Science for Communities

Sources of Pollution in the Waiau Freshwater Management Unit

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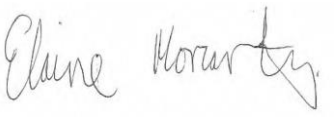

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EXECUTIVE SUMMARY

Environmental waters may be impacted by faecal contamination from human and animal sources, including the discharge of municipal sewage or animal effluents, seepage from septic tanks, stormwater and urban run-off, agricultural run-off, and direct deposition by animals, including birds, wildlife, and livestock (where access permits). Water that is contaminated by faeces may contain microbial pathogens (disease-causing bacteria, viruses or protozoa), and as such, may pose a health risk to people using the water for drinking water, recreation or mahinga kai. Because of difficulties in monitoring waters for the presence of pathogens, microbial water quality is routinely assessed by monitoring the presence of faecal indicator organisms such as faecal coliforms and *Escherichia coli*. These organisms are not themselves harmful to humans, but are present in high concentrations in faeces and thus indicate the possibility of contamination. However, whilst the detection of faecal indicators is important in highlighting that there is a risk of faecal pathogens being present, it does not identify the source(s) of the contamination. Being able to discriminate between different faecal sources (e.g. human, livestock, wildfowl) is an important aspect of effective water quality management, as the risk to human health may differ between different faecal sources. The identification of a faecal source can also assist in designing and prioritising targeted mitigation efforts.

This report details the results of a study of faecal pollution sources at five freshwater sites within the Waiau Freshwater Management Unit (FMU) in Southland. Faecal coliform and *E. coli* concentrations in water samples from these sites were determined as indicators of faecal pollution being present. *Campylobacter* was enumerated as a pathogen of faecal origin. Where *Campylobacter* was detected, isolates were analysed using molecular techniques including polymerase chain reaction (PCR) and multiplex ligation-dependent probe amplification-binary typing (MBiT) to determine their species, estimates of virulence, and a likely source. Microbial faecal source tracking (FST) tools were also utilised to characterise the pollution sources for each site.

The freshwater sites sampled in this study were variable in their water quality, with two sites in particular being vulnerable to high levels of faecal contamination. These two sites – Orauea River at Orawia Pukemaori Road and Lill Burn at Lill Burn Monowai Road – recorded *E. coli* concentrations of more than 7,000 colony-forming units (cfu) per 100 ml of water. Thirty-four percent of all water samples collected across all sites exceeded 1,000 cfu/100 ml. For comparison, the current national Microbiological Guidelines for Freshwater Recreation Areas state that at *E. coli* concentrations above 550 cfu/100 ml, the local council and health authority must advise the public that the water is unsuitable for recreation, due to the elevated health risk. *E. coli* concentrations were nominally higher following rainfall, although additional sampling would provide greater certainty in understanding the impacts of rainfall on water quality at these sites.

Ruminant animals (both cattle and sheep) and wildfowl were important sources of faecal pollution in these waterways, and sites were often impacted by multiple sources (e.g. Figure 1). Both wildfowl and ruminant signatures were commonly detected under both base and high flow conditions, however, wildfowl pollution was the dominant faecal source under base flow conditions, with ruminant pollution dominant following rainfall. Direct deposition into and immediately adjacent to waterways is the likely route of transmission for wildfowl

contamination. Ruminant contamination likely enters waterways via direct deposition and possibly effluent discharge during base flow conditions, with additional inputs via overland flow and artificial tile drains following rainfall. No human faecal contamination was detected.

Campylobacter was detected in 80% of all samples, representing 4 of 5 sites. *Campylobacter jejuni* was recovered from all *Campylobacter*-positive samples, with an unspeciated thermophilic *Campylobacter* additionally recovered in one sample. Wildfowl were determined to be the most common source of *Campylobacter*, followed by ruminants (sheep, cattle or deer), and poultry; however, the prevalence of different sources varies depending on rainfall.

