Faecal Source Tracking in the Taruheru River, Gisborne

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DISCLAIMER

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Abbreviations

BacR	Ruminant PCR marker
ESR	Institute of Environmental Science and Research
FC	Faecal coliform
FIB	Faecal indicator bacteria
FST	Faecal source tracking
GDC	Gisborne District Council
GenBac3	General faecal PCR marker
GFD	Avian PCR marker
MfE	Ministry for the Environment
MoH	Ministry of Health
PCR	Polymerase chain reaction

EXECUTIVE SUMMARY

In this study faecal source tracking (FST) tools were used to investigate likely sources of faecal contamination of the Taruheru River, Gisborne, under base flow conditions. The Taruheru River travels from bush clad foothills flowing predominantly southeast through land used for agricultural and horticultural purposes before flowing through the city of Gisborne (Tūranga-nui-a-Kiwa) and converging with the Waimata River to become the Turanganui River, which enters Poverty Bay (Te Moana-a-Toi).

Samples were taken in June and July of 2017 from eight sites on the Taruheru River and two tributaries of the same river. Sampling for this envirolink project was delayed due to sewer overflows into the Taruheru River proceeding heavy rainfall events. Sampling occasions, therefore, were timed to occur at least a month post-sewer overflow events and a week post-significant rainfall events. This timing ensured baseflow conditions for sampling where there was minimal risk of sewer overflows into the urban river system, and less likelihood of overland runoff and sediment disturbance impacting the FST results.

This current study forms stage-2 of an initial study of the wider Gisborne catchment, which investigated 16 locations on seven waterways in the Gisborne District (Devane and Williamsom, 2014). The 2014 study sampled the rivers during falling-high river conditions proceeding a heavy rainfall event, whereas the current study focussed on faecal source tracking during base flow river conditions. The Waimata and Turanganui Rivers, which also flow through Gisborne City, were not sampled during this 2017 FST study.

The FST tools used were faecal indicator bacteria (FIB) (*Escherichia coli*, faecal coliforms and enterococci), and polymerase chain reaction (PCR) markers (all sites had general, human, ruminant, and avian faecal PCR markers assayed). The river sites studied (Appendix A.3) in order of upstream to downstream were:

Waihirere Stream in the Waihirere Domain
Harper Road
King Road Bridge
Haisman Road Tributary
Tuckers Road Bridge
Gaddum Tributary
Cemetery Western Boundary
Lytton Road Bridge
Stanley-Oak Street footbridge
Wi Pere Pipe

Overall, nine of the ten sites were impacted by elevated levels of *E. coli* above the water quality guidelines Alert level of 260 *E. coli*/100 mL (Ministry for the Environment (MfE) and Ministry of Health (MoH), 2003) on at least one sampling occasion (Table 1). Only at the most upstream site in the Waihirere Domain were *E. coli* levels (<31 CFU/100 mL) below the Alert level during both sampling events. Eight of 21 water samples had concentrations above the Action level of 550 *E. coli*/100 mL. Two of these samples also exceeded the national bottom line of 1000 *E. coli*/100 mL (Table 6) and both samples originated from the two tributaries of Haisman Road and Gaddum. Eight water samples had elevated enterococci concentrations, with the lower tidal reaches having enterococci concentrations higher than the Action level (280 enterococci/100 mL) for marine waters.

In general, FIB levels were lowest in the upstream section of the Taruheru River with concentrations peaking in the two tributaries at Haisman Road and Gaddum. Concentrations of FIB decreased again in the lower reaches of the river, except for enterococci levels above the Action level during the July sampling event at the tidal site of Wi Pere pipe.

The sites at Haisman Road tributary and Stanley-Oak Street Footbridge were the only sites to record human pollution (June sampling). Ruminant and avian faecal sources were identified at all sites at various levels although avian sources were not identified in all samples. Most sites reported a ≤50% contribution from ruminant faecal sources, except at the two most upstream sites of Waihirere Domain and Harper Road (however, the ruminant signal at Waihirere Domain was in association with very low levels of *E. coli*). Ruminant contributions of less than 50% may be indicative of aged faecal sources from overland runoff or it signifies that the pollution has travelled some distance from its source.

This base river flow study identified ruminant and avian as the principal faecal signals in both rural and urban areas of the Taruheru River. This finding contrasts with a number of international studies, which have identified that during base river flow conditions there is little or no run-off from agricultural land (e.g., Kay et al. (2010)), and therefore, in urban environments, the major faecal sources would be derived from urban pollution. It is apparent that in the Taruheru River catchment that under base flow conditions, agricultural activities continue to impact the urban sections of the river. These findings are similar to the 2014 study of the Taruheru River under falling-high river conditions. This current study of FST data during winter conditions has also confirmed that human faecal contamination can be an intermittent source to this urban area.

Recommendations:

- Identify the sources of human faecal contamination at Haisman Road Bridge and Stanley-Oak Street Footbridge by conducting a sanitary survey at these locations to identify if there are septic tanks/other human faecal sources in the area where leakage/overflows of sewerage could be occurring.
    - Faecal sterol analysis alongside additional PCR marker analysis of the water and/or stream sediment (sterols only in sediment) may be helpful at these two sites to detect human inputs from recent/aged sources. The sanitary survey could inform a targeted approach to identify sites for detection of critical sources of human faecal contamination.
- At Gaddum Tributary, based on the FST assessment of PCR marker abundance, it would appear that ruminant and avian pollution sources are not the total sources of the elevated FIB. Therefore, it would be worthwhile to reanalyse the DNA extracts from the water samples at Gaddum Tributary for dog PCR markers to identify contributions from these faecal sources. At this site there are known dog kennels and a pig farm in the vicinity. Unfortunately we do not currently have a pig PCR marker for identifying pig pollution.
- Conduct discharge flow calculations for the two tributaries (Haisman Road Bridge and Gaddum) to understand whether these tributaries are acting as critical sources of faecal pollution as indicated by their high FIB counts.
- A long-term aim could be the investigation of sediments in these urban waterways, including the Turanganui and Waimata Rivers to understand the deposition and accumulation patterns of sewage markers (faecal indicator bacteria and faecal sterols) entering this environment.

Table 1: Summary of the microbial results and faecal sources identified in the Taruheru River and selected tributaries.

Location	Month 2017	*E. coli* (CFU/100mL)	¥Entero (CFU/100mL)	Faecal source(s) identified	Location	Month 2017	*E. coli* (CFU/100mL)	¥Entero (CFU/100mL)	Faecal source(s) identified
Waihirere Stream @ Domain	*June	9.8	1.6	Not tested	Gaddum Trib.	June	**820	370	Low level ruminant and avian
	*July	30	1.6	Ruminant		July	1200	66	Low level ruminant and avian
Harper Road	June	250	48	Ruminant and low level avian	Cemetery Western Boundary	June	540	100	Ruminant and low level avian
	July	290	28	Ruminant and low level avian	Lytton Road Bridge	June	570	170	Ruminant and avian
King Road Bridge	June	480	110	Ruminant and avian		July	710	130	Ruminant and low level avian
	July	370	43	Ruminant and low level avian	Stanley-Oak Street Footbridge	June	240	76	Low level human pollution; avian and low level ruminant
Haisman Road Trib	June	1300	270	Human pollution, and low level ruminant and avian		July	540	150	Ruminant
	July	660	210	Low level ruminant and avian	Wi Pere Pipe	June	120	54	Avian and low level ruminant
	July	620	110	Ruminant		July	410	440	Ruminant
Tuckers Road Bridge	June	640	230	Ruminant and avian		July	420	330	Ruminant and low level avian
	July	390	60	Ruminant and low level avian					

*Samples were collected on 21 June 2017 and 24 July 2017; ¥Entero = enterococci **Green colour coding = below Alert levels for FIB; orange colour coding = above Alert levels for FIB; red colour coding = above the Action levels for FIB.

1. INTRODUCTION

The focus of this study was to identify the likely sources of the elevated levels of faecal indicator bacteria (FIB) recorded in the Taruheru River, which flows through rural areas impacted by agricultural and horticultural activities before entering the city of Gisborne (Tūranga-nui-a-Kiwa). Ten rural and urban sites along the Taruheru River were chosen to evaluate FIB concentrations (*Escherichia coli*, faecal coliforms and enterococcus) in the water. Water samples with elevated FIB levels were further analysed using a suite of Polymerase Chain Reaction (PCR) markers for faecal source tracking (FST). PCR markers evaluated in this study were indicative of human, animal and wildfowl faecal pollution sources. The study focussed on faecal source tracking during base flow river conditions. Gisborne District Council (GDC) provided data collected on rainfall, river flow, and the following water quality parameters: temperature, pH, salinity, conductivity, dissolved oxygen, and either clarity (June sampling) or turbidity (July).

In 2014, to ensure resources were targeted to effectively mitigate faecal contamination of surface waters, GDC and the Institute of Environmental Science and Research Limited (ESR) developed stage-1 of a baseline sampling programme for two major catchments near Gisborne City and applied FST tools to 16 locations on seven waterways in the Gisborne District (Devane and Williamsom, 2014). Several Gisborne rivers, including the Taruheru frequently have levels of faecal indicator bacteria (FIB) elevated above the Alert level (260 *E. coli*/100 mL) of the water quality guidelines for recreational activity. Surface waters that experience elevated or sporadically elevated levels of FIB may pose public health risks to recreational users of these waters. Within the Gisborne district the sources of these bacteria are not always clear, although agricultural activities and human wastewater are often suspected. In the case of the Taruheru River, sewage overflows may occur after heavy rainfall events. 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.

Robust baseline FST data are required to identify the most likely sources of faecal inputs and the times when different reaches of waterways are likely to represent the greatest public health risk. To collect these data, optimum sampling is required at different river flow conditions, preferably on the rising-high, high, falling-high, base and low river flow conditions.

The collection of Stage-1 baseline FST data for Gisborne was deliberately initiated by sampling after sustained heavy rainfall as the river levels were returning to base flow (falling-high flow) and satisfied one of the river conditions for establishing reference point FST data. The overland run-off from these rain events in 2014 will have transported faecal contamination into the waterways and disturbed sediment reservoirs of FIB. Sampling of the falling-high river flow in 2014 did not identify human faecal contamination, while animal, including some avian faecal contamination was detected. In the 2014 study, five sites on the Taruheru River were sampled and avian faecal sources were the dominant sources identified in the river water with dog and ruminant as secondary faecal sources. The exception was an urban site named middle urban where it was the ruminant signal that dominated with avian and dog markers as the secondary sources.

The current FST study, referred to as Stage-2 of the GDC-ESR sampling programme, targets eight sites on the Taruheru River and two tributaries of the same river. Two sampling events were carried out in June and July of 2017 to collect water samples from the sites

under base flow conditions. Sampling for this envirolink project was delayed due to sewer overflows into the Taruheru River proceeding heavy rainfall events. Sampling occasions, therefore, were timed to occur at least a month post-sewer overflow events and a week post-significant rainfall events. This timing ensured baseflow conditions for sampling where there was minimal risk of sewer overflows into the urban river system, and less likelihood of overland runoff and sediment disturbance impacting the FST results.

1.1 GISBORNE DISTRICT AND THE TURANGANUI CATCHMENT

Gisborne is located on the East Coast of the North Island in an area known as East Cape and Poverty Bay (Te Moana-a-Toi). Gisborne District Council (GDC) is responsible for 8386 square kilometres of land[1] and a population of approximately 44,000[2], according to the 2013 NZ census. Gisborne is known as the city of rivers. For this study we focused on water quality in the Taruheru River. The ten sites evaluated for FIB and faecal source tracking markers were distributed between the headwater site of the Waihirere Domain and the most downstream site at Wi Pere Street, and included the Haisman Road and Gaddum Tributaries. The site at Wi Pere Street is upstream of the Botanical Gardens and above the confluence of the Taruheru and Waimata Rivers, which join to form the Turanganui River flowing into Poverty Bay (Te Moana-a-Toi). Turanganui River, which is 1.2 km in length, qualifies as the shortest river in New Zealand. Descriptions, survey notes and photographs of each sampling site were provided by GDC and are presented in Appendix 3.

