Faecal Source Investigation Tryphena, Great Barrier Island

Prepared for The Great Barrier Island Local Board and Auckland Council

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WILKS ENVIRONMENTAL CONSULTING
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Limitations:

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Executive Summary

The aim of this investigation was to use faecal source tracking tools to investigate likely sources of faecal contamination in three streams within the Tryphena catchment; Blackwell Stream, Mulberry Grove Stream and Garden Road Stream. All three streams frequently have levels of faecal indicator bacteria elevated above the alert level of the water quality guidelines for recreational activity (>260 PN/100ml; Mfe/MoH 2003).

Surface waters that experience elevated or sporadically elevated levels of the faecal indicator bacteria *Escherichia coli* may pose public health risks to recreational users of these waters and shellfish harvesting areas. The sources of these bacteria are not always clear, although agricultural activities and human wastewater are often suspected. In many cases it is likely that more than one faecal source will be present and that some of the faecal inputs to rivers will be weather related.

In this study, analysis of *Escherichia coli* was performed on 43 water samples. Samples were collected on two occasions, once in March 2017 during 'dry weather' conditions and once during May 2017 following rainfall events, or 'wet weather' conditions. Samples were taken after rainfall as many contaminants are entrained in overland runoff, stormwater (e.g. bird faeces from stream banks, roads and roofs) and wastewater overflows which is then discharged into the rivers.

Of the 43 water samples analysed for *Escherichia coli*, 25 were analysed for Faecal Source Tracking. The Polymerase Chain Reaction (PCR) type of faecal source tracking analysis was used in this investigation as it detects bacterial contamination from a wide range of sources using DNA markers. A total of four bacterial sources were positively identified in this investigation; ruminant, avian, dog and human. Ruminant sources were predominately identified in Blackwell stream during both the dry and wet sampling events. Human source was also detected in Blackwell Stream, however only one PCR marker was identified and two Human PCR markers are required for a positive human faecal source result. Avian sources were weakly found in Mulberry Grove Stream, with the several water samples being 'not identified' despite high *Escherichia coli* concentrations. Human faecal contamination was found in Garden Road Stream, while other samples from this stream were also returned as 'not identified'. The PCR method has limitations and the source of bacterial contamination was not able to be identified at some sites, potentially due to degraded, aged or partially-treated sources such as from septic tanks.

1.0 Introduction

Contamination of waterways with faecal pollution is a major cause of reduced water quality in New Zealand and puts people at risk through contact with this water, as well as through the consumption of contaminated shellfish. In particular, areas frequently used for recreational activity pose a heightened health risk, due to the possibility of accidental immersion and consumption of contaminated water. Poor recreational water quality, identified by high levels of faecal bacteria often leads to closures of bathing waters such as rivers and beaches and highly valued shellfish harvesting areas. Indicator bacteria such as *Escherichia coli* (*E. coli)* are used to detect the presence of faecal material, and therefore potentially pathogenic organisms. A number of factors influence whether any pathogens will actually be present including the source of the faeces, time since excretion and the influence of various attenuation factors including sunlight, predation, and sedimentation.

Faecal Source Tracking (FST) is a set of methods which can be used to determine the host (different animals or Human) that contributes faecal pollution. The Polymerase Chain Reaction (PCR) type of faecal source tracking analysis was used in this investigation as it detects bacterial contamination from a wide range of sources using DNA markers. Total DNA is extracted from a water sample and the sample is examined using the PCR for DNA from source-specific organisms. The presence of certain microorganisms indicates the source of the faecal contamination.

In response to community concern over poor water quality flowing into the Tryphena Harbour and the closure of receiving bathing and recreational beach areas (Pah Beach, Gooseberry Beach and Mulberry Beach), the Great Barrier Island Local Board funded a year-long water quality monitoring programme in April 2015. The purpose was to analyse key parameters and determine whether there were any consistent water quality issues, and if so, recommend future targeted investigations or action, where required. Results suggested that faecal contamination, (indicated by *E.coli* concentrations) in Blackwell Stream, Mulberry Grove Stream and Garden Road Stream was occurring. All three streams frequently exceeded ‘alert’ (260 MPN/100 mL) and ‘action’ (550 MPN/100 mL) guideline values for contact recreation (MfE/MoH 2003) at various times throughout the 2015-2016 investigation (Buckthought 2016). The Great Barrier Island Local Board therefore decided to investigate the sources of such *E.coli* exceedances using Faecal Source Tracking techniques in order to inform future management options.

1.1 Scope

The aim of this investigation was to use faecal source tracking tools to investigate likely sources of faecal contamination in three streams within the Tryphena catchment; Blackwell Stream, Mulberry Grove Stream and Garden Road Stream (location map Appendix A1). In order to gain a better understanding of the sources of *E.coli* this investigation applied faecal source tracking tools in order to:

- determine the sources and location (if possible) of *E.coli* entering the three streams of interest (Blackwell Stream, Mulberry Grove Stream and Garden Road Stream); and
- Determine whether or not *E.coli* levels and sources vary during dry conditions (when most people use the waterways and beaches) and wet conditions (water-lodged soils, poor drainage and direct overflow path to waterways).

2.0 Background Information

2.1 Physical Description, Blackwell Stream

Blackwell Stream flows out of a forested catchment, down through low intensity farming and boarder's low intensity residential and commercial premises (i.e., café and pub) before reaching the ocean. The stream flows out into Pah Beach, a popular bathing/recreational beach and shellfish gathering area (Appendix A1 and A2). The upper reaches of the stream appear to visually be in a healthy state, with good algal cover, macroinvertebrate community, good diversity in flow patterns with thick riparian vegetation. Through the middle to lower reach riparian vegetation was patchy and low numbers of cattle are grazed either side of the stream throughout the year. There is no fencing along the stream margin from the mouth to 2 km upstream. During the March, dry weather sampling event cattle were observed in the paddocks on both sides of the stream and freely had access to the stream channel. No cattle were observed in the paddocks immediately adjacent to the stream or in the stream during the wet weather sampling in May.

The lower section of the stream often has high sedimentation due to the tidal nature where scouring of the stream bankside and sand deposition occurs. Deposition of seaweed in the lower tidal reach can also be high and over summer months often results in a very pungent odour of decaying organic matter. This smell can often be confused with the smell of raw human sewage. The riparian vegetation consists of thick grass cover (no trees) near the mouth and above the tidal wedge. This would provide ideal habitat for native Inanga spawning and the upper reaches of the stream have premium freshwater habitat with good riparian shading, undercut banks, woody debris and leaf detritus and healthy macroinvertebrate communities to feed on. Longfin Eels (*Anguilla dieffenbachia)* have been observed in the lower reach, Banded Kokopu (Galaxias fasciatus) in the upper reach and ducks can be seen throughout in the stream from time to time. During this investigation four potential barriers to fish passage were identified and are discussed further in Wilks, T 2016.

Residential and commercial properties are located between 60 to 200m from the stream margin on the true left side of the stream (Appendix A2). Due to the topography and geology of this area, excess water accumulating during rainfall events on these properties and those in the Blackwell Drive community, is able to flow via several cut out drains into a small wetland before entering Blackwell stream. Due to the shallow topsoil and impermeable clay soil profile beneath, retention time is short (particularly when water logged), thus filtration and processing of contaminants is brief before runoff enters the stream. In addition to the close proximity of residential and commercial properties and stormwater overflow pathways, onsite-waster water systems used in the area could also be contributing to fecal contamination in the stream if they are performing poorly (Ambury 2017).