Molecular MBiT analysis of *Campylobacter* isolates revealed a high diversity of genotypes across the FMU, and that there was no separation of these to particular sites. Forty-two percent of the isolates obtained from waters in the Waiau FMU were found to overlap (i.e. be indistinguishable from) human clinical isolates from the Southland area. These genotypes are thus possible sources of waterborne human infection. Of these overlapping isolates, only 14% were found to be of wildfowl origin, suggesting that wildfowl may be a minor source of illness within the community compared with other sources (e.g. human or ruminant faeces), however their risk should not be discounted. Although the presence of other faecal pathogens (e.g. *E. coli* O157, *Cryptosporidium*) was not assessed, the prevalence of *Campylobacter* suggests additional pathogens may be present in the environment.

Options for management and mitigation are discussed. In addition to the source attribution work that has been undertaken in this report, detailed site visits may provide additional information regarding possible routes for the transmission of faecal materials to adjacent waterways (e.g. terrain, stock management, fencing, unconsented discharge activity). Mitigation options may include additional fencing, construction of riparian buffer strips or wetlands, reduced stock densities on land that is prone to overland and/or subsurface flow, stock rotation during inclement weather, irrigation management, wastewater treatment, and avian deterrent (‘scaring’) devices or population control. One mitigation strategy will not be effective at all sites; a site-specific risk assessment that considers the interaction between faecal source, land topography, soil type and the influence of climate variables, together with water quality modelling, will yield the greatest improvements in water quality. The protection of public health should be at the forefront of this decision making, which should also include consultation with landowners and the public.

Figure 1. An example of land with multiple sources of faecal pollution (sheep and wildfowl). The photograph was taken within the Aparima FMU, Southland. Credit: Brent Gilpin, ESR.

1. BACKGROUND

1.1 MICROBIAL WATER QUALITY

Environmental waters may be impacted by faecal contamination from a number of different sources, including the discharge of municipal sewage, seepage from septic tanks, agricultural effluents, stormwater and urban runoff, and direct deposition from birds or domestic or wild animals. The contamination of waterways with faecal material may result in the introduction of enteric pathogens (disease-causing bacteria, viruses or protozoa that live in the gut), such as *Campylobacter*, *Salmonella,* norovirus, *Cryptosporidium* or *Giardia* (MfE and MoH, 2003; Field and Samadpour, 2007; WHO, 2011; Wood et al., 2016). Human contact with contaminated water, for example through recreational activities, collection of mahinga kai or consumption of drinking water, may result in pathogen ingestion and illness. Illness usually presents as self-limiting gastroenteritis (vomiting, diarrhoea) or respiratory or skin infections. The risk and severity of illness depends on the specific pathogen and dose ingested, and the overall health of the consumer; the risk is greatest for individuals with low immunity, including young children, the elderly, pregnant women, and people who are otherwise immunocompromised (MfE and MoH, 2003; Wood et al., 2016). The risk may also differ based on the source of contamination; faecal contamination of human origin is considered to pose the greatest risk to human health due to the host-specificity of any pathogens, particularly viruses, that are present. However, enteric pathogens from ruminant animals (e.g. cows and sheep) and wildfowl are also known to present a risk to human health (i.e. to be zoonotic) (Field and Samadpour, 2007; Soller et al., 2010; Atwill et al., 2012; Devane and Gilpin, 2015).

Direct routine monitoring for the presence of pathogens in waterways is impractical, as pathogens tend to be present in the water at only low levels and are often unevenly distributed, making detection difficult. Further, specific testing for each potential pathogen is expensive and time-consuming, and some pathogens cannot be cultured within the laboratory (EPA, 2006; Field and Samadpour, 2007; Greening and Lewis, 2010). A simpler and accepted approach to assess microbiological water quality is to monitor the presence of indicator organisms. Indicator organisms are not usually pathogenic themselves, but are indicative of faecal contamination, and therefore the potential presence of faecal pathogens. The most commonly used indicators of faecal contamination are faecal coliforms, *E. coli* and enterococci – bacteria which live in the intestinal tract of humans and warm-blooded animals, and are found in elevated concentrations in their faeces (MfE and MoH, 2003; Field and Samadpour, 2007; Wood et al. 2016). Collectively, these bacteria are referred to as faecal indicator bacteria (FIB). In contrast with pathogen monitoring, the presence of FIB is quick and inexpensive to test. *E. coli* is the preferred indicator organism for monitoring freshwaters (MfE and MoH, 2003).