The soils in the Gisborne catchments, while of variable geology, are mainly derived from sandstone and mudstone and are highly susceptible to erosion. This means that the rivers, particularly in the Waipaoa catchment, have a sediment load that increases dramatically during storm events. Stop banks have been constructed to protect Gisborne city and its outlying suburbs from flooding that occurs as a consequence of the raised river bed level.

The headwater of the **Taruheru River** is in the Waihirere Domain, which is reserve land that has allowed the water quality within the reserve to maintain high quality. From the Waihirere Reserve the Taruheru River flows through a highly modified environment, which includes horticultural and agricultural land and residential properties. The lower reaches are tidal. The Taruheru River eventually combines with the Waimata River and flows into the Turanganui River, which enters Poverty Bay (Te Moana-a-Toi).

The **Waimata River** flows from forestry and bush-covered steep gullies and hill country sheep and cattle farming areas then through inner Gisborne city. Near the city, the river is used for recreational purposes and local parks provide access to the public for water activities. The lower reaches of the Waimata River are tidal. The Waimata River eventually combines with the Taruheru River and flows into the Turanganui River. The Waimata River was not sampled in the current survey.

The **Turanganui River** has the honour of being the shortest river in New Zealand at 1.2 km in length. The Turanganui is formed by joining of the Taruheru and Waimata Rivers. It is a well-used recreational river and flows through the lower industrial area and commercial areas of Gisborne before entering Poverty Bay (Te Moana-a-Toi). It is strongly tidal. The Turanganui River was not sampled in the current survey.

[1] http://www.gdc.govt.nz/our-district/
[2] http://www.stats.govt.nz/Census/2013-census/profile-and-summary-reports/quickstats-about-a-place.aspx?request_value=13991&tabname=Populationanddwellings

1.2 HUMAN FAECAL CONTAMINATION

Raw human sewage can contain a number of pathogenic organisms including *Campylobacter* spp., *Escherichia coli* O157, *Cryptosporidium* spp., *Giardia* spp., and viruses such as enterovirus and norovirus (Allos, 2001; Coia, 1998; Fayer et al., 2000; Graczyk et al., 2007; Hafliger et al., 2000). If ingested, these enteric pathogens can cause a range of illnesses from mild to severe; including vomiting, diarrhoea, kidney failure, haemolytic uraemic syndrome and, in some cases, death. When untreated sewage contaminates rivers or oceans, waterborne transmission of these pathogens can occur to those who use the water for swimming, boating, fishing and shellfish-gathering activities, or if the water is used as a source of drinking-water. Secondary transmission of these pathogens to humans can occur via animals or birds exposed to the water.

Wastewater treatment removes or inactivates some variable portion of these pathogens before they enter rivers or oceans. Each wastewater treatment system and process within a system will have different removal effectiveness, and may exhibit seasonal variations, such as for oxidation pond wastewater treatment. The microorganisms in sewage may remain suspended in the water column or they may be deposited into the river bed sediment at some point along the flow path (Devane et al. 2014). Pathogens not removed or inactivated during wastewater treatment will be transported along the river and eventually into the estuaries, with some unknown degree of inactivation along the way. Even wastewater treated using land-based disposal may contribute some unknown proportion of pathogens to surface water and groundwater due to run-off in rain events and preferential flow through the soil. The process of deposition and re-suspension of microorganisms to and from sediments is poorly understood. There is limited information about the rates of microbial survival in sediments, although reduced oxygen levels and protection from sunlight may allow microorganisms to survive longer in sediments than the overlying water (Davies et al., 1995).

Most recreational water activities disturb sediments, mobilising microorganisms from the riverbed, as do heavy rainfall, increased river flows, and the activity of animals, birds and fish in the river (Devane et al., 2014). Disturbance of the stream sediments is also likely to progressively enrich downstream sediments with microorganisms.

Microbial water quality is measured primarily by testing for the faecal indicator bacteria (FIB) such as *E. coli* (in freshwater) and enterococci (in saline waters). These bacteria usually do not cause disease themselves, but they are prevalent in faecal material and sewage, and therefore indicate the potential presence of pathogenic organisms that can be transmitted by the faecal-oral route. FIB are also easy (and therefore relatively cheap) to detect in water. The *Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas* (MfE & MoH, 2003) recommend that freshwater contains fewer than 260 *E. coli* per 100 mL, and marine water contains fewer than 140 enterococci per 100 mL.

In fresh raw sewage, *E. coli* and enterococci are good indicators of potential risk to human health from pathogenic bacteria, protozoa and viruses. However, a range of physical and environmental factors including, river dilution, movement within a river, storage in sediments, and the intrinsic characteristics of the microorganisms may, over time, alter the relationship between these indicator bacteria and the pathogens of concern (Sobsey, 1989).

1.3 ANIMAL FAECAL CONTAMINATION

There is growing concern about the pathogens present in animal faeces that may be transmitted to humans. Agricultural animals are major contributors of faecal pollution to surface water through direct deposition in waterways or overland run-off from faecal deposits on paddocks (Sobsey et al., 2006; Wang et al., 2004). Faecal pollution in waterways from agricultural sources are known to impact human health (Soller et al., 2010). There are a wide variety of pathogenic microorganisms in faecal material that are able to cause disease in humans, these microbes are termed zoonoses (Sobsey et al., 2006). Zoonoses include viruses (e.g., enterovirus), bacteria such as *E. coli* O157:H7, *Campylobacter* and *Salmonella*; and protozoan groups (e.g., *Cryptosporidium*) (Moriarty et al., 2011b; Moriarty et al., 2008; Sobsey et al., 2006). The intensification of animal farming in New Zealand has resulted in large quantities of faecal waste in relatively small areas, which require effective management to mitigate the potential effects on public health.

An outbreak of campylobacteriosis in a small rural township of Darfield, NZ, occurred in 2012, where 29 cases were confirmed as due to *C. coli* or *C. jejuni* and one case due to *Giardia*, while a further 138 cases were defined as probable cases of gastroenteritis. This episode was linked to the failure of the township's drinking water supply after a period of heavy rainfall (Bartholomew et al., 2014). Contamination was suspected to result from unprotected bore well heads in paddocks where sheep grazed, or from pasture runoff into the river from which the well drew source water. Faecal specimens from local sheep were identified as carrying subtypes of *Campylobacter* that were closely related to those identified in clinical specimens.

Feral animals and birds are also suspected to contribute to pollution in waterways (Devane et al., 2013; Devane et al., 2015; Fogarty et al., 2003). The public health risk from feral and avian sources of pollution, however, is less certain. A risk assessment by Soller et al. (2010) has suggested a lower pathogen potential for avian species compared with humans and agricultural animals. There is mounting evidence, however, of pathogen carriage by waterfowl and other avian species. Potential pathogens identified in avian faecal droppings include campylobacters (Moriarty et al., 2012; Ryu et al., 2014), pathogenic *E. coli* (Wallace et al., 1997), *Salmonella* serovars (Refsum et al., 2002), avian influenza viruses (Brown et al., 2006; Gilbert et al., 2006) and clinically relevant antibiotic resistance bacteria (Bonnedahl et al., 2014).

1.4 FAECAL SOURCE TRACKING (FST)

Human wastewater may discharge into rivers deliberately from wastewater treatment outfalls, accidentally from sewer overflows, or may occur without the contributor's knowledge through failed on-site wastewater disposal systems (septic tanks). Other faecal sources can contribute to microbial indicators and pathogens in a water body. In urban areas, faeces from dogs and waterfowl are common, while in rural areas livestock, wildlife and waterfowl are usual contributors, in addition to human faecal/wastewater inputs. A range of FST tools can identify whether faecal pollution is from human, dog, livestock or wildfowl sources (Devane et al., 2008; Devane et al., 2007; Devane et al., 2006; Gilpin et al., 2008; Gilpin et al., 2013). In this study, FST tools were applied to 21 surface water samples taken from eight locations on the Taruheru River and two tributaries that flow into the Taruheru., Gisborne. Water sampling from these ten sites occurred on 21 June and 24 July 2017.

There are an increasingly large number of methods available that can be used to identify possible sources of faecal pollution. The suite of tools available from ESR include: molecular markers, faecal sterols and fluorescence whitening agents (Devane et al., 2008; Devane et al., 2013; Devane et al., 2006). ESR doesn't necessarily use all these on every sample, as we have found there are sample types and contexts where the analyte is less likely to be present or present at such low concentrations that it may not be value for money.

1.4.1 Polymerase Chain Reaction (PCR) markers for FST

There is a range of microorganisms other than faecal coliforms, *Escherichia coli* and enterococci present in faeces that are specific to each animal host. Difficulties in culturing and identifying these organisms have limited their useful application to faecal source identification. An alternative approach is to extract total DNA from a water sample and examine the sample using the polymerase chain reaction (PCR) for DNA from source-specific organisms. PCR assays targeting predominantly human, ruminant and avian markers have been applied to the samples in this study (Table 2). Additional assays for specific hosts such as sheep, cows, possum and canine (referred to as dog-associated) can also be applied to these samples as the DNA is stored in the freezer for a limited time, and therefore, available for retesting as appropriate based on the initial results.

Table 2 PCR markers used in this study and their target host species.

Target Group	Assay abbreviation	Reference
General faecal marker	GenBac3	Siefring et al. (2008)
Human	BacH	Reischer et al. (2007)
	BiAdo	Matsuki et al. (2004)
Ruminant: cows, sheep, deer, goats	BacR	Reischer et al. (2006)
Avian: gulls, geese, chickens, ducks	GFD	Green et al. (2012)

2. METHODS 1

2.1 SAMPLE COLLECTION

All water samples collected for analysis were taken as grab samples from the river bank or bridges/jetties. The water samples were kept on ice and returned to the laboratory to be processed for microbial enumeration and application of FST tools within 24 h of collection. Timing of sampling events was staged to occur when rainfall was minimal in the week prior to sampling, and when there were no recorded sewer overflows in the month preceding the sampling dates. Please note that on 24th July, two water samples were collected at Haisman Road Tributary and Wi Pere pipe, and no sample was collected from the Cemetery Western Boundary site.

2.2 DETERMINATION OF PHYSICAL AND CHEMICAL VARIABLES

A multi-analytes probe (Aquaprobe, Aqualink/Aquameter no 1041-00189 Rev C, Aquaread Ltd) was used to measure the water quality parameters: pH, temperature, dissolved oxygen, turbidity and salinity and conductivity. Rainfall and river flow were also measured for the months of June and July and sediment loads (June only). This data was kindly provided by GDC.

2.3 ENUMERATION OF FAECAL INDICATOR BACTERIAL (FIB)

Enumeration of FIB was performed by Watercare Laboratory services www.watercarelabs.co.nz

2.3.1 Escherichia coli

Membrane filtration was used to enumerate *E. coli* concentrations in the water using USEPA Method 1603 with a detection limit of 2 cfu/100 mL.

2.3.2 Faecal coliforms

Membrane filtration was used to enumerate faecal coliform (FC) concentrations in the water samples using APHA (online edition) 9222 D, with a detection limit of 2 cfu/100 mL.

2.3.3 Enterococcus

Membrane filtration was used to enumerate enterococci concentrations in the water samples using method APHA (online edition) 9230 C with a detection limit of 2 cfu/100 mL.

2.3.4 Alert and Action levels for Faecal indicator bacteria (FIB)

The Alert (or amber) mode is triggered when a single sample is greater than 140 enterococci per 100 mL for marine waters and 260 *E. coli* per 100 mL for freshwaters. The second level of response to a single-sample exceedance is Action (or red) mode. In the case of marine waters this is triggered when two consecutive samples are greater than 280 enterococci per 100 mL, and for freshwaters when a single sample exceeds 550 mL *E. coli* per 100 mL (MfE & MoH, 2003).

2.4 FAECAL SOURCE TRACKING METHODS

2.4.1 PCR markers

Molecular biology tools (e.g., PCR) are sensitive and usually permit detection of low concentrations of a target, often less than 20 copies of DNA (representing ~4 bacteria cells if based on 16S rRNA as the target molecule) when several replicate reactions are carried out.

