
Copy Number
0 10 20 30 40 50
Antigua
Antigua
Antigua
Antigua - Rain
Antigua - Rain
Antigua - Rain
Kerrs
Kerrs
Kerrs
Kerrs - Rain
Kerrs - Rain
Kerrs - Rain
Owles
Owles
Owles
Owles - Rain
Owles - Rain
Owles - Rain
Humphreys
Humphreys
Humphreys
Humphreys - Rain
Humphreys - Rain
SNBP
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SNBP -Rain
SNBP -Rain
SNBP -Rain
Ferniehurst
Ferniehurst
Ferniehurst
Ferniehurst - Rain
Ferniehurst - Rain
Ferniehurst - Rain
Bowenvale
Bowenvale
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Bowenvale - Rain
Bowenvale - Rain
Bowenvale - Rain
Waltham
Waltham
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Waltham - Rain
Waltham - Rain
Waltham - Rain
Catherine
Catherine
Catherine
Catherine - Rain
Catherine - Rain
Catherine - Rain

3.3.1 Interpretation of quantitative comparison of PCR markers in water

Human indicative markers were observed at all sites following rainfall events, with the Antigua Boatsheds and Waltham sites recording the highest levels. Outside rain events, Kerrs Reach and Catherine sites were the only sites to have human indicators in the absence of rainfall.

The canine faecal marker followed a similar pattern to human indicative markers, with this marker recorded at all the sites sampled following rainfall.

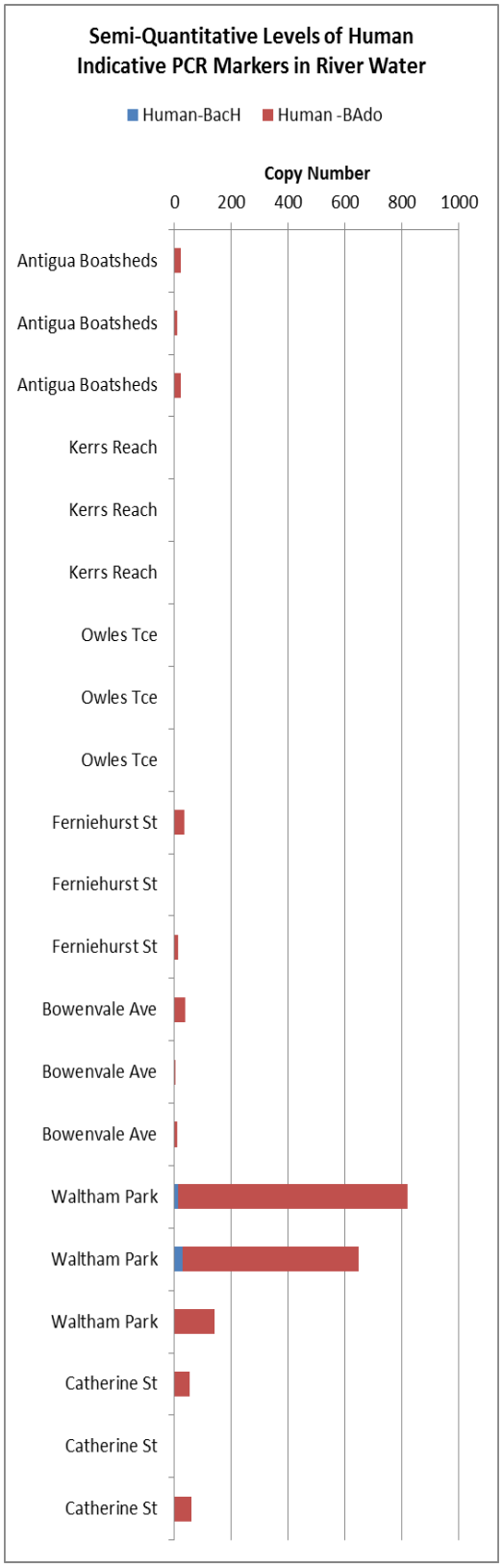
In contrast, the wildfowl markers and in particular the E2 marker, which is more commonly associated with ducks, had higher concentrations detected at base flow compared with rainfall impacted events. While this maker was recorded at all sites during both base flow and rainfall sampling, the Antigua Boatsheds site was clearly the most impacted by wildfowl faecal pollution.