1.2 SOURCES OF POLLUTION AND ROUTES OF TRANSMISSION

Land use surrounding a waterway and across the wider catchment is known to have major impacts on microbial water quality. A review of the pathways and mechanisms by which faecal microorganisms may enter a waterway was carried out Pattis (2017). Some of the most significant faecal sources and associated pathways for transmission are summarised below.

1.2.1 Animal faeces

It is well recognised that grazing livestock are an important source of diffuse faecal contamination of freshwaters. In New Zealand, concentrations of *E. coli* in agricultural streams are typically 20 times higher than streams in forested catchments (Davies-Colley et al., 2004), with the presence of zoonotic pathogens has also being demonstrated in impacted waterways (Till et al. 2008).

Cattle

A number of studies have measured the presence and concentration of faecal indicators and pathogens in the faeces of dairy and beef cattle, and have demonstrated a link between cattle farming and degraded microbial quality of local surface and ground waters (Collins, 2004; Davies-Colley et al., 2004; Close et al., 2008; Moriarty et al., 2008). For example, *Campylobacter* has been reported in cattle faeces at sites throughout New Zealand, with the percentage of positive animals varying between 11 and 81% (Fakir, 1986; Meanger and Marshall, 1989; Ahmed, 1999; Wu, 2001; Adhikari et al., 2004; Gilpin et al., 2008). Devane et al. (2005) reported that 98 and 94% of composite samples collected from five dairy and five beef cattle farms contained *Campylobacter*. Studies have also reported the presence of *Salmonella enterica* (Callaway et al., 2005; Sinton et al., 2007; Kunze et al., 2008), Shiga toxin-producing *E. coli* (STEC; Bunic and Avery, 1997; Cookson et al., 2006), *Cryptosporidium* (Grinberg et al., 2005) and *Giardia* (Learmonth et al., 2003) in bovine faeces. In a survey of New Zealand dairy farms, Moriarty et al. (2008) reported median bacterial counts of 10^6 *E. coli* and 10^5 *Campylobacter* per gram of faeces, although counts were highly variable for individual samples. Low levels of STEC, *Cryptosporidium* and *Giardia* were also detected.

Sheep

In New Zealand, an estimated 32 million sheep graze on open pasture (Moriarty et al. 2011), and have been implicated as significant contributors to the microbial loading of freshwaters (MfE and MoH, 2003; Davies et al., 2004; Devane et al., 2005; McDowell, 2006). It has been suggested that in some instances, the total *E. coli* burden per hectare of pasture is higher for land being grazed by sheep than by cattle (Wilcock, 2006). Sheep are known to harbour a range of microbial pathogens, including *Campylobacter* (Jones et al., 1999; Bailey et al., 2003; Oporto et al., 2007; Milnes et al., 2008), STEC (Kudva et al., 1998), *Giardia* (Castro-Hermida et al., 2007; Santin et al., 2007), and *Cryptosporidium* (Castro-Hermida et al., 2007; Santin et al., 2007; Milnes et al. 2008; Quilez et al., 2008). There is some evidence that many of the ovine *Cryptosporidium* and *Giardia* genotypes may not be zoonotic (Ryan et al. 2005).