DNA was extracted from up to 1000 mL of water sample by filtering through a Supor 200, 0.2 μM PES filter (Pall Corp. Washington Port, NY, USA). The filter was transferred to a 50 mL falcon tube, and 1 mL of guanidine isothiocyanate (GITC) buffer (5 M guanidine isothiocyanate, 0.1M EDTA, 10% sarcosyl) was added, after which the filter was frozen at -20°C. DNA extraction was performed on the filter using the Qiagen DNeasy Kit (QIAGEN Valencia, CA) following the manufacturer's instructions. Briefly, 700 μL AL buffer (supplied by manufacturer) was added to the filter, and the mixture was vortexed and incubated for 5 minutes at room temperature. The supernatant was added to a spin column from the DNeasy kit, and the column centrifuged for 1 min at 13,000 rpm. The supernatant was then discarded. The filter was then washed using the kit's reagents and the DNA eluted in 100 μL of elution buffer. During each sample extraction procedure, a blank of purified water was extracted to monitor for DNA contamination. Real-time PCR conditions for all bacterial PCR assays used the LightCycler 480 (Roche Diagnostics Ltd.) and an annealing temperature of 60°C. Detailed procedures are described in Devane et al. (2013). The general, human and ruminant and avian faecal PCR markers are reported as copy number/100 mL based on the volume filterd for DNA extraction.

Selected samples were tested for inhibition of the PCR assays, by dilution of the DNA extract 10–fold prior to the PCR assay. No inhibition was detected in any of the samples, suggesting that high turbidity in some samples was not interfering with the PCR.

2.5 CAVEATS

It is important to recognise that the absence of detected FST markers or of components of the FST assays does not necessarily mean that these targets were absent in a sample. A "not detected" (ND) result means that no target was detected by the assay on the day that assay was carried out. For some assays, possible outcomes are "not detected", and "below the limit of quantitation (LOQ)". Each outcome depends on the detection limits and quantification limits of the individual assays, which will vary dependent on the volume of water sample filtered.

3. RESULTS AND DISCUSSION

3.1 DESCRIPTIVE STATISTICS FOR MICROBIAL RESULTS

The general descriptive statistics for the faecal indicator bacteria (FIB) are presented in Table 3. Enterococci concentrations were similar between the June and July samplings, and *E. coli* concentrations were also similar between the two sampling events. Both FIB had mean concentrations that were above the Alert levels and maximum concentrations greater than their respective Action levels (section 2.3.4 and Ministry for the Environment (MfE) and Ministry of Health (MoH), 2003). However, it should be noted that enterococci Alert and Action levels are guidelines for marine and coastal waters, and therefore, relevant for the lower tidal reaches of the Taruheru River. In the case of *E. coli*, two samples also exceeded the national bottom line of 1000 *E. coli*/100 mL and both of these samples originated from the two tributaries of Haisman Road and Gaddum (Figure 1). Nine of the ten sites were impacted by elevated levels of *E. coli* above the Alert Level of (260 *E. coli*/100 mL) for the recreational water quality guidelines on at least one sampling occasion (MfE & MoH, 2003). Sixteen of the 21 water samples collected from the ten sites recorded *E. coli* levels above the Alert Level.

Compared with the other FIB, there was greater variability for the mean of faecal coliforms between June and July sampling events. *E. coli* is a subset of the faecal coliforms making up approximately >90% of the faecal coliform group in fresh faeces. Differences between *E. coli* and FC in the same sample may indicate that the source of *E. coli* is aged faeces or faecal material that has travelled some distance from its source. The lower levels of *E. coli* suggest die-off of this bacterium compared with the other faecal coliform members.

Table 3: Descriptive statistics for faecal indicator bacteria during June and July 2017.

Faecal indicator bacteria	Month	No. of samples	Mean	Standard deviation	Median	Minimum	Maximum
Escherichia coli	June	10	497	378	510	10	1300
	July	12	513	297	420	30	1200
Faecal coliforms	June	10	1042	1161	635	28	3900
	July	12	621	408	580	50	1700
Enterococci	June	10	143	116	105	2	370
	July	12	143	136	110	2	440

The FIB levels at sites along the Taruheru River are represented graphically in Figure 1 and in Figure 2. FIB levels were lowest in the upstream section of the Taruheru River with concentrations peaking in the two tributaries at Haisman Road and Gaddum. Concentrations of FIB decreased again in the lower reaches of the river except for enterococci levels above the Action level during the July sampling event at Wi Pere pipe.

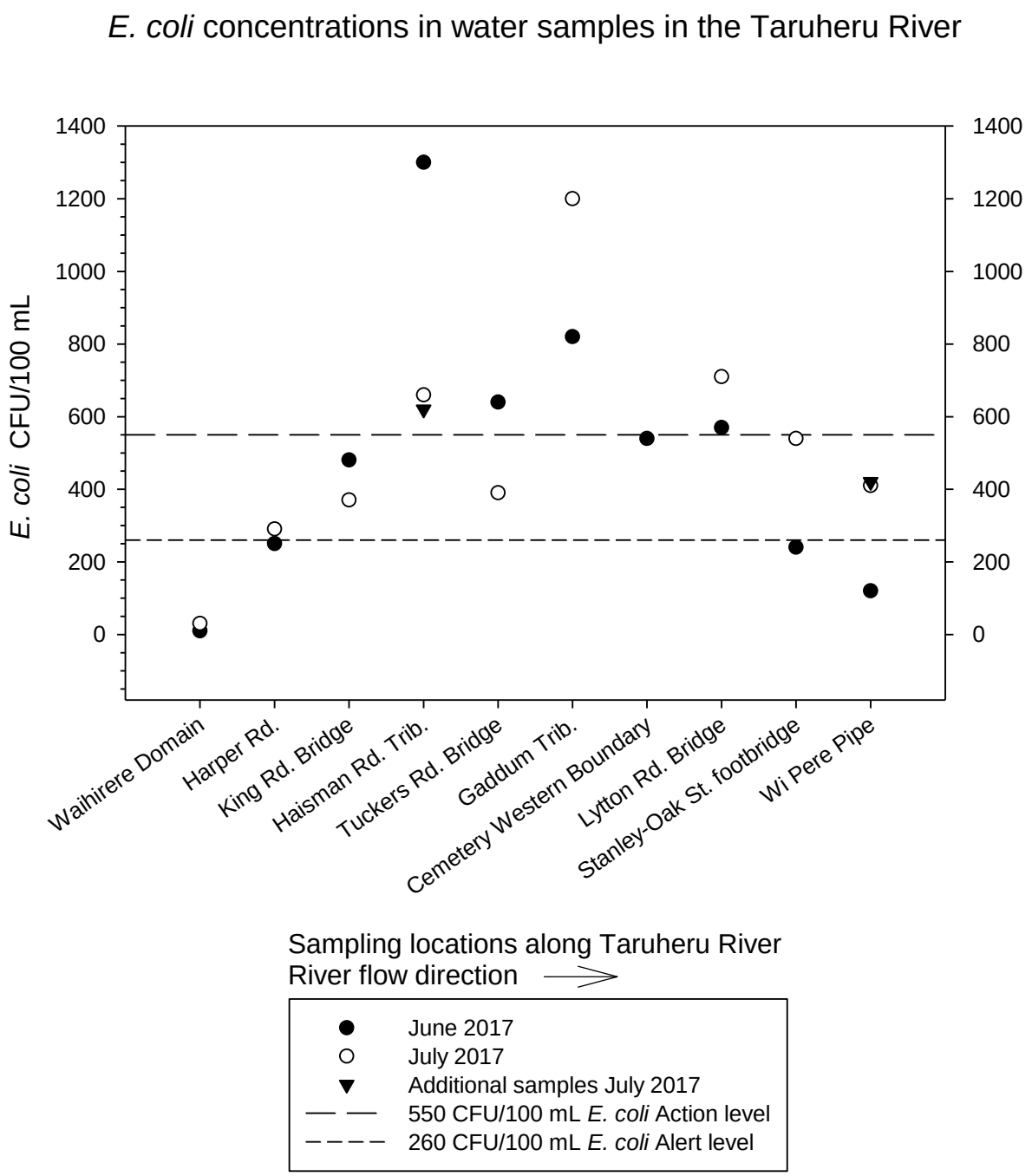

Figure 1: *E. coli* concentrations in water samples from the Taruheru River and its tributaries during June and July of 2017.

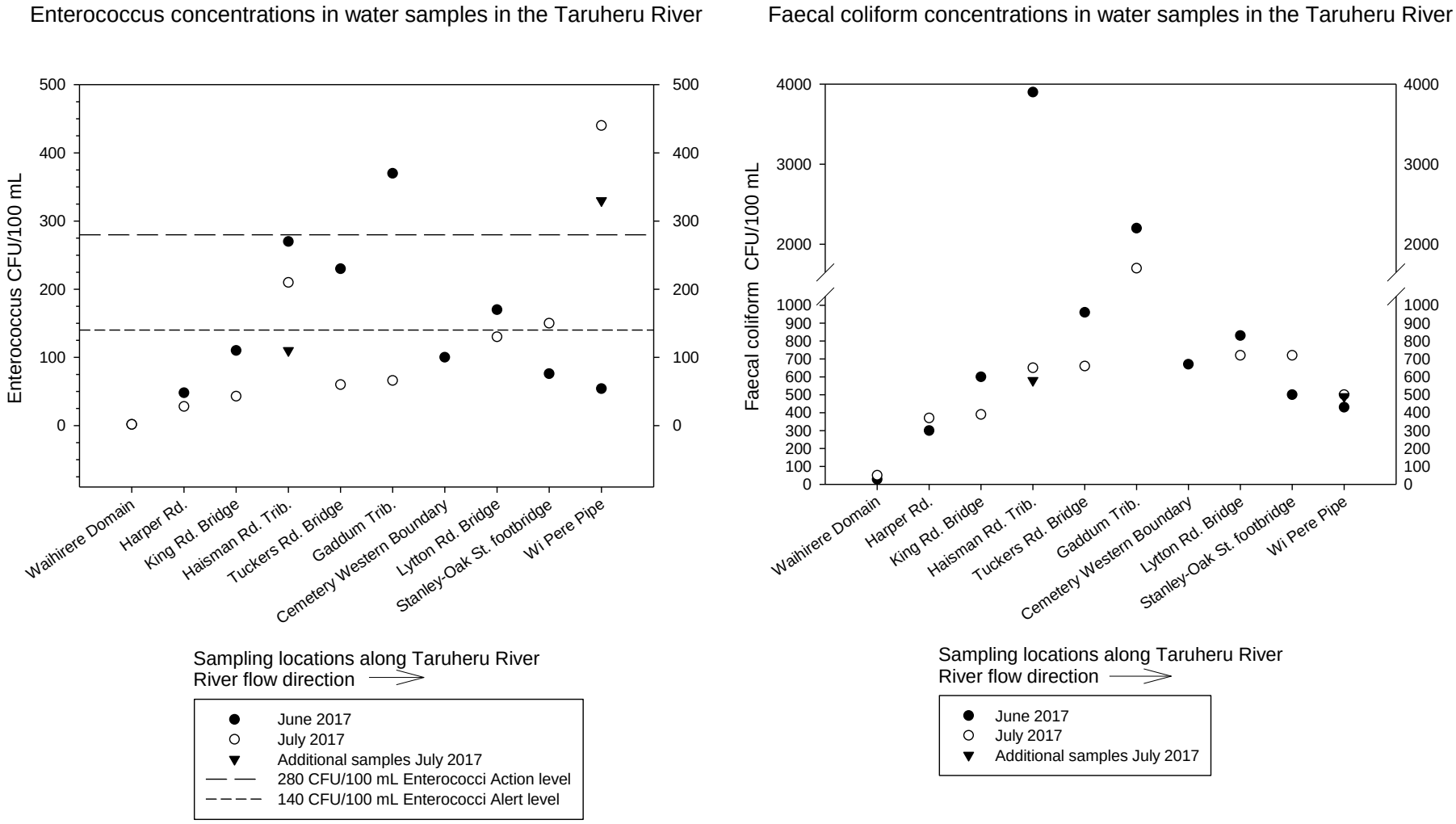

Figure 2: Enterococci and faecal coliform concentrations in water samples from the Taruheru River and its tributaries during June and July of 2017.

