Faecal Source Tracking at Recreational Bathing Locations in Northland

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Client Report

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Executive Summary

Northland Regional Council (NRC) requested assistance from ESR's faecal source tracking (FST) team to establish the source of elevated levels of *Escherichia coli* consistently detected in waterways in 10 Northland locations. Identification of the faecal source is necessary to mitigate the pollution – this requires a range of tests referred to as the FST toolbox. The analytes tested by the toolbox were fluorescent whitening agents (FWAs) which detect human faecal contamination derived from sewage input and faecal sterol (FS) analysis which can detect both human and herbivore contamination. In addition, six polymerase chain reaction (PCR) markers were tested on the samples. One of the PCR markers is a general marker to confirm the presence of faecal contamination and the other five markers are specific for human, ruminant, wildfowl, pig or possum contamination.

The FST toolbox was applied to the water samples which were collected from each location over three sampling events at fortnightly intervals. The following records the results from each of the sites. The last two sites at Ruahuia and Mangere Streams were contracted on a commercial basis by NRC and the results included in this report.

Results

Raumunga Stream

Raumunga Stream is a river system with urban, agricultural and native bush land uses impacting on its environment. The FST toolbox identified wildfowl faecal pollution in Raumunga Stream. There was no evidence of human faecal pollution.

Whangarei Falls

Whangarei Falls is a river system with urban and agricultural land uses impacting on its environment. Wildfowl and ruminant faecal pollution were detected at Whangarei Falls. There was no evidence of human faecal pollution.

Lang's Beach Stream

Identification of the source of faecal contamination was not conclusive at this location. Lang's Beach is a slow flowing wetland type habitat surrounded by scrub. Birds and septic tanks are expected to be the major inputs to this waterway. There was no evidence of human faecal pollution. The ruminant PCR marker was positive at Lang's Beach on the 25 February 2008 sampling event (which occurred after a heavy rainfall event) at both the Below the Toilet and Middle Beach sites. This result was unexpected due to the nature of the environment. These two samples were not sent for FS analysis and therefore, ruminant faecal contamination cannot be confirmed.

High *E. coli* and Total *Bacteroidetes* numbers at Lang's Beach did not result in significant detection of the other faecal markers, apart from a weak signal for the bird PCR marker at Middle Beach. If pollution is due to bird faeces, this may reflect the low specificity of the E2 marker for wildfowl species other than ducks. FS analysis did not suggest faecal contamination at the Lang's Beach sites, but high levels of plant sterols were identified and the ratio 24-ethylcholesterol/ 24-ethylcoprostanol suggested plant decay or vegetation runoff as a source of sterols. Identification of faecal contamination was not conclusive by FST markers used in this study. Further work is required to elucidate the source of contamination including applying novel wildfowl PCR markers and determining if particular subtypes of *E. coli* are persisting in the environment aided by the high plant concentrations identified by FS analysis. It may also be more cost-effective to identify the human health risk associated with this watercourse by employing methods that determine the presence/absence of pathogens relevant to human health.

Ocean Beach Stream

The Ocean Beach Stream is a slow flowing, small stream moving through native bush and low intensity agriculture. Birds and septic tanks are thought to be the major inputs to this site. Faecal pollution at Ocean Beach Stream was confirmed by FS analysis. Identification of faecal contamination was not conclusive by FST markers used in this study however; there was no evidence of human faecal pollution. No significant sources of animal/bird faecal contaminations were identified by the suite of PCR markers which would explain

the high *E. coli* numbers recorded at Ocean Beach Stream. If pollution is due to bird faeces, this may reflect the low specificity of the E2 PCR marker for wildfowl species other than ducks. Further research and additional tools are required to determine the source of faecal contamination at Ocean Beach Stream.

Otamure Bay Stream

Otamure Bay stream is a slow flowing, wetland type habitat with low intensity agriculture in the surrounding environment. Birds were suspected to be a major input to this waterway with farming regarded as a minor input. Ruminant and wildfowl faecal contamination were detected at Otamure Bay Stream on all three sampling occasions. No human faecal pollution was identified.

Waiharakeke Stream

Waiharakeke is a slow flowing river system with a wetland habitat upstream. Exotic forestry and agriculture are expected to be the major inputs to this system. Wildfowl and ruminant faecal contamination were identified on separate sampling events at Waiharakeke Stream and possum faecal contamination was identified at very low levels on two sampling occasions. There was no evidence of human faecal pollution.

Wairoa at Ahipara

Wairoa is a river system where agriculture is expected to be the major input from the surrounding environment. There was no evidence of human faecal pollution. Herbivore (such as the ruminants, cattle and sheep), and wildfowl faecal contamination was detected on both sampling occasions at Wairoa.

Otiria Stream

Otiria is a slow flowing river system with a wetland habitat upstream. Exotic forestry and agriculture are expected to be the major inputs to this system. There was no evidence of human faecal pollution. Strong signals for animal and bird faecal contamination were

detected at Otiria Stream. There was also an unconfirmed possibility of possum and pig contamination at this site.

Ruahuia Stream

The water samples were collected at the headwaters of Ruahuia stream where the stream travels through native bush. Feral animals are expected to be the major inputs to this system. There was no evidence of human faecal pollution. A weak ruminant PCR signal was detected on the 25 February 08 sampling event after heavy rainfall. FS analysis could not be performed on this sample as a filtered sample was not received.

Mangere Stream

Mangere Stream is a slow flowing river system where the major inputs are expected to be from agriculture and the surrounding native bush, with the possibility of septic tanks impacting the waterway. There was no evidence of human faecal pollution. Wildfowl faecal contamination was detected in the Mangere Stream samples. FS analysis was not performed on the samples received as requested by NRC.

Conclusions

Total *Bacteroidetes*, indicating non-specific faecal contamination, were identified within the range 10^5–10^7 CFU/ml, in all water samples tested.

Human faecal contamination was not identified by the FST markers employed in this study at any of the 10 sites sampled. These included the human marker, FWAs, which were not detected in any of the waters analysed, and therefore, may not be a cost-effective marker for future investigations of these particular water sites. FWAs are only associated with sewage where there is mixing of grey water with the sewage effluent as occurs in most household plumbing systems. FWAs will not be detected in sewage systems where the laundry detergents used for washing do not contain FWAs as a laundry brightener.