Moriarty et al. (2011c) undertook a survey of microbial indicators and pathogens in the faeces of New Zealand sheep and lambs. They determined that lamb faeces contain 10-100 times the concentration of *E. coli*, enterococci and *Campylobacter* than sheep faeces. Further, the prevalence of *Campylobacter*, *Salmonella* and STEC was higher in lambs than in sheep. For example, *Campylobacter* was present in 81% and 30% of lambs and sheep, respectively, with mean concentrations of 10^5 and 10^3 per gram of faeces. Further, 29% and 4% of lamb and sheep samples were positive for *Cryptosporidium*, while mean *E. coli* loads were 10^8 per gram for lambs and 10^7 per gram for sheep.

Other ruminants

Compared with other ruminants, information as to the microbial burden of equine faeces is limited. Several studies have enumerated *E. coli* in horse faces: Weaver et al. (2005) reported a mean concentration of 3.0 x 10^5 cfu/g wet weight, while Moriarty et al. (2015) reported a concentration of 1.2 x 10^5 cfu/g dry weight. Other studies have isolated potentially zoonotic strains of *Cryptosporidium* spp. and *Giardia* spp. (Grinberg et al., 2009; Smith et al., 2010, Perrucci et al., 2011; Traversa et al., 2012, Santin et al., 2013), *Salmonella* spp. (Wittum et al., 2012; Jay-Russell et al., 2014), STEC (Pichner et al., 2005; Pritchard et al., 2009) and *Campylobacter* spp. (Hurcombe et al., 2009; Moriarty et al., 2015). The prevalence of zoonotic microorganisms in horse faeces varies significantly between pathogens, as well as between studies (eg, <1% STEC, Pichner et al., 2005; 20% *Cryptosporidium*, Smith et al., 2010).

Few studies have investigated the microbial content of deer faeces. Pattis et al. (2017) reported that in a survey of faecal samples from red deer, *E. coli* was present in all samples, with an average concentration of 10^8 cfu/g wet weight. *Campylobacter* was isolated in 13% of samples. *Yersinia* and *Cryptosporidium* have also been associated with deer populations (Ball and Till, 1998), suggesting that deer may be a significant source of faecal contamination of surface waters. Indeed, the concentrations of *E. coli* and *Campylobacter* have been reported to be between 2 and 10 times higher downstream of deer farms than upstream (Eyles et al., 2002), and deer wallows connected to waterways have been shown to adversely affect microbial water quality (McDowell and Paton, 2004; McDowell, 2009).

Routes of transmission

The contamination of surface waters with livestock faeces may result from the delivery of faecal materials through overland or subsurface flow, or where access permits, direct defecation into a waterbody (Collins, 2004; Davies-Colley et al., 2004; McDowell, 2006; Close et al., 2008; Moriarty et al., 2008; Moriarty et al., 2011c).

The direct deposition of faecal matter into waterways by livestock may be a significant source of faecal contamination under base-flow conditions and may occur where stock can freely access streams, or at herd crossings (Davies-Colley et al., 2004; Wilcock et al., 2006). In these cases, faecal material reaches the water immediately with no opportunity for microbial die-off or attenuation, so any pathogens present are likely to be in their most infectious state. Bagshaw (2002) observed that in a cattle herd with free access to streams, approximately 4% of total daily defecation occurred in the stream or riparian zone (within 2m of the riverbank), of which half was deposited directly into the stream. Sheep tend to spend little time in or around

flowing water compared to cattle, although they may still be associated with significant faecal deposition around the riparian zone, which may impact water quality via wash-in (Wilcock, 2006; Robson et al., 2015).