3.2 WATER QUALITY PARAMETERS, RAINFALL AND RIVER FLOW

Water quality parameters were collected for each water sample and descriptive statistics are presented in Table 4 for the June sampling event and in Table 5 for the July event. Figures for water quality parameters are presented in the Appendices in Section A.2. Water samples were collected on the incoming tide in the lower reaches in June, and at low tide in the July sampling event, which may account for some of the differences observed in Figure 3 to Figure 5. Temperature ranges were between 7.6°C and 11.3°C at all sites in June and between 9.5°C and 12.3°C in July (Figure 3). The pH range in the water for all samples was between 7.3 and 8.6 (Figure 3). Conductivity and salinity measures increased markedly from Lytton Road Bridge downstream, signifying tidal influences at these locations (Figure 4).

For all sites, mean values of dissolved oxygen (DO) were 10.8 mg/L and 9.8 mg/L (June and July, respectively) (Figure 5), with levels typically decreasing as salinity increased. In June, Haisman Road Tributary recorded high DO levels of 19 mg/L and Gaddum Tributary the lowest value of 8.0 mg/L compared to the river values. The high DO at Haisman Road Tributary was accompanied by 1300 *E. coli* and 3900 FC/ 100 mL. DO can be affected by temperature, salinity and atmospheric pressure. The increase in DO at this site was not associated with temperature differences as fluctuations in temperature between sites was minimal for the June sampling (Figure 3). Overall, dissolved oxygen levels were well above the 5-6 mg/L level where oxygen may become limiting for aquatic populations.

Clarity (cm) was measured during the first sampling event but in July turbidity was measured (in nephelometric units (NTU)). High variability was noted for both clarity and turbidity measures, with the two tributaries of Haisman Road and Gaddum having lower clarity and high turbidity. The July sample at Waihirere had the highest turbidity reading at 32 NTU, however there was no clarity recorded for this site in June to help decide if this was a singular event or turbidity is an issue at this site. Overall, clarity decreased downstream and excluding Waihirere, turbidity increased downstream of King Road Bridge and remained within the range of 10-12 NTU in the main Taruheru River but higher in the tributaries.

There was no significant rainfall or changes in river flow in the seven days prior to collection of samples for either the June or July sampling events (Appendix A.2: Figure 6 and Figure 7 and Appendix A.3.1). Sediment load data was only obtained for July 2017 at Courtneys Bridge, and there were several increases in sediment load to the catchment during July. The last increase in sediment load occurred ten days prior to the July sampling event during a heavy rainfall event. The sediment load returned to approximate baseline levels one day after this rainfall event, and therefore, would not be expected to impact water quality for the July sampling.

Table 4: Descriptive statistics for WQ parameters collected in June 2017.

June	Sample size	Mean	*Std. Dev.	Maximum	Minimum	Median
Temperature (°C)	10	9.1	1.4	11.3	7.6	8.6
DO %	10	94.0	22.1	152.1	68.4	90.2
DO (mg/L)	10	10.8	2.8	18.1	8.0	10.0
Conductivity (µs/cm)	10	3529	6023	17965	529	742
Salinity (ms/cm)	10	2.00	3.57	10.64	0.26	0.37
pH	10	8.2	0.4	8.6	7.3	8.3
Clarity (cm)	10	34.0	17.6	69.0	19.0	27.0

*Std. Dev = standard deviation

Table 5: Descriptive statistics for WQ parameters collected in July 2017.

July	Sample size	Mean	Std. Dev.	Maximum	Minimum	Median
Temperature (°C)	12	10.9	1.1	12.3	9.5	10.5
DO %	12	89.9	5.1	99.6	79.5	90.9
DO (mg/L)	12	9.8	0.9	11.2	8.4	10.2
Conductivity (µs/cm)	12	4039	6187	15821	444	618
Salinity (ms/cm)	12	2.30	3.63	9.25	0.22	0.30
pH	11	7.9	0.2	8.3	7.7	7.8
NTU	12	14.3	8.6	32.0	3.9	11.5

3.3 FAECAL SOURCE TRACKING

The results of the faecal source tracking analysis on the ten sites along the Taruheru River are presented in Table 6, with photos of the sampling sites presented in Appendix A.3.2 and A.3.3. In general, the general faecal PCR marker (GenBac3) that targets all faecal pollution was tenfold higher in samples collected during June 2017, and this was a statistically significant difference (Mann-Whitney Rank Sum Test, P = <0.001). There was no substantial rainfall in the seven days prior to collection of samples in either June or July (Figure 6 and Figure 7). Therefore, higher PCR marker concentrations in June would not be attributed to increased rainfall and land runoff into the river. River flow at Tuckers Road Bridge was significantly different between June and July (Mann-Whitney Rank Sum Test, P = 0.049) and at Courtneys Road Bridge (P = <0.001). Median River volumes were approximately two fold higher in July compared with June, which may account for the lower GenBac3 PCR marker concentrations in the July water samples. In addition, in June the water samples were collected as the tide was coming in, while in July the water samples were collected at low tide.

In the following dialogue the sites are analysed individually in relation to their FIB levels and faecal source tracking markers. Action and Alert levels for enterococci are commented upon where there are exceedances, however, it should be noted that these Action and Alert levels are guidance values for marine/coastal waters rather than freshwater.

The contribution of ruminant pollution (BacR, PCR marker) has been estimated based on its ratio to the general faecal marker (GenBac3). Research at ESR has shown that the %BacR/GenBac3 was >15% in all cowpat runoff, indicating that fresh inputs of cow faeces to a waterway may produce a similar ratio (Devane, 2016). Estimations of the ruminant contribution have been given in Table 6, and where the estimation is attributed to less than 50-100% ruminant pollution, it may indicate aged faecal sources that have travelled a long distance down river, or land runoff from irrigated effluent or faecal deposits (eg cowpats). It should also be noted that avian species carry lower abundance of the bacteria targeted by GenBac3 so it is possible to identify an estimated100% contribution from ruminant sources with avian pollution still being identified. The ratio between *E. coli* and faecal coliforms can also be another useful marker of aged sources of faecal material in a waterway. Generally *E. coli* are at least 90% of the faecal coliforms in fresh faeces. If this ratio is reduced in freshwater then it may be indicative of aged sources where there has been a higher die-off of *E. coli* in the environment compared with the other faecal coliforms.

The water samples were tested with the GFD avian PCR marker, which targets a wide range of wildfowl including ducks. ESR can also test for a duck specific PCR marker, which may identify higher faecal contributions where duck sources predominate.

Site descriptions, survey notes and photographs of each sampling site were provided by GDC and presented in the Appendices in Section A.3.

3.3.1 Waihirere Stream @ Domain

Low levels of *E. coli* (<31 CFU/ 100 mL) and negligible enterococci were detected at this location where the Waihirere stream flows through the Waihirere Domain. There is bush on either side of this sampling site and no obvious sources of runoff into the stream. Due to the low *E. coli* concentrations only one of the samples was tested and returned a positive detection of the ruminant PCR marker, which seemed to be the main contributor of faecal pollution. There is a sheep fence above the true right of the stream, therefore, sheep may be a source of this ruminant PCR marker but other possibilities for ruminant faecal contamination include feral goats and deer (if present in the upstream catchment). ESR can

test for a sheep specific PCR marker, however, the low levels of the ruminant marker suggests a low likelihood of detecting the specific ruminant species in this water sample.

3.3.2 Harper Road

Moderate levels of *E. coli* (250-290/100 mL), which spanned the Alert level of 260 *E. coli*/100 mL, were detected in the water samples collected at Harper Rd. Low levels of enterococci <50 CFU/100 mL were also identified. Ruminant pollution was identified as the major contributor (up to 50-100% from ruminant) to these elevated *E. coli* levels with lower secondary contributions from avian sources.

3.3.3 King Road Bridge

Concentrations of *E. coli* were between the Alert and Action levels for the recreational water quality standards at King Rd Bridge and enterococci were below the Alert level. PCR markers in both June and July water samples registered a 10-50% contribution from ruminant faecal sources, and avian sources were also detected at lower levels.

3.3.4 Haisman Road Tributary

At the Haisman Road Tributary the levels of *E. coli* were above the Action level of 550 *E. coli*/100 ML, and in June, they were above the national bottom line of 1000 *E. coli*/100 mL and just below the Action level for enterococci. The elevated levels of *E. coli* (1300 CFU/100 mL) during the June sampling were associated with the detection of human pollution, low level ruminant and avian sources. Turbidity readings were not undertaken during the June sampling but clarity measurements were not high when compared with other sites recorded during the June event, however, there are no other clarity data in this stream for comparison.

The July levels of *E. coli* were still elevated at 660 and 620 CFU/100mL but only ruminant and avian sources were detected in these two water samples collected seven minutes apart. Turbidity was high in these July samples (23 NTU), which may be suggestive of stream sediment re-suspension or additional drain clearing. In the second of these July samples, ruminant sources were identified at 10-50 % contribution (and no avian source) compared with the lower levels of ruminant sources in the other two samples.

In addition, the *E. coli* concentration was 45% of the faecal coliform concentration in the June water, suggestive of less recent faecal inputs. In general, the faecal coliforms in fresh animal and human faeces are largely comprised of *E. coli* (90% and higher). In the July samples, the counts between *E. coli* and faecal coliforms were similar suggesting fresher faecal inputs.

This site warrants further investigation to detect the source of the human pollution detected in the June sample. The human pollution and high *E. coli* and FC concentrations in June were in association with very high dissolved oxygen levels (19 mg/L, Figure 5). It was noted (in the site descriptions) that this stream had just undergone drain clearance which may have impacted the elevation in DO and FIB levels during the June sampling event. No other water quality parameters (including clarity) were noted as markedly different to other sites during this June event. An investigation of the location of septic tanks in this area may provide information on sites of potential leakage into the Haisman Road Tributary. Faecal sterol analysis of the water and/or stream sediment may be helpful at this site to detect human inputs from aged sources.

3.3.5 Tuckers Road Bridge

Tuckers Rd Bridge is at the approximate upstream limit of tidal influence. At this site both of the water samples were above the Alert level for *E. coli* with the June sample above the

Action level for *E. coli* and above the Alert level for enterococci. A contribution of 10-50% from ruminant sources was identified in both June and July water samples with secondary sources of avian faecal pollution.

3.3.6 Gaddum Tributary

At Gaddum Tributary, concentrations of *E. coli* exceeded the Action level for *E. coli* and enterococci in the June water sample and for *E. coli* in the July water. *E. coli* levels in the July water also exceeded the national bottom line at 1200 CFU/100 mL, however enterococci were below the Alert level. Although the faecal indicator bacteria (FIB) concentrations were high in the two samples, no human PCR markers were detected and low levels of both ruminant and avian sources were detected with the general faecal PCR marker also reduced compared to other sites in the June sampling. The concentrations of faecal coliforms were high for both sampling events and in June the *E. coli* were only 37% of the faecal coliform suggesting less recent sources of faecal *E. coli* (Table 7). A pig farm and dog kennels are located in the vicinity of Gaddum Tributary.

Based on the FST assessment of PCR marker abundance, it would appear that ruminant and avian pollution sources are not the total sources of the elevated FIB. Therefore, it would be worthwhile reanalysing the current water sample DNA extracts for dog PCR markers at Gaddum Tributary to identify contributions from these faecal sources. Unfortunately, we do not currently have a pig PCR marker to assay these samples.

3.3.7 Cemetery Western Boundary

The single sample collected in June from the Cemetery Western Boundary had an elevated *E. coli* concentration just under the Action level. Ruminant faecal sources were identified and a low level of avian pollution. The ruminant sources are consistent with sheep and cattle on fields adjacent to this location, but at 10-50% contribution of the ruminant PCR marker to sources this may indicate aged pollution for example, runoff from land or pollution travelled from further upstream.

3.3.8 Lytton Road Bridge

The samples from Lytton Road Bridge had *E. coli* concentrations above the Action level and enterococci above and just below the Alert level. Ruminant sources appeared to contribute 10-50% of the pollution with avian pollution a secondary source. As this site is downstream of an historic meatworks plant, it may be prudent to use the faecal sterols to test for leachate from the meatworks, which could be identified by a dominant cholesterol signature.

3.3.9 Stanley-Oak Street Footbridge

Low level human pollution was identified at Stanley-Oak Street Footbridge in the June sampling. The human signal was in association with *E. coli* and enterococci below the Alert level and the *E. coli* percentage of faecal coliforms was low at 48%, perhaps suggestive of aged faecal sources. Avian sources and low level ruminant pollution were also identified in the June sample. A human faecal signal was not identified in the July water sample, which contained a 10-50% contribution from ruminant sources and *E. coli* near the Action level and enterococci above the Alert level.