High levels of sterols were identified at all sites allowing for confident interpretation of sterol ratios. At all sites, except Lang's Beach, faecal sterols were identified at levels

which indicated faecal contamination, but excluded human-derived contamination as a source. FS analysis on samples from Lang's Beach excluded faecal contamination and suggested that sterol concentrations measured were derived from plant decay and/or vegetative runoff from land. An ongoing review of the statistical analysis of the faecal sterol results may contribute further knowledge to source identification specific to animals and birds for these water sites. If further information is gained from advanced statistical analyses, this information will be relayed to NRC.

Ocean Beach Stream recorded faecal contamination but a specific source was not detected by the FST markers. The major faecal contributor at Ocean Beach Stream and Lang's Beach is thought to be bird pollution. The wildfowl PCR marker used in this study is based on the prevalence of a *Desulfovibrio*-like bacterium that is found in 76% of ducks (Mallards and Grey Ducks) and occurs at a lower prevalence in other waterfowl species. Therefore, the non-identification of bird pollution may be due to the low prevalence of the target bacterium in the host birds associated with these two environments. Identification of additional bird markers specific to other waterfowl species, such as gulls, may clarify the source of *E. coli* and Total *Bacteroidetes* at Ocean Beach Stream and Lang's Beach.

Based on the results of this study, the faecal sources contributing to the high *E. coli* levels at all 10 sites are not likely to be human derived. The confidence in these results is increased by the multisampling approach where most sites were sampled on three occasions at fortnightly intervals. This sampling regime encompassed water collection after both dry conditions and periods of heavy rainfall. Although the faecal contamination was not derived from humans, the heath risk associated with these recreational waters remains unknown. An understanding of the risk associated with human activities such as recreational swimming and shellfish gathering in these contaminated waters would, therefore, require an assessment of the presence of pathogens relevant to human health.

Recommendations

- Extensive use of FWA analysis is not useful in these particular locations as all analyses in this study reported non-detectable levels of FWAs.

- Future work requires alternative approaches which could focus on wildfowl, farm and feral animals and possibly environmental sources of microbial indicators such as *E. coli* persisting in the environment.
    - One approach would be to target specific sources comparing populations (i.e. different or similar subtypes) of *E. coli* found in water (e.g. at Lang's Beach) with faecal scats from known animal/bird hosts found in the same environment. This could be done by applying phenotypic (e.g. antibiotic resistance analysis) or genotypic techniques (e.g. Repetitive Extragenic Palindromic PCR (REP-PCR)]. This approach would also investigate whether particular subtypes of *E. coli* were persisting in the water environment such as sediments, and contributing to the high levels of microbial indicators.
    - Additional wildfowl PCR markers have been identified by an international research group which are specific to gulls and geese. When the method details become available we will apply the novel markers to the DNA extracted from these water samples (e.g. Ocean Beach and Lang's Beach Streams) to see if they give us greater detail about the species of bird pollution identified.
- Determination of the health risk posed by these contaminated waters such as in association with the human activities of recreational water contact and shellfish gathering, would require an assessment of the presence/absence of pathogens relevant to human health.

1. INTRODUCTION

Faecal source tracking (FST) in surface waters is an important step in the management of New Zealand's environmental waters. Identification of high levels of the microbial faecal indicators such as *Escherichia coli* during routine sampling of waterways must be followed up to determine the source of the faecal contamination. Mitigation of the pollution requires identification of the faecal source. This step has become more complex as the number of tools available for analysis has increased, and has led to a need for clear guidance on the use of these tools and the interpretation of the results they generate.

Northland Regional Council (NRC) sought advice on the identification of the source of high levels of *E. coli* recorded from water samples taken from several Northland recreational bathing sites. The source of bacterial contamination in these locations is unclear, thus making it difficult to implement effective environmental management plans. Identification of the sources of faecal contamination will enable NRC to prioritise resources within these catchments to achieve the greatest improvements in water quality. The second beneficial outcome would be determining what types of faecal discrimination tests are most useful for identifying the source of contamination in Northland's waterways. Once the source(s) is identified the associated environmental risks to humans and stock can be gauged and the public informed as appropriate. Currently, the selected sites have permanent signs erected warning people against swimming. It is predicted that the advice from ESR will show what is required to improve water quality e.g. stock exclusion, riparian planting, improvements in onsite systems, or bird control. Remediation of the contaminated waterways will give the public access to areas with appropriate water quality for swimming, shellfish gathering and stock water.

Background

Microbial faecal indicators such as *E. coli* identify the presence of faecal contamination, but do not identify whether the source is from birds, farm animals, humans or other animals e.g. feral species. A range of tests has been developed that aid the discrimination of faecal sources. They are referred to as a "toolbox" and include molecular and chemical markers that distinguish between animal, bird and human faecal sources. Proposed indicators include genetic markers based on host-specific micro-organisms, and chemical

indicators, including faecal sterols (FS) and fluorescent whitening agents (FWAs). Each one of these tools is relevant for a particular purpose and when used in conjunction with other tools, can increase the certainty that pollution is derived from a specific source.

Objective

- To identify the source of high numbers of *E. coli* in the waterways at 10 different locations in Northland.
    - Identify the presence/absence of faecal contamination
    - Determine the source(s) of animal/bird/human faecal contamination by employing a range of tests from the Faecal Source Tracking (FST) Toolbox.

Methods

Indicators evaluated

E. coli enumeration by Colilert® (IDEXX) was performed on all water samples prior to arrival at ESR. Processing of samples for FST markers continued if *E. coli* results were above 200 MPN/100 ml. Water and faecal samples were sent on ice to ESR within 24 hours of collection and analysed for PCR markers and FWAs. Subsequently, FS analysis was performed on selected samples based on the initial test marker results. The FST markers used in this study are outlined.

Fluorescent Whitening Agents

FWAs are common constituents of washing powders that adsorb to fabric and brighten clothing. There are a range of FWAs, but only one (4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)-amino]stilbene-2,2'-disulfonate) is used in New Zealand. Most household plumbing mixes effluent from toilets with "grey water" from washing machines. As a consequence, FWAs are usually associated with human faecal contamination in both septic tanks and community wastewater systems (Devane et al., 2006; Managaki et al., 2006). FWAs can be extracted from water samples, and quantified by HPLC (High Pressure Liquid Chromotography).