2.1.1 Sampling Site Descriptions, Blackwell Stream

Seven sampling sites were identified along the reach of interest in Blackwell Stream. The upper most sampling location BW6 was located above the main residential housing and agricultural landuse practices. This site was selected in order to provide an indication of 'normal' background *E.coli* concentrations for Blackwell Stream. Several small tributary creeks and cut out drains which collect runoff from surrounding hillside farmland and residential housing along Blackwell Drive were sampled below BW6 (BW5, BW4, BW3, BW2, BW7; Appendix A2) down to the lowest sampling location near the stream mouth (BW1; Appendix A2). BW5 was located at the outlet of a cut out drain which collects runoff from a property on the true right side of the stream, up near the uppermost sampling location, BW6. This drain has been observed to have a pale orange-yellow type algal scum near the outflow into Blackwell Stream. Sampling location BW4, located above

the main housing areas of Blackwell Drive, was selected to be representative of farming land where cattle have access to the stream and stream margins (Figure 2A). Lower down the stream catchment, BW7 is located in the main stem, just below the entrance of a cut out drain. This drain (and another further downstream above BW1) collects runoff from residential properties and stormwater infrastructure during high rainfall events along Blackwell Drive (Appendix A2). A small pond approximately 30 m from the stream edge also feeds into this cut out drain before entering the stream.

A  B 

Figure 2. Blackwell Stream: A) Red dot indicates sampling location BW4. B) Red dot indicates sampling location BW1.

2.2 Physical Description, Mulberry Grove Stream

Mulberry Grove Stream flows out of a forested catchment, down through low intensity residential housing and two commercial properties (i.e., café, motel and laundromat) (Appendix A3). The stream flows out into Mulberry Grove Beach, a popular bathing/recreational beach and shellfish gathering area (Appendix A1 and A3). The upper reaches of the stream have premium freshwater habitat with riparian shading, undercut banks, woody debris, leaf detritus and a healthy macroinvertebrate community. Large longfin eels (*Anguilla dieffenbachia)* (> 1m in length) were observed throughout the channel during both sampling events. One potential barrier to fish passage was identified in the upper reach and is discussed further in Wilks, T 2016. There is a small area along the middle to upper reach where low numbers of sheep (15-20) frequently graze. There is no fencing along the true right side of the stream. Ducks can be observed in the stream and along the margin at various times throughout the year. The lower tidal section of the stream particularly in the summer months has a notably different algal composition to the rest of the stream, with long green-yellow filamentous algae. This area can have high levels of seaweed deposition also. A reduction in water level in the lower stream reach appears to occur over the summer months, potentially as a result of water takes further upstream.

Houses along Mulberry Grove Stream are extremely close to the stream edge (true left side of the stream), with their property boundaries immediately adjacent to the stream margin. Sections are generally small which can inhibit the effectiveness of wastewater discharge fields (Ambury 2017). There has been significant erosion to property boundaries through washouts from previous high flow events and from overflowing stormwater infrastructure along the upper reach of Mulberry Grove Stream. From the stream mouth to just above the last house along Mulberry Grove Road, numerous pipes (Figure 3), water takes and outlet drains were identified along the stream margin. These appeared to be coming from residential and commercial properties, and stormwater infrastructure.

2.2.1 Sampling Site Description, Mulberry Grove Stream

Seven sampling sites were identified along the reach of interest in Mulberry Grove Stream. The upper most sampling location MG6 was selected in order to provide an indication of 'normal' background *E.coli* concentrations for Mulberry Grove Stream as it is located above the main residential housing and agricultural landuse practices. MG7 was located in a cut out drain below the uppermost residential house on the true right hand side (Appendix A3). Sampling locations MG5 (Figure 3A), MG4 (Figure 3B), MG3, MG2 and MG1 were all below piped outlets entering the stream from either residential properties, commercial or stormwater infrastructure pipes. Sampling location MG5 collects runoff from 3 properties on the true left stream bank and during wet weather the stormwater network diverts water from six of the properties on the topside of Mulberry Grove Road down into Mulberry Grove Stream. There were also two large back ribbed pipes on the true left side of the stream bank above MG5. One black ripped pipe was seeping water during both sampling events and had a fine layer of orange algae (suspected iron bacteria) present on the substrate surface (Figure 3A). Of the piped outlets, only MG3 (during the wet weather sampling event) visually had a discharge coming out and was directly sampled. The most seaward sampling location MG1, was located below 6 white plastic pipes concreted into the wall. These pipes drain from a property on the true right side. Site location map provided in Appendix A3.

A  B 

Figure 3. A) Large black ribbed pipe, with fine layer of orange algae above sampling location MG5. B) Two Large pipes on stream edge above sampling location MG4.

2.3 Physical Description, Garden Road Stream

Garden Road Stream is of similar quality to Blackwell and Mulberry Grove Stream. It flows out of a forest catchment, down through low intensity residential housing before reaching the ocean (Appendix A4). The stream flows out into Mulberry Grove Beach, a popular bathing/recreational beach and shellfish gathering area (Appendix A1 and A4). The upper reaches of the stream appear visually to be in a healthy state, with good algal cover, macroinvertebrate community, good diversity in flow patterns with thick riparian vegetation. A large plantation of pine forest in the middle reach of this waterway has recently been cleared. It is unknown if any stream monitoring (e.g., for sedimentation) was undertaken during or post removal. Large longfin eels (*Anguilla dieffenbachia)* (> 1m in length) were observed throughout the channel during both sampling events. Ducks can generally be observed in the lower reach year round. Residential housing occurs on both sides of the stream along the

lower 250 – 300 m reach, with Mulberry Grove Primary School located at the bottom of the stream. Primary school students regularly undertake contact recreational activities at Mulberry Grove Beach so improving poor water quality should be a high priority for this area. Similar to Mulberry Grove Stream, properties on the true left side of the stream boarder immediately adjacent to the stream edge, section sizes are generally small and there are numerous pipes, water takes and drains entering the stream.

2.3.1 Sampling Site Description, Garden Road

Seven sampling sites were identified along the reach of interest in Garden Road Stream. The upper most sampling location GR8 was selected in order to provide an indication of 'normal' background *E.coli* concentrations for Garden Road Stream, as it is located above the main residential housing and agricultural landuse practices. Sampling locations GR7, GR5 and GR2 were all below piped outlets entering the stream from either residential properties or stormwater infrastructure. Water was flowing out the white piped outlet at GR2 during both sampling events and was directly sampled (Figure 4A). GR6 was located below a suspected failing wastewater discharge channel entering the stream from the uppermost property boarding the true right side of the stream. This property is a holiday house was vacant during both of the sampling events. GR4 was located below the uppermost permanent residential property with a suspected failing wastewater system on the true left side of the stream (Figure 4A). GR3 was located below a culvert on the true right side which collects runoff from several properties on Rosalie Bay Road. GR1 was the lowest sampling location in the catchment and also located below a cut out drain which collects runoff from the lowest residential property on the true left bank. Site location map provided in Appendix A4.

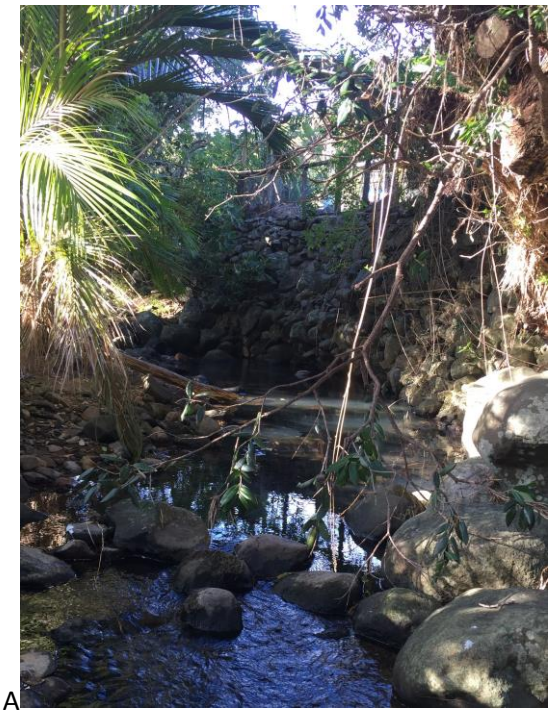
A  B 

Figure 4. A) Sampling location GR4. B) Pipe entering the stream from a residential property, above sampling site GR2.