This site warrants further investigation to detect the source of the human pollution detected in the June sample.

3.3.10 Wi Pere Pipe

In addition to the June water sample, two samples were collected at Wi Pere Pipe for the July sampling. The June sample had FIB concentrations below the Alert levels. For the two

July water samples, which were collected nine minutes apart, the *E. coli* concentrations were above the Alert level and enterococci were above the Action level, which is relevant for this brackish water environment. Avian and ruminant pollution at varying levels were detected on both sampling occasions.

3.4 CONCLUSIONS AND RECOMMENDATIONS

In this study, FIB concentrations and faecal source tracking (FST) tools were used to investigate likely sources of faecal contamination of the Taruheru River, Gisborne under base flow conditions. Sixteen of the 21 water samples collected from the ten sites recorded *E. coli* levels above the Alert Level of (260 *E. coli*/100 mL) with nine of the ten sites sampled on the Taruheru being impacted by elevated levels of *E. coli* above the recreational water quality guidelines (MfE & MoH, 2003). Only at the most upstream site in the Waihirere Domain were *E. coli* levels at <31 CFU/100 mL, below the Alert level on both sampling occasions. Eight of 21 water samples had concentrations above the Action level of 550 *E. coli*/100 mL). Two of these samples also exceeded the national bottom line of 1000 *E. coli*/100 mL (Table 6) and both samples originated from the two tributaries of Haisman Road and Gaddum. Eight water samples also had exceedances of enterococci concentrations, which included samples from the two tributaries.

In general, FIB levels were lowest in the upstream section of the Taruheru River with concentrations peaking in the two tributaries at Haisman Road and Gaddum. Concentrations of FIB decreased again in the lower reaches of the river except for enterococci levels above the Action level during the July sampling event at the tidally influenced Wi Pere pipe.

The sites at Haisman Road tributary and Stanley-Oak Street Footbridge were the only sites to record human pollution (June sampling). Ruminant and avian faecal sources were identified at all sites at various levels although avian sources were not identified in all samples. Most sites reported a ≤50% contribution from ruminant faecal sources, except at the two most upstream sites of Waihirere Domain and Harper Road (however, the ruminant signal at Waihirere Domain was in association with very low levels of *E. coli*). Ruminant contributions of less than 50% may be indicative of aged faecal sources from overland runoff or it signifies that the pollution has travelled some distance from its source.

It is well-established that all PCR markers used for faecal source tracking have some level of cross-reactivity where they may be identified in other non-target animals/birds. Therefore, it is worthwhile, where additional host-specific PCR markers exist, to test more than one marker for a specific faecal source. In the case of human pollution, therefore, both PCR markers needed to be positive to provide confidence in the finding of human pollution sources. It may be prudent to exclude possum faecal pollution as the source of the human PCR signal at Haisman Road tributary and Stanley-Oak Street Footbridge, as possum populations in NZ can also carry the human-specific bacteria targeted by the PCR markers (Devane et al., 2013). Studies at ESR, however, have concluded that the BiAdo PCR marker has a very low likelyhood of detecting possum faecal contamination in a waterway. Therefore, when BiAdo and BacH human PCR markers are detected simultaneously in a water sample it is likely to be indicative of human faecal sources.

Low levels of the avian GFD PCR marker were identified in this study. Higher levels of avian pollution may have been obtained if the duck specific marker was assayed where duck populations dominated the wildfowl in a particular location. This factor would only be relevant where *E. coli* concentrations were elevated and no other faecal sources were identified and for this study, avian pollution was always identified in association with the other faecal sources human and/or ruminant.

If the specific detection of sheep or cow faecal contamination was required rather than ruminant pollution sources then this would require the collection of larger volumes of water.

This step would be necessary because the cow and sheep specific PCR markers are present in lower concentrations in the host animals compared with the ruminant PCR marker.

The two tributaries entering the Taruheru River have high levels of FIB exceeding the MfE and MoH 2003 Action levels for *E. coli* on all sampling occasions. It would be worthwhile to perform discharge flow calculations for the two tributaries to see if their high FIB counts are based on a flow that would markedly impact the main river flow or if dilution is significant on entering the Taruheru River. Furthermore, Haisman Road Tributary warrants further investigation to identify the source of the human pollution detected in the June sample, in addition to the ruminant and avian sources detected. The sampling event in June occurred after drain clearage of this stream, which may have disturbed sediments harbouring aged faecal contamination. July samples did not contain human indicators of faecal pollution, rather there were ruminant and avian sources detected.

International research has shown that during base flow conditions there is little or no run-off from agricultural land (e.g., Kay et al. 2010). Thus, during base and low flow conditions, the dominant input of faecal contamination in urban areas would be expected to be urban pollution not agricultural pollution. This does not seem to be the case for the urban reaches of the Taruheru River, which were predominately impacted by ruminant and avian sources.

Further investigation of the two sites where human faecal sources were identified during the June sampling will reveal the point sources at these two locations but as human pollution was not identified at other locations, nor during the July sampling event, then these human signals may be localised and not signify major critical sources of human contamination. The consistent ruminant and avian faecal signatures identified in the Taruheru River, however, does suggest that the water quality is compromised for recreational activities. Pathogens such as *Campylobacter*, pathogenic *E. coli* (for example *E. coli* O157:H7) and protozoa (for example *Cryptosporidium*) can be associated with faecal sources derived from agricultural livestock (Moriarty et al., 2011a; Moriarty et al., 2005; Moriarty et al., 2011c; Moriarty et al., 2008; Moriarty et al., 2012).

Similar to the 2014 findings of FST analysis in the Taruheru River under falling-high flow river conditions, this base river flow study identified ruminant and avian as the predominant faecal signals. The ruminant faecal signal may be due to transport from surrounding agricultural activities. This current study of FST data during winter conditions has also confirmed that human faecal contamination can be an intermittent source to this urban area.

Recommendations:

- Identify the sources of human faecal contamination at Haisman Road Bridge and Stanley-Oak Street Footbridge by conducting a sanitary survey at these locations to identify if there are septic tanks/other human faecal sources in the area where leakage/overflows of sewerage could be occurring.
    - Faecal sterol analysis alongside additional PCR marker analysis of the water and/or stream sediment (sterols only in sediment) may be helpful at these two sites to detect human inputs from recent/aged sources. The sanitary survey could inform a targeted approach to identify sites for detection of critical sources of human faecal contamination.
- At Gaddum Tributary, based on the FST assessment of PCR marker abundance, it would appear that ruminant and avian pollution sources are not the total sources of the elevated FIB. Therefore, it would be worthwhile to reanalyse the DNA extracts

from the water samples at Gaddum Tributary for dog PCR markers to identify contributions from these faecal sources. At this site there are known dog kennels and a pig farm in the vicinity. Unfortunately we do not currently have a pig PCR marker for identifying pig pollution.

- Conduct discharge flow calculations for the two tributaries (Haisman Road Bridge and Gaddum) to understand whether these tributaries are acting as critical sources of faecal pollution as indicated by their high FIB counts
- A long-term aim could be the investigation of sediments in these urban waterways, including the Turanganui and Waimata Rivers to understand the deposition and accumulation patterns of sewage markers (faecal indicator bacteria and faecal sterols) entering this environment

Table 6: Faecal source tracking results for the Taruheru River and selected tributaries.

ESR Number	Location	Month 2017	*E. coli*	¥Entero	PCR markers / 100 mL						Conclusion
			(CFU/100mL)		GenBac3	BacH	BiADO	BacR	Proportion Ruminant	Avian	
CMB171067	Waihirere Stream @ Domain	*June	9.8	1.6	NA	NA	NA	NA	NA	NA	Not tested
CMB171208		*July	30	1.6	25,000	32	ND	2,300	50 - 100%	ND	Ruminant
CMB171063	Harper Road	June	250	48	320,000	ND	90	21,000	50 - 100%	200	Ruminant and low level avian
CMB171209		July	290	28	58,000	35	ND	1,600	10 - 50%	36	Ruminant and low level avian
CMB171064	King Road Bridge	June	480	110	390,000	ND	ND	15,000	10 - 50%	520	Ruminant and avian
CMB171211		July	370	43	42,000	60	ND	930	10 - 50%	32	Ruminant and low level avian
CMB171062	Haisman Road Trib	June	1300	270	420,000	690	300	2,000	1 - 10%	59	Human pollution, and low level ruminant and avian
CMB171217		July	660	210	82,000	ND	ND	1,200	1 - 10%	41	Low level ruminant and avian
CMB171218		July	620	110	98,000	ND	ND	1,800	10 - 50%	ND	Ruminant
CMB171066	Tuckers Road Bridge	June	640	230	370,000	74	ND	14,000	10 - 50%	310	Ruminant and avian
CMB171212		July	390	60	43,000	39	ND	750	10 - 50%	32	Ruminant and low level avian

Table continued	Location	Month 2017	*E. coli*	¥Entero	PCR markers / 100 mL						Conclusion
ESR Number			(CFU/100mL)		GenBac3	BacH	BiADO	BacR	Proportion Ruminant	Avian	
CMB171061	Gaddum Trib.	*June	**820	370	43,000	ND	ND	420	1 - 10%	92	Low level ruminant and avian
CMB171210		*July	1200	66	30,000	ND	ND	130	1 - 10%	35	Low level ruminant and avian
CMB171068	Cemetery Western Boundary	June	540	100	200,000	ND	ND	5,500	10 - 50%	220	Ruminant and low level avian
CMB171065	Lytton Road Bridge	June	570	170	340,000	51	ND	5,500	10 - 50%	440	Ruminant and avian
CMB171213		July	710	130	37,000	27	ND	570	10 - 50%	28	Ruminant and low level avian
CMB171070	Stanley-Oak Street Footbridge	June	240	76	350,000	290	57	4,700	1 - 10%	320	Low level human pollution; avian and low level ruminant
CMB171216		July	540	150	36,000	46	ND	710	10 - 50%	<18	Ruminant
CMB171069	Wi Pere Pipe	June	120	54	320,000	200	ND	3,400	1 - 10%	400	Avian and low level ruminant
CMB171214		July	410	440	29,000	48	ND	770	10 - 50%	<18	Ruminant
CMB171215		July	420	330	80,000	100	ND	1,900	10 - 50%	36	Ruminant and low level avian

*Samples were collected on 21 June 2017 and 24 July 2017; ¥Entero = enterococci (faecal coliform concentrations can be found in Table 7)
**Green colour coding = below Alert levels for FIB; orange colour coding = above Alert levels for FIB; red colour coding = above the Action levels for FIB.