Faecal Sterols

Sterols are neutral lipids that have important biological functions, in plants and animals, such as for cell membrane structure (e.g. cholesterol). The sterol profile in 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 endogenous biosynthesis of sterols (humans on a low cholesterol diet synthesise cholesterol). Perhaps the most important factor is the anaerobic bacteria in the animal gut which biohydrogenate sterols to stanols of various isomeric configurations. For example, 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. Consequently, analysis of sterol composition of animal faeces generates a sterol fingerprint which can be distinctive (Leeming et al., 1996). Some generalisations or guidelines to the individual sterols are outlined in Table 1.

Table 1: Sterols and their relevance to faecal contamination

Sterol	Properties of sterol
Coprostanol	Principal human biomarker, high relative amounts indicate fresh human faecal material. Constitutes 60% of the total sterols found in human faeces. Only anaerobic bacteria can hydrogenate cholesterol to coprostanol, therefore, coprostanol is not generally found in unpolluted fresh or marine waters unless anaerobic sediments are present.
Epicoprostanol	Found in trace amounts (relative to coprostanol) in human faeces. Increases in concentration during sewage treatment processes as coprostanol is converted to epicoprostanol.
24-ethylcoprostanol	Principal herbivore* indicator
24-ethylepicoprostanol	Usually also present in herbivore faeces, often at a similar level to 24-ethylcoprostanol
Cholesterol	Precursor to coprostanol and epicoprostanol. Also comes from domestic waste, food scraps, algae etc.
Cholestanol	The most stable isomer of coprostanol and formed from the reduction of cholesterol in the environment and, therefore, occurs in unpolluted environments.
24-methylcholesterol	Plant sterol
24-ethylcholesterol	Plant Sterol. Precursor to 24-ethylcoprostanol and 24-ethylepicoprostanol
Stigmasterol	Plant sterol

* For the purposes of this study, an herbivore is an animal whose principal diet consists of vegetation. Members of the ruminant group are a subset of herbivores and include cattle, sheep, deer and goats.

FS analysis generates a lot of data, the interpretation of which can be quite complex. The absolute levels of each sterol or stanol can depend on many factors. The ratios of sterols are, however, less concentration dependent, and due to the equivalent stability of stanols, are fairly stable. Therefore, sterol interpretation relies upon the analysis of various sterol ratios as outlined below.

Key sterol ratios

In general, analysis of sterols requires a total sterol concentration of greater than 2000 parts per trillion (ppt). If this level of sterols is detected in a water sample, then it is important to initially determine if there is faecal contamination in the water course. Detection of general faecal contamination from an unspecificed source(s) is identified by using the following ratios:

- **Coprostanol/cholestanol:**
  - If ratio is less than 0.5 then the stanols may not be of herbivore/human faecal origin
- **24-ethylcoprostanol/24-ethylcholestanol:**
  - If ratio is less than 0.5 then the stanols may not be of herbivore/human faecal origin

If faecal pollution is indicated then it is determined whether contamination is of human origin using the following ratios, which are based on the higher concentration of coprostanol found in human faeces compared with other animals/birds.

- **%Coprostanol/Total sterol concentration**
  - > 5-6% suggests human faecal contamination
- **Coprostanol/(coprostanol + cholestanol)** also called 5β/(5β+5α stanols)
  - > 0.7 suggests human faecal contamination
- **Coprostanol/24-ethylcoprostanol**
  - < 1.0 suggests herbivore; ≥ 1.0 suggests human faecal contamination

If human faecal pollution is indicated, determine if it is from fresh/untreated faecal inputs or from aged/treated sewage.

- **Coprostanol/epicoprostanol**
  - > 2.0 suggests fresh or untreated human faecal contamination
    The concentration of epicoprostanol increases during sewage treatment processes as coprostanol is converted to epicoprostanol

If herbivore pollution is suspected then apply

- **%24-ethylcoprostanol/total sterols**
  - > 5–6% suggests herbivore contamination
- **24-ethylcholesterol/24-ethylcoprostanol**
  - < 1.0 suggests herbivore faecal contamination; > 4.0 suggests plant decay and/or runoff from vegetation

If a mixed pollution event is suspected then apply the following ratio:

- **%Coprostanol/(coprostanol/24-ethylcoprostanol)**
    - < 30% suggests a 100% herbivore source for the faecal contamination; > 75% suggests a 100% human source; percentages in between require a more complex equation to attribute to mixed pollution events from herbivore and human sources

PCR Markers

Polymerase chain reaction (PCR) assays amplify specific DNA sequences, producing banding patterns and other signals which can be detected either visually or spectrophotometrically. The differences between DNA sequences of closely related bacteria, mean that PCR markers can be designed to distinguish between species of bacteria that are almost impossible to tell apart using phenotypic (e.g. biochemical) tests. More importantly, PCR enables the detection of microbes that have so far resisted attempts to grow them in the laboratory (e.g. the anaerobic *Bacteroidetes*). If the target species of enteric microbes are highly host specific, i.e., are resident in only humans or particular animal species, then a PCR assay of the DNA can be a useful FST tool.

The PCR markers used in this study are outlined in Table 2 and are reported as being specific for their host target such as human or ruminant PCR markers.

Bacteroidetes *PCR markers*

The *Bacteroides-Prevotella* group of bacteria resides exclusively in the gut of warm-blooded animals. They are strict anaerobes, which mean they are highly unlikely to replicate in the environment. They are excreted in higher numbers than the faecal coliforms and are therefore a universal PCR marker designed to detect all of the members of the *Bacteroides-Prevotella* group (termed Total *Bacteroidetes*). This universal marker is a useful indicator of the presence of non-specific faecal pollution. Confirmation of a faecal contamination event may have increasing relevance as the debate over environmental replication of traditional bacterial indicators continues. When this PCR marker is used to detect general faecal contamination it is supplemented by sterol ratio analysis as outlined in the sterol section above.

In addition, differences within the DNA of members of the *Bacteroidetes* group can be exploited, leading to the design of PCR assays based on these sequence differences or 'markers' in bacteria that are specific to a host animal.

Wildfowl PCR marker

The E2 PCR marker is based on the 16S rRNA DNA sequence of a *Desulfovibrio*-like bacteria isolated from duck faeces and found to be prevalent (76%, n = 42) in ducks, with lower prevalence in other wildfowl species (Devane et al., 2007). The ducks identified in this study as the host for the E2 PCR marker are the Mallard and its hybrid form with the Grey Duck. Both duck species belong to the genus, *Anas*.