2.3.2 Stormwater Outflow Pipes

Three stormwater outflow pipes were selected for sampling as they directly enter two of the three beaches of concern; Pah Beach and Gooseberry Beach (Appendix A1). Stormwater outflow pipe located at the southern end of Gooseberry Beach (STRM1), Stormwater outflow

pipe located at the northern end of Gooseberry Beach (STRM2) and a Stormwater outflow pipe located, near the public toilets at the Pah Beach (STRM3) (Appendix X). STRM3 was dry during the March sampling event, and STRM2 was inaccessible during the May sampling event. All three stormwater outflows collect runoff from the upper catchment residential housing and roading infrastructure; STRM3 collects the upper northern end of Blackwell Drive, STRM2 collects the upper southern end of Blackwell Drive and STRM1 also collects runoff from houses above Shoal Bay Road and Omanawa Lane.

2.4 Potential Sources of Faecal Contamination

There are a few key sources of faecal contamination in the Tryphena catchment, some or all of which may be contributing to the observed high *E.coli* in Blackwell Stream, Mulberry Grove Stream and Garden Road Stream. These include livestock (i.e., cattle, sheep), horses, dogs, cats, avian fauna (i.e., ducks) and human.

A recent wastewater education project undertaken in the Tryphena catchment (Ambury 2017) suggests that poorly performing on-site wastewater systems and discharge fields may be widespread. Ambury (2017) suggest that many of the septic tanks are old (i.e., installed more than 20 years ago), and many are serviced infrequently or not at all. In addition to septic tanks, several other methods of wastewater management were identified in the Tryphena catchment. These include secondary treatment systems, long drops, and greywater diversion to gardens or landscaped areas 9Ambury 2017). In the Tryphena catchment, there are also two public long drop toilet facilities owned and operated by Auckland Council, one located 3-5 m from Pah Beach and one located approximately 50 m at Mulberry Grove. These methods and facilities were not inspected during the education programme. However, as some of these methods pose contamination risks (especially long drops and greywater diversion), and merit investigation in the future, especially if shown to be an issue.

3.0 Methodology

Project Overview:

Stage One: A stream walk from the mouth to above residential housing and farming areas was undertaken at all three streams known to have *E.coli* contamination issues; Blackwell stream, Mulberry Grove stream and Garden Road stream. This was to identify hot-spots where bacterial contamination was most likely and to identify future sample locations. Each potential sampling location was captured using a GPS device and photographed for a later prioritisation exercise to confirm the sites to be sampled. A letter drop was provided to residents and landowners immediately adjacent to those streams informing them that a stream walk would be conducted.

Stage two: During low tide, water samples were taken from the selected locations and sent off for *E.coli* analysis (analysed by Aqualab NZ) and FST analysis (analysed by ESR)(Section 4.2). Samples were collected once in dry weather, (no rain for at least two weeks) in March 2017 and once in wet weather, May 2017. The wet weather sampling event occurred after a several rainfall events and during very light drizzle the day of sampling (Section 4.1). Close attention was made to any presence of animals i.e., cattle, sheep, dogs, cats, avian fauna or suspect wastewater discharges during both sampling events.

3.1 Site Selection and Location

A total of twenty-four sites were selected for sampling during the investigation (Table 4.1; Appendix A). Twenty sites were sampled in March 2017; five on Blackwell Stream, seven on Mulberry Grove Stream and six on Garden Road stream and two stormwater outflow pipes entering Gooseberry Beach (Table 4.1). Twenty-three sites were sampled in May 2017; seven on Blackwell Stream, seven on Mulberry Grove Stream and seven on Garden Road stream and two stormwater outflow pipes, one entering Pah Beach and One entering Gooseberry Beach (Table 4.1). Detailed site descriptions are provided below, along with sampling locations in Table 4.1, and location maps in Appendix A1-A4.

Table 4.1. Water Quality Sampling Locations, Tryphena Great Barrier Island

Stream Name	Site ID	Dry	Wet	Easting	Northing
Blackwell Stream	BW1	Yes	Yes	1823339	5979804
Blackwell Stream	BW2	No	Yes	1823415	5979847
Blackwell Stream	BW3	No	Yes	1823316	5979925
Blackwell Stream	BW4	Yes	Yes	1823425	5979935
Blackwell Stream	BW5	Yes	Yes	1823492	5980171
Blackwell Stream	BW6	Yes	Yes	1823503	5980246
Blackwell Stream	BW7	Yes	Yes	1823386	5979865
Mulberry Grove Stream	MG1	Yes	Yes	1823860	5978741
Mulberry Grove Stream	MG2	Yes	Yes	1823923	5978754
Mulberry Grove Stream	MG3	Yes	Yes	1823948	5978760
Mulberry Grove Stream	MG4	Yes	Yes	1824080	5978785
Mulberry Grove Stream	MG5	Yes	Yes	1824109	5978769
Mulberry Grove Stream	MG6	Yes	Yes	1824186	5978914
Mulberry Grove Stream	MG7	Yes	Yes	1824149	5978831
Garden Road Stream	GR1	Yes	Yes	1824013	5978411
Garden Road Stream	GR2	Yes	Yes	1824059	5978387
Garden Road Stream	GR3	Yes	Yes	1824093	5978372
Garden Road Stream	GR4	Yes	Yes	1824106	5978344
Garden Road Stream	GR6	No	Yes	1824165	5978342
Garden Road Stream	GR7	Yes	Yes	1824167	5978247
Garden Road Stream	GR8	Yes	Yes	1824195	5978223
Stormwater outflow -South end Gooseberry Flat	STRM1	Yes	Yes	1823584	5979348
Stormwater outflow -North end Gooseberry Flat	STRM2	Yes	No	1823614	5979284
Stormwater outflow -Pah Beach	STRM3	No	Yes	1823456	5979668

3.2 Sample collection and Analysis

Water samples were collected at the locations outlined above (Table 4.1). Site location maps are provided in Appendix A. Samples were collected on two occasions in March and May 2017. March was representative of dry conditions, where it had not rained for at least two weeks prior sampling. Sampling also occurred in May after rainfall (Table 4.1) in order to see if *E.coli* concentrations increased as many contaminants are entrained in overland flow such as stormwater runoff (e.g. bird faeces from roads and roofs) and wastewater overflows which is then discharged into rivers.

3.2.1 *Escherichia coli* Analysis

The *E. coli* samples were collected in sterile 100 ml bottles and sterile 1 L bottles were filled to allow filter-freezing for the bacterial source samples. The samples were stored at less than 4°C and couriered to Aqualab NZ for analysis within 24 hours of sample collection. The *E. coli* samples were analysed using the Colilert-quantitray method and reported as Most Probable Number (MPN) per 100ml. The 1 L samples were passed through a 0.45µm filter and frozen.

Once all water sampling and *E.coli* analysis was complete, those samples that exceeded the MoH/MfE guidelines for recreational water quality (>260 MPN/100ml) or close to that value were couriered to the Environmental Science and Research (ESR) laboratory in Christchurch for bacteria source analysis.

For the purposes of the current report, the MoH/MfE (2003) guidelines for recreational water quality have been used. Whilst only one of the three streams is used for swimming (Mulberry Grove Stream), all three stream flow into the popular swimming beaches. Therefore the 'alert' (260 MPN/100ml) and 'action' (550 MPN/100 ml) guideline values are considered appropriate when reviewing *E.coli* results.