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APPENDIX A: MICROBIAL CONCENTRATIONS IN WATER SAMPLES

A.1 MICROBIAL RESULTS

Table 7: Microbial concentrations of faecal indicator bacteria in water samples from Taruheru River

Microbial concentrations CFU/100 mL	¥Waihirere Stream @ Domain		Gaddum trib		Harper Road		King Rd Bridge		Tuckers Rd Bridge	
Date	21 June 2017	24 July 2017	21 June 2017	24 July 2017	21 June 2017	24 July 2017	21 June 2017	24 July 2017	21 June 2017	24 July 2017
Time	**0929**	**1025**	**0855**	**1050**	**0950**	**1006**	**1004**	**0954**	**1022**	**0943**
*enterococci	1.6	1.6	370	66	48	28	110	43	230	60
**E. coli*	9.8	30	820	1200	250	290	480	370	640	390
*Faecal coliform	28	50	2200	1700	300	370	600	390	960	660

Microbial concentrations CFU/100 mL	Haisman Rd Trib			Cemetery Western Boundary		Lytton Rd Bridge		Stanley-Oak St footbridge		Wi Pere Pipe		
Date	21 June 2017	24 July 2017		21 June 2017	24 July 2017	21 June 2017	24 July 2017	21 June 2017	24 July 2017	21 June 2017	24 July 2017	
Time	**1034**	**0934**	****0941**	**1058**	**-**	**1122**	**0917**	**1140**	**0906**	**1153**	**0820**	****0829**
*enterococci	270	210	110	100	¥NT	170	130	76	150	54	440	330
**E. coli*	1300	660	620	540	NT	570	710	240	540	120	410	420
*Faecal coliforms	3900	650	580	670	NT	830	720	500	720	430	500	490

¥This sample was not tested for PCR markers as *E. coli* concentration was too low; ¥NT = sample not collected

**Extra samples taken at these sites due to low *E. coli* at Wi Pere Pipe and Waihirere Stream Domain on first sampling 21 June 2017

A.2 WATER QUALITY PARAMETERS

Table 8: Water quality parameters collected on 21 June 2017

Taruheru River sites	Time	Temperature	DO %	DO mg/L	Conductivity µs/cm	Salinity ms/cm	pH	Clarity (cm)	Water Type
Waihirere Domain	9:29	7.6	100.8	12.04	529	0.26	8.60		fresh
Harper Rd.	9:50	8.0	91.7	10.84	608	0.3	8.43	69	fresh
King Rd. Bridge	10:04	8.1	92.7	10.92	622	0.3	8.31	44	fresh
Haisman Rd. Trib.	10:34	7.7	152.1	18.11	729	0.36	8.39	27	fresh
Tuckers Rd. Bridge	10:22	8.6	89.7	10.44	684	0.34	8.25	33	fresh
Gaddum Trib.	8:55	8.6	68.4	7.96	861	0.4	8.37	19	fresh
Cemetery Western Boundary	10:58	9.3	80.9	9.27	755	0.37	8.43		brackish
Lytton Rd. Bridge	11:22	10.8	86.3	9.52	1451	0.73	8.17	21	brackish
Stanley-Oak St. footbridge	11:40	11.1	87.0	9.17	11084	6.32	7.30		saline
Wi Pere Pipe	11:53	11.3	90.7	9.3	17965	10.64	7.76	25	saline

Table 9: Water quality parameters collected on 24 July 2017

Taruheru R sites	Time	Temperature	DO %	DO mg/L	Conductivity µs/cm	Salinity ms/cm	pH	Turbidity (NTU)	Water Type
Waihirere Domain	10:25	10.0	99.6	11.23	444	0.22	8.27	32.0	fresh
Harper Rd.	10:06	10.4	91.9	10.27	549	0.27	7.76	3.9	fresh
King Rd. Bridge	9:54	10.4	90.9	10.15	576	0.28	7.76	5.2	fresh
Haisman Rd. Trib.	9:34	9.5	92.9	10.59	582	0.28	7.89	23.3	fresh
	9:41	9.5	92.8	10.60	571	0.24	7.87	23.1	fresh
Tuckers Rd. Bridge	9:43	10.5	91.3	10.17	618	0.30	7.80	11.5	brackish
Gaddum Trib.	10:50	11.8	86.8	9.38	732	0.36	7.73	15.8	fresh
Lytton Rd. Bridge	9:17	11.8	84.6	9.12	1046	0.52	7.84	10.7	brackish
Stanley-Oak St. footbridge	9:06	11.8	79.5	8.38	7714	4.29		11.8	brackish
Wi Pere Pipe	8:20	12.3	89.7	9.06	15779	9.25		9.9	brackish
	8:29	12.3	89.2	9.07	15821	9.23		9.8	brackish