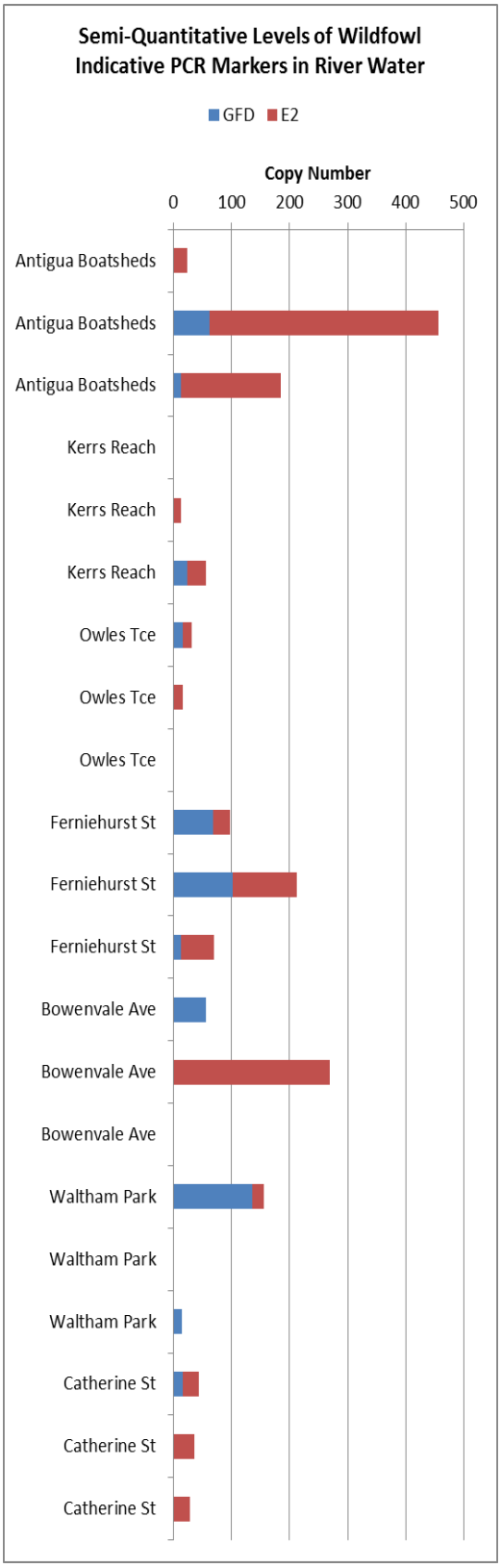
A very low level of ruminant faecal pollution is present in the Avon River under rainfall conditions, which appears to be attributed primarily to sheep. However it should be noted that the sheep marker is more sensitive than the cow marker, which means at these very low levels of ruminant pollution, cows faecal pollution can't be exclude.

In contrast, the Heathcote River has much higher levels of the ruminant marker following rainfall, with very low levels during base flow. Both cow and sheep markers were detected in Heathcote River samples.

3.4 QUANTITATIVE COMPARISON OF PCR MARKERS IN SEDIMENTS

Human (at most sites) and wildfowl (at all sites) markers were detected in sediment samples, as well as canine at Owles Terrace in the Avon River. Levels were much lower than in water and are presented in the following graphs. Of the human markers only BAdo was present at appreciable levels in Waltham samples. The waterfowl markers were highest in concentration at the Antigua Boatsheds, Ferniehurst Street and Bowenvale Avenue sites.


Semi-Quantitative Levels of Human Indicative PCR Markers in River Water
Human-BacH
Human -BAdo
Copy Number
0
200
400
600
800
1000
Antigua Boatsheds
Antigua Boatsheds
Antigua Boatsheds
Kerrs Reach
Kerrs Reach
Kerrs Reach
Owles Tce
Owles Tce
Owles Tce
Ferniehurst St
Ferniehurst St
Ferniehurst St
Bowenvale Ave
Bowenvale Ave
Bowenvale Ave
Waltham Park
Waltham Park
Waltham Park
Catherine St
Catherine St
Catherine St


Semi-Quantitative Levels of Wildfowl Indicative PCR Markers in River Water
GFD
E2
Copy Number
0
100
200
300
400
500
Antigua Boatsheds
Antigua Boatsheds
Antigua Boatsheds
Kerrs Reach
Kerrs Reach
Kerrs Reach
Owles Tce
Owles Tce
Owles Tce
Ferniehurst St
Ferniehurst St
Ferniehurst St
Bowenvale Ave
Bowenvale Ave
Bowenvale Ave
Waltham Park
Waltham Park
Waltham Park
Catherine St
Catherine St
Catherine St


Semi-Quantitative Levels of Wildfowl
Indicative PCR Markers in River Water
Canine
Copy Number
0
5
10
15
20
25
Antigua Boatsheds
Antigua Boatsheds
Kerrs Reach
Kerrs Reach
Kerrs Reach
Owles Tce
Owles Tce
Owles Tce
Ferniehurst St
Ferniehurst St
Ferniehurst St
Bowenvale Ave
Bowenvale Ave
Bowenvale Ave
Waltham Park
Waltham Park
Waltham Park
Catherine St
Catherine St
Catherine St

4. DISCUSSION

In this study, six water samples were collected from each of the three sites in the Avon River, four in the Heathcote River, and two in the estuary between 16th April 2015 and 11th September 2015. Three samples were collected from each site during base flow conditions, and three were collected following rainfall events of approximately 10mm or more of rain in the previous 24 hours, with elevated flow conditions.

4.1 WATER SAMPLING

E. coli concentrations in the water samples were typically elevated, exceeding recreational water guideline values on a number of occasions during base flow, and after rainfall almost all samples exceeded MfE and MoH (2003) guideline values.

E. coli concentrations were typically lower than enterococci concentrations during base flow, but on occasions following rainfall, enterococci exceeded the concentration of *E. coli.* This could have been due to re-suspension of enterococci from the sediment. However, we found low concentrations of enterococci in sediments, and almost always below the concentration of *E. coli.* Alternatively, the increase may be due to the concentrations of enterococci in faecal sources such as dog faeces which were often detected following rainfall. Dog faeces have not been well characterised for indicator organisms, nor for quantitative levels of many pathogens. A third possibility is that the enterococci reflected an aged faecal source, where enterococci may persist longer than *E. coli.*

AC/TC faecal ageing ratio analysis suggested fresh sources of faeces present in almost all samples, and therefore, did not support aged pollution.

Campylobacter were found in all but one of the collected river water samples, and at levels of up to 240 per 100 ml during base flow and up to 460 per 100 ml following rainfall (Table 26). Speciation and genotyping of the *Campylobacter* suggested that base flow isolates were consistent with a wildfowl source. Following rainfall, wildfowl genotypes were still present, but supplemented by isolates more likely to come from ruminant or poultry sources. As isolates from ruminant and poultry sources are frequently found among human clinical cases, on the basis of *Campylobacter* genotyping these *Campylobacter* could also be from human sewage. Wildfowl genotypes are rarely found among human clinical cases. This may be because they are less able to cause disease in humans, or perhaps because the exposure route to humans occurs less often.

Table 26 Maximum concentrations of *Campylobacter* (MPN per 100 ml) and the source of the isolates in water samples

Area	River	Base flow	Rainfall
Avon	Antigua Boatsheds	4.3 (Wildfowl)	15 (Wildfowl & other)
	Kerrs Reach	4.3 (Wildfowl)	93 (Wildfowl & other)
	Owles Tce	21 (Wildfowl)	24 (Wildfowl)
Heathcote	Ferniehurst St	240 (Ruminant, wildfowl & other)	460 (Ruminant, wildfowl & other)
	Bowenvale Ave	150 (Wildfowl)	93 (Ruminant, wildfowl)
	Waltham Park	46 (Wildfowl)	150 (Wildfowl, ruminant, poultry)
	Catherine St	4.3 (Wildfowl)	240 (Wildfowl, poultry)
Estuary	South New Brighton Park	4.3 (Wildfowl)	24 (Wildfowl)
	Humphreys Drive	ND	9.3 (Wildfowl, other)

Additional FST analysis was undertaken using molecular markers and faecal sterols (Table 27). These supported wildfowl as a dominant faecal source during base flow at all the sites sampled, although at Kerrs Reach and Catherine Street, human sources were detected.

Following rainfall, human sources were detected at much higher frequency at all of the sites sampled, along with canine sources at all of the sites sampled, and in Heathcote River, ruminant sources of faecal pollution were recorded at many sites.

A semi-quantitative analysis of PCR markers would indicate strongest human signals at the Waltham and Antigua Boatsheds sites after rainfall. The canine signal is also driven by rainfall events. In contrast, the highest levels of wildfowl markers were observed during base flow, with the highest levels observed at the Antigua Boatsheds. In the Heathcote River, the highest levels of wildfowl markers were observed at the Ferniehurst site.

The highest levels of ruminant markers were observed after rainfall in the Heathcote River samples. Very low levels of ruminant marker were detected in the Avon River sites, with the levels decreasing as sites moved downstream. This suggests ruminant inputs further upstream from sampling sites.

Table 27 Summary of faecal source tracking

Area/River	Location	Base flow	Rainfall
Avon	Antigua Boatsheds	Wildfowl dominant source	Wildfowl, human, canine sources
	Kerrs Reach	Human and wildfowl sources	Human, wildfowl, canine sources
	Owles Tce	Wildfowl dominant sources	Wildfowl, human, canine sources
Heathcote	Ferniehurst St	Wildfowl, canine sources	Human, ruminant (both cow and sheep), wildfowl, and canine sources
	Bowenvale Ave	Wildfowl dominant sources	Human, ruminant (both cow and sheep), wildfowl, and canine sources
	Waltham Park	Wildfowl sources	Human, ruminant (both cow and sheep), wildfowl, and canine sources
	Catherine St	Human and wildfowl sources	Human, ruminant (both cow and sheep), wildfowl, and canine sources
Estuary	South New Brighton Park	Wildfowl dominant sources	Wildfowl dominant sources
	Humphreys Drive	Wildfowl dominant sources	Human, wildfowl, canine sources

4.2 SEDIMENT SAMPLES

Analysis of sediment samples indicated relatively low levels of *E. coli* in most samples, and *Campylobacter* were detected only once (Table 28). The sediment sampling methodology also samples overlying water, so it is possible that microorganisms in the sediment are only transiently present. Certainly this set of sampling suggests that the sediments are not a large reservoir of microorganisms. Faecal source tracking suggested primarily wildfowl sources although on occasional samples human and canine sources were indicated. Wildfowl sources are likely to be continuous input, whereas other sources may be more transient. The human sterol signature in one sample did not correlate with other markers, and suggested a historical source of faecal pollution as sterols are more resistant to degradation once incorporated into sediments. The BAdo marker in sediments may suggest that the *Bifidobacterium adolescentis* is able to persist or even grow in the sediments. In either case avoidance of sediment resuspension before sampling for sterols or PCR markers is recommended.

Table 28 Summary of sediment analysis

River	Site	*E. coli* cfu/100 ml	*Campylobacter* MPN/100 ml	FST
Avon	Antigua Boatsheds	109	<0.3	Wildfowl
	Kerrs Reach	40	<0.3	Wildfowl
	Owles Tce	35	<0.3	Wildfowl
Heathcote	Ferniehurst St	120	Up to 1.5, wildfowl	Wildfowl
	Bowenvale Ave	133	<0.3	Wildfowl
	Waltham Park	575	<0.3	Wildfowl, Human
	Catherine St	84	<0.3	Wildfowl

4.3 COMPARISONS WITH PREVIOUS STUDIES

Ongoing monitoring of the Avon and Heathcote Rivers is undertaken by the Christchurch City Council. Since 2008 there has been no statistical change in *E. coli* levels in river samples, with a number of elevated levels of *E. coli* over many years (Margetts & Marshall, 2015).

Comparison of the faecal source tracking results suggests that in the Avon River the situation has now returned to a similar situation to that prior to the earthquakes with wildfowl dominated sources during base flow, and the input of canine sources during rainfall events. In the Heathcote River, no previous faecal source tracking results are available for comparison.

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