Table 2: PCR markers used in the Envirolink study

Host Target group	Bacterial Target	Reference
General indicator of faecal contamination	*Bacteroides-Prevotella* group (Total *Bacteroidetes*)	Dick and Field (2004)
Human	A bacterial species belonging to *Bacteroides-Prevotella* group	Bernhard et al. (2003)
Ducks	*Desulfovibrio*-like species	Devane et al. (2007)
Ruminants*	A bacterial species belonging to *Bacteroides-Prevotella* group	Bernhard et al. (2003)
Possums	A bacterial species belonging to *Bacteroides-Prevotella* group	Unpublished results
Pigs	A bacterial species belonging to *Bacteroides-Prevotella* group	Dick et al. (2005)

* Ruminants are a subset of herbivore animals and include sheep, cattle, deer and goats.

Sampling Methodology

Ten sites were sampled over three sampling events with an interval of two weeks between each of the sampling periods. Water samples were collected on two occasions in February 2008 and once during March 2008. *E. coli* levels were determined for all samples, but if the numbers were too low (< 200 MPN/100 ml) then further testing was discontinued

where appropriate. All samples with significant numbers of *E. coli* were subjected to FWA analysis by HPLC and PCR marker detection using human-, herbivore-, wildfowl-, possum- and pig- specific PCR markers. It should be noted that the possum and pig PCR markers are under development and therefore results from these tests should be treated with caution unless confirmed by other FST tools such as FS analysis. FS analysis was performed on a subset of the samples and where possible (dependent on receiving a filter and high *E. coli* levels) on at least one sample from each site, apart from the two sites Ruahuia and Mangere Stream which were requested for analysis on a commercial basis.

Rainfall data (courtesy of NRC) recorded during January and February 2008 are presented in the Appendix (Table 6). It will be noted that the first sampling that occurred on 12 February 08 followed a period of dry weather, whereas the 25 February 08 sampling event occurred after heavy rainfall on the 23 and 24 of February 08.

The sites sampled for this Envirolink are listed in Table 3 along with information on their land usage and the potential contamination inputs for each site.

Table 3: Land use information for sites chosen for sampling

Sites sampled	Site No.	Land use	Catchment potential inputs (major)	Catchment potential inputs (minor)	Habitat type
Raumunga Stream	103246	urban/agriculture/native bush	farming, septic tanks	reticulated sewage system	river system
Whangarei Falls	105972	Urban/agriculture	farming, septic tanks	reticulated sewage system	river system
Lang's Beach Stream (at toilets)	100686	mostly shrub	birds, septic tanks		slow flowing, wetland type habitat
Ocean Beach Stream	102077	native bush/low intensity agriculture	birds, septic tanks	farming	slow flowing and small
Otamure Bay Stream	108859	low intensity agriculture	birds	farming, odd septic tank	slow flowing, wetland type habitat
Waiharakeke Stream	108921	exotic forestry/agriculture	farming, wetlands upstream	septic tanks	slow flowing river system
Wairoa Stream (Ahipara)	105053	agriculture	farming	septic tanks	river system
Otiria Stream	105376	exotic forestry/agriculture	farming, wetlands upstream	septic tanks	slow flowing river system
Ruahuia	106991	native bush	wild animals		headwater stream
Mangere	101625	agriculture/native bush	farming, septic tanks, wild animals		slow flowing river

Results

Overall, there was no indication of human faecal pollution detected at any of the sites based on the analysis of PCR markers, FWAs and sterol ratios. No FWAs were detected in any samples over the three sampling intervals. Total *Bacteroidetes*, indicating non-specific faecal contamination, was identified in all water samples tested and the concentration range was 10^5–10^7 CFU/ml. The results and the conclusions drawn for individual sites are summarised below. Table 4 contains the results from analysis for *E. coli*, PCR markers and FWAs, and Table 5 reports on the sterol concentrations and the significance of sterol ratio analysis on each of the water samples.

Raumunga Stream

Raumunga Stream is a river system with urban, agricultural and native bush land uses impacting on its environment.

Conclusion: Wildfowl faecal pollution was detected at Raumanga Stream. There was no evidence of human faecal pollution.

The water sample from March 08 was not tested further as *E. coli* levels were too low (131 MPN/100 ml). Of the other two samples analysed:

- No human markers were detected
  - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
  - No FWAs were detected.
  - FS analysis excluded human pollution.
- Wildfowl faecal pollution was detected
  - Wildfowl pollution was detected by the PCR marker in both samples.
  - FS analysis of the water sample from 25 February 08 is consistent with either avian or herbivore pollution
- The other markers were not indicative of other animal faecal sources.

Whangarei Falls

Whangarei Falls is a river system with urban and agricultural land uses impacting on its environment.

Conclusion: Wildfowl and ruminant faecal pollution were detected at Whangarei Falls. There was no evidence of human faecal pollution.

The water sample from March 08 was not tested further as *E. coli* levels were too low (85 MPN/100 ml). Of the other two samples analysed:

- No human markers were detected

    - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
    - No FWAs were detected.
    - FS analysis of the water sample from 25 February 08 excluded human pollution.
- Wildfowl and ruminant faecal pollution was detected
    - Wildfowl and ruminant PCR markers were detected on both sampling occasions.
    - FS analysis of the water sample from 25 February 08 was indicative of herbivore and/or bird faecal contamination.
- A weak possum signal was detected by the PCR marker in the water sample from 25 February 08 which was sent for FS analysis. FS analysis did not support the identification of possum contamination.
- The markers were not indicative of other animal faecal sources

Lang's Beach Stream

Lang's Beach is a slow flowing wetland type habitat surrounded by scrub. Birds and septic tanks are expected to be the major inputs to this waterway

Conclusion: **Identification of sources of faecal contamination was not conclusive for this location; however there was no evidence of human faecal pollution. The ruminant PCR marker was positive at Lang's Beach Below the Toilet and at Middle Beach in the water samples from 25 February 08 after a heavy rainfall event. This result was unexpected due to the nature of the environment where septic tanks and birds were suggested as contaminants. These two samples were not sent for FS analysis and therefore, ruminant faecal contamination cannot be confirmed.**

High *E. coli* and Total *Bacteroidetes* numbers at Lang's Beach did not result in significant detection of the other faecal markers, apart from a weak signal for the bird PCR marker at Middle Beach. FS analysis did not suggest faecal contamination at the Lang's Beach sites, but high levels of plant sterols were identified and the ratio 24-ethylcholesterol/ 24-ethylcoprostanol suggested plant decay or vegetation runoff as a source of sterols. If pollution is due to bird faeces, this may reflect the low specificity of the E2 marker for wildfowl species other than ducks. Further research and additional tools are required to determine the source of faecal contamination at Langs Beach.

A total of five samples were collected from Lang's Beach over the three sampling intervals from Middle Beach and the site Below the Toilets. Only the sample from March 08 from Below the Toilets recorded low *E. coli* levels (84 MPN/100 ml) – it was therefore excluded from further analysis. The other four samples had *E. coli* levels ranging from 800–2755 MPN/100ml.

- No human markers were detected
    - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
    - No FWAs were detected.
    - FS analysis of the samples from 12 February and March 08 excluded human pollution.
- Wildfowl faecal pollution was detected

    - The wildfowl PCR marker reported a weak positive on both sampling occasions at Middle Beach.
    - FS analysis on the Middle Beach sample (March 08) did not support faecal contamination.
- Ruminant faecal pollution was detected
    - Ruminant PCR marker was detected at the Middle Beach and Below the Toilet sites on the 25 February 08 sampling event which occurred after heavy rainfall. This result was unexpected due to the nature of the environment where septic tanks and birds were suggested as contaminants. These two samples were not sent for FS analysis; therefore, ruminant faecal contamination cannot be confirmed.
- FS analysis on samples from Middle Beach (March 08) and Lang's Beach stream (12 February) did not suggest faecal contamination, but high levels of plant sterols were identified and the ratio 24-ethylcholesterol/ 24-ethylcoprostanol (38.5 and 47.0, respectively) suggested plant decay or runoff from vegetation.
- The pig PCR markers reported a weak positive at Middle Beach in samples from 25 February 08
    - This was the sampling period proceeding heavy rainfall, therefore, it may be wise not to interpret these results for pig as positive.
    - Pig contamination cannot be detected by the other FST tools and therefore the result should be interpreted with caution.

Ocean Beach Stream

The Ocean Beach Stream is a slow flowing, small stream moving through native bush and low intensity agriculture. Birds and septic tanks are thought to be the major inputs to this site.

Conclusion: Identification of sources of faecal contamination was not conclusive for this location. Faecal pollution at Ocean Beach Stream was confirmed by FS analysis; however, there was no evidence of human faecal pollution. No significant sources of animal/bird faecal contaminations were identified by the PCR markers which would explain the high *E. coli* numbers recorded at Ocean Beach Stream. If pollution is due to bird faeces, this may reflect the low specificity of the E2 PCR marker for wildfowl species other than ducks. Further research and additional tools are required to determine the source of faecal contamination at Ocean Beach Stream.

- No human markers were detected
    - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
    - No FWAs were detected.
    - FS analysis of 25 February 08 sample excluded human pollution.
- Apart from a weak signal from the possum PCR marker on the sample from 25 February 08, no other markers were indicative of other animal or bird faecal sources
    - FS analysis on the sample from 25th February 08 confirmed faecal contamination, but not from a human source. The FS analysis was consistent with bird and/or animal contamination. Ongoing research is attempting to refine our interpretation of animal/bird contamination through advanced statistical analyses.

Otamure Bay Stream

Otamure Bay stream is a slow flowing, wetland-type habitat with low intensity agriculture in the surrounding environment.

Conclusion: Ruminant and wildfowl faecal contamination were detected at Otamure Bay Stream on all three sampling occasions. No human faecal pollution was identified.

- No human markers were detected
  - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
  - No FWAs were detected.
  - FS analysis of the sample from 12 February 08 excluded human pollution.
- Wildfowl and ruminant faecal pollution were detected on all three sampling occasions
  - Wildfowl and ruminant PCR markers were positive on all three sampling occasions.
  - FS analysis of the sample from 12 February 08 confirmed faecal contamination, but excluded a human source. The FS analysis was consistent with bird and/or animal contamination. Ongoing research is attempting to refine our interpretation of animal/bird contamination through advanced statistical analyses.

Waiharakeke Stream

Waiharakeke is a slow flowing river system with a wetland habitat upstream. Exotic forestry and agriculture are expected to be the major inputs to this system.

Conclusion: Wildfowl and ruminant faecal contamination were identified on separate sampling events at Waiharakeke Stream and possum faecal contamination was identified at very low levels on two sampling occasions. There was no evidence of human faecal pollution.

The sample from March 08 sample was not tested as it recorded low numbers of *E. coli*. Of the other two samples analysed:

- No human markers were detected
  - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
  - No FWAs were detected.
  - FS analysis excluded human pollution.
- Ruminant faecal contamination was detected in the sample from 25 February 08
  - Ruminant pollution was detected by the PCR marker
  - The level of 24-ethylcoprostanol (main herbivore sterol) in of the sample from 25 February 08 sent for FS analysis was suggestive of herbivore faecal contamination; this was supported by the ratio of 24-ethylcholesterol/24-ethylcoprostanol (2.3) which indicates herbivore faecal contamination rather than plant decay. In addition the ratio of 24-ethylcoprostanol to total sterols was indicative of herbivore faecal contamination.

- Wildfowl pollution was detected in the sample from12 February 08
    - Wildfowl pollution was detected by the PCR marker.
    - FS analysis on the sample from 25 February 08 reported high levels of faecal contamination which although indicative of herbivore does not exclude avian pollution.
- Possum faecal contamination was detected
    - Both sampling occasions reported a very weak positive for the possum PCR marker, however this was not supported by FS analysis on the sample from the 25 February.

Wairoa at Ahipara

Wairoa is a river system where agriculture is expected to be the major input from the surrounding environment.

Conclusion: There was no evidence of human faecal pollution.
Herbivore (such as the ruminants, cattle and sheep), and wildfowl faecal contamination was detected on both sampling occasions at Wairoa.

This site was only sampled on the two occasions in February 08 and both samples were analysed for faecal sterols.

- No human markers were detected
    - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
    - No FWAs were detected.
    - FS analysis of both samples from February 08 excluded human pollution.
- Wildfowl and ruminant faecal pollution was detected
    - Wildfowl and ruminant PCR markers were detected on both sampling occasions
    - FS analysis supported the detection of faecal contamination, but both samples excluded the possibility of human faecal contamination. Faecal contamination, specifically from herbivores such as the ruminants, cattle and sheep was detected. This is consistent with farming being the major land use in the area. Avian faecal contamination cannot be confirmed by FS at this time but further investigation of FS statistical techniques is in progress.
- The possum PCR marker was weakly detected in the sample from 25 February 08, but this was not supported by FS analysis.

Otiria Stream

Otiria Stream is a slow flowing river system with a wetland habitat upstream. Exotic forestry and agriculture are expected to be the major inputs to this system.

Conclusion: There was no evidence of human faecal pollution.
Strong signals for animal and bird faecal contamination were detected at Otiria Stream. There was also an unconfirmed possibility of possum and pig contamination at this site.

- No human markers were detected
  - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
  - No FWAs were detected.
  - FS analysis of the sample from 12 February 08 excluded human pollution. The percentage of coprostanol over total sterols was marginally above the 5% threshold for detection of human contamination (5.2%), but this may be a consequence of a strong faecal contamination signal from herbivores which also produce coprostanol as well as high levels of 24-ethylcoprostanol. Human contamination was not supported by the other FS ratios, FWA or PCR analysis.
- Wildfowl and ruminant faecal pollution were detected
  - Wildfowl and ruminant PCR markers were detected in the sample from 12 February 08, but for the other two sampling events only a weak ruminant signal was detected in the sample from 25 February 08.
  - FS analysis supported the detection of faecal contamination that was not of human origin. Faecal contamination, specifically from herbivores such as the ruminants, cattle and sheep was detected as indicated by the 24-ethylcoprostanol/total sterol percentage (22%) and 24-ethylcholesterol/ 24-ethylcoprostanol ratio (0.85). Avian faecal contamination cannot be confirmed by FS at this time but further investigation of faecal sterol statistical techniques is in progress.
- A weak possum PCR marker signal was detected in all three sampling occasions. This was not supported by FS analysis; again, this may reflect the high levels of faecal contamination due to herbivore contamination where coprostanol and 24-ethylcoprostanol were both detected at high levels.
- The pig marker was positive in the sample from 12 February 08, but pig contamination cannot be detected by the other FST tools and therefore the result should be interpreted with caution.

Ruahuia Stream

The water samples were collected at the headwaters of Ruahuia stream where the stream travels through native bush. Feral animals are expected to be the major inputs to this system.

Conclusion: There was no evidence of human faecal pollution.
A weak ruminant PCR signal was detected on the sampling event (25 February 08) after heavy rainfall. FS analysis could not be performed on this sample as a filtered sample was not received.

- No human markers were detected
  - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
  - No FWAs were detected.
  - FS analysis was not performed on any samples.
- Ruminant faecal contamination was detected on one sampling occasion.
  - A weak ruminant PCR marker signal was detected in the sample from 25 February 08. A filter of this sample was not received and therefore FS analysis could not be performed on this sample.
- No other PCR markers indicative of animal/bird sources were detected.
- Three faecal scats collected along the edge of the Ruahuia Stream on 25 February 2008 were negative for the wildfowl, pig, ruminant and possum PCR markers.

Mangere Stream

Mangere Stream is a slow flowing river system where the major inputs are expected to be from agriculture and the surrounding native bush, with the possibility of septic tanks impacting the waterway.

Conclusion: There was no evidence of human faecal pollution.
Wildfowl faecal contamination was detected in the Mangere Stream samples.

Mangere Stream was sampled on the two sampling events in February 08.

- No human markers were detected
  - Human indicative *Bacteroidetes* PCR marker was below the detection limit.
  - No FWAs were detected.
  - FS analysis was not performed on the samples as requested by NRC.
- Wildfowl faecal contamination was detected on both sampling occasions.
  - The wildfowl PCR marker signal was detected on both sampling occasions in February.
- No other PCR markers indicative of animal sources were detected.

Table 4: Results of bacterial counts, FWA and PCR markers for animal/bird/human contamination

Client Ref No.	ESR Ref No.	Date Received	Sample Site	*Total Coli-forms	*E. coli	TotalBac	Wildfowl	Ruminant	Possum	Pig	FWA ppb	Human
20080831	CMB08233	12/2/2008	Raumanga		240	positive	strong pos	ND**	ND	ND	<0.01	ND
081139	#CMB08255	25/2/2008			933	positive	weak pos	ND	ND	ND	<0.01	ND
081323	CMB08272	11/3/2008		>24192	131	NT‡	NT	NT	NT	NT	NT	NT
20080832	CMB08234	12/2/2008	Whangarei Falls		563	positive	strong pos	positive	ND	ND	<0.01	ND
081142	CMB08258	25/2/2008			857	positive	weak pos	positive	weak pos	ND	<0.01	ND
081328	CMB08277	11/3/2008		>24192	85	NT	NT	NT	NT	NT	NT	NT
20080815	CMB08235	12/2/2008	Lang's Beach		959	positive	ND	ND	ND	ND	<0.01	ND
081135	CMB08251	25/2/2008	Lang's BT#		801	positive	ND	positive	ND	ND	<0.01	ND
081136	CMB08252	25/2/2008	Lang's MB¥		1483	positive	weak pos	positive	ND	weak positive	<0.01	ND
081321	CMB08270	11/3/2008	Lang's BT	>24192	84	NT	NT	NT	NT	NT	NT	NT
081322	CMB08271	11/3/2008	Lang's MB	>24192	2755	positive	weak pos	ND	ND	ND	<0.01	ND
20080798	CMB08236	12/2/2008	Ocean Beach		4,106	positive	ND	ND	ND	ND	<0.01	ND
081141	CMB08257	25/2/2008			798	positive	ND	ND	weak pos	ND	<0.01	ND
081320	CMB08269	11/3/2008		>24192	252	positive	ND	ND	ND	ND	0.01	ND
20080788	CMB08237	12/2/2008	Otamure Bay		1,607	positive	strong pos	positive	ND	ND	<0.01	ND
081140	CMB08256	25/2/2008			1046	positive	weak pos	pos	ND	ND	<0.01	ND
081327	CMB08276	11/3/2008		>24192	1126	positive	positive	positive	ND	ND	<0.01	ND
20080838	CMB08238	12/2/2008	Waiharakeke		228	positive	strong pos	ND	weak pos	ND	<0.01	ND
081153	CMB08260	25/2/2008			1169	positive	ND	positive	weak pos	ND	<0.01	ND
081325	CMB08274	11/3/2008		>24192	72	NT	NT	NT	NT	NT	NT	NT
20080840	CMB08239	12/2/2008	Wairoa	>600		positive	strong pos	strong pos	ND	ND	<0.01	ND
081152	CMB08259	25/2/2008			1500	positive	weak pos	pos	weak pos	ND	<0.01	ND
20080841	CMB08240	12/2/2008	Otiria		2,909	positive	strong pos	strong pos	weak pos	positive	<0.01	ND
081154	CMB08261	25/2/2008			1336	positive	ND	weak pos	weak pos	ND	<0.01	ND
081326	CMB08275	11/3/2008		>24192	311	positive	ND	ND	weak pos	ND	<0.01	ND
20080829	CMB08241	12/2/2008	Ruahuia		413	positive	ND	ND	ND	ND	<0.01	ND
081137	CMB08253	25/2/2008			1067	positive	ND	ND	ND	ND	<0.01	ND
081324	CMB08273	11/3/2008		>24192	132	positive	ND	weak pos	ND	ND	<0.01	ND
20080830	CMB08242	12/2/2008	Mangere		573	positive	strong pos	ND	ND	ND	<0.01	ND
081138	CMB08254	25/2/2008			1989	positive	weak pos	ND	ND	ND	<0.01	ND

* MPN/100ml; ND** not detected; # samples tested by FS analysis; NT‡ Not tested, as *E. coli* numbers too low; BT# Below the Toilet; MB¥ Middle Beach

Table 5: Sterol concentrations and sterol ratio analysis

	CMB08255 Raumanga Stream 25/2/2008	CMB08258 Whangarei Falls 25/2/2008	CMB08235 Lang's Beach stream 12/2/2008	CMB08271 Lang's Stream middle beach 11/3/2008	CMB08257 Ocean Beach Stream 25/2/2008	CMB08237 Otamure Bay Stream 12/2/2008	CMB08260 Waiharakeke Stream 25/2/2008	CMB08259 Wairoa Stream @Ahipara 25/2/2008	CMB08239 Wairoa 12/2/2008	CMB08240 Otiria 12/2/2008
Sterol concentration										
coprostanol	48	51	*31*	*18*	87	117	313	139	199	634
24-ethylcoprostanol	153	154	39	29	208	467	1552	716	886	2730
epicoprostanol	*13*	*15*	*16*	*6*	*21*	28	73	*32*	42	145
cholesterol	1839	2058	3460	2368	1853	3384	5063	2550	3410	2395
cholestanol	211	169	131	293	190	410	481	270	276	418
24-methylcholesterol	570	446	551	413	497	947	1352	811	734	514
24-ethylepicoprostanol	*44*	*35*	*6*	*4*	*31*	103	334	186	190	844
stigmasterol	829	624	1571	347	640	1144	2305	1274	1462	570
24-ethylcholesterol	2290	1307	1517	1376	1278	2801	3573	2631	1999	2328
24-ethylcholestanol	217	186	95	88	155	453	1087	590	510	1686
total sterol concentration (ppt)	6215	5043	7418	4942	4959	9853	16134	9200	9710	12264
Sterol ratio analysis										
coprostanol/cholestanol	0.23	0.30	0.24	0.06	**0.46**	0.28	**0.65**	**0.52**	**0.72**	**1.52**
24-ethylcoprostanol/24-ethylcholestanol	**0.70**	**0.83**	0.41	0.33	**1.34**	**1.03**	**1.43**	**1.21**	**1.74**	**1.62**
%Coprostanol/total sterols	0.8%	1.0%	0.4%	0.4%	1.8%	1.2%	1.9%	1.5%	2.1%	**5.2%**
5β/(5β+5α stanols)	0.19	0.23	0.19	0.06	0.31	0.22	0.39	0.34	0.42	0.60
coprostanol/24-ethylcoprostanol	0.32	0.33	0.79	0.61	0.42	0.25	0.20	0.19	0.23	0.23
coprostanol:epicoprostanol	3.60	3.48	2.00	2.96	4.19	4.15	4.29	4.42	4.71	4.38
coprostanol/coprostanol+24-ethylcoprostanol	24.0%	24.9%	44.3%	37.7%	29.5%	20.0%	16.8%	16.3%	18.4%	18.9%
% 24-ethylcoprostanol/total sterols	2.46%	3.04%	0.53%	0.59%	4.19%	4.74%	**9.62%**	**7.79%**	**9.13%**	**22.26%**
24-ethylcholesterol/24-ethylcoprostanol	14.97	8.51	**38.51**	**47.09**	6.16	6.00	2.30	3.67	2.25	**0.85**

Sterol ratio analysis	**Interpretation of sterol ratios**
coprostanol/cholestanol	>0.5 indicates faecal contam.
24-ethylcoprostanol/24-ethylcholestanol	>0.5 indicates faecal contam.
%Coprostanol/total sterols	>5-6% suggests human contam.
5β/(5β+5α stanols)	>0.7 suggests human contam.
coprostanol/24-ethylcoprostanol	>1.0 suggests human contam.
coprostanol:epicoprostanol	>2.0 suggests fresh/untreated sewage

Sterol ratio analysis	**Interpretation of sterol ratios**
coprostanol/coprostanol+24-ethylcoprostanol	>75% suggests 100% human contribution
% 24-ethylcoprostanol/total sterols	>6% suggests herbivore contam.
24-ethylcholesterol/24-ethylcoprostanol	<1.0 suggests herbivore contam.; >4.0 suggests plant decay/vegetative runoff

Conclusions

Total *Bacteroidetes*, indicating non-specific faecal contamination, were identified within the range 10^5–10^7 CFU/ml, in all water samples tested.

Human faecal contamination was not identified by the FST markers employed in this study at any of the sites. These included the human marker, FWAs, which were not detected in any of the waters analysed, and therefore, may not be a cost-effective marker for future investigations of these particular water sites. FWAs are only associated with human sewage where there is mixing of grey water with the sewage effluent as occurs in most household plumbing systems. FWAs will not be detected in sewage systems where the laundry detergents used for washing do not contain FWAs as a laundry brightener.

High levels of sterols were identified at all sites allowing for confident interpretation of sterol ratios. At all sites, except Lang's Beach, FS were identified at levels which indicated faecal contamination, but excluded human-derived contamination as a source. An ongoing review of statistical analysis of the FS results may contribute further knowledge to source identification specific to animals and birds for these water sites. If further information is gained from advanced statistical analyses then this information will be relayed to NRC.

In addition, unpublished international research has identified further bird PCR markers for other wildfowl species such as gulls. When they become available these markers may be useful for such sites as Ocean Beach Stream where faecal contamination was identified by sterol analysis but none of the PCR markers in the FST toolbox were detected. The current wildfowl PCR marker is identified in 76% of ducks (Mallard and Grey Ducks) but at a much lower prevalence in other wildfowl species. Application of the new bird PCR markers may further knowledge about the faecal pollution source(s) at Ocean Beach Stream and also Lang's Beach where bird pollution was suspected as being a major source of *E. coli*.

From the results of this study, the faecal sources contributing to the high *E. coli* levels are not likely to be human derived. The confidence in these results is increased by the

multisampling approach where most sites were sampled on three occasions at fortnightly intervals. This sampling regime encompassed water collection after both dry conditions and periods of heavy rainfall. Although the faecal contamination was not identified as human related, the heath risk associated with recreational water contact and shellfish consumption from these waters remains unknown. An understanding of the health risk posed by these contaminated waters would, therefore, require an assessment of the presence of pathogens relevant to human health.

Recommendations

- Extensive use of FWA analysis is not useful in these locations as all analyses in this study reported non-detectable levels of FWAs.
- Future work requires alternative approaches which could focus on wildfowl, farm and feral animals and possibly environmental sources such as *E. coli* persisting in the environment.
    - One approach would be to target specific sources comparing populations of *E. coli* found in water (e.g. at Lang's Beach) with faecal scats from known animal/bird hosts found in the same environment. This could be done by applying phenotypic (e.g. antibiotic resistance analysis) or genotypic techniques [e.g. Repetitive Extragenic Palindromic PCR (REP-PCR)]. This approach would also investigate whether particular subtypes of *E. coli* were persisting in the water environment (e.g. sediments) and contributing to the high levels of microbial indicators.
    - Additional wildfowl PCR markers have been identified by an international research group. When the method details become available we will apply the novel markers to the DNA extracted from these water samples to see if they give us greater detail as to the species of bird pollution identified.
- Determination of the health risk posed by these contaminated waters, associated with recreational water contact and shellfish consumption, would require an assessment of the presence/absence of pathogens relevant to human health.

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Appendix

Table 6: Rainfall at selected Northland locations during January and February 2008

	Rainfall in Northland (mm)			
	Site 531207 Awanui	Site 533817 Ohaeawai	Site 547339 Waiarohia	Site 640436 Ahuroa
Date rainfall mm	at School Cut	at Ohaeawai Auto	at NRC Water St	at Brynderwyn
2/1/2008	0.0	0.0	0.0	0.0
3/1/2008	0.0	0.0	0.0	0.0
4/1/2008	0.0	0.0	0.0	0.0
5/1/2008	0.0	0.0	0.0	0.0
6/1/2008	0.0	0.0	0.0	0.0
7/1/2008	0.0	0.0	0.0	0.5
8/1/2008	0.0	0.0	0.0	0.0
9/1/2008	25.5	27.0	19.0	23.5
10/1/2008	5.0	30.5	12.0	4.5
11/1/2008	0.0	5.0	1.5	0.0
12/1/2008	0.0	0.0	0.0	0.5
13/1/2008	0.0	0.0	0.0	0.0
14/1/2008	0.0	0.0	0.0	0.0
15/1/2008	0.0	0.0	0.0	0.0
16/1/2008	0.0	0.0	0.0	0.0
17/1/2008	0.0	5.0	0.0	0.0
18/1/2008	0.0	0.0	0.0	0.0
19/1/2008	0.0	0.0	0.0	0.0
20/1/2008	0.0	0.5	0.0	0.0
21/1/2008	2.5	13.0	3.5	1.0
22/1/2008	5.5	27.5	42.5	68.5
23/1/2008	1.0	2.5	1.0	11.5
24/1/2008	0.0	0.0	0.5	0.0
25/1/2008	0.0	0.0	2.0	0.0
26/1/2008	0.0	1.5	0.0	0.0
27/1/2008	0.0	0.0	0.0	0.0
28/1/2008	0.0	0.0	0.0	0.0
29/1/2008	0.0	0.0	0.0	0.0
30/1/2008	0.0	0.0	0.0	0.0
31/1/2008	0.0	0.0	0.0	2.0
1/2/2008	0.0	0.0	0.0	0.0
2/2/2008	0.0	0.0	0.0	0.0
3/2/2008	0.0	0.0	0.0	0.0
4/2/2008	0.0	0.0	0.0	0.0
5/2/2008	0.0	0.0	0.0	0.0
6/2/2008	0.0	0.0	0.0	0.0
7/2/2008	0.0	0.0	0.0	0.0
8/2/2008	0.0	0.0	0.0	0.0
9/2/2008	0.0	0.0	0.0	0.0
10/2/2008	0.0	0.0	0.0	0.0
11/2/2008	9.0	10.0	15.0	9.5
12/2/2008	0.0	0.0	0.0	0.0
13/2/2008	0.0	0.0	0.0	0.0
14/2/2008	0.5	0.0	0.0	0.0
15/2/2008	28.5	24.0	22.0	14.5
16/2/2008	1.0	1.5	0.5	4.0
17/2/2008	4.0	1.0	1.0	2.0

Rainfall in Northland (mm)

Date rainfall mm	Site 531207 Awanui at School Cut	Site 533817 Ohaeawai at Ohaeawai Auto	Site 547339 Waiarohia at NRC Water St	Site 640436 Ahuroa at Brynderwyn
18/2/2008	0.0	0.0	0.0	0.0
19/2/2008	0.0	0.0	0.0	0.0
20/2/2008	0.0	0.5	1.0	0.0
21/2/2008	0.0	0.0	0.0	0.0
22/2/2008	0.0	0.0	1.0	0.5
23/2/2008	57.5	105.5	33.5	12.5
24/2/2008	26.5	95.5	67.5	78.5
25/2/2008	0.0	0.5	6.0	27.0