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Sources of Faecal pollution in Selected Waikato Rivers - July 2015

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July 2015

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CONTENTS

LIST OF TABLES

EXECUTIVE SUMMARY

The water quality in five streams in Waikato was assessed for the concentration of *Escherichia coli* (*E. coli*) and for the source of faecal pollution. The water samples were collected during dry weather and following greater than 10mm of rainfall in the previous 24 hours. The concentrations of *E. coli* in all rivers increased following rainfall, with the highest concentrations detected greater than 24,000 *E. coli* /100ml (Mangaone, Mangawhero and Komakorau Streams). The best water quality was detected in samples from the Karapiro stream under baseflow. Faecal Source Tracking (FST) was carried out on all samples and the markers for human, ruminant and avian pollution were applied. Ruminant and avian pollution was detected in almost all samples. Ruminant pollution was generally more dominant following rainfall. No human pollution was detected at any of the sites.

The levels of PCR markers detected are lower than would normally be observed in samples contaminated the same levels of *E. coli* from a fresh source. This may indicate that the pollution sources are partially aged, treated or have undergone transport processes. This may suggest a lower health risk than fresh faecal material. Further work would be required to validate this suggestion.

When the historical data for the sites was compared against the National Objectives Framework it was found that all sites failed to meet the Primary Contact Guidelines. This study has identified faecal pollution sources in the rivers and allows for mitigation options to be put inplace and the improvement in water quality to be measured against this baseline data.

1. INTRODUCTION

1.1 WATER QUALITY

The microbial quality of a river may be impacted in several ways including defecation from birds, septic tanks and wastewater treatment plants and agricultural practices. Land-use surrounding a river is known to have a major impact on the microbial quality of a river with water quality in agricultural catchments of inferior water quality to forested areas (1). Livestock farming whereby animal faecal material enters the river can result in water which contains a number of pathogenic microorganisms including *Cryptosporidium*, *Salmonella*, *Campylobacter* and *E. coli*. Several routes of transmission exist for animal faeces including direct deposition, overland flow of faecal material during rainfall, discharge of effluent collected on farm etc. When people come into contact with this water through either consumption for drinking water, food gathering or recreational contact it can result in human illness with outcomes including vomiting, diarrhoea and in some cases death.

1.2 SOURCES OF POLLUTION

1.2.1 Animal faeces

Several studies have measured the presence and concentration of faecal indicators and pathogens in the faeces of dairy cattle in NZ (2-6). The zoonotic organisms which would cause disease in humans excreted by cattle include *Campylobacter*, *E. coli* O157, *Salmonella*, and *Cryptosporidium.* Sheep are also recognised as excreting a number of zoonotic microorganisms. Several studies worldwide have quantified the indicator and pathogen loading from sheep (7-11). A New Zealand study comparing the microbial loading of sheep and lambs found that lambs excrete a significantly higher quantity of *E. coli* and enterococci and *Campylobacter* than sheep. The study reported a prevalence of 80.9% for *Campylobacter* in lambs faeces, but this reduced to 30.4% for sheep in the study (12).

1.2.2 Avian faeces

Concern has arisen over the contribution of waterfowl to the microbial loadings of surface waters, and their subsequent impacts on bathing water quality (13).Waterfowl, such as mallard ducks (*Anas platyrhynchos*), Canada geese (*Branta canadensis*), black swans (*Cygnus atratus*), and species of gull are abundant in New Zealand. Mallard duck numbers are estimated at 4.5 million (14) and Canada geese and black swan numbers are each estimated at less than 100,000 (15).There are no published national totals of gull numbers. These birds live on and near coastlines, estuaries, rivers, streams, wetlands and lakes, and they are also found on and around waste stabilisation ponds.

Waterfowl harbour a range of potentially pathogenic microorganisms (16) (17), and as such, are important reservoirs of nonpoint sources of faecal contamination.

1.2.3 Human Sources

Human wastewater contains a high concentration of *E. coli* (approximately 10^6 per 100 ml) as well as a range of zoonotic pathogens including *Norovirus, Campylobacter* and *Giardia.*

1.2.4 Pathways for transmission

A study "Assessing the relative importance of faecal pollution sources in rural catchments" (18) examined the key sources of faecal pollution to waterways. They determined that the loadings to land are greatest when stocking rates of dairy cattle are highest and incorporated in systems such as wintering pads, block grazed pasture and feed pads (18). Rainfall driven overland flow from Dairy farms has been identified as the largest pathway of faecal microbial losses from agricultural catchments (19-21).

A United Kingdom (UK) study referred to farmyards as an overlooked source for highly contaminated runoff (22). They found that farmyard runoff was extremely variable (10^4 – 10^7 faecal coliforms per 100 ml) and showed significantly higher concentrations relative to roof water. A study of a stream by a farm found that the faecal coliform concentration increased by 15,600 cfu/100ml when samples downstream were compared to upstream samples of the farm (23).

Few studies have quantified the *E. coli* losses from pasture due to sheep grazing. A study of a catchment in Otago estimated the loss at 8.6 x 10^9 *E. coli* per hectare per year when the pasture was grazed by sheep (1, 24). A New Zealand study comparing the contribution from sheep and cattle to pollution noted that sheep grazing at 5 animals / ha may deliver an *E. coli* loading rate that is an order of magnitude higher than dairy or beef cattle grazing at a typical stocking rate 3 animals/ha (18).

A study of a stream in the Peak District of the UK noted that as the quality of the land increased through a stream catchment, and the number of sheep grazing increased, the quality of the water decreased significantly. Also, as stocking densities of sheep increased in summer and decreased in winter the same seasonal pattern was noted in the streams in relation to indicator bacteria (25, 26).

Human wastewater may enter a waterway in a number of ways. This may occur directly when wastewater is pumped into a river due to a broken/overloaded wastewater system. It may also enter via storm water following heavy rainfall when the wastewater treatment plant (WWTP) is unable to deal with the volume of waste being produced. Some places have combined sewer overflows (CSOs) whereby wastewater and storm water flow in the same

pipe to the WWTP. On occasions when the volumes are too great from the CSOs WWTP to treat they discharge directly to rivers. Finally, wastewater may enter a river due to a failing septic tank. Wastewater can seep from the site to the river and the level of treatment depends on the distance travelled and the soil type.

2. FAECAL SOURCE TRACKING

2.1 FST METHODS

There are an increasingly large number of methods available that can be used to identify the possible sources of faecal pollution. In this study molecular markers were examined in each of the water samples.

2.1.1 Molecular Markers

There are a range of microorganisms other than faecal coliforms, *Escherichia coli* and enterococci present in the faeces, which are specific to animal hosts. Difficulties in culturing and identifying these organisms have limited their useful application to faecal source identification. An alternative approach is to extract total DNA from a water sample and examine the sample using the polymerase chain reaction (PCR) for DNA from source-specific organisms. Eight assays have been applied to the samples in this study. The first targets the Bacteroidales group of bacteria, and is not source specific. The remaining assays target more source specific bacteria which are indicative of faecal pollution. Microorganisms targeted by these assays and their specificities are listed in Tables 1 and 2.

Table 1 Summary of PCR Markers, Sensitivity and Microbial Targets

Marker Assay	Sensitivity	Target
General GenBac	High	Bacteroides 16S rRNA
Human BiADO	Medium - less sensitive than BacH	*Bifidobacterium adolocentis*
Human BacH	Medium - most sensitive human assay	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 2 Specificity of PCR Markers

Marker Assay	Detected in faeces from:	Negative in faeces from:
General GenBac	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, 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

3. MATERIAL AND METHODS

3.1 SAMPLING SITES

Five sites in the vicinity of Hamilton were chosen by Dairy NZ to sample in this study. They were chosen due to the elevated concentration of *E. coli* in the water samples in routine Environment Waikato testing. Sampling occurred both during dry weather for base-flow sources and following heavy rainfall.

- Karapiro Stream
- Komakorau Stream
- Mangaone Stream
- Mangaonua Stream
- Mangawhero Stream

3.2 MICROBIAL WATER QUALITY

Water samples were collected by Dairy NZ staff from each of the 5 locations. A stainless steel bucket attached to a rope was dropped into the stream and the bucket was filled. This water was swirled around the bucket, and poured back into the river. The bucket was re-filled and the water collected was used to fill three collection bottles (2 L volume each). Samples were stored in chilly bin containing ice before shipping to Christchurch. Samples were received at the laboratory in Christchurch within 24 hours of collection.

3.2.1 *E. coli* analysis

Water samples, upon receipt in the laboratory, were analysed for *E. coli* using Colilert assay as per the manufacturer's instructions.

3.2.2 FST PCR Markers

Water samples (150 mL) were filtered and DNA extracted as described previously (27). Real-time polymerase chain reaction (PCR) was performed using the qPCR reagent and

cycling conditions outlined in Devane et al 2007 (27). The PCR assays applied to water samples are listed in Table 1.

Each qPCR assay run included a non-template control (NTC), and an extraction blank of purified water to monitor for DNA contamination and standard concentrations of each target. The standard curve was generated from 10-fold serial dilutions as outlined in Devane, Robson (27)). SYBR green assays were subjected to melting curve (T_m) analysis and amplicons checked that they were within 0.3°C of the T_m of positive controls on each LightCycler 480® run. All samples and controls were analysed in duplicate, samples that registered a cyclic threshold (Cp) value above 40 were considered to be below the detection limit.

The General Bac marker is reported on a semi-quantative scale of Very Strong Positive, Strong Positive, Positive, Weakly Positive and not detected. Samples with Positive or Weakly Positive result may not have sufficient levels of markers to be able to detect more specific markers.

The Ruminant specific markers are reported using a percentage value. The percentage values given are based on levels of this marker relative to the general indicator in fresh ruminant faeces.

- Samples reported as up to 100% ruminant are consistent with all of the general faecal marker having come from a ruminant source.
- Lower levels (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 of less than 10% may indicate that ruminant pollution was only a minor contributor.

All other assays are reported as present/absent, or at low levels (if below the normal limit of detection).

3.3 DATA ANALYSIS

All historical *E. coli* data along with matching rainfall data supplied by Dairy NZ was analysed using Excel. This was to determine if rainfall was a significant factor resulting in elevated *E. coli* counts in the river water.

4. MICROBIAL WATER QUALITY RESULTS

4.1 KARAPIRO STREAM

Under base flow conditions the water quality at this site was of good quality with a maximum of 190 *E. coli* /100ml (Table 3). FST analysis was carried out on two of these samples with ruminant pollution detected as the dominant source of faecal pollution at this site. The cow marker was detected in one of these samples, with levels in the other sample too low for further discrimination.

Following rainfall an increase in *E. coli* was observed, with the last two samples exceeding water quality guidelines by a significant margin. In these samples ruminant pollution dominates, with cow and sheep markers detected in both samples. Relative levels suggest that cow pollution dominates. Wildfowl pollution was also detected in the rainfall impacted samples. The BiADO marker in the first rainfall impacted sample may reflect a very weak human pollution signal, or a seagull source. But without detection of the other human marker, we would conclude that there is not a significant human source of pollution.

Table 3 *E. coli* and faecal source tracking results for the Karapiro Stream

River Condition		Base flow			Rainfall Impacted		
Date Sampled		4 May	20 May	11 June	13 April	20 April	28 April
Indicator Bacteria	Total coliforms mpn/100ml	2200	2900	2800	>24000	>24000	>24000
	E. coli mpn/100ml	74	170	190	440	2100	7300
PCR Markers	**General Bac**	*E. coli* too low for FST analysis	strong positive	strong positive	very strong positive	very strong positive	very strong positive
	Human-BacH		ND	ND	ND	ND	ND
	Human - BiADO		ND	ND	present	ND	ND
	Ruminant		Up to 50%	Up to 50%	10-50%	Up to 50%	Up to 100%
	Cow		ND	present	ND	present	present
	Sheep		ND	ND	ND	present	present
	Wildfowl - GFD		ND	ND	Low levels	present	present
	Wildfowl - E2		ND	present	Low levels	present	present

4.2 KOMAKORAU STREAM

Ruminant pollution is present in all base flow samples at varying concentrations (Table 4). Although the first base flow sample contained the highest *E. coli* concentration of the base flow samples, it has the lowest level of ruminant pollution. Under base flow wildfowl pollution was present in one sample, but absent in the samples containing a lower concentration of *E. coli*.

Under flood conditions high concentrations of *E. coli* were detected in the river water. Ruminant pollution was detected in all three samples, with a higher concentration detected in the last two samples. The first flood sample, which contained a low concentration of ruminant pollution was also positive for one of the human pollution markers (BacH). As the second marker was missing, it is unlikely that human pollution is present. Wildfowl pollution was detected on all three flood occasions.

Table 4 *E. coli* and faecal source tracking results for the Komakorau Stream

River Condition		Base flow			Rainfall Impacted		
Date Sampled		4 May	20 May	11 June	13 April	20 April	28 April
Indicator Bacteria	Total coliforms mpn/100ml	16000	10000	4900	>24000	>24000	>24000
	E. coli mpn/100ml	1700	1000	440	>24000	7300	6900
PCR Markers	**General Bac**	strong positive	positive	strong positive	very strong positive	very strong positive	very strong positive
	Human-BacH	ND	ND	ND	present	ND	ND
	Human - BiADO	ND	ND	ND	ND	ND	ND
	Ruminant	Up to 10%	10-50%	Up to 50%	Low, up to 1%	Up to 50%	Up to 50%
	Cow	ND	ND	ND	ND	present	present
	Sheep	ND	ND	ND	ND	ND	ND
	Wildfowl - GFD	ND	ND	ND	present	ND	present
	Wildfowl - E2	present	ND	ND	present	present	present

4.3 MANGAONE RIVER

Under base flow the *E. coli* concentrations were relatively low on each sampling occasion (Table 5). Ruminant pollution was a minor source of pollution with only 10% of the pollution attributed to it. Wildfowl was determined to be the dominant faecal source under base flow and was detected in each of the three base flow samples.

Following heavy rainfall the Mangaone River was significantly higher in *E. coli* concentration with concentrations greater than 24,000 per 100 ml. Ruminant pollution was detected on all three occasions, with bovine specific pollution detected on the first two occasions. Wildfowl pollution was present on all occasions and accounted for a significant load of the microbial pollution following rainfall.

Table 5 *E. coli* and faecal source tracking results for the Mangaone Stream

River Condition		Base flow			Rainfall Impacted		
Date Sampled		4 May	20 May	11 June	13 April	20 April	28 April
Indicator Bacteria	Total coliforms mpn/100ml	2700	9200	6900	>24000	>24000	20000
	E. coli mpn/100ml	320	380	250	>24000	2000	2000
PCR Markers	**General Bac**	very strong positive	strong positive	strong positive	very strong positive	very strong positive	very strong positive
	Human-BacH	ND	ND	ND	ND	ND	ND
	Human - BiADO	ND	ND	ND	ND	ND	ND
	Ruminant	< 1%	Up to 10%	Up to 10%	10-50%	Up to 100%	Up to 10%
	Cow	ND	ND	ND	present	present	ND
	Sheep	ND	ND	ND	ND	ND	ND
	Wildfowl - GFD	present	present	present	present	present	present
	Wildfowl - E2	present	present	present	present	present	present

4.4 MANGAONUA

Elevated *E. coli* levels were detected at Mangaonua Stream under base flow (Table 6). On the first sampling occasion low level ruminant pollution was detected which contained a strong sheep indicative marker. One the second sampling occasion up to 100 % of the detected pollution was ruminant with bovine pollution detected. While on the final sampling ruminant pollution was detected in the absence of the bovine or ovine marker. Wildfowl was detected strongly on all sampling occasions.

Very high levels of *E. coli* were detected on all occasions following heavy rain at Mangaonua Stream. One the first occasion low level ruminant pollution was detected. On the two remaining sampling occasions, up to 100% of the faecal pollution detected was of ruminant origin, with the bovine marker also detected. Both wildfowl markers were detected on all three sampling occasions.

Table 6 *E. coli* and faecal source tracking results for the Mangaonua Stream

River Condition		Base flow			Rainfall Impacted		
Date Sampled		4 May	20 May	11 June	13 April	20 April	28 April
Indicator Bacteria	Total coliforms mpn/100ml	3900	9800	5500	>24000	>24000	>24000
	E. coli mpn/100ml	770	590	610	1600	3100	7300
PCR Markers	**General Bac**	very strong positive	very strong positive	very strong positive	very strong positive	very strong positive	very strong positive
	Human-BacH	ND	ND	ND	ND	ND	ND
	Human - BiADO	ND	ND	ND	ND	ND	ND
	Ruminant	< 10%	Up to 100%	Up to 50%	Up to 10%	Up to 100%	Up to 100%
	Cow	ND	present	ND	ND	present	present
	Sheep	present	ND	ND	ND	ND	ND
	Wildfowl - GFD	present	present	present	present	present	present
	Wildfowl - E2	present	present	present	present	present	present

4.5 MANGAWHERO

The *E. coli* concentration on the first base flow sample is very high relative two the other two samplings events (Table 7). Wildfowl pollution is the dominant faecal source detected at Mangawhero Stream under base flow. Low level ruminant pollution was detected on the first and third sampling occasions.

Elevated *E. coli* was detected in all three water samples following heavy rainfall. Ruminant pollution was detected in the first sample accounting for 10 to 50% of the faecal pollution. In the second sample ruminant pollution dominated, with ovine and bovine pollution sources detected. While, on the last sampling only a low level of ruminant pollution was detected. Both wildfowl markers were detected on each of the sampling occasions.

Table 7 *E. coli* and faecal source tracking results for the Mangawhero Stream

River Condition		Base flow			Rainfall Impacted		
Date Sampled		4 May	20 May	11 June	13 April	20 April	28 April
Indicator Bacteria	Total coliforms mpn/100ml	7300	16000	6500	>24000	>24000	20000
	E. coli mpn/100ml	2100	230	360	>24000	2800	2600
PCR Markers	**General Bac**	very strong positive	very strong positive	very strong positive	very strong positive	very strong positive	very strong positive
	Human-BacH	ND	ND	ND	ND	ND	ND
	Human - BiADO	ND	ND	ND	ND	ND	ND
	Ruminant	< 1%	ND	1 - 10%	10-50%	Up to 100%	< 1%
	Cow	ND	ND	ND	ND	present	ND
	Sheep	ND	ND	ND	ND	present	ND
	Wildfowl - GFD	present	present	present	present	present	present
	Wildfowl - E2	present	present	present	present	present	present

4.6 E. COLI AND RAINFALL DATA ANALYSIS

Analysis of historical rainfall and *E. coli* data provided by DairyNZ, analysis was carried out to determine if rainfall was a significant contributing factor to elevated *E. coli* concentrations in the rivers. Standard statistical analysis was carried out on the data using Excel as detailed in Table 8. The first step of analysis was to split the data into two categories - less than or equal to 540 *E. coli* per 100 ml and greater than or equal to 540 *E. coli* per 100 ml.

Table 8 Statistical Analysis on Water Testing Results from Streams

		Karapiro Stream	Komakorau Stream	Mangaone Stream	Mangaonua Stream	Mangawhero Stream
E. coli ≤540	Median	260	470	410	495	365
	Mean	307	370	408	495	351
	min	90	140	330	490	150
	max	520	500	480	500	500
	count	19	3	4	2	10
E. coli >540	Median	1000	1100	1000	1700	1500
	Mean	2700	1588	1397	2242	1666
	min	600	600	570	700	590
	max	8000	4700	6200	7100	3900
	count	7	24	21	24	15

The Karapiro Stream contained the greatest number of samples which contained less than 540 *E. coli* per 100 ml (19/26 samples), and does show that acceptable water quality is achievable over 70% of the time. For this stream targeting the elevated levels of *E. coli* would achieve significant improvement. Mangawhero Stream achieved values less than 540 *E. coli* per 100 ml 40% of the time, while for the other streams, very low quality water results were common.

The next analysis examined the impact of >10 mm of rainfall in the previous 24 hours on levels of *E. coli* (Table 9). While only a small number of data points were observed, these results suggest that at Karapiro Stream, rainfall does not result in or explain the elevated levels of *E. coli*. The other streams were almost always contaminated which limits the application of this analysis, but clearly rainfall events are only explaining a small number of the elevated *E. coli* levels (maximum of 17% of samples taken). When it rains levels of *E. coli* almost always get elevated, while there are also plenty of other elevations in E. coli levels in the absence of rainfall.

Table 9 Analysis of the number of water samples with >540 *E. coli* /100ml that are observed when >10mm of rainfall in the previous 24 hours.

Criteria	Karapiro Stream	Komakorau Stream	Mangaone Stream	Mangaonua Stream	Mangawhero Stream
≤540	2	1	0	0	1
>540	0	4	2	2	1
Positive Predictive Value (PPV)	0%	80%	100%	100%	50%
% of samples>540	0%	17%	10%	8%	7%

The data supplied was then graded against the National Objectives Framework (NOF) for Freshwater Quality for Primary and Secondary Contact. Primary contact is graded using the 95th percentile, while for secondary contact the annual median is employed. Due to the low number of data points supplied, the data was grouped into one dataset of 2009-2013. The data is colour-coded based on the values.

Green is A grade <260 *E. coli* for Primary and Secondary Contact

Yellow is B grade >260 <540 *E. coli* per 100 ml for Primary and Secondary Contact

Amber is C grade >540 <1000 *E. coli* per 100 ml for Secondary Contact

Red is D grade >1000 *E. coli* per 100 ml for Secondary Contact (National Bottom Line) and >540 *E. coli* for Primary contact. Only two grades exist for Primary Contact A and B.

Table 10 Comparison of the Karapiro Stream data against the NOF

2009-2013	All Data	Non-rainfall Impacted Data
Count	26	22
Median	410	440
Mean	951	1069
95%	7200	7400

Table 11 Comparison of the Komakorau Stream data against the NOF

2009-2013	All Data	Non-rainfall Impacted Data
Count	27	22
Median	1000	1050
Mean	1452	1479
95%	3935	4160

Table 12 Comparison of the Mangaonua Stream data against the NOF

2009-2013	All Data	Non-rainfall Impacted Data
Count	26	22
Median	1700	1650
Mean	2107	2140
95%	6940	6980

Table 13 Comparison of the Mangawhero Stream data against the NOF

2009-2013	All Data	Non-rainfall Impacted Data
Count	25	21
Median	600	600
Mean	1140	1060
95%	3600	3680

Table 14 Comparison of the Mangaone Stream data against the NOF

2009-2013	All Data	Non-rainfall Impacted Data
Count	25	21
Median	900	1000
Mean	1239	1270
95%	3275	4055

All sites failed to reach the National bottom line of 1000 *E. coli* per 100 ml for Primary Contact. The Mangaone and the Mangawhero both attained C grade status for Secondary contact while the remaining three rivers were graded as being below the National bottom line. The exclusion of samples obtained following rainfall did not seem to make a significant difference to the grade the rivers would receive under the NOF.

5. DISCUSSION

This study explored the microbial water quality in five streams in Waikato by assessing the concentration of *Escherichia coli* (*E. coli*) and the sources of faecal pollution using PCR based methods.

The concentration of *E. coli* increased in the rivers on all occasions following rainfall. Analysis of long-term data found that while the concentration increased following rainfall, elevated concentrations were also present in dry weather. Therefore rainfall was a poor indicator of water quality with a maximum of 17% of elevated *E. coli* concentrations predicted by previous rainfall. The Karapiro Stream has the best water quality of the five streams sampled. The *E. coli* concentration was low under base flow, but only two samples processed for FST due to the low level of contamination present.

When the long term data was analysed for the use of rainfall as an predictor of poor water quality it was found to not to be effective with elevated counts present in the absence of rainfall. This is demonstrated in the data when analysed against the 95th percentile. River generally exceeded the National bottom line of 1000 E. coli per 100 ml regardless of whether the rainfall impacted data was included or excluded. The 95th percentile of the Karapiro Stream data was determined to be 7,200 *E. coli* per 100ml when all data was included in the analysis and 7,400 *E. coli* per 100ml when rainfall impacted data was removed. The increase in the 95th percentile value following the removal of rainfall impacted data highlights the lack of value of rainfall as an indicator of poor water quality.

Ruminant pollution was detected in all streams, typically at greater levels following rainfall. Waterfowl pollution was also present at all sites with Komakorau Stream the least impacted by birds, with the markers detected on only one of the six sampling occasions. The Karapiro Stream changed considerably following rainfall with an increase in the presence of ruminant (cow) pollution. No human pollution was detected at any of the sites either under base flow or rainfall impacted conditions. The levels of PCR markers detected are lower than might have been indicated based on *E. coli* levels. This may indicate that the pollution sources are partially aged, treated or have undergone transport processes.

As no pathogen testing such as *Campylobacter* has been carried out on these samples, no judgement on the human health risk from contact with the rivers can be attained. The sampling does show variation in sources and strength of the sources in dry weather and following rainfall. For example in the Mangawhero River ruminant pollution is detected in

base flow and following rainfall. However the level of ruminant pollution increases following rainfall accounting for up to 100% of the faecal pollution detected in the water sample.

Future work that could be carried out in relation to these rivers is to walk along the river and assess the presence of fencing and the areas where livestock may have access to the rivers. Further microbial analysis could be carried out at different locations along the rivers to determine if there are "hot spots" of pollution arising from a particular location or land-use along the river. The waters could also be tested for the pathogen *Campylobacter* to determine if it is present in the river and to determine the source of it.

6. GLOSSARY

PCR	Polymerase Chain Reaction
FST	Faecal Source Tracking
NOF	National Objectives Framework
qPCR	Quantitative PCR
mpn	Most Probable Number
cfu	Colony Forming Unit
cp	cyclic threshold

REFERENCES

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