3.2.2 Faecal Source Tracking (FST) Analysis

A range of microorganisms are present in faeces, which are specific to their animal hosts. Total DNA is extracted from a water sample and the sample is examined using the polymerase chain reaction (PCR) method for DNA from source-specific organisms, by comparing the sample DNA to a DNA-library. The presence of certain microorganisms indicates the source of the faecal contamination. Assays specific for humans, herbivores, dogs and wildfowl are available. ESR laboratory used a total of six PCR markers based on the likely sources of bacterial contamination in the catchment. The PCR markers included:

- General marker (GenBac) representative of either Human, Cow, Sheep, Deer, Goat, Pig, Rabbit, Possum, Cat, Dog, Horse, Duck, Swan, Seagull, Geese, Chicken
- Ruminant (BacR) representative of Cattle, Sheep, Deer or Goat
- Canine (DogBac),
- Avian (GFD) representative of Duck, Swan, Seagull, Geese, Chicken; and
- Two human markers (BacH and BiADO)

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 - 100 % ruminant should be interpreted as an entirely ruminant source. Samples reported between 1 – 50 % 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 or human faecal sources. When identified as the Avian PCR marker it is indicative of either duck, swan, seagull, geese and/or chicken.

Results for all other animal sources can only be reported as present or absent. In several marker assays "human municipal sewage" is detected at low levels. Because of the nature and source of municipal sewage ESR cannot be certain it is a pure human source. A positive result seen in the assays may in fact be a true positive for the target marker (ESR 2017).

4.0 Results

4.1 Rainfall

The size and topography of Great Barrier Island can often result in it raining on one side of the island or one end of the island not the rest. The only calibrated rainfall data is collected in Claris, Great Barrier Island by Auckland Council. Whilst this is only 20 km from the study area, rainfall patterns can vary substantially. Therefore, the following rainfall data has been used as an indicator of rainfall for Tryphena only, rather than specific rainfall measurements. This data is further supported by field staff notes.

There were no rainfall events for two weeks prior the Dry weather sampling in March 2017. During April 2017, the island had experienced two periods of heavy down-poor, resulting in widespread flooding. There was a 2 week stand down period before sampling to allow for stream water levels and clarity to return to normal. There had been light rainfall for 48 hours prior to the wet weather sampling in May 2017 and it was lightly drizzling during the actual sampling event.

Note: Although outside of the scope of this project, a brief review was undertaken on rainfall data and water quality results from the safe swim monitoring programme where faecal indicator bacteria are monitored over summer months at two recreational beaches in Tryphena. There appeared to be a strong relationship between rainfall and faecal indicator bacteria exceedances of the contact recreational guidelines at Pah Beach and Mulberry Beach.

4.2 *E.coli* and Faecal Source Tracking

E.coli Analysis was performed on 43 water samples, 12 from Blackwell Stream, 14 from Mulberry Grove, 13 from Garden Road and 4 from stormwater drains. Where samples exceeded the 'alert' guideline for recreational water quality (260 MPN/100ml; MoH/MfE 2003) or close to that value, samples were analysed for faecal bacterial sources using the PCR method. Of the 43 water samples analyses, 25 were analysed using the PCR method. Raw *E.coli* results and PCR marker analysis results are presented in Appendix B and Appendix C, with a detailed breakdown of results presented below.

It is important to note that the following results are based off two sampling events only and provide an indication 'snap shot' of faecal contamination present at the time.

4.2.1 Blackwell Stream

E.coli concentrations increased from the upper sampling location (BW6) down to the stream mouth (BW1; Figure 5, Appendix B). Nine of the eleven results (82 %) exceeded the alert level and six (54 %) exceeded the action level for contact recreation (MfE/MoH 2003).

Three key locations of faecal contamination were identified; BW1, BW7 and BW4. All three of those sites had high *E.coli* concentrations (> 800 MPN/100ml) during both dry and wet weather sampling (Figure 5, Appendix B). The increase in *E.coli* from BW7 to BW1 which are approximately 60 m apart during the wet weather sampling, suggests that there is a source of contamination coming in between the two sampling points (Figure 5). This is most likely from a cut out drain immediately above BW1 which collects runoff from residential and commercial properties (Appendix A2). There was no distinct pattern in *E.coli* concentrations between dry weather and wet weather sampling events. *E.coli* concentrations were higher during dry weather at BW7 and BW4 than during wet weather sampling and higher at BW1 during wet weather than dry weather sampling (Figure 5).

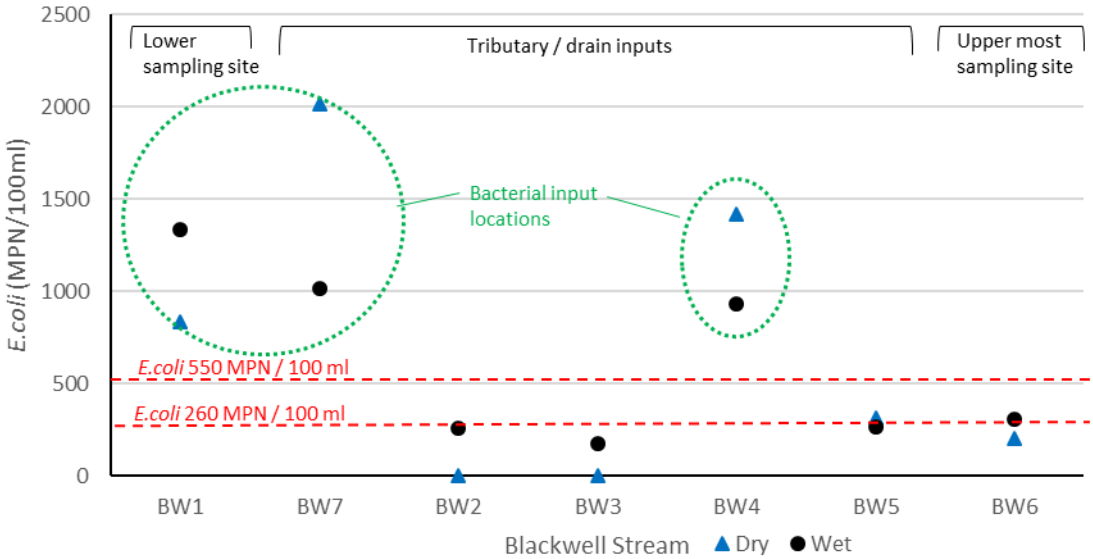


Figure 5. *E.coli* concentrations at Blackwell Stream, taken during March "Dry" and May "Wet" weather conditions 2017. Red dashed lines indicate guideline for recreational water quality (alert 260 and action 550 MPN/100ml; MoH/MfE 2003).

FST was undertaken on 10 of the 12 water samples (Table 5.2.1; Appendix C). All samples analysed indicated the presence of ruminant faecal matter and some avian (Table 4.2.1; Appendix C). The ruminant marker is representative of Cattle, Sheep, Deer and Goat and the Avian is representative of Duck, Swan, Seagull, Geese and Chicken. The only ruminant species present along Blackwell Stream are cattle, therefore results suggest that cattle and to a much lesser extent Avian fauna (most likely ducks) appear to be the dominant sources of bacterial or faecal contamination in Blackwell Stream. Cattle and ducks were observed in and around the sample area during the dry weather event. No animals were observed during the wet weather sampling event in or along the stream.

Human faecal source was detected at the lowest sampling location BW1 (Appendix C) and in a cut out drain near the upper most sampling location, BW5 during the dry weather sampling (Appendix C), however only one PCR marker was identified and two Human PCR markers are required for a positive human faecal source result. The source of bacterial contamination was not able to be identified, most likely due to degraded, aged or partially-treated sources such as from wastewater systems (i.e., septic tanks).

Table 5.2.1 Blackwell Stream Bacterial Source Summary

Site	Sampled	Event	E.coli (MPN/100ml)	Bacterial Source
BW1	22/03/2017	DRY	836	Ruminant, some avian
BW1	1/05/2017	WET	1334	Ruminant, some avian
BW2	2/05/2017	WET	256	Avian
BW4	22/03/2017	DRY	1421	Ruminant, some avian
BW4	1/05/2017	WET	932	Ruminant
BW5	22/03/2017	DRY	315	Weak ruminant
BW5	1/05/2017	WET	262	Ruminant, some avian
BW6	1/05/2017	WET	309	Weak ruminant
BW7	22/03/2017	DRY	1014	Ruminant, some avian
BW7	1/05/2017	WET	2014	Ruminant, some avian

4.2.2 Mulberry Grove Stream

The concentrations of *E.coli* varied substantially between sites, increasing exponentially from the upper most sampling location MG6 to the lowest sampling location MG1 during both dry and wet weather sampling events (Figure 6). Results suggest multiple bacterial input locations (Figure 6). Ten of the fourteen sample results (71 %) exceeded the alert level and six (43 %) exceeded the action level for contact recreation (Figure 6; MfE/MoH 2003). The lowest *E. coli* level recorded was 41 MPN/100ml at site MG6 (the upper most sampling location) and the highest *E. coli* level recorded was 1046 MPN/100ml at site MG5 and 1017 MPN/100ml at the lowest sampling site MG1 (Figure 6; Appendix B).

There was a notable difference in results between the dry and wet sampling events, with *E.coli* concentrations almost doubling during the wet sampling event (Figure 6; Appendix B). Dry weather sampling results suggest there were four main areas of faecal contamination, increasing between sampling locations MG4, MG3, MG2 to MG1 and wet weather sampling results suggest there were multiple faecal contamination locations downstream of MG6 (Figure 6). During the wet weather sampling, concentrations increased from MG3 (576 MPN/100ml) to MG2 (960 MPN/100ml). These sites are 30 m apart and are located within the same property. Both dry and wet weather results for MG1 were high (MG1: dry 836 MPN/100ml and wet 1334 MPN/100ml) and higher than the upstream site MG2 (Figure 6, Appendix B).

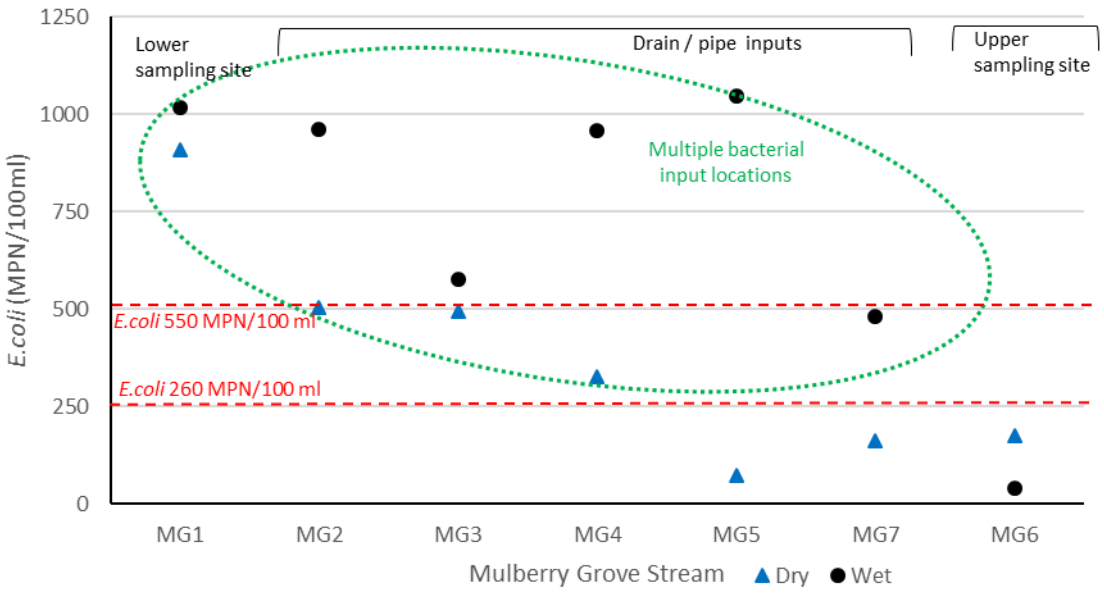


Figure 6. *E.coli* concentrations at Mulberry Grove Stream, taken during March "Dry" and May "Wet" weather conditions 2017. Red dashed lines indicate guideline for recreational water quality (alert 260 and action 550 MPN/100ml; MoH/MfE 2003).

FST analysis was undertaken on 10 of the 14 water samples (Table 5.2.2). FST results were largely non-conclusive, and despite high *E.coli* values (Table 5.2.2, Appendix C) the majority of samples were either 'not identified' or 'weak avian' at very low levels (Appendix C). The four samples collected during the dry weather event were not identified (MR1, MG2, MG3, and MG4) and five of the samples (MG1, MG2, MG4, MG5) collected during wet weather returned 'weak avian source' and only one site, MG3 was strongly positive for 'avian source' (Appendix C). The source of bacterial contamination was not able to be identified, most likely due to degraded, aged or partially-treated sources such as from wastewater systems. Note: no avian fauna was observed in or near the stream during either sampling event.

Table 5.2.2 Mulberry Grove Bacterial Source Summary

Site	Sampled	Event	E.coli	Bacterial Source
MG1	22/03/2017	DRY	909	Not Identified
MG1	1/05/2017	WET	1017	Weak avian
MG2	22/03/2017	DRY	504	Not Identified
MG2	1/05/2017	WET	960	Weak avian
MG3	22/03/2017	DRY	495	Not Identified
MG3	1/05/2017	WET	576	Avian
MG4	22/03/2017	DRY	327	Not Identified
MG4	1/05/2017	WET	959	Weak avian
MG5	1/05/2017	WET	1046	Weak avian
MG7	1/05/2017	WET	480	Weak avian

4.2.3 Garden Road Stream

E.coli concentrations were generally low in Garden Road Stream (Figure 7, Appendix B) with the exception of three samples. *E.coli* concentrations increased at two sites during the wet weather sampling, GR3 (450 MPN/100ml) and GR2 (809 MPN/100ml) (Figure 6, Appendix B). The highest *E.coli* concentration (and of the whole study) was obtained at GR4 during the dry weather sampling (2613 MPN/100ml; Figure 6, Appendix B).

The upper most sampling location and lowest sampling location returned similar low values (Figure 7) on both dry and wet sampling events. This suggests that there was a reasonable level of dilution occurring within the stream on those occasions when high *E.coli* concentrations at GR2 and GR4 occurred.

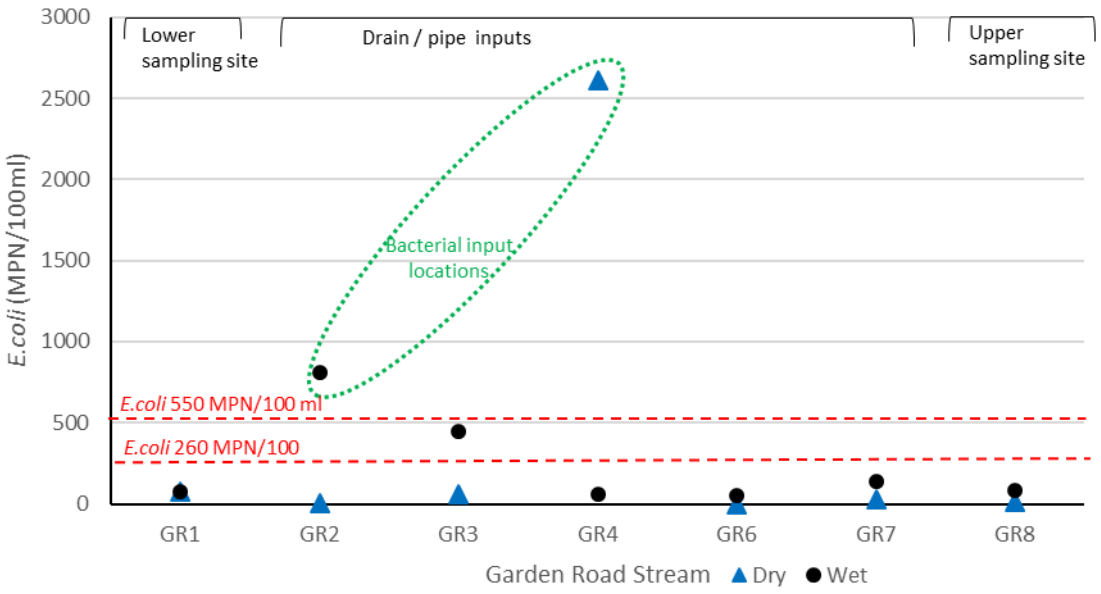


Figure 7. *E.coli* concentrations at Garden Road Stream, taken during March "Dry" and May "Wet" weather conditions 2017. Red dashed lines indicate guideline for recreational water quality (alert 260 and action 550 MPN/100ml; MoH/MfE 2003).

Of the 14 *E.coli* samples taken, 3 were analysed for Faecal source tracking analysis (Table 5.2.3, Appendix C). Results indicated that at GR2 (wet sampling event; *E.coli* = 809 MPN/100ml) the source was of human origin with weak evidence of dog. The faecal source of both GR3 (wet sampling; *E.coli* 450 MPN/100ml) and GR4 (dry sampling; *E.coli* 2613 MPN/100ml) was not able to be identified. Human faecal matter was detected at GR3, however only one PCR marker was identified and two Human PCR markers are required for a positive human faecal source result (Appendix C). This can be due to degraded, aged or partially-treated sources such as poorly performing wastewater systems and discharge fields.

Table 5.2.3 Garden Road Bacterial Source Summary

Site	Sampled	Event	E.coli	Bacterial Source
GR2	1/05/2017	WET	809	Human, weak dog
GR3	1/05/2017	WET	450	Not Identified
GR4	22/03/2017	DRY	2613	Not Identified
STRM1	22/03/2017	DRY	345	Avian
STRM2	22/03/2017	DRY	399	Avian

4.2.4 Stormwater Outflow

E.coli concentrations were higher at STRM1 during dry (345 MPN/100ml) weather than wet (197 MPN/100ml) weather sampling (Appendix B). STRM2 was similar to STRM1 during dry weather, recording *E.coli* at 399 MPN/100ml. No water sample was able to be obtained from STRM2 during wet weather as previous flooding has made the upper channel inaccessible without a grabber and the lower end of the outflow pipe had been completely covered in sand. STRM3 was dry during the dry weather sampling, and *E.coli* was below the alert guideline values for contact recreation (260 MPN/100ml) during the wet weather sampling (STRM3 = 213 MPN/ 100ml).

FST analysis was undertaken on two samples from STRM1 and STRM2 taken during the dry weather sampling event (Table 5.2.3). Both results came back positive for Avian faecal source (Appendix C).

5.0 Summary

1. All three streams, Blackwell Stream, Mulberry Grove Stream and Garden Road stream experience high levels of faecal contamination from various sources.
2. Faecal bacterial contamination often increases considerably after rainfall.
3. A total of four bacterial sources were positively identified in this investigation; ruminant, avian, dog and human. The source of bacterial contamination was not able to be identified at some sites, potentially due to degraded, aged or partially-treated sources such as septic tanks.
4. Ruminant bacterial contamination was the dominant source of *E.coli* during the investigation in Blackwell Stream. The widespread ruminant bacterial contamination during the dry weather sampling in Blackwell Stream indicates livestock access to the stream (primarily cattle) and lack of riparian fencing may be an issue.
5. Avian bird species, most likely ducks, were a weak contributing factor to faecal contamination in Mulberry Grove Stream. Despite high *E.coli* concentrations, the source of many samples were unable to be identified. This is potentially a result of semi-treated human bacterial contamination from poorly performing or failing on-site wastewater systems.
6. Human bacterial contamination was found at one site in Garden Road Stream during wet weather. The most likely cause of human bacterial contamination in this area is failing on-site wastewater systems (septic tanks) (Ambury 2017). Further work is required to identify where the pipe at site GR2 is specifically coming from (i.e., wastewater system or diverted household greywater) and cause/source of faecal contamination further up the stream.
7. Similarly to Mulberry Grove Stream, despite high levels of E.coli concentrations, faecal sources were not able to be identified in the upper reaches of Garden Road Stream.
8. A limitation of the PCR bacterial source method is that faecal 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 some sites, one cannot rule out that 'unidentified' sources could be an aged or partially treated human source, especially where a very strong positive general marker and high *E. coli* are found (Appendix C). An additional investigation would be required to determine if human bacterial sources are present at those site. However, given the knowledge of old wastewater systems, and potentially poorly performing wastewater systems it would it prudent that focus be given to adequately assessing and remediating those where required.

6.0 Recommendations

1. Visit property owners along Blackwell Stream without livestock exclusion to discuss mitigation options and riparian fencing. Riparian fencing alone would greatly reduce the bacterial contamination in Blackwell Stream, particularly during dry weather. It is recommended that funding (such as the Auckland Council Waterway Protection Fund) be allocated for livestock exclusion fencing along Blackwell Stream.

2. Good riparian management with a 10m set-back has been shown to reduce *E. coli* concentrations in New Zealand streams (Collins and Rutherford, 2004). Riparian buffer widths between 1 and 10 m 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. It is recommended that funding (such as the Auckland Council Waterway Protection Fund) be allocated for riparian planting along the lower reaches of Blackwell Stream to help improve soil filtration capability.

3. Inspect onsite wastewater systems at all properties boarding Blackwell Stream, Mulberry Grove Stream and Garden Road Steam and provide free inspections of long drops with the aim of identifying if any pose contamination risk. Long drops may be an acceptable solution if they are located and constructed appropriately. However, if they are poorly located or constructed, they could pose a contamination risk to the waterways.

4. There are two public long drop toilet facilities owned and operated by Auckland Council, one located 3-5 m from Pah Beach and one located approximately 50 m from Mulberry Grove Beach. Given the close proximity of these to the recreational areas it recommended that the maintenance log and structural integrity of these are reviewed.

5. The wastewater education project (Ambury 2017) found that it was relatively common for Tryphena residents to divert their greywater away from their on-site wastewater system (e.g., by directing pipes from their bathroom, laundry or kitchen, on to gardens or landscaped areas). While residents often assume that greywater poses no risk to the environment, greywater can have high faecal coliform bacteria concentrations. This may be the case for Mulberry Grove Stream and Garden Road Stream (GR2) were several pipes entering the streams from property boundaries were identified and high *E.coli* concentrations in the receiving waters. Therefore, further investigation is warranted and/or more education around the potential contamination issue with greywater diversion into waterways.

6. Several Tryphena businesses have high wastewater volumes (e.g., cafes, pubs and schools) and make use of secondary treatment systems which are located in close proximity to stream margins. While these systems are serviced annually, it is particularly important to ensure that they provide adequate treatment. For this reason, it is recommended that periodic monitoring of local businesses with high wastewater volumes occurs, to ensure that their treatment systems and disposal fields are operating correctly.

7. *E.coli* concentrations were generally shown to be higher after the wet weather sampling event. Rainfall can influence concentrations of faecal indicator bacteria when stormwater overwhelms wastewater systems, and given the poor drainage of clay soil, soils quickly become waterlogged causing rapid overland surface flow. Additionally, tidal flows may wash faecal bacteria back towards the beach. Therefore, raising general health messages within the community is recommend, and having appropriate signage when required:
    - avoid swimming in high-risk areas such as near outfalls and stream mouth
    - avoid swimming for up to 48 hours after rain events

7.0 Limitations

It is important to note that there were limitations of this study, primarily low sample numbers. The number of samples collected was small (two occasions; one dry, one wet). However, given this limitation, a number of common bacterial sources and potential solutions have been identified which could 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. Faecal Source Tracking (FST) technology is a rapidly developing science and the assays are constantly improving.

8.0 References

Ambury, A., (2017.) Onsite Wastewater Education Programme, Tryphena Great Barrier Island. Whiterock Consulting. *Prepared for:* Auckland Council and the Great Barrier Local Board.

Buckthought, L. (2016). Freshwater monitoring programme: Tryphena Harbour Streams, Great Barrier Island.

Collins, R and Rutherford, K. 2004. Modelling bacterial water quality in streams draining pastoral land. *Water Research,* 38**,** 700-712

Ministry for the Environment and Ministry of Health (MfE/MoH) 2002/2003. *Microbiological water quality guidelines for marine and freshwater recreational areas.* Ministry for the Environment. Wellington.

Wilks, T., (2017). Culvert Remediation Validation Monitoring, Great Barrier Island. Wilks Environmental Consulting. *Prepared for:* The Great Barrier Local Board and Auckland Council.

APPENDIX A1: Overview Map, Tryphena

Sample Locations
Great Barrier Island

0 40 80 120
Meters

Scale @ A4
= 1:9,383

Date Printed:
13/07/2017

Auckland Council
Te Kaunihera o Tāmaki Makaurau

APPENDIX A2: Blackwell Stream Site Location Map

Sample Locations
Blackwell Stream & Stormwater outlet pipes
Great Barrier Island

0 25 50 75
Meters

Scale @ A4
= 1:5,000

Date Printed:
13/07/2017

Auckland Council
Te Kaunihera o Tāmaki Makaurau

APPENDIX A3: Mulberry Grove Stream Site Location Map

Sample Locations
Mulberry Grove Stream
Great Barrier Island

0 10 20 30
Meters

Scale @ A4
= 1:2,729

Date Printed:
13/07/2017

Auckland Council
Te Kaunihera o Tāmaki Makaurau

APPENDIX A4: Garden Road Stream Site Location Map

Sample Locations
Garden Road Stream
Great Barrier Island

0 10 20 30
Meters

Scale @ A4
= 1:3,234

Date Printed:
13/07/2017

Auckland Council
Te Kaunihera o Tāmaki Makaurau

Document Path: U:\COO\ES\ESU\Geospatial_ESU\Projects\01_Biodiversity\2016\01_0073_16_MattGBIculverts_Dec2016\3_Workspaces\Sample locations GBI.mxd

APPENDIX B: Raw *Escherichia coli* Results

Key: Red indicates those samples that were analysed for FST, NS = not sampled

Stream Name	Site ID	DRY	WET
		E.coli MPN/100ml	
Blackwell Stream	BW1	836	1334
Blackwell Stream	BW7	2014	1014
Blackwell Stream	BW2	NS	256
Blackwell Stream	BW3	NS	171
Blackwell Stream	BW4	1421	932
Blackwell Stream	BW5	315	262
Blackwell Stream	BW6	201	309
Mulberry Grove Stream	MG1	909	1017
Mulberry Grove Stream	MG2	504	960
Mulberry Grove Stream	MG3	495	576
Mulberry Grove Stream	MG4	327	959
Mulberry Grove Stream	MG5	73	1046
Mulberry Grove Stream	MG7	161	480
Mulberry Grove Stream	MG6	173	41
Garden Road Stream	GR1	75	74
Garden Road Stream	GR2	5	809
Garden Road Stream	GR3	63	450
Garden Road Stream	GR4	2613	63
Garden Road Stream	GR6	NS	52
Garden Road Stream	GR7	31	142
Garden Road Stream	GR8	10	84
Stormwater drain Sth Gooseberry Beach	STRM1	345	197
Stormwater drain Nrth Gooseberry Beach	STRM2	399	NS
Stormwater drain Pah Beach	STRM 3	NS	213

APPENDIX C: Faecal Source Tracking Report

13 June 2017

To: Rhianna Drury
Senior Healthy Waters Specialist
Auckland Council
AUCKLAND

Rhianna.drury@aucklandcouncil.govt.nz

From: ESR Christchurch Science Centre
PO Box 29181
CHRISTCHURCH 8540

Email: faecalsource@esr.cri.nz

REPORT ON FAECAL SOURCE TRACKING ANALYSIS

The following samples were received on 9th May 2017 and were analysed for faecal source PCR markers. The samples had been pre-filtered prior to sending to ESR.

ESR Number	Client Reference	Date Sampled	Site Description	*E.coli* MPN/100mL
CMB170738	22145/01	22/03/2017	BW1	836
CMB170739	22145/02	22/03/2017	BW4	1421
CMB170740	22145/03	22/03/2017	BW5	315
CMB170741	22145/05	22/03/2017	MG1	909
CMB170742	22145/06	22/03/2017	MG2	504
CMB170743	22145/07	22/03/2017	MG3	495
CMB170744	22145/08	22/03/2017	MG4	327
CMB170745	22145/13	22/03/2017	GR4	2613
CMB170746	22145/18	22/03/2017	STRM1	345
CMB170747	22145/19	22/03/2017	STRM2	399
CMB170748	22145/20	22/03/2017	BW7	2014
CMB170749	22272/02	1/05/2017	GR2	809
CMB170750	22272/03	1/05/2017	GR3	450
				Cont.

ESR Number	Client Reference	Date Sampled	Site Description	*E.coli* MPN/100mL
CMB170751	22272/08	1/05/2017	MG1	1017
CMB170752	22272/09	1/05/2017	MG2	960
CMB170753	22272/10	1/05/2017	MG3	576
CMB170754	22272/11	1/05/2017	MG4	959
CMB170755	22272/12	1/05/2017	MG5	1046
CMB170756	22272/14	1/05/2017	MG7	480
CMB170757	22272/15	1/05/2017	BW1	1334
CMB170758	22272/16	2/05/2017	BW2	256
CMB170759	22272/18	1/05/2017	BW4	932
CMB170760	22272/19	1/05/2017	BW5	262
CMB170761	22272/20	1/05/2017	BW6	309
CMB170762	22272/21	1/05/2017	BW7	1014

Results of faecal source PCR Marker Analysis:

Please refer to the appendix for guidance on interpretation of these results

ESR Number	Client Reference	Site	*E. coli* (MPN/ 100mL)	General GenBac / 100 mls	Human BacH / 100 mls	Human BiADO / 100 mls	Ruminant BacR / 100 mls	Proportion Ruminant	Dog DogBac / 100 mls	Avian GFD / 100 mls	Conclusion
CMB170738	22145/01	BW1	836	630,000	detected, <LOQ	ND	130,000	50 - 100%	ND	300	Ruminant source (50-100%) + some avian
CMB170757	22272/15	BW1	1334	95,000	ND	ND	7,200	50 - 100%	ND	190	Ruminant source (50-100%) + some avian
CMB170758	22272/16	BW2	256	19,000	ND	ND	ND	ND	ND	770	Avian source
CMB170739	22145/02	BW4	1421	760,000	ND	ND	110,000	50 - 100%	ND	390	Ruminant source (50-100%) + some avian
CMB170759	22272/18	BW4	932	37,000	ND	ND	4,600	50 - 100%	ND	ND	Ruminant source (50-100%)
CMB170740	22145/03	BW5	315	44,000	detected, <LOQ	ND	480	1 - 10%	ND	ND	Weak ruminant source (1-10%) or aged ruminant source
CMB170760	22272/19	BW5	262	37,000	ND	ND	2,500	50 - 100%	ND	180	Ruminant source (50-100%) + some avian
CMB170761	22272/20	BW6	309	19,000	ND	ND	480	10 - 50%	ND	ND	Ruminant source (10-50%)
CMB170748	22145/20	BW7	2014	510,000	ND	ND	68,000	50 - 100%	ND	160	Ruminant source (50-100%) + some avian
CMB170762	22272/21	BW7	1014	51,000	ND	ND	4,000	50 - 100%	ND	140	Ruminant source (50-100%) + some avian

Cont.

ESR Number	Client Reference	Site	*E. coli* (MPN/ 100mL)	General GenBac / 100 mls	Human BacH / 100 mls	Human BiADO / 100 mls	Ruminant BacR / 100 mls	Proportion Ruminant	Dog DogBac / 100 mls	Avian GFD / 100 mls	Conclusion
CMB170741	22145/05	MG1	909	56,000	ND	ND	ND	ND	ND	ND	Faecal source not identified
CMB170751	22272/08	MG1	1017	34,000	ND	ND	ND	ND	ND	detected, <LOQ	Weak evidence avian source
CMB170742	22145/06	MG2	504	51,000	ND	ND	ND	ND	ND	ND	Faecal source not identified
CMB170752	22272/09	MG2	960	40,000	ND	ND	ND	ND	ND	detected, <LOQ	Weak evidence avian source
CMB170743	22145/07	MG3	495	44,000	ND	ND	ND	ND	ND	ND	Faecal source not identified
CMB170753	22272/10	MG3	576	57,000	ND	ND	ND	ND	ND	100	Avian source
CMB170744	22145/08	MG4	327	49,000	ND	ND	ND	ND	ND	ND	Faecal source not identified
CMB170754	22272/11	MG4	959	28,000	ND	ND	ND	ND	ND	detected, <LOQ	Weak evidence avian source
CMB170755	22272/12	MG5	1046	19,000	ND	ND	ND	ND	ND	detected, <LOQ	Weak evidence avian source
CMB170756	22272/14	MG7	480	34,000	ND	ND	ND	ND	ND	detected, <LOQ	Weak evidence avian source
CMB170749	22272/02	GR2	809	370,000	400	1,800	ND	ND	detected, <LOQ	ND	Human source + weak evidence dog source
CMB170750	22272/03	GR3	450	15,000	detected, <LOQ	ND	ND	ND	ND	ND	Faecal source not identified
CMB170745	22145/13	GR4	2613	32,000	ND	ND	ND	ND	ND	ND	Faecal source not identified

Cont.

ESR Number	Client Reference	Site	*E. coli* (MPN/ 100mL)	General GenBac / 100 mls	Human BacH / 100 mls	Human BiADO / 100 mls	Ruminant BacR / 100 mls	Proportion Ruminant	Dog DogBac / 100 mls	Avian GFD / 100 mls	Conclusion
CMB170746	22145/18	STRM1	345	370,000	ND	ND	ND	ND	ND	710	Avian source
CMB170747	22145/19	STRM2	399	250,000	ND	ND	ND	ND	ND	360	Avian source

Abbreviations: NA = sample was not analysed for this marker.
Detected, <LOQ = the marker was detected but at a level less than the limit of quantitation (LOQ)
ND = not detected, sample was analysed, but the marker was not detected.
NC = not calculated, sample contained a low level of ruminant marker and at this low level a ratio calculation is not valid

Notes:
Brief details of the methods of analysis are available on request.
These results relate to samples as received.
This report may not be reproduced except in full.

Paula Scholes
Laboratory Operations Coordinator

Beth Robson
Senior Technician

APPENDIX: Assay Interpretation Guidance Notes

PCR Marker interpretation notes

- Each marker is strongly associated with, but not exclusive to the source tested for. They each have some degree of non-specificity.
- Each marker is a separate test and the levels of the various markers within the same sample cannot be compared. For example, if sample A has a BacH result of 1,000 and a BacR of 100 it is not valid to say there is more human contamination than ruminant in sample A.
- Levels of the same marker in different samples can be compared. For example;
    - If sample A has a BacH result of 1,000 and sample B has a BacH of 10,000 it is valid to conclude there is more human faecal contamination in sample B than in sample A; or
    - If site H sampled in January has a GFD result of 500 and when sampled in February has a GFD result of 10,000, it is valid to conclude the level of avian faecal contamination in February is greater.
    - To be classified as a significantly greater or lesser result the level of marker should vary be a factor of 10.
- Both Human markers are required to be present for a positive human result.
- Ruminant specific markers are reported using a percentage value based on levels of this marker relative to the general marker in fresh ruminant faeces.
    - Samples reported as 50-100% ruminant are consistent with all of the general faecal marker having come from a ruminant source.
    - The lower levels reported (10-50%) may be a consequence of the presence of other sources of pollution, or in fact ruminant sources may still account for all the pollution, but this may include aged faecal material where relative levels of the ruminant marker decline more rapidly than the general marker.
    - Levels less than 10% ruminant suggest a very minor contribution from ruminant sources.

The quantiation limits of these methods are:

General GenBac / 100 ml	Human BacH / 100 ml	Human BiADO / 100 ml	Human HumM3 / 100 ml	Ruminant BacR / 100 ml	Schill Sheep / 100 ml
110	83	110	63	91	100

CowM2 / 100 ml	DogBac / 100 ml	Avian GFD / 100 ml	Avian E2 / 100 ml	Gull- 2	
88	79	72	99	presence / absence test	

Note:

- These quantitation limits are based on testing a sample volume of 250 mls, if more or less water than this is tested the quantitation limit will be lower or higher accordingly.
- For some markers the detection limit is lower that this quantitation limit. Where samples fall into this case they are reported as “detected, < LOQ” and indicates that the marker is present but at very low levels.
- These quantitation limits are the same as those previously used to differentiate “present / ND” reporting. For example, a sample previously reported as “present” is now reported as a number / 100 ml and a sample previously reported as ND is now reported as either “detected, < LOQ” or “ND”.

FWA interpretation notes

The analysis of FWAs in septic tank and community wastewater consistently identifies levels between 10 and 70 µg/L. In previous analysis of water samples levels of FWA greater than 0.1 µg/L suggest human sewage, with levels greater than 0.2 µg/L strongly indicative of human sewage. Levels greater than 0.1 µg/L correlate well with other indicators of human pollution and indicate a local or recent source of pollution. FWAs degrade under sunlight exposure and will undergo dilution. Levels lower than 0.1 µg/L may be indicative of dilute or distant sources of human pollution.

Reference: Devane M., Saunders D. and Gilpin B. (2006). Faecal sterols and fluorescent whiteners as indicators of the source of faecal contamination. Chemistry in New Zealand 70(3), 74-7.
http://www.nzic.org.nz/CiNZ/articles/Devane_70_3.pdf

Faecal sterol Intepretation Notes:

Faecal sterol ratios must be interpreted with consideration to the levels of sterols, and relative to one another. For example H1 is typically also above 5-6% in ruminant faeces. Human and ruminant sources generally require at least two of three ratios to reach thresholds.
Plant sterols and mixed sources also have differing effects on sterol interpretations which must be considered.

Conclusions are the best interpretation of sterols in our opinion. Conclusions in **bold** are highly supported by the sterol data, conclusions in brackets are supported by sterol data with some variation from a pure source, or with a lower degree of certainty.

Ratio Key:

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