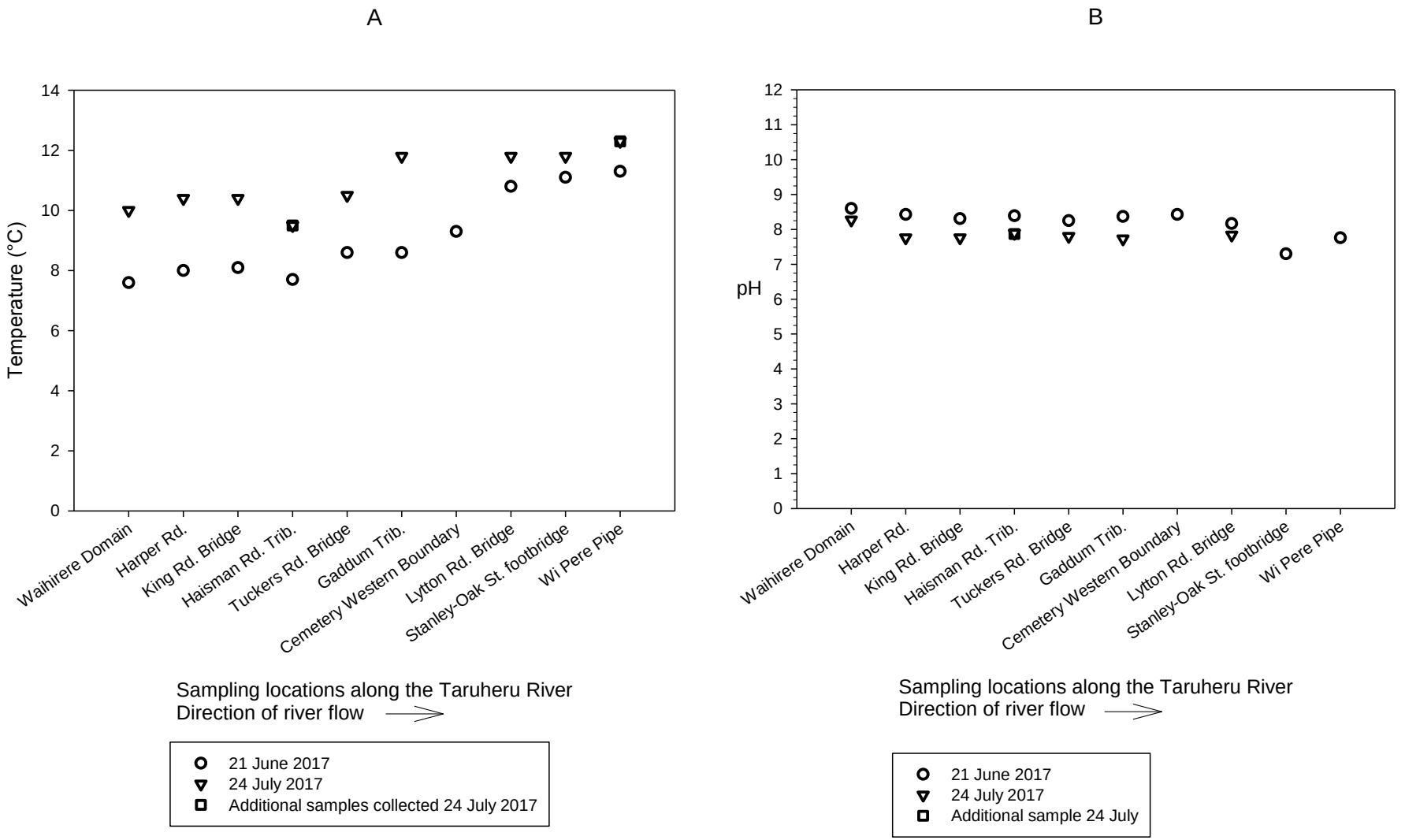


Figure 3: Temperature (A) and pH (B) measurements in the Taruheru River

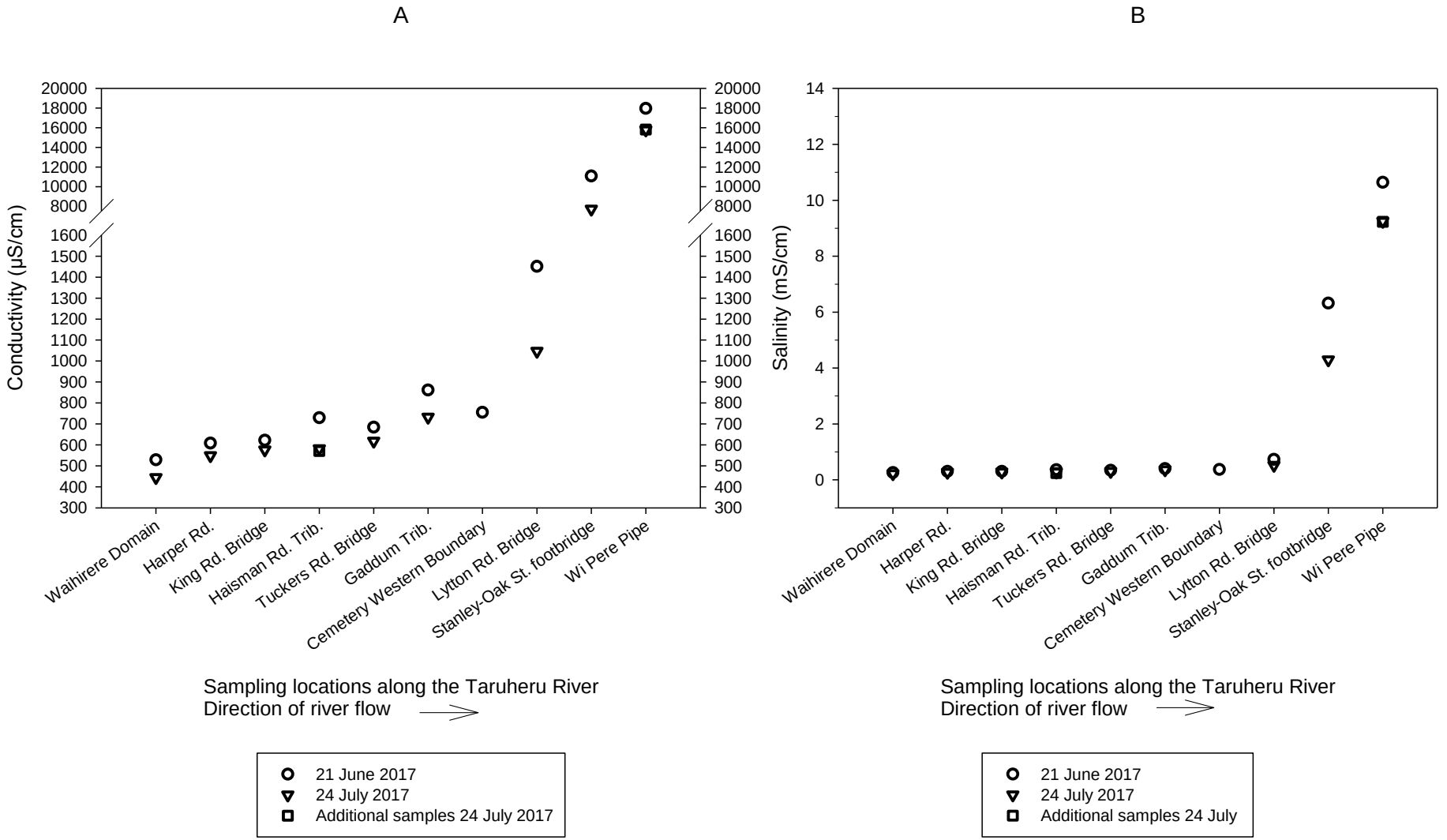


Figure 4: Conductivity (A) and salinity (B) measurements in the Taruheru River

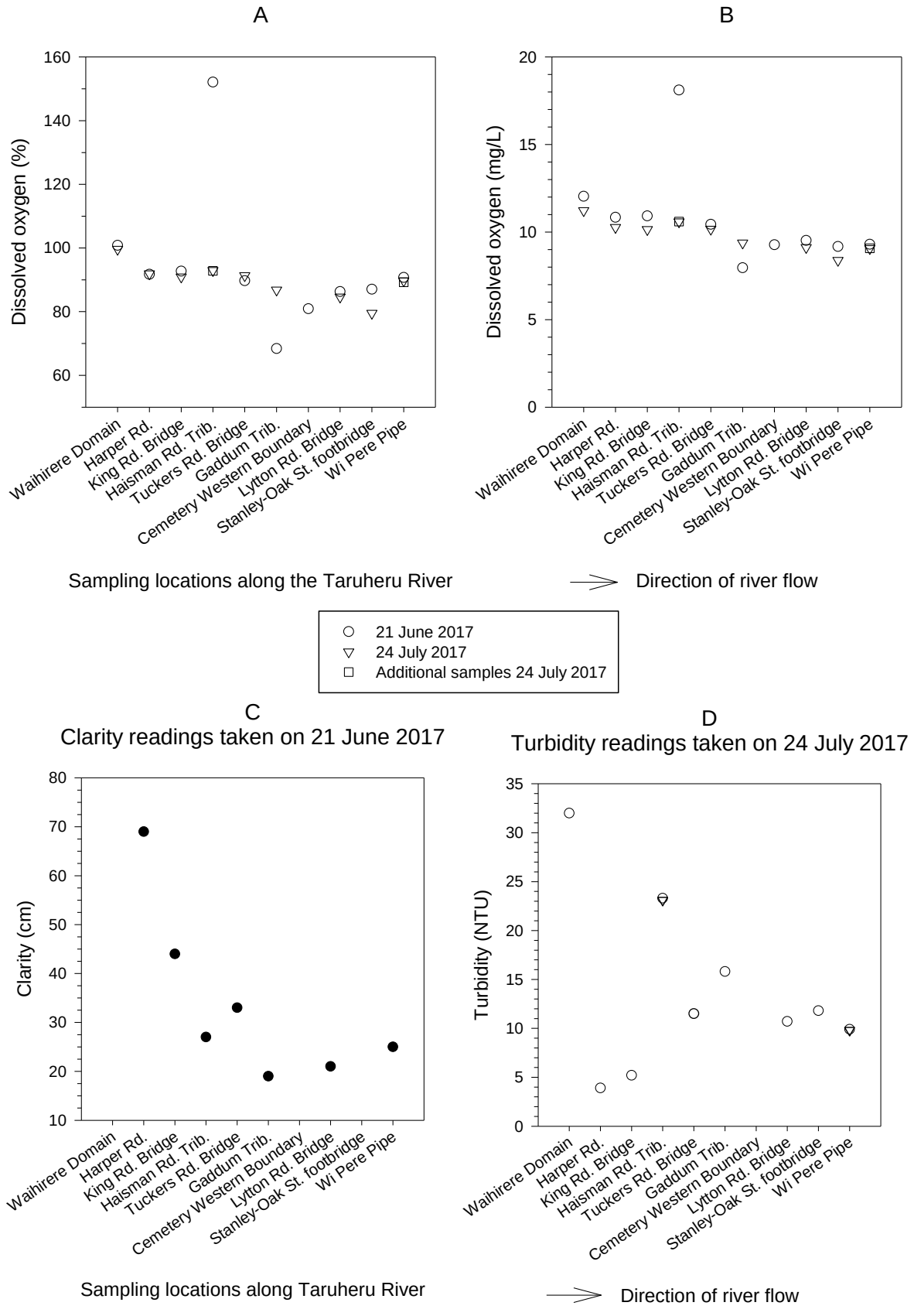


Figure 5: Dissolved oxygen (DO) measurements in the Taruheru River A) percentage of DO and B) DO in mg/L. Visual clarity measurements were taken as C) Clarity in the June sampling and D) Turbidity readings of nephelometric turbidity units (NTU) in July 2017; (two samples were taken at Haisman Rd. Trib. and Wi Pere pipe on 24 July).

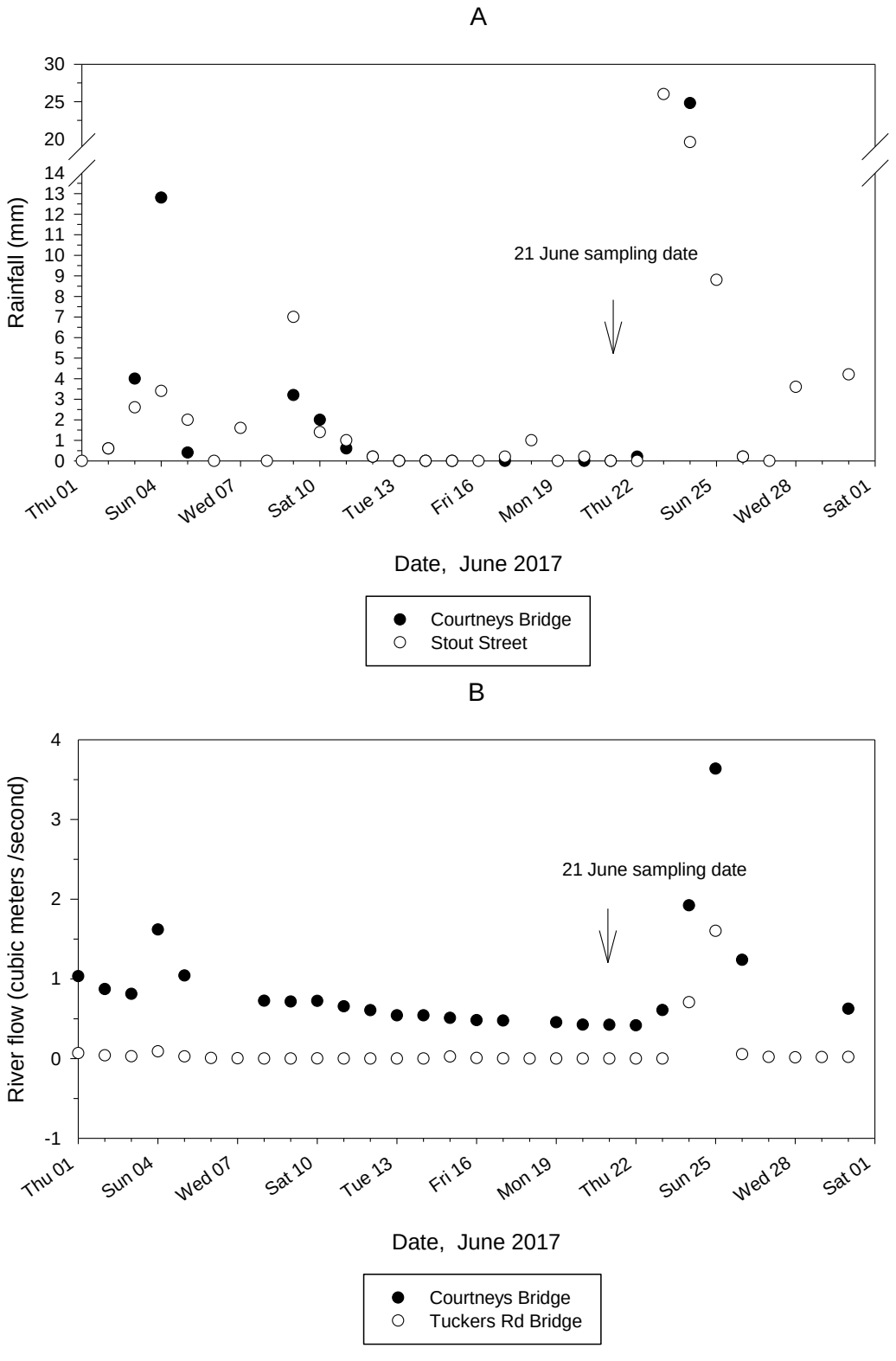


Figure 6: Rainfall (A) and river flow (B) during June 2017 at selected sites along the Taruheru River. Courtney's Bridge is downstream of Waihirere Domain and Tuckers Road Bridge is downstream of the Haisman Road tributary. Stout Street is to the North East of the Lytton Road Bridge Site.

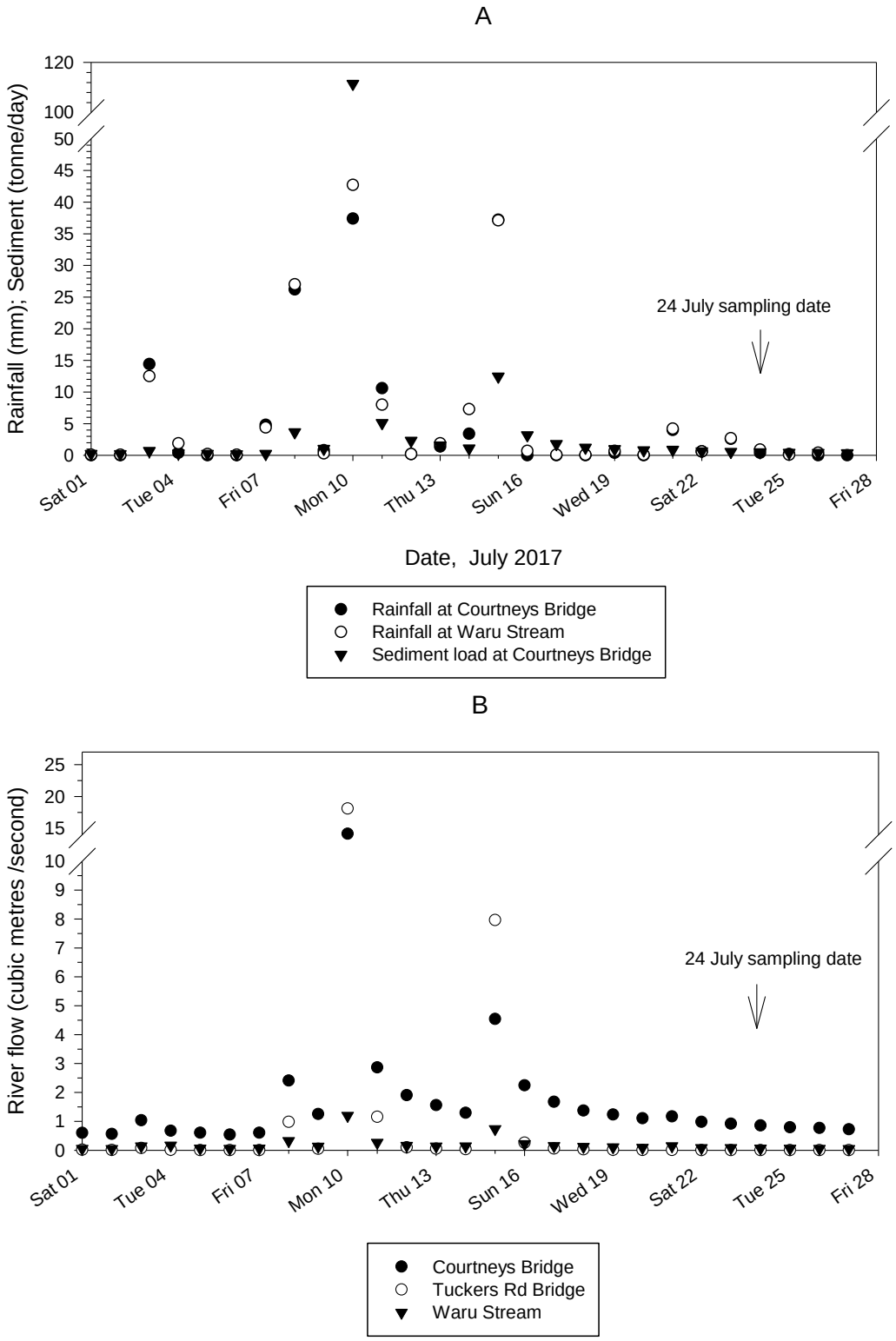
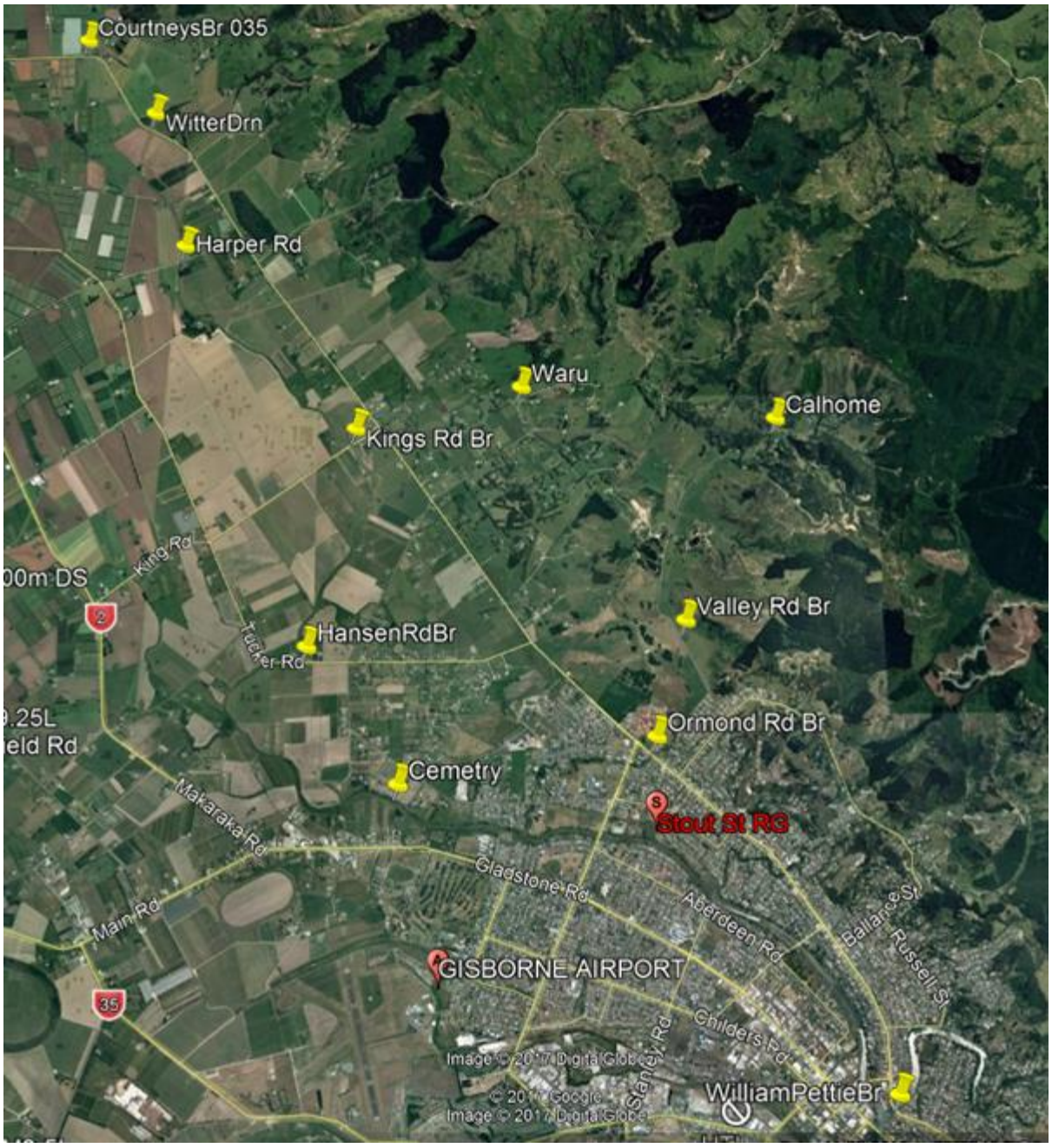


Figure 7: Rainfall and sediment loads (A); and River flow (B) during July 2017 at selected sites along the Taruheru River. Courtney's Bridge is downstream of Waihirere Domain and Tuckers Road Bridge is downstream of the Haisman Road tributary. Waru stream is a tributary of the Taruheru, which is downstream of the King Road sampling site, and the monitoring site is at McLaurin Bridge on the Waru stream.

A.3 DESCRIPTIONS AND PHOTOGRAPHS OF THE SAMPLING SITES OF THE TARUHERU AND ITS TRIBUTARIES

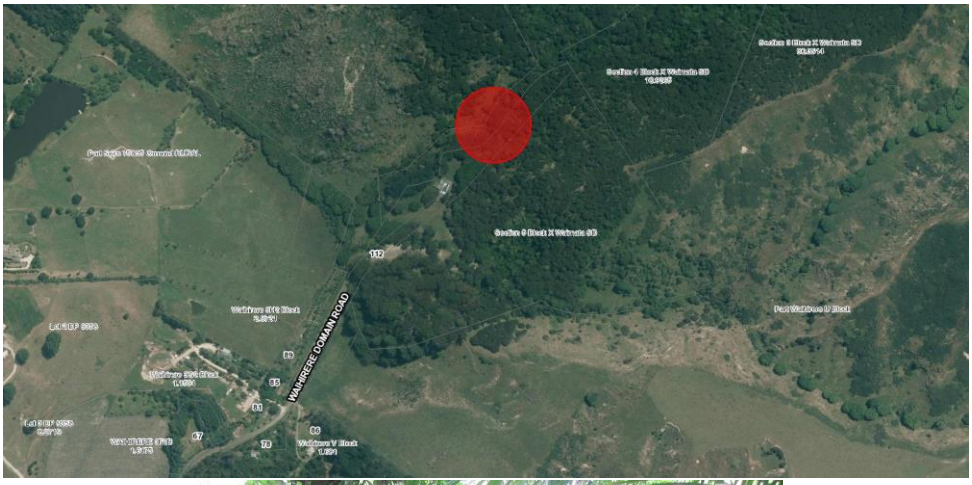
A.3.1 Location of some sampling sites and rain weather stations along the Taruheru River



A.3.2 All Taruheru River sites sampled for this envirolink study

A.3.3 Taruheru Faecal Source Tracking Site Descriptions

Site Name	Waihirere Domain
Land Owner	Gisborne District Council
Contact	
GPS Location	2030959E 5719095N
Site notes & potential *E. coli* sources Shaded area, small pools, rocks. Sheep fence on top bank of TRHS. No visible runoff.	



Site Name	Harper Road Bridge
Land Owner	Gisborne District Council
Contact	
GPS Location	2031277E 5715956N
Site notes & potential *E. coli* sources Large amount of grass on the bank edges along this stretch of the river – grazed periodically but sheep. There is a roadside drain that comes into the river from the eastern side. Intensive horticulture occurs on the western side throughout the year. Sheep present when sampling	

Site Name	King Road Bridge
Land Owner	Gisborne District Council
Contact	
GPS Location	2032951E 5714134N
Site notes & potential *E. coli* sources Two road side drains enter the River at this point, from both sides. Horticulture occurs on the TRHS throughout the year. Moderate farming occurs on the TLHS. Cattle grazing occurs periodically through the year also, with cattle camping under the bridge. Small amounts of rubbish in the river.	



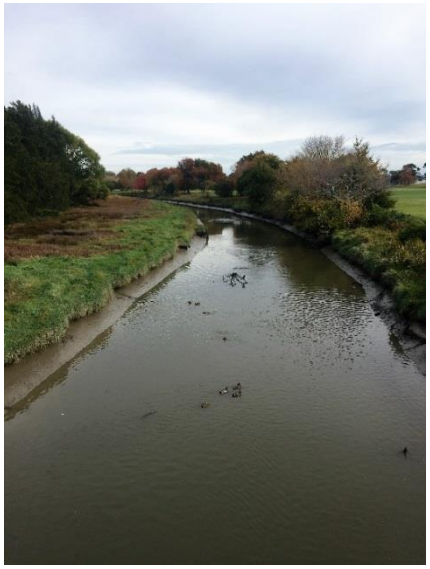
Site Name	Haisman Road Trib
Land Owner	Peter Franks
Contact	06 8686280
GPS Location	2032520E 5712098N
Site notes & potential *E. coli* sources This tributary has just been 'drain cleared' by the GDC. Orchard/Olives on the TLHS, small farm-let on the TLHS with several donkeys and a horse grazing the paddock. Upstream there is moderate farm use and some horticulture.	


Site Name	Tuckers Road Bridge
Land Owner	Gisborne District Council
Contact	
GPS Location	2032520E 5712098N
Site notes & potential *E. coli* sources Approximate limit for tidal influence. Both sides of the river are grazed by sheep and sometimes cattle. Pigs from the neighboring property also graze infrequently. Small debris dams have been seen below the bridge, which cause build up in rubbish and impeded flow. Haisman Road Trib 550m above the bridge has been "drain cleared" (March 2017) Mass eel death has been recorded 700m below the bridge (results unknown). Major horticulture on both sides upstream of Tuckers Road bridge. Outgoing tide	

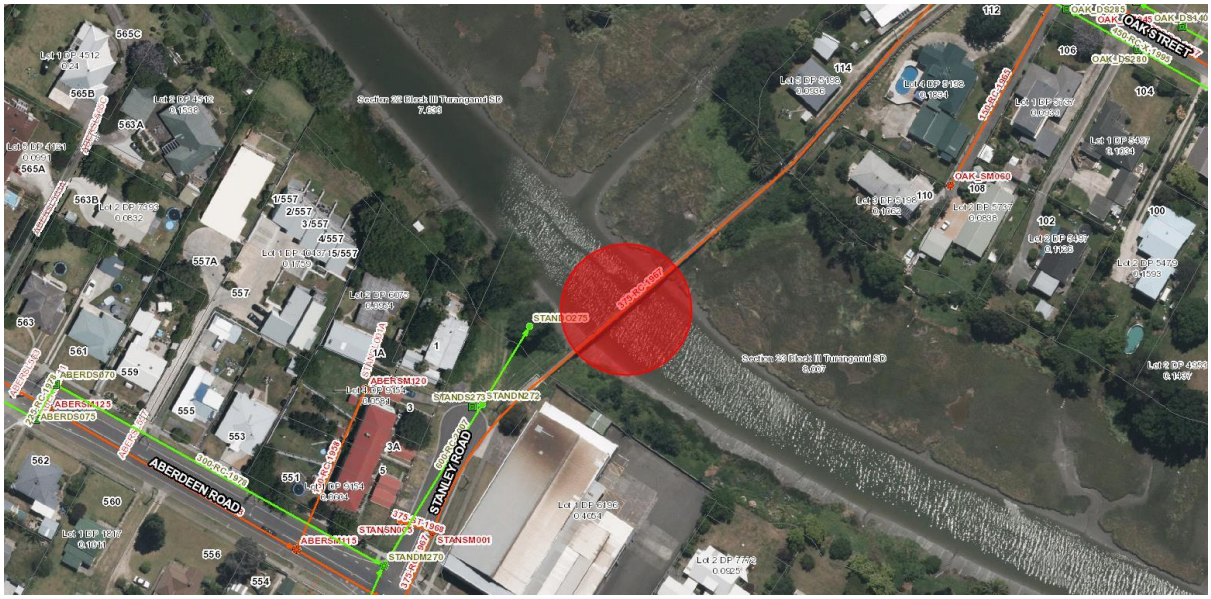
Site Name	Gaddum Trib
Land Owner	Cody & Rachael Langlans
Contact	0273677127
GPS Location	2031522E 5711363N
Site notes & potential *E. coli* sources Soft sedimentary. Kiwifruit and Persimmon orchard on TRHS bank. Grazed by sheep on the TLHS. Small pig farm and dog kennel facility upstream. Citrus and horticulture further upstream. Outgoing tide	

Site Name	618 Nelson Road, Cemetery site
Land Owner	Gisborne District Council – access through Donna and Kevin Williams property
Contact	0274483478
GPS Location	2033006E 5710894N
Site notes & potential *E. coli* sources Soft eroded banks. Cattle and sheep grazing in the paddocks close to the river, fenced off. Cattle feed spread on the paddock adjacent. Intense horticulture on TLHS upstream. Last site before storm water drains – utilities enter the waterway. Upstream of the cemetery. Bubbles and foam noticed or the surface. Incoming tide	

Site Name	Lytton Road Bridge
Land Owner	Gisborne District Council
Contact	
GPS Location	2035232E 5710513N
Site notes & potential *E. coli* sources Ducks present prior to sampling. Swift inflow from the tide. Soft sedimentary bottom. Rubbish and other household items in the river. This is downstream of the historic meat works. Urban area begins. Park and rec area on TLHS. Downstream of cemetery. Incoming Tide	



Site Name	Stanley Road Bridge
Land Owner	Gisborne District Council
Contact	
GPS Location	2036258E 5709942N
Site notes & potential *E .coli* sources Ducks and geese present prior to sampling. Small trib upstream on TLHS. Stormwater drain entering upstream on the TRHS. This site has a lot of discarded rubbish and house hold items. Foot bridge that is used regularly – Incoming Tide	



Site Name	Wi Pere Street Bridge
Land Owner	Gisborne District Council
Contact	
GPS Location	2037000E 5709556N
Site notes & potential *E. coli* sources Ducks present prior to sampling. Soft sedimentary bottom. Small amount of debris build up downstream. Stormwater incoming both sides upstream and downstream. Large botanical gardens downstream on TRHS. Playground, aviary and duck pond. Incoming Tide	

Key for map of stormwater and wastewater at Wi Pere Street Bridge:

Stormwater Mains – Green

Stormwater Drain - Blue

Wastewater lines – Orange

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