Overland flow is an important route of indirect transmission of microorganisms from livestock to waterways, and is one of the largest sources of diffuse pollution in New Zealand (Collins et al., 2003; McLeod et al., 2005; Kay et al.,2008; Monaghan et al., 2008; Muirhead and Monaghan, 2012). Overland flow occurs during rainfall or irrigation, where the infiltration rate of the soil is exceeded and/or soils have become saturated (Hughes and Wilson, 2016). Microorganisms associated with faecal material on the land are transferred via the flow of water over the land surface to the surrounding waterways. The risk of overland flow depends on factors including the gradient of the land, soil type and management practices such as stocking density (Wilcock, 2006). Rainfall-driven overland flow from dairy farms has been identified as the largest pathway of faecal microbial losses from agricultural catchments (Kay et al., 2008; Muirhead and Monaghan, 2012). In Otago, *E. coli* losses from pasture associated with sheep grazing were estimated at 10^9 *E. coli* per hectare per year (McDowell and Wilcock, 2008). A UK study reported farmyard runoff to contain 10^4-10^7 faecal coliforms per 100 ml (Edwards et al., 2008). Hedley et al. (2004) reported surface runoff from dairy pasture contained >10^5 MPN *E. coli* and 10^3 MPN *Campylobacter* per 100ml.

Faecal contaminants may also be transferred to waterways via bypass or preferential flow routes. These routes may be natural, such as areas of cracking, subsurface erosion or root channels, or artificial, such as mole and tile drainage systems (Hughes and Wilson, 2016). Preferential flow channels allow for contaminants to bypass the soil matrix, reducing or almost completely removing the opportunity for attenuation of contaminants within the soil.

Finally, animal wastes may be discharged directly to surfaces waters during the discharge of agricultural effluents, such as those from fairy sheds. The discharge of effluents to surface waters requires a resource consent. Alternatively, such wastes may be discharged to land (where it may in turn be subject to overland or subsurface flow).

1.2.2 Avian faeces

Wildfowl species may contribute to the microbial loading of surface water with concomitant impacts on recreational water quality. In New Zealand, birds including mallard ducks (*Anas platyrhynchos*), Canada geese (*Branta canadensis*), black swans (*Cygnus atratus*) and several species of gull are abundant (Heather and Robertson, 2005; Moriarty et al., 2011a). The birds live on and around coastlines, estuaries, rivers, streams, wetlands and lakes, and are also found in the vicinity of waste stabilisation ponds. They may defecate directly into the water or along banks and verges, and can represent an important local source of faecal pollution. Direct deposition by birds is considered to be an important source of faecal contamination under base flow conditions (Wilcock, 2006).

A range of potentially zoonotic pathogens have been isolated from the faeces of wildfowl. For example, *Campylobacter*, *Cryptosporidium*, *Bacillus cereus* and *Clostridium perfringens* have been recovered from New Zealand ducks (Murphy et al., 2003; Moriarty et al., 2011a). *Salmonella*, *Vibrio*, *Listeria* and *Campylobacter* have been recovered from various gull species (Hatch, 1996; Moore et al., 2002; Moriarty et al., 2011a), and *Campylobacter* and *Cryptosporidium* from black swans (Rohela et al., 2005; Moriarty et al., 2011a). *Salmonella*,

Giardia, *Cryptosporidium* and *Campylobacter* have been isolated from Canada geese (Whalstrom et al., 2003; Jellison et al., 2004; Kassa et al., 2004; Zhou et al., 2004; Moriarty et al., 2011a); Moriarty et al. (2011a) reported that 40% of Canada geese faecal samples collected were positive for *Campylobacter*, at concentrations up to 10^5 MPN/g dry weight.

1.2.3 Human sources

Human sewage contains high concentrations of indicator organisms, including *E. coli* (approximately 10^6-10^8 per 100 ml). A range of pathogenic microorganisms, including *Campylobacter*, *Salmonella*, *Shigella*, norovirus, rotavirus, adenovirus, *Cryptosporidium* and *Giardia* may also be present if these are present in the source population (Yang et al., 2014; Marin et al., 2015; Kitajima et al., 2014; Haramoto et al., 2015).

Most human waste in New Zealand is treated by municipal sewage treatment systems before being discharged to the environment, typically a waterway or the coastal marine environment. Waste may also be treated in on-site septic systems. Untreated or partially-treated human waste may enter the environment through inadequate treatment, or via urban runoff or combined sewer overflows (CSO), where both sewage and stormwater flow in the same pipe to the treatment plant; after heavy rainfall, their combined volume may exceed the capacity of the plant and be discharged directly to the environment. Waste may also enter waterways from failing septic tanks (e.g. through leaking systems or ineffective treatment) or leaking sewerage pipes, and subsequent subsurface flow through the soil. A report prepared for the Ministry for the Environment (MfE, 2008) estimated that between 15 and 50% of septic tanks, particularly aging systems, are susceptible to failure.

Estimating the prevalence and abundance of pathogens in human sewage is complex, and dependent on whether the sewage is raw or treated, and the type of treatment that has been undertaken (Soller et al., 2010). The level of contamination that may reach a waterway via the subsurface (e.g. from a failing septic tank system or broken sewerage pipe) depends on the distance contaminants must travel, as well as soil type and saturation.

1.3 FAECAL SOURCE TRACKING

Whilst the detection of FIB provides an indication that water is contaminated with faecal material, and thus there is a risk of pathogens being present, it does not identify the source(s) of contamination. Discriminating between human and non-human sources of faecal contamination, and/or the subsequent identification of the animal species are essential components of effective water quality management (Gourmelon et al., 2010; Cornelisen et al., 2011; Pantos, 2017). Faecal source attribution allows for risk assessment and targeted mitigations. For example, human contamination is considered to pose a greater risk than wildfowl contamination. The ‘toolbox’ of analyses involved in determining the origin of faecal contamination is known as Faecal Source Tracking (FST), and includes microbial and chemical methods (Scott et al., 2002; Field and Samadpour, 2007; Harwood et al., 2014).

Microbial methods look to identify the presence of microorganisms that are specific to the gut of a certain host animal. There is a wide range of microorganisms other than the traditional

faecal indicators (i.e. coliforms, *E. coli* and enterococci), that are present in animal faeces, and some of these are specific to certain animals. Although these organisms are often difficult to culture in the laboratory, it is possible to extract the total DNA from a water sample and use polymerase chain reaction (PCR) to identify gene fragments ('markers') that are unique to these host-associated microorganisms. However, while many markers are strongly associated with an animal source, they each have a degree of non-specificity (Devane et al., 2013; Harwood et al., 2014). Chemical FST methods include analysis of faecal sterol and stanol fingerprints, which differ between human and animal sources, and compounds associated with anthropogenic pollution, such as caffeine, synthetic drugs (e.g. contraceptives) and fluorescent whitening agents (Scott et al., 2002; Hewitt and Williamson, 2014).

1.4 *CAMPYLOBACTER*

Campylobacter is the most commonly reported bacterial cause of human gastroenteritis in New Zealand, with over 6,000 notified cases each year (a rate of >135 cases per 100,000 persons; peaking at 15,873 cases in 2006) – one of the highest reported incidences in the developed world (Savill et al. 2001; Till and McBride, 2004; Devane et al., 2005; ESR, 2007, 2017). The contamination of drinking and recreational waters with *Campylobacter* has been associated in a number of outbreaks, including Havelock North (DIA, 2017). *Campylobacter* spp. are found in a range of animal reservoirs including cows, sheep, deer, poultry and wildfowl, and are readily recoverable from environmental water samples in New Zealand. For example, in a national microbiological survey of freshwater, McBride et al. (2002) reported the presence of *Campylobacter* in 60% of samples collected. Savill et al. (2001) also reported the detection of *Campylobacter* in 60% of samples collected from five New Zealand rivers. *Campylobacter* is therefore a priority waterborne pathogen in New Zealand.

Beyond the initial detection and enumeration of *Campylobacter*, speciation is important, since different species and strains may differ in their pathogenicity. *Campylobacter jejuni* and *Campylobacter coli* are frequently implicated in human disease, while other thermotolerant species such as *Campylobacter lari* and *Campylobacter upsaliensis* are not commonly reported among notified cases. Methