

Papakura Stream Faecal Source Investigation

August 2015

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Papakura Stream Faecal Source Investigation

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Executive summary

The Papakura Stream flows from Brookby to the Manukau Harbour. The upper rural Papakura Stream has one of the highest *E. coli* levels in Auckland, and reducing the contamination in this catchment is a priority for the Environmental Services Unit (ESU). Before we could identify and prioritise remedial actions to improve water quality, we needed to identify the sources of high bacterial contamination.

Four sub-catchments investigated for *E.coli* and bacterial sources

We investigated a total of seven sites in the rural Papakura Stream catchment above Porchester Road. The seven sites were located in four sub-catchments of the Papakura Stream on unnamed tributaries between Alfriston and Brookby. Water samples were collected for *E. coli* and Microbial Source Tracking (MST) using PCR markers. Samples were collected at each site on four occasions in dry and wet weather from January to April 2014.

Livestock exclusion would reduce widespread ruminant bacterial contamination

Ruminant bacterial contamination from cows and sheep was the most frequently identified bacterial source in this investigation. A lack of riparian fencing to exclude livestock is likely the cause of the widespread ruminant bacterial contamination in the Papakura Stream. Increasing the extent of riparian fencing in the rural Papakura Stream catchment may therefore greatly reduce *E. coli* levels. All riparian fencing and planting should be documented and used to report on the success of the Waterway Protection Fund and other interventions in the Papakura Stream catchment.

Sources of human bacteria need to be isolated

Human bacterial contamination was found in one of the Papakura Stream tributaries investigated. This hints at one or more failing on-site wastewater systems in the area, but further work is needed to isolate the cause. Microbial Source Tracking (MST) methods should be used rather than just *E. coli* to isolate the sources of human bacterial contamination in this sub-catchment.

Birds were not a significant contributor

Ducks or other bird species directly accessing a tributary were a likely source of avian bacterial contamination identified on one occasion. However, birds were not a significant contributor of bacterial contamination in the upper Papakura catchment in this investigation and no specific management is recommended.

Livestock exclusion, on-site wastewater system inspections and education are needed

A number of management recommendations have been made as a result of the findings of this investigation. These include site visits to properties without stock excluded from their waterways and a review of effluent management on dairy farms in the catchment to reduce widespread ruminant faecal contamination. In addition, On-Site Wastewater System (OSWS) inspections and education would be beneficial to minimise the risk of human faecal contamination entering waterways in the catchment.

Table of contents

Executive summary	v
1.0 Introduction	1
1.1 Papakura Stream begins north-east of Brookby	1
1.2 Papakura Stream has one of the highest <i>E. coli</i> levels in Auckland.....	1
1.3 Papakura Stream is a priority for the Environmental Services Unit.....	2
1.4 We needed to identify all sources of high <i>E. coli</i>	2
2.0 Methodology	4
2.1 Site selection and location.....	4
2.2 Sample collection and analysis	5
3.0 Results.....	7
3.1 Rainfall and river-flow low apart from one event in mid-April.....	7
3.2 Site specific <i>E. coli</i> levels	8
3.3 Ruminant bacterial source found at every site	9
4.0 Discussion.....	12
4.1 Livestock exclusion may reduce ruminant bacterial sources.....	12
4.2 Land management in the sub-catchment with highest <i>E. coli</i> levels	12
4.3 The sources of human bacteria need to be identified	12
4.4 No correlation between identification of bacterial source and <i>E. coli</i>	13
4.5 <i>E. coli</i> levels were lower than in previous investigations.....	13
4.6 Fewer bacterial sources identified in this investigation.....	13
4.7 Bacterial sources in other rural sub-catchments are likely to be similar	14
4.8 Monitoring and evaluation is needed to measure success	14
4.9 The limitations of this study were low sample size and PCR method	15
5.0 Conclusions	16
6.0 Recommendations.....	17
7.0 References	18
Appendix A Papakura Faecal Source Testing (Van Duivenboden, 2008 unpublished)....	19
Appendix B Aerial map showing location of sampling sites in 2014 investigation	26
Appendix C Investigation data.....	27
Appendix D ESR Bacteria Source Report	29
Appendix E Sampling sites and bacterial source	34

List of figures

Figure 1-1 Map of the Papakura Stream catchment in the Auckland region.....	1
Figure 2-1 Map showing the location of the sites sampled in this investigation.	4
Figure 3-1 Mean daily flow of Papakura Stream at Great South Rd.	7
Figure 3-2 Total daily rainfall at Longford Park.....	7
Figure 3-3 Boxplot showing the distribution of <i>E. coli</i> data	8

List of tables

Table 2-1 Location of Papakura Stream tributary sampling sites	5
Table 3-1 Total rainfall at Longford Park, 48 and 72 hours prior to sample collection	8
Table 3-3 Sources of bacterial contamination at Papakura Stream tributary sampling sites .	10

1.0 Introduction

1.1 Papakura Stream begins north-east of Brookby

The Papakura Stream has its headwaters in native bush north-east of Brookby and flows through rural Alfriston and urban areas including Takanini (Figure 1-1). The Papakura Stream then discharges to the Pahurehure Inlet of the Manukau Harbour. Land use in the upper catchment is predominately rural, with mostly sheep and beef farms, some equestrian, lifestyle properties and three dairy farms. The lower Papakura Stream catchment is urban residential with some commercial and industrial areas in Takanini.

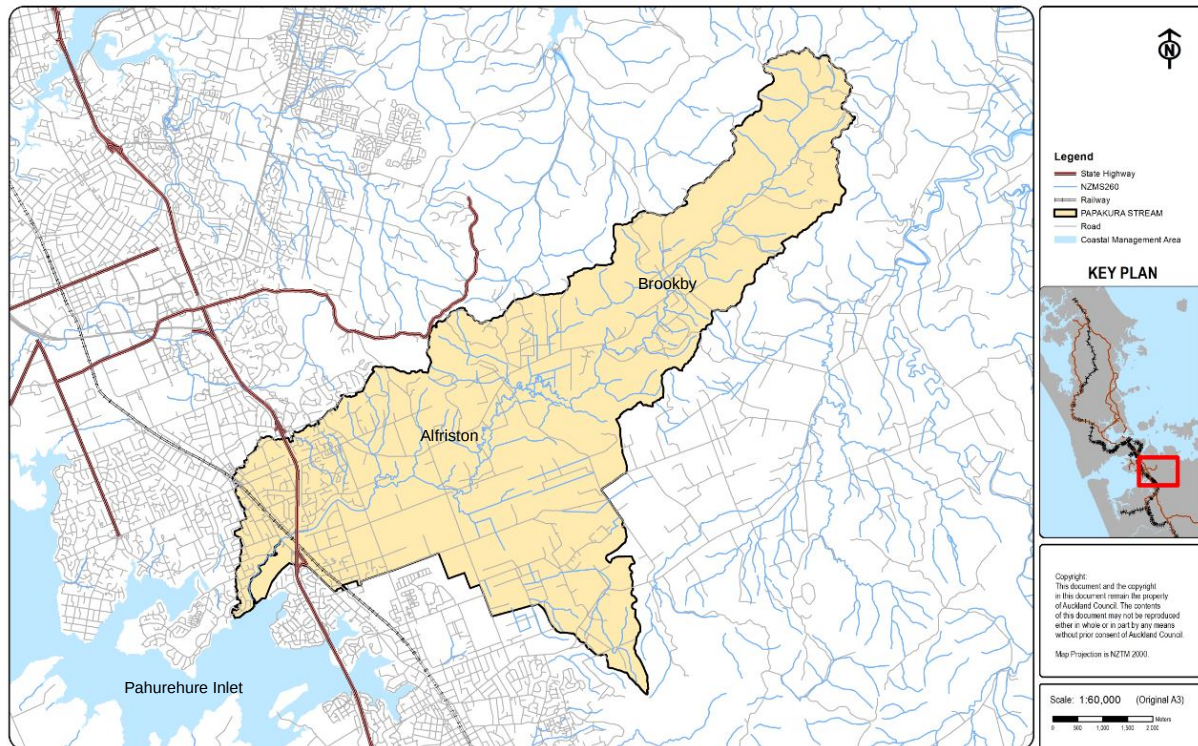


Figure 1-1 Map showing the location of the Papakura Stream catchment in the Auckland region. The Papakura catchment originates north-east of Brookby and flows to the Pahurehure Inlet.

1.2 Papakura Stream has one of the highest *E. coli* levels in Auckland

Bacterial contamination in the rural Papakura Stream catchment is high, and has been for some time (Scarsbrook, 2007). Long-term Auckland Council water quality monitoring at the Porchester Road site from 2004 to 2013 shows that *E. coli* concentrations in the Papakura Stream are stable. However, *E. coli* concentrations are consistently high (median 1,225 cfu/100ml) (LAWA, 2015), below the *National Policy Statement for Freshwater Management 2014* national bottom line (1,000 *E. coli*/100ml) (MfE, 2014) and above national guidelines for primary contact recreation (550 *E. coli*/100ml) (MfE, 2003).

The Porchester Road site recorded the highest *E. coli* (65,000 cfu/100ml) out of 36 monitored Auckland Council sites in 2013 (Lockie and Neale, 2014). Nationally, levels of *E. coli* at the Porchester Road site rank among the worst 25% of rural sites in New Zealand (LAWA, 2015).

A previous investigation in the Papakura Stream found high *E. coli* levels above recreational water quality guidelines at all 42 sites sampled during wet weather, showing that bacterial contamination in the catchment is widespread (Bull et al., 2008). A range of water quality variables including *E. coli* were sampled during one dry and two wet weather events (>10mm rainfall in previous 24 hours) in 2008. The highest *E. coli* levels were found during wet weather with sites 4 (Porchester Road), 7, 16 and 19 recording the highest *E. coli* in both dry and wet weather (Bull et al., 2008)

Microbial Source Tracking (MST) at five of these sites identified a range of sources of bacterial contamination including human, ruminant, equine, canine, possum and avian (Van Duivenboden, 2008). A total of 6 sites were monitored by Van Duivenboden (2008) to identify sources of bacterial contamination including one dry and one wet weather event (>10mm rainfall in previous 24 hours). The highest *E. coli* levels were found during wet weather with sites 16 and 19 recording the highest *E. coli* in both dry and wet weather (Van Duivenboden, 2008). Further details about this investigation can be found in Appendix A.

Although the Papakura Stream is not widely used for swimming or other contact recreation activities, the stream flows to the Pahurehure Inlet and Manukau Harbour where people regularly fish, swim, and collect shellfish. Improvements in water quality in the Papakura Stream will therefore safeguard public health and recreation in these downstream receiving environments.

1.3 Papakura Stream is a priority for the Environmental Services Unit

Auckland Council's Environmental Services Unit (ESU) has recently published an Environmental Services Operational Strategy (ESOS) 2015-2018 (ESU, 2014). The ESOS defines the unit's intended environmental outcomes that will be used to align work programmes. One of the priority environmental outcomes stated in the ESOS is 'healthy waterways and harbours.' This outcome supports the need to improve water quality in the Papakura Stream.

The current 'Sustainable Catchments' ESU programme is working with landowners and community groups in eight priority Auckland stream catchments to improve water quality. The Papakura Stream is a focus catchment for this programme and a number of activities are occurring. One example is the Waterway Protection Fund that is available to landowners to support livestock exclusion fencing and riparian planting to improve water quality in the Papakura Stream and neighbouring Ngakaroa Stream catchments.

A previous study by the Research Investigations and Monitoring Unit (RIMU) in 2012 recorded the extent of riparian fencing in the rural Papakura Stream following a standard methodology used in other Auckland studies (Neale et al., 2009). While this study did not include an assessment of farming practices, it did identify the need for livestock exclusion fencing and riparian planting in the upper Papakura Stream catchment (Mike McMurtry, pers. comm.).

1.4 We needed to identify all sources of high *E. coli*

Even though a water quality investigation in the Papakura Stream had previously identified the sources of bacterial contamination at some sites, we didn't know the sources of contamination at all sites. Before we could identify and prioritise remedial actions to improve water quality, we needed to identify all sources of high bacterial contamination in the Papakura Stream catchment. Resources for this investigation were limited so the sources of bacterial contamination were identified at the rural sites with the highest *E. coli* levels as measured by Bull et al. (2008).

This report is intended for a range of audiences, including Auckland Council staff, the Franklin and Papakura Local Boards and landowners in the rural Papakura Stream catchment.

2.0 Methodology

2.1 Site selection and location

A total of seven sites were selected in the rural Papakura Stream catchment above Porchester Rd (Figure 2-1). Sampling sites were selected because they were identified as having a 'poor' or 'very poor' Water Quality Rating in Bull et al. (2008). The sources of bacterial contamination at these sites were either not identified by Van Duivenboden (2008) (Sites 12 and 25), or further investigation was recommended by Van Duivenboden (2008) to explore the sources of contamination in more detail (Sites 16 and 19). Thus, we investigated sites with high *E. coli* levels, for which a source had not been identified or where further investigation was required.

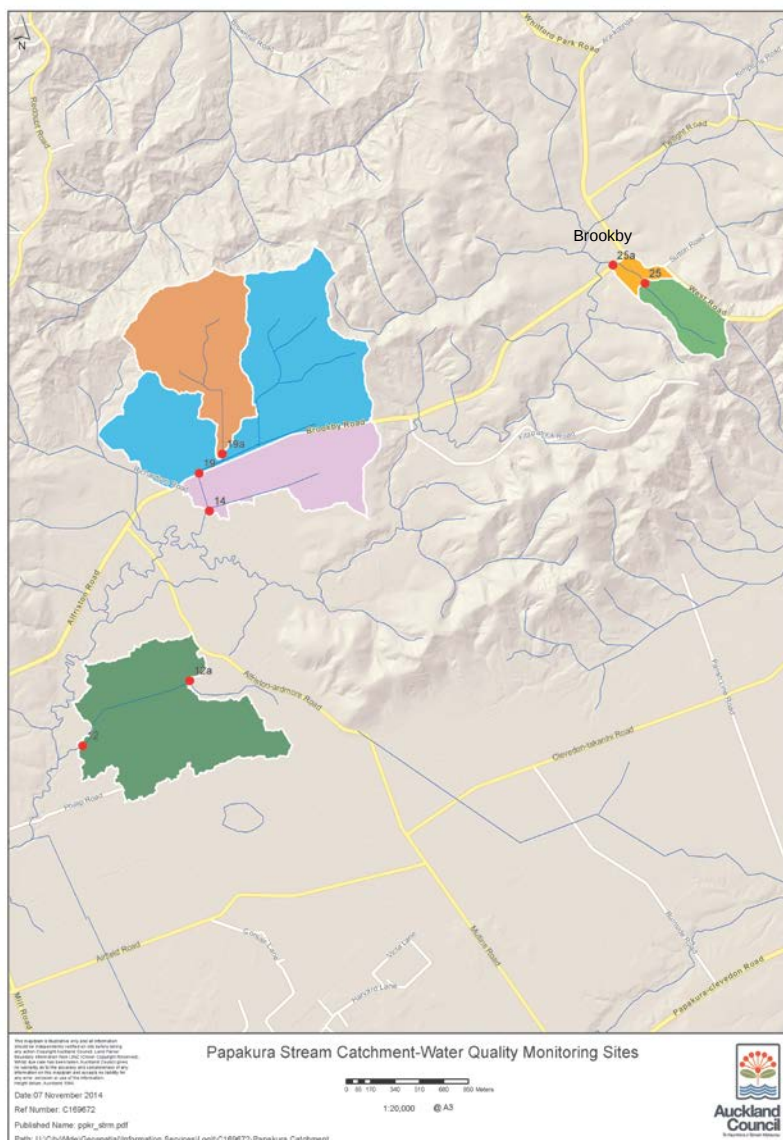


Figure 2-1 Map showing the location of the sites sampled in this investigation.

The coloured areas represent the sub-catchments above each site. Note that the sub-catchment above site 12a is not able to be displayed due to the low lying topography of this area. Also note that the site 19 sub-catchment also incorporates the area above site 19a. Site numbering is consistent with Bull et al. (2008). Sites with an 'a' or 'b' were added as additional sites to improve spatial resolution.

The seven sites were located in four sub-catchments of the Papakura Stream on unnamed tributaries between Alfriston and Brookby. Originally, nine sites were selected but two of these were dry and were not able to be sampled. Site numbering is consistent with Bull et al. (2008) and an 'a' or 'b' was added to denote a new site in the same sub-catchment. Site location details are also presented in Table 2-1 and an aerial map of the sampling locations is presented in Appendix B.

Table 2-1 Location of Papakura Stream tributary sampling sites

Sub-catchment	Location	Site Number ¹	Location (NZTM)	
			Easting	Northing
1	Alfriston Rd	12	1773721	5901320
1	Alfriston Rd	12a	1774466	5901774
2	Brookby Rd	14	1774596	5902966
2	Brookby Rd	16b	Dry – not sampled	Dry – not sampled
3	Brookby Rd	19	1774532	5903216
3	Brookby Rd	19a	1774713	5903293
4	Twilight Rd	25	1777638	5904537
4	Twilight Rd	25a	1777414	5904663
4	Twilight Rd	25b	Dry – not sampled	Dry – not sampled

¹ Site numbering is consistent with Bull et al. (2008). Sites with an 'a' or 'b' were added as additional sites to improve spatial resolution

2.2 Sample collection and analysis

Water samples were collected at each site on four occasions from January to April 2014. Sampling was carried out in dry conditions as recommended by Van Duivenboden (2008) on three of the four sampling occasions. The fourth sampling occasion was completed during a rainfall event to give an indication of the sources of bacterial contamination at the sites during wet weather.

We analysed all samples for *E. coli* and faecal bacteria sources. The *E. coli* samples were collected in sterile 100ml bottles and 2l bottles were used for the bacteria source samples. The samples were stored at less than 4°C and couriered to Aqualab NZ for analysis within 24 hours of sample collection. Field measurements were also taken for water temperature, conductivity and dissolved oxygen using a YSI Pro2030 multi-parameter meter.

The *E. coli* samples were analysed using the Colilert-quantitray method, consistent with Bull et al. (2008) and Van Duivenboden (2008) and are reported as Most Probable Number (MPN) per 100ml. All samples greater than the laboratory detection limit of 10 MPN/100ml *E.coli* were filtered for bacteria source analysis, known as Microbial Source Tracking (MST).

The Polymerase Chain Reaction (PCR) type of MST analysis was used in this investigation as it detects bacterial contamination from a wide range of sources using DNA markers, including ruminant (cows, sheep, goat, deer), equine (horse), canine (dog), avian (bird), possum, human and a range of other animal sources.

The bacteria source samples in this investigation were passed through a 0.45µm filter by Aqualab NZ and the filters were frozen. Once all water sampling was complete, the frozen filters were couriered to the Environmental Science and Research (ESR) laboratory in Christchurch for bacteria source analysis.

Bacteria sources were identified using Polymerase Chain Reaction (PCR) and compared to an existing DNA library. A total of seven PCR markers were analysed based on the likely sources of bacterial contamination in the catchment. The PCR markers included ruminant (BacR), canine (DogBac), avian (GFD), equine (Horse), two human markers (BacH and BiADO) and a general marker (GenBac).

Ruminant results are reported as a percentage of the ruminant marker relative to the general marker in fresh ruminant faeces. Therefore, samples reported as 50 per cent or 100 per cent ruminant should be interpreted as an entirely ruminant source. Samples reported between one and 50 per cent are more difficult to interpret. These samples can be entirely ruminant with a proportion of aged ruminant faecal material, or can be a mix of ruminant and other animal faecal sources.

Results for all other animal sources can only be reported as present or absent. This is because the analysis for determining specific percentage contribution has only been developed for the ruminant PCR marker at this stage (Brent Gilpin, pers. comm.).

3.0 Results

3.1 Rainfall and river-flow low apart from one event in mid-April

River flows were low at the Papakura Stream at Great South Rd flow-monitoring site (lower Papakura catchment) for the majority of the sampling period (Figure 3-1). There were periods of sustained low flow (minimum of 34l/s) from mid-February to mid-March. The flow peaked at 1.417m³/s in mid-April after a 60mm rainfall event recorded at the Longford Park site on 18 April 2014 (Figure 3-2).

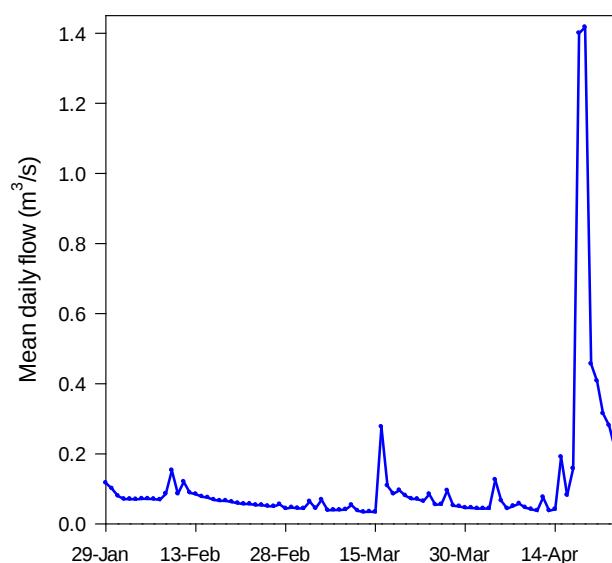


Figure 3-1 Mean daily flow of Papakura Stream at Great South Rd.

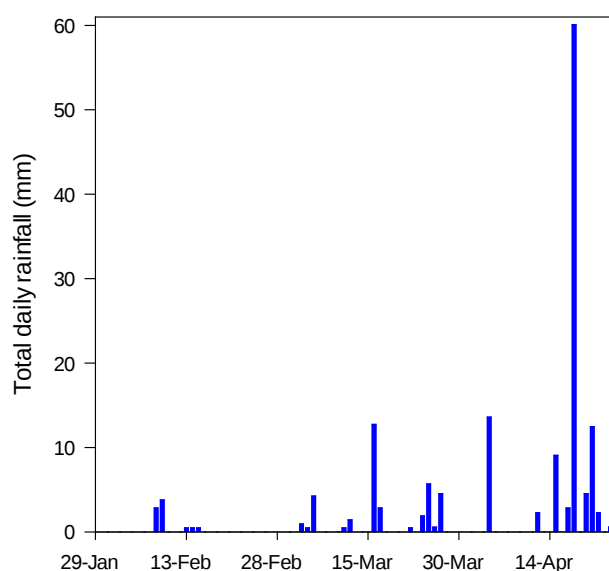


Figure 3-2 Total daily rainfall at Longford Park

Three of the four water quality samples were collected during dry weather on 31 January, 12 March and 10 April. Total rainfall in the 72 hours prior to sampling was 0.0, 1.9 and 0.0mm, respectively (Table 3-1). The fourth sampling run was conducted four days after a 60mm

rainfall event and with 14.7 and 19.3mm of rain recorded in the previous 48 and 72 hours, respectively.

Table 3-1 Total rainfall at Longford Park, 48 and 72 hours prior to water quality sample collection

Sampling date (2014)	Rainfall in previous 48 hours (mm)	Rainfall in previous 72 hours (mm)
31 January	0.0	0.0
12 March	1.9	1.9
10 April	0.0	0.0
22 April	14.7	19.3

3.2 Site specific *E. coli* levels

The concentrations of *E. coli* varied substantially between sites, ranging from a median of 35 MPN/100ml at site 12a to 2,000 MPN/100ml at Site 19a (Figure 3-3). The lowest *E. coli* levels recorded were less than the laboratory detection limit of 10 MPN/100ml at sites 12a and 25. The highest *E. coli* level recorded was 4,600 MPN/100ml at site 19a, below the national bottom line of 1,000 *E. coli*/100ml (MfE, 2014). Site-specific *E. coli* results are described below and all raw data can be found in Appendix C.

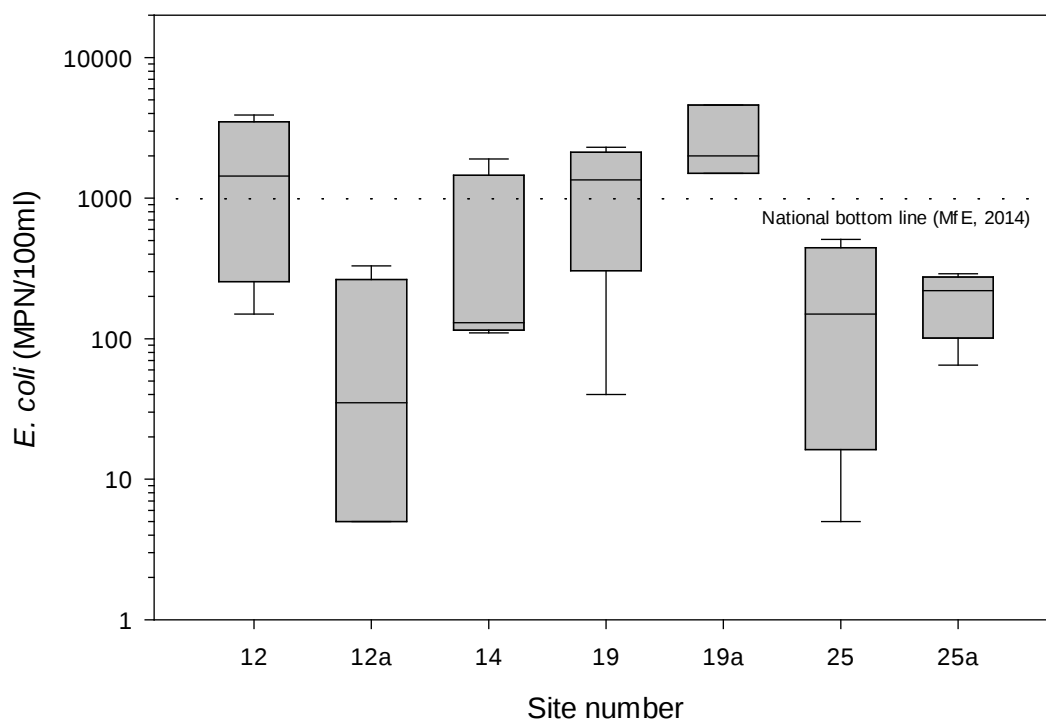


Figure 3-3 Boxplot showing the distribution of *E. coli* data for each site from Jan to Apr 2014. Boxes represent the interquartile range, the midline represents the median value and the upper and lower error bars represent the highest and lowest values, respectively.

3.2.1 Site 12 *E.coli* levels were higher than Site 12a

Site 12 had the greatest variation in *E. coli* levels over the sampling period, ranging from 150 to 3,900 *E. coli* MPN/100ml (Figure 3-3). *E. coli* was below the national bottom line of 1,000

E. coli/100ml on two of the four sampling occasions (MfE, 2014). The highest *E. coli* level recorded at this site (3,900 MPN/100ml) was during wet weather.

Site 12a had low levels of *E. coli* over the sampling period, ranging from <10 to 330 *E. coli* MPN/100ml (Figure 3-3). All samples were better than the national bottom line of 1,000 *E. coli*/100ml (MfE, 2014). The highest *E. coli* level recorded at this site (330 MPN/100ml) was during dry weather.

3.2.2 The highest *E. coli* level at site 14 was in dry weather

At site 14, *E. coli* ranged from 110 to 1,900 MPN/100ml over the sampling period (Figure 3). *E. coli* was below the national bottom line of 1,000 *E. coli*/100ml on just one occasion (MfE, 2014). The highest *E. coli* level recorded at this site (1,900 MPN/100ml) was during dry weather.

3.2.3 Highest *E. coli* level recorded in this investigation was at Site 19a

Site 19 had an *E. coli* range of 40 to 2,300 MPN/100ml over the sampling period (Figure 3-3). *E. coli* was below the national bottom line of 1,000 *E. coli*/100ml on three of the four sampling occasions (MfE, 2014). The highest *E. coli* level recorded at this site (2,300 MPN/100ml) was during dry weather.

Site 19a had an *E. coli* range of 1,500 to 4,600 MPN/100ml over the sampling period (Figure 3-3). *E. coli* was below the national bottom line of 1,000 *E. coli*/100ml on all three sampling occasions (this site was dry and not sampled on one occasion) (MfE, 2014). This site recorded the highest *E. coli* level of all sites (4,600 MPN/100ml) and this was during dry weather.

3.2.4 *E. coli* levels were below bathing guidelines at Sites 25 and 25a

Site 25 had low levels of *E. coli* over the sampling period, ranging from <10 to 510 MPN/100ml (Figure 3-3). All samples were better than the national bottom line of 1,000 *E. coli*/100ml (MfE, 2014). The highest *E. coli* level recorded at this site (510 MPN/100ml) was during wet weather.

Site 25a had the least variation in *E. coli* over the sampling period, with levels ranging from 65 to 290 MPN/100ml (Figure 3-3). All samples were better than the national bottom line of 1,000 *E. coli*/100ml (MfE, 2014). This site recorded the lowest *E. coli* maximum of all sites (290 MPN/100ml) and this was during dry weather.

3.3 Ruminant bacterial source found at every site

A total of three different bacterial sources were identified in the upper Papakura Stream during this investigation: ruminant, human and avian (Table 3-3). The source of bacterial contamination was not able to be identified at some sites on some sampling occasions, most likely due to degraded, aged or partially-treated sources. The most prevalent source of *E. coli* was ruminant, which was found at all sites on at least one occasion. Site-specific bacteria source results are described below and the detailed ESR laboratory report can be found in Appendix D.

Table 3-2 Sources of bacterial contamination at Papakura Stream tributary sampling sites

Site number	Sampling date	<i>E. coli</i> (MPN/100ml)	General marker	Bacteria source ²
12	31/01/14	150	strong positive	Unidentified
12	12/03/14	2,300	strong positive	Ruminant 100%
12	10/04/14	570	very weak positive	Unidentified
12	22/04/14	3,900	very strong positive	Ruminant 100%
12a	10/04/14	330	very strong positive	Unidentified
12a	22/04/14	65	strong positive	Ruminant 10%
14	31/01/14	1,900	strong positive	Unidentified
14	12/03/14	130	weak positive	Unidentified
14	10/04/14	130	strong positive	Unidentified
14	22/04/14	110	positive	Ruminant 100%
19	31/01/14	40	strong positive	Unidentified
19	12/03/14	2,300	strong positive	Unidentified
19	10/04/14	1,600	very strong positive	Unidentified
19	22/04/14	1,100	very strong positive	Human
				Ruminant 10-50%
19a	31/01/14	1,500	positive	Unidentified
19a	10/04/14	4,600	positive	Unidentified
19a	22/04/14	2,000	positive	Ruminant 100%
25	31/01/14	50	positive	Unidentified
25	12/03/14	250	positive	Unidentified
25	22/04/14	510	strong positive	Ruminant <10%
25a	31/01/14	290	very strong positive	Ruminant <10%
25a	12/03/14	65	very strong positive	Unidentified
25a	10/04/14	210	very strong positive	Avian
25a	22/04/14	230	very strong positive	Ruminant 1-10%

² Ruminant bacterial sources are expressed as a percentage of the general marker for fresh sources.

3.3.1 Ruminant contamination was sole source at sites 12 and 12a

A fresh ruminant source was found to comprise 100 per cent of the general bacterial marker at Site 12 for two of the four sampling occasions (Table 3-3). A bacteria source was not able to be identified in the remaining two samples. The general bacterial marker ranged from very weak positive to very strong positive at this site.

A fresh ruminant source was found to comprise 10 per cent of the general bacterial marker at Site 12a on one of the two sampling occasions which were above 10 *E. coli* MPN/100ml (Table 3-3). A bacteria source was not able to be identified in the remaining sample. The general bacterial marker ranged from strong positive to very strong positive at this site.

3.3.2 Ruminant source identified once at site 14

A fresh ruminant source was found to comprise 100 per cent of the general bacterial marker at Site 14 on one of the four sampling occasions (Table 3-3). A bacteria source was not able to be identified in the remaining three samples. The general bacterial marker ranged from weak positive to strong positive at this site.

3.3.3 Human and ruminant sources at sites 19 and 19a

Human and ruminant bacterial sources were identified at Site 19 on one of the four sampling occasions. A fresh ruminant source comprised 10-50 per cent of the general bacteria marker, with a human bacterial source also identified (Table 3-3). A bacteria source was not able to be identified in the remaining three samples. The general bacterial marker ranged from strong positive to very strong positive at this site.

A fresh ruminant source was found to comprise 100 per cent of the bacterial contamination at Site 19a on one of the three sampling occasions (this site was dry and not sampled on one occasion) (Table 3-3). A bacteria source was not able to be identified in the remaining two samples. The general bacterial marker was positive at this site.

3.3.4 Ruminant and avian sources at sites 25 and 25a

A fresh ruminant source comprised <10 per cent of the bacterial contamination at Site 25 on one of the three sampling occasions (this site had an *E. coli* level which was <10 MPN/100ml on one occasion and could not be analysed for bacterial sources) (Table 3-3). A bacteria source was not able to be identified in the remaining two samples. The general bacterial marker ranged from positive to strong positive at this site.

A fresh ruminant source comprised <10 per cent and 1-10 per cent of the bacterial contamination at Site 25a on two of the four sampling occasions (Table 3-3). An avian source was identified at this site on one occasion and a bacteria source was not able to be identified in the remaining sample. The general bacterial marker was very strong positive at this site.

4.0 Discussion

4.1 Livestock exclusion may reduce ruminant bacterial sources

A ruminant source of bacterial contamination was identified at all sites on at least one occasion. A ruminant source was identified at every site during the one-off wet weather sampling event. Possible causes of this include direct livestock access to waterways, overland flow of contaminated water or poor dairy effluent management. Dairy farms which supply milk to Fonterra are required to have riparian fencing in place; however, it would be useful to confirm this for the three dairy farms in the upper Papakura catchment.

The Bull et al (2008) study found stock access was available at approximately 90 per cent of 32 sites studied. Further, stock access by cattle and some sheep and horses was recorded by Bull et al (2008). Stock access was observed in this 2014 investigation by cattle and horses. These results show that riparian fencing to physically exclude livestock is likely to reduce faecal contamination in these waterways. Vegetated riparian buffers established on properties with ruminant livestock (sheep, beef, dairy and goats) would assist in reducing bacterial contamination in the Papakura Stream.

Two sites showed a ruminant bacterial source during dry weather (Sites 12 and 25a). Direct stock access was observed in both of these sub-catchments on at least one occasion during the 2014 investigation, with dairy cows observed in the site 12 sub-catchment and cattle in the site 25a sub-catchment. Riparian fencing alone would greatly reduce the bacterial contamination in these two sub-catchments during dry weather. It is recommended that the Waterway Protection Fund prioritises livestock exclusion fencing in the Site 12 and 25a sub-catchments.

Good riparian management with a 10m set-back has been shown to reduce *E. coli* concentrations in New Zealand streams, with modelled simulations predicting a reduction in *E. coli* concentrations between three and 82 per cent (Collins and Rutherford, 2004). Riparian buffer widths between one and 10m can remove sediment-associated faecal microbes entering streams, with the width and efficacy dependent on land slope, soil drainage, stocking rates and magnitude of rainfall events (NIWA, 2006).

4.2 Land management in the sub-catchment with highest *E. coli* levels

Site 19a recorded the highest *E. coli* level of all monitored sites over the investigation period from January to April 2014 (4,600 MPN/100ml). Site 19a also had the highest median *E. coli* (2,000 MPN/100ml) showing that bacterial contamination is consistently high in this sub-catchment. Although a fresh ruminant bacterial source was identified at site 19a on one occasion, no sources were identified on the other two occasions indicating that aged, degraded or partially-treated bacterial contamination from unknown sources are also present.

It is recommended that one property above site 19a (a dairy farm) is inspected to determine the cause/s of ruminant bacterial contamination on this property. An assessment of farm management practices would also be useful to identify improvements which could be made on the other two dairy farms in the wider Papakura Stream catchment.

4.3 The sources of human bacteria need to be identified

Human bacterial contamination was identified once during this investigation, at Site 19 during wet weather. The most likely cause of human bacterial contamination in rural areas is failing on-site wastewater systems (septic tanks). Further investigation is needed to identify the

source or sources of human bacterial contamination in the sub-catchment above site 19. It is recommended that these property owners (approximately five properties) are contacted and their on-site wastewater systems inspected.

A limitation of the PCR bacterial source method is that sources cannot always be identified if they are aged, degraded or partially treated, such as by a septic tank. So while human bacterial contamination was not identified at other sites, we cannot rule out that 'unidentified' sources at other sites could be an aged or partially treated human source, especially where a 'very strong positive' general marker and high *E. coli* are found. An additional investigation would be required to determine if human bacterial sources are present at other sites.

4.4 No correlation between identification of bacterial source and *E. coli*

A bacterial source was identified in a sample with *E. coli* as low as 65 MPN/100ml in this investigation. Conversely, no source was identified in a sample with the highest *E. coli* level (4,600 MPN/100ml). This shows that there was no correlation between *E. coli* level and the likelihood of identifying a bacterial source in this investigation.

The PCR method is most suitable for identifying bacterial sources in samples with 'fresh' (recent) effluent. However, a more effective method is needed to identify aged, degraded or partially-treated sources, as this is likely to be the cause of the lack of correlation between *E. coli* and bacterial source identification. Faecal sterol analysis (an existing tool) could be more successful than the PCR method at detecting aged sources (Devane, 2015) and this method may be worth trialling in the future.

4.5 *E. coli* levels were lower than in previous investigations

The *E. coli* levels in this study were lower than in previous studies, showing that the magnitude of *E. coli* contamination is highly variable, ranging from several thousand to several million MPN/100ml. Bull et al. (2008) recorded 2,000,000 and 388,000 MPN/100ml at sites 16 and 19, respectively. The *E. coli* concentrations found in Bull et al. (2008) were up to three orders of magnitude higher than the *E. coli* levels monitored in this investigation during wet weather.

The *E. coli* concentrations from Van Duivenboden (2008) were up to one order of magnitude higher than the *E. coli* levels monitored in this investigation during wet weather, with Van Duivenboden recording up to 19,000 and 35,000 MPN/100ml at sites 16 and 19, respectively.

These previous studies show that *E. coli* levels in the rural Papakura Stream catchment were highest during wet weather. Therefore, management actions should focus on minimising the mobilisation of faecal contamination in wet weather and during the winter months when greatest rainfall is recorded. Potential actions include wide vegetated riparian buffers, careful land application of dairy effluent and regular septic tank maintenance to prevent overflows in wet weather.

4.6 Fewer bacterial sources identified in this investigation

One of the four sub-catchments monitored in the 2014 investigation (site 19) was also investigated by Van Duivenboden (2008), allowing for the comparison of bacterial source results. A total of four bacterial sources were found at site 19 in the 2008 study (human, avian, ruminant, dog), compared to two bacterial sources identified in the 2014 investigation (human, ruminant) (Appendix E). This result may be due to improvements in this sub-

catchment since 2008 or it could be due to the low number of sampling occasions in both investigations (two in 2008, four samples in 2014).

A human source was identified at site 19 in the 2008 and 2014 investigations during wet weather. This suggests persistent on-site wastewater contamination in the site 19 sub-catchment. It is recommended that the source or sources of on-site wastewater contamination be identified and remedied through further water quality investigation or property inspections.

No equine or canine bacterial sources were identified in the 2014 investigation; however, these sources were found at a number of sites in the Van Duivenboden (2008) study. Equine bacterial contamination was identified in the 2008 study at sites 16 and 4 (SOE site) in wet weather. While access by horses was possible above site 14 during the 2014 investigation, animals were not observed directly accessing the tributary. Even though equine bacterial sources were not identified in this investigation, it is recommended that riparian fencing and vegetated buffers be established to reduce the chance of water contamination in the site 14 sub-catchment, especially during wet weather.

Avian bacterial sources were identified at site 25a in the 2014 investigation during dry weather. While avian bacterial contamination was not widespread in this investigation, the 2008 study identified avian bacterial sources at a number of sites including site 4 (Porchester Rd), 6, 7, 16 and 19 (Van Duivenboden, 2008). It was not clear which bird species contributed to the avian bacterial contamination at these sites; however, ducks are a likely source as they were observed by Van Duivenboden (2008) and several duck ponds can be found in the catchment.

4.7 Bacterial sources in other rural sub-catchments are likely to be similar

Bacterial sources were identified at a total of 12 sites from monitoring during the 2014 and Van Duivenboden (2008) investigations. However, Bull et al. (2008) reported exceedances of the 'action' guideline for recreational waters (MfE, 2003) at 29 of the 42 sites monitored during dry weather and at all 42 sites during wet weather.

While 11 of the 42 Bull et al. (2008) sites were located in the urban area downstream of the Porchester Rd site (site 4), further bacterial source investigation in the remaining rural sub-catchments could be undertaken. Alternatively, it could be reasonably assumed that the sources of bacterial contamination in the remaining rural sub-catchments are likely to be similar to the bacterial sources identified in this study (predominately ruminant, with some avian and human sources).

4.8 Monitoring and evaluation is needed to measure success

The Research, Investigations and Monitoring Unit (RIMU) at Auckland Council undertook stream walks in the Papakura Stream catchment in 2012, to identify areas of bank erosion and map the extent of riparian vegetation and fencing (Mike McMurtry pers. comm.) Although this information has not been formally reported, data showing the location and extent of riparian fencing in the catchment could be used as a baseline for measuring the progress of current and future fencing initiatives.

In recent years an Auckland Council Waterway Protection Fund (WPF) has been available to landowners in the Papakura Stream catchment. The WPF is increasing livestock exclusion, but it is important to know how much fencing is being added annually. Correlating increased livestock exclusion with improvements in water quality as measured at the Porchester Rd site is also needed.

4.9 The limitations of this study were low sample size and PCR method

There are several limitations of this investigation. The number of samples collected was small (four occasions; three dry, one wet) in four sub-catchments. However, given this limitation, a number of common bacterial sources and solutions were identified which could be applied to the entire catchment to improve water quality. These include the need for riparian fencing to exclude livestock and further investigation or inspection of on-site wastewater systems in the catchment.

Another limitation of this investigation is the PCR library-based bacterial source methodology. It is difficult to identify aged, degraded or partially treated human bacterial sources with the library-based PCR methodology. This means that sites with a 'very strong positive' general marker, but where no source was identified, could be due to aged, degraded or partially treated bacterial sources. Microbial Source Tracking (MST) technology is a rapidly developing science and the assays are constantly improving.

Two methods could possibly be used to determine if aged bacteria sources are present in a sample (Devane, 2015). An AC/TC faecal ageing ratio is a fast and cost-effective method which could be used alongside *E. coli* at the early stages of an investigation. This, together with a sterol faecal ageing ratio test could provide insight into the presence of fresh or historical 'aged' human bacterial contamination (Devane et al. submitted).

5.0 Conclusions

1. Widespread ruminant bacterial contamination in the upper Papakura Stream catchment hints at livestock access to these streams and a lack of riparian fencing.
2. The highest *E. coli* was recorded at site 19a, near Brookby Rd.
3. Human bacterial contamination found at site 19 on one occasion is likely to be a result of failing on-site wastewater system(s) but further work is needed to isolate the cause.
4. Ducks or other bird species were not a significant contributor of bacterial contamination. Avian sources were only found on one occasion in the tributary between sites 25 and 25a.
5. *E. coli* levels in the Papakura Stream from the 2014 investigation were lower than results from previous investigations conducted in the catchment in 2008.
6. Fewer sources of bacterial contamination were identified in this investigation compared to a previous bacterial source investigation in 2008.
7. Further investigation may be warranted to identify sources of bacterial contamination in the remaining rural Papakura Stream sub-catchments.

6.0 Recommendations

- Visit all property owners in the rural Papakura Stream catchment without livestock exclusion to discuss riparian fencing and the Waterway Protection Fund. Properties in the site 12 and 25a sub-catchments should be the initial priority as these sites showed ruminant bacterial sources during dry weather, which hints at livestock access.
- Document the extent of riparian fencing and planting and report this to track success of the Waterway Protection Fund and other initiatives in the Papakura Stream catchment.
- Visit all dairy farms to discuss effluent management and mitigation options to reduce ruminant bacterial contamination in the Papakura Stream.
- Isolate the location of human contamination in the sub-catchment above site 19 near Brookby Rd.
- Inspect On-Site Wastewater Systems (OSWS) at the five properties in the sub-catchment above site 19 near Brookby Rd.
- Educate all property owners in the rural Papakura Stream catchment about On-Site Wastewater System (OSWS) maintenance and management.
- Trial Microbial Source Tracking (MST) methods which can positively identify aged, degraded or partially treated bacterial sources.

7.0 References

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Appendix A Papakura Faecal Source Testing (Van Duivenboden, 2008 unpublished)

Faecal source testing.

Draft FINAL REPORT

The Brief

Auckland Regional Council wished to investigate significant microbiological results observed in the Long Term Baseline surveys and more recent water quality surveys. Faecal source identification is required to better understand the sources of *E.coli* and thereby identify potential management responses.

Background

The ARC's State of the Environment (SOE) monitoring and recent water quality studies have identified elevated levels of faecal indicator bacteria in the Papakura Stream. SOE time series analysis confirms a significant deterioration in water quality (Scarsbrook, 2007). ARC has commissioned an intensive terrestrial and aquatic survey of the stream for catchment management purposes, which has included microbiological sampling. Further investigation is required to make meaningful conclusions about the above microbiological data. Source identification will reveal the appropriate management response to be sought via the ICMP process.

Fiscal constraints limited this investigation to the worst few sites identified in the aquatic survey. All microbiological data should be subject to source identification in order to enable risk assessment and correctly identify appropriate management responses. Most sites in the Papakura Stream have recorded elevated levels of *E.coli*, many of which are of considerable concern.

General

At ARC's request, sampling was undertaken in both wet and dry weather. A single samples was requested at each of five sites. The ARC's SOE reporting site (Porchester Road Bridge) was included to give a possible anchor between the surveys. A significant inflow immediately upstream of the bridge was included in this study in order to characterise its quality. This was undertaken because it has the potential to affect water quality at the SOE site, due to its attributes of flow, proximity and mixing.

The dry weather survey was undertaken on 9 July 2008. Preceding weather was dry for at least the last two days. pH readings made using test strips, showed low pH values at Porchester Bridge (pH 5.5) and the Porchester Road Drain (pH 5.0).

Observations made on the day of sampling include:

On-site effluent disposal fields in close proximity to the stream at Site 6.

Two large ponds with attendant wild and domestic ducks, goats, dogs, Pukeko and livestock occur immediately above Site 7.

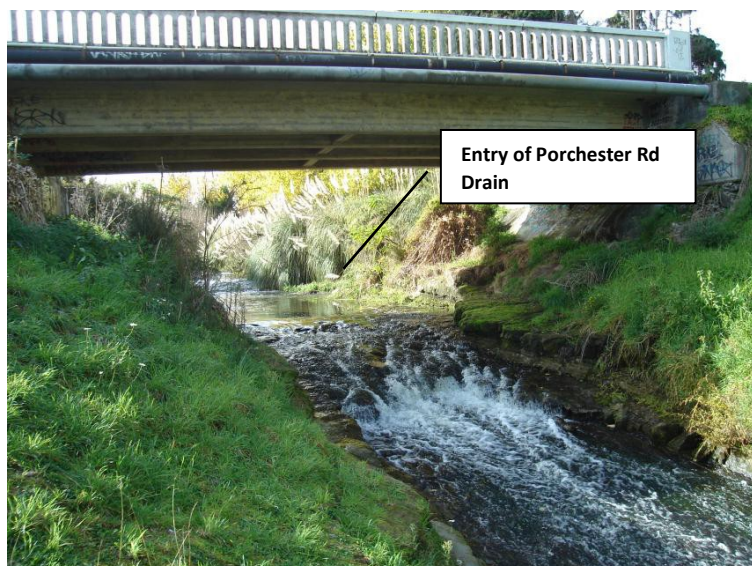
Site 7 could not be sampled as per information provided, due to free running dogs.

Samples were taken immediately downstream, on the southern side of the road.

No other significant stream inputs were observed between this sampling point and the original Site 7.

The wet weather survey was conducted after more than 10mm of rain in the preceding 24 hours. There was a significant amount precipitation previously, and flows were noticeably higher and more turbid at most sites.

Sampling sites



Porchester Rd bridge LTB site (Site 4) showing close proximity of the Porchester Road Drain input.



Site 6. Note proximity of wastewater treatment plant (arrowed)



Site 7: The lower of the two ponds. Note ducks.



Tributary feeding in between Sites 19 and 16.

Potential equine and human source in wet weather.

Results

7.1.1 Site 6

Site	<i>E.coli</i>	Human	Avian	Ruminant	Possu	Dog	Equine
Site 6 Dry Wx	9.80E+01	weak positive	positive	Non-detect	ndetc	ndetc	ndetc

ndetc = no detection.

7.1.2 Site 7

Site	<i>E.coli</i>	Human	Avian	Ruminant	Possu	Dog	Equine
Site 7 Dry Wx	3.60E+02	weak positive	positive	ndetc	ndetc	ndetc	ndetc

Site 19

Site	<i>E.coli</i>	Human	Avian	Ruminant	Possu	Dog	Equine
Site 19 Dry Wx	3.60E+03	ndetc	weak positive	V. strong positive	ndetc	ndetc	ndetc

Site 16

Site	<i>E.coli</i>	Human	Avian	Ruminant	Possu	Dog	Equine
Site 16 Dry Wx	7.70E+03	strong positive	v. weak positive	Strong positive	ndetc	ndetc	ndetc

Site 4 (LTB Site) and Porchester Road Drain.

Site	<i>E.coli</i>	Human	Avian	Ruminant	Possu	Dog	Equine
Site 4 Dry Wx	1.70E+03	weak positive	positive	Weak positive	ndetc	ndetc	ndetc
Drain Dry Wx	7.50E+01	ndetc	ndetc	ndetc	ndetc	Weak positive	ndetc

Wet weather survey

Site	<i>E.coli</i>	Human	Avian	Ruminant	Possu	Dog	Equine
Site 6 Wet Wx	2.00E+0	positive	ndetc	ndetc	ndetc	w. positive	ndetc
Site 7 Wet Wx	6.40E+0	positive	weak positive	ndetc	ndetc	w. positive	Ndetc
Site 19 Wet Wx	3.50E+0	weak positive	v. weak positive	ndetc	ndetc	w. positive	ndetc
Site 16 Wet Wx	1.90E+0	positive	v. weak positive	ndetc	ndetc	positive	weak positive
Site 4 Wet Wx	4.90E+0	positive	weak positive	ndetc	v.weak positive	positive	weak positive
Site 4 Wet Wx Internal Duplicate	5.80E+0	positive	v. weak positive	ndetc	v.weak positive	positive	weak positive
Porchester Drain Wet Wx	7.50E+0	positive	ndetc	ndetc	ndetc	ndetc	ndetc

Discussion

The sites were jointly decided upon by RMpro and ARC after consideration of the first two rounds of a catchment wide ecological study. That study included microbiological sampling for traditional indicators. On the day of sampling for this project, *E.coli* results were considerably lower across the board than those found in the other surveys. This may indicate the degree of variability and complexity in the catchment. Restricted one- off sampling, under two very different scenarios, impedes accurate descriptions of catchment contaminant sources. However, the following observations can be made:

Site 6 had a principal source of avian derived contamination in dry weather, although human sewage was also present. A catchment inspection of on-site effluent disposal fields is probably warranted. In the wet weather sample, this site became principally human derived contamination.

Site 7 results probably accurately reflect the expected. Large ponds with numerous ducks and geese etc, produced the likely principal source. Human derived contamination is also indicated. Moderate rainfall reversed the predominant sources but retained avian influences.

Site 19 indicated heavy ruminant pollution in dry weather. This is as might be expected with intense dairying in this small sub catchment. The wet weather changes to the principal source indicated are difficult to interpret from a single sample.

Site 16 is downstream of Site 19 and confirms the predominant ruminant contamination in dry weather. However, a consistent human signature is present and remains in the wet weather sample. Equine influences occur only in the wet weather sample and would appear to corroborate the potential influence of the equestrian activities to the west. A further study in this sub catchment is probably warranted in order to establish the degree of influence of the horse arena and any potential intermittent human sewage impacts.

Potential sources of faecal contamination at LTB Site 4 include mixtures of human, ruminant and avian. In wet weather, a greater range of sources was identified. This is unsurprising as the catchment sources will be many and varied by this point and probably

subject to greater temporal fluctuation. This is not dissimilar in effect to the 'time of concentration' phenomenon in stormwater practice.

The Porchester Road Drain was generally of good microbiological quality in either survey. However, dog and human sources are indicated, even at relatively low *E.coli* levels.

Where indicated sources do not match the expected or intuitive sources, caution must be exercised due to the low sample numbers for each weather type.

Recommendations

Where results indicate faecal sources from species of special concern, additional sampling can be undertaken. This is recommended to focus initially on more dry weather information and to then to investigate rainfall variations on top of the established picture. The sporadic nature of significant rain events and consequent sporadic sampling, will increase the complexity of interpretation. However, this would be overcome with sufficient sampling effort over wetter months. To determine how principal sources change over time and with flow, a targeted investigation will be required. A thorough review of water chemistry and biological data of any subject sites would provide a valuable precursor to further investigations.

In order to better interpret the LTB microbiological data, source identification should be implemented regularly at LTB Site 4. This would enable a clearer picture of whether elevated FC levels observed occasionally since 2000, are attributable to any one source. Once the source is identified, appropriate mitigation can be undertaken.

RMpro Limited 2008

Appendix 1
Full results

ESR Ref No	Client Ref No	<i>E.coli</i> mpn	Human HumBac Probe	Avian E2	Ruminant	Possum	Dog	Horse
CMB08338	PAP 6	9.80E+01	Weak positive	Positive	Nd	nd	nd	Nd
CMB08337	PAP 7	3.60E+02	Weak positive	Positive	Nd	nd	nd	Nd
CMB08336	PAP 19	3.60E+03	Nd	Weak positive	v. strong positive	nd	nd	Nd
CMB08335	PAP 16	7.70E+03	Strong positive	v. weak positive	Strong positive	nd	nd	Nd
CMB08339	PAP4 LTB	1.70E+03	Weak positive	Positive	Weak positive	nd	Nd	Nd
CMB08340	Porchester	7.50E+01	nd	nd	nd		w. positive	nd
CMB08355	PAK WA = 6	2.00E+03	Positive	nd	Nd	nd	w. positive	Nd
CMB08361	PAK WB = 7	6.40E+03	positive	weak positive	nd		w. positive	nd
CMB08357	PAK WC= 19	3.50E+04	Weak positive	v. weak positive	Nd	nd	w. positive	Nd
CMB08356	PAK WD= 16	1.90E+04	Positive	v. weak positive	Nd	nd	Positive	Weak positive
CMB08358	PAK Porchester	7.50E+02	Positive	nd	Nd	nd	Nd	Nd
CMB08359	PAK W4 LTB	4.90E+04	Positive	Weak positive	Nd	v. weak	Positive	Weak positive
CMB08360	Internal Duplicate	5.80E+04	positive	v. weak positive	Nd	v. weak	Positive	Weak positive

Papakura Stream faecal source investigation



Appendix C Investigation data

Table 1 Median, standard deviation, minimum and maximum *E. coli* values for each site

Site Number	<i>E. coli</i> (MPN/100ml)	
	Median $\pm$ SD	Min-Max
12	1435 $\pm$ 1720	150-3,900
12a	35 $\pm$ 155	<10-330
14	130 $\pm$ 888	110-1,900
19	1350 $\pm$ 951	40-2,300
19a	2000 $\pm$ 1664	1,500-4,600
25	150 $\pm$ 230	<10-510
25a	220 $\pm$ 95	65-290

Table 2 Raw *E. coli* data for each site

Site	Date	Time	<i>E. coli</i> (MPN/100ml)
12	31-Jan-14	930	150
12	12-Mar-14	950	2,300
12	10-Apr-14	930	570
12	22-Apr-14	1010	3,900
12a	31-Jan-14	1000	<10
12a	12-Mar-14	1010	<10
12a	10-Apr-14	945	330
12a	22-Apr-14	1035	65
14	31-Jan-14	1045	1,900
14	12-Mar-14	1030	130
14	10-Apr-14	1030	130
14	22-Apr-14	1215	110
19	31-Jan-14	1125	40
19	12-Mar-14	1045	2,300
19	10-Apr-14	1000	1,600
19	22-Apr-14	1140	1,100
19a	31-Jan-14	1110	1,500
19a	12-Mar-14	dry	dry
19a	10-Apr-14	1015	4,600
19a	22-Apr-14	1150	2,000
25	31-Jan-14	1210	50
25	12-Mar-14	1130	250
25	10-Apr-14	1110	<10
25	22-Apr-14	1250	510
25a	31-Jan-14	1140	290

25a	12-Mar-14	1100	65
25a	10-Apr-14	1040	210
25a	22-Apr-14	1230	230

Appendix D ESR Bacteria Source Report

10 June 2014

To

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Purchase order: 3000131005

From:

Dr Brent Gilpin
ESR Christchurch Science Centre
PO Box 29181
CHRISTCHURCH

AMENDED REPORT ON FAECAL SOURCE TRACKING ANALYSIS – PAKAKURA SITE

This report amends the PCR results table on pages 3 and 4. The sampling data for sample CMB140403 and the *E.coli* level for sample CMB140413 have been corrected. This report replaces the previous report dated 6 June 2014

The following water samples were received on 30th April 2014 and were analysed for faecal source PCR markers.

ESR Number	Client Reference	Sample Details	E.coli MPN/100ml
CMB140393	18643/1	Papakura Stream, site 12	150
CMB140394	18643/3	Papakura Stream, site 14	1900
CMB140395	18643/4	Papakura Stream, site 19a	1500
CMB140396	18643/5	Papakura Stream, site 19	40
CMB140397	18643/6	Papakura Stream, site 25a	290
CMB140398	18643/7	Papakura Stream, site 25	50
CMB140399	18792/1	Papakura Stream, site 12	2300
CMB140340	18792/3	Papakura Stream, site 14	130
CMB140341	18792/4	Papakura Stream, site 19	2300
CMB140342	18792/5	Papakura Stream, site 25a	65

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ESR Number	Client Reference	Sample Details	E.coli MPN/100ml
CMB140403	18792/6	Papakura Stream, site 25	250
CMB140404	18914/1	Papakura Stream, site 12	570
CMB140405	18914/2	Papakura Stream, site 12a	330
CMB140406	18914/3	Papakura Stream, site 14	130
CMB140407	18914/4	Papakura Stream, site 19	1600
CMB140408	18914/5	Papakura Stream, site 19a	4600
CMB140409	18914/7	Papakura Stream, site 25a	210
CMB140410	18947/1	Papakura Stream, site 12	3900
CMB140411	18947/2	Papakura Stream, site 12a	65
CMB140412	18947/3	Papakura Stream, site 14	110
CMB140413	18947/4	Papakura Stream, site 19	1100
CMB140414	18947/5	Papakura Stream, site 19a	2000
CMB140415	18947/6	Papakura Stream, site 25a	230
CMB140416	18947/7	Papakura Stream, site 25	510

ESR No	Sampled	<i>E.coli</i>	General GenBac	Human BacH	Human BiADO	Ruminant BacR	Dog DogBac	Bird GFD	Horse	Conclusion
<u>Site 12</u>										
CMB140393	31/01/2014	150	strong positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140399	12/03/2014	2300	strong positive	ND	ND	present – 100%	ND	ND	ND	Faecal contamination – ruminant source
CMB140404	10/04/2014	570	very weak positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140410	22/04/2014	3900	very strong positive	ND	ND	present – 100%	ND	ND	ND	Faecal contamination – ruminant source
<u>Site 12a</u>										
CMB140405	10/04/2014	330	very strong positive	ND	ND	present / ND - < 1%	ND	ND	ND	Unidentified faecal source
CMB140411	22/04/2014	65	strong positive	ND	ND	present 10%	ND	ND	ND	Faecal contamination – ruminant source
<u>Site 14</u>										
CMB140394	31/01/2014	1900	strong positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140400	12/03/2014	130	weak positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140406	10/04/2014	130	strong positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140412	22/04/2014	110	positive	ND	ND	present 100%	ND	ND	ND	Faecal contamination – ruminant source
<u>Site 19</u>										
CMB140396	31/01/2014	40	strong positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140401	12/03/2014	2300	strong positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140407	10/04/2014	1600	very strong positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140413	22/04/2014	1100	very strong positive	ND	present	present 10 – 50%	ND	ND	ND	Faecal contamination – human & ruminant sources

ESR No	Sampled	E.coli	General GenBac	Human BacH	Human BiADO	Ruminant BacR	Dog DogBac	Bird GFD	Horse	Conclusion
<u>Site 19a</u>										
CMB140395	31/01/2014	1500	positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140408	10/04/2014	4600	positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140414	22/04/2014	2000	positive	ND	ND	present 100%	ND	ND	ND	Faecal contamination – ruminant source
<u>Site 25</u>										
CMB140398	31/01/2014	50	positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140403	12/03/2014	250	positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140416	22/04/2014	510	strong positive	ND	ND	present up to 10%	ND	ND	ND	Faecal contamination – ruminant source
<u>Site 25a</u>										
CMB140397	31/01/2014	290	very strong positive	ND	ND	present up to 10%	ND	ND	ND	Faecal contamination – ruminant source
CMB140402	12/03/2014	65	very strong positive	ND	ND	ND	ND	ND	ND	Unidentified faecal source
CMB140409	10/04/2014	210	very strong positive	ND	ND	ND	ND	present	ND	Faecal contamination – avian source
CMB140415	22/04/2014	230	very strong positive	ND	ND	present 1 – 10%	ND	ND	ND	Faecal contamination – ruminant source

Abbreviations:

NA = sample was not analysed for this determinant.

ND = sample was analysed, but the determinant was not detected.

PCR Marker Interpretation Guidance Notes:

General marker

- The general PCR marker was detected in all samples.
- In samples where it was detected at very strong or strong levels we would expect source specific markers to be detected if the contamination was a recent event

Where the general marker was detected more weakly - this suggests a more diluted or aged source and thus source specific markers would be less likely to be detected.

Human markers

Where human markers were detected they were not at “high levels”.

Where human indicative markers was not detected in both assays this gives a higher level of confidence to conclude that a human source is not present.

There is little evidence for human faecal contamination in this stream. Human markers were only detected once – at site 19 on 22nd April.

Ruminant marker

Where ruminant marker was detected, the percentage values given are based on levels of this marker relative to the general marker in fresh ruminant faeces.

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

The lower levels reported (up to 50%, up to 10% and up to 1%) 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 marker.

Bird Marker

The avian specific marker GFD detects duck, swan, seagull, geese and chicken faecal sources. It was detected once – at site 25a on 10th April

Dog and Horse Markers

Neither the dog or horse markers were detected in any of the samples from this stream.

Notes:

PCR Markers: 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 1.00E+03, or 1.00x10³.

Brief details of the methods of analysis are available on request. These results relate to samples as received.

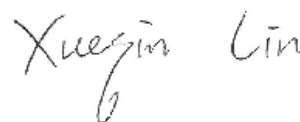
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Paula Scholes
Laboratory Manager

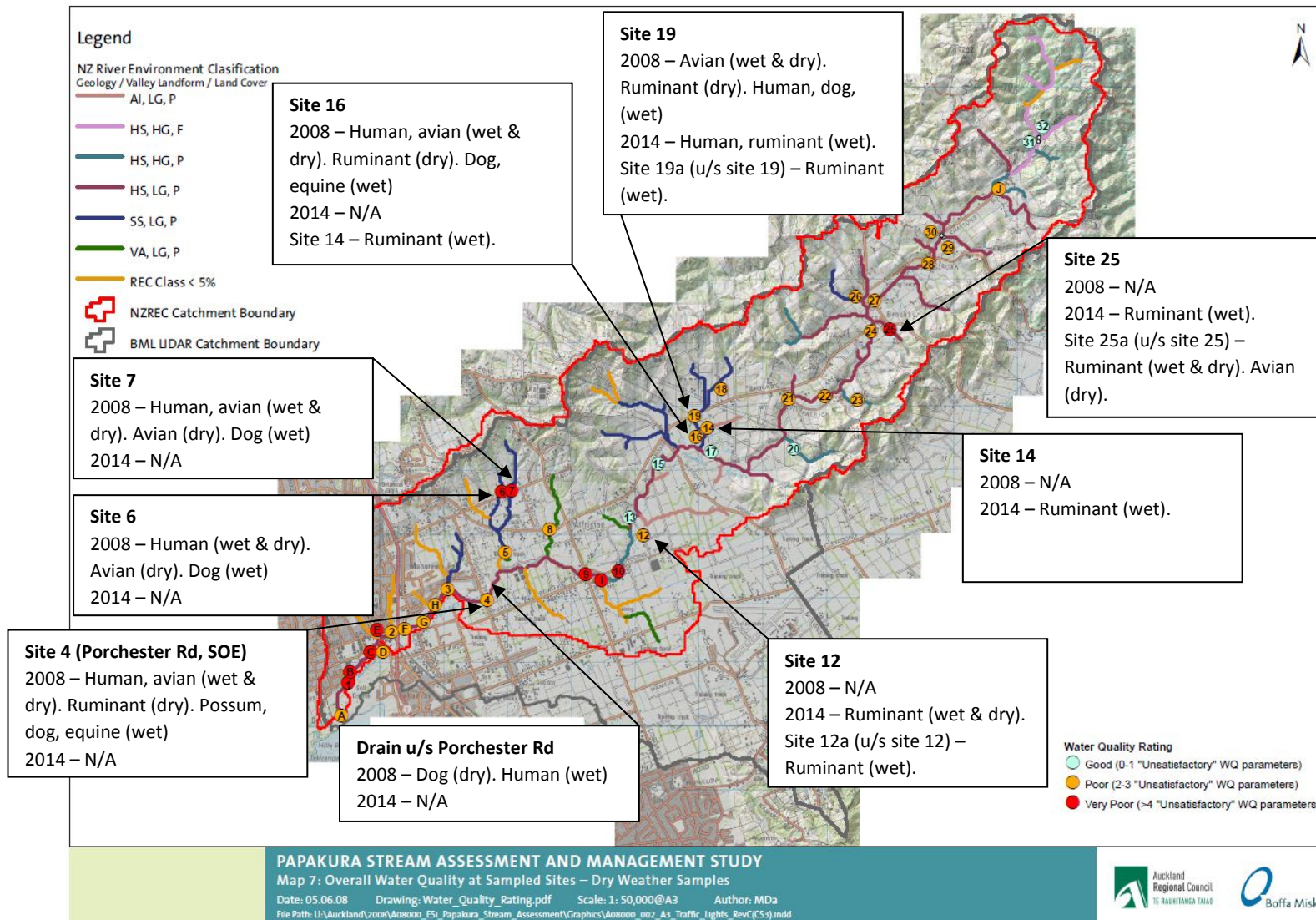


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Appendix E Sampling sites (Bull et al., 2008) & bacterial source (Van Duivenboden, 2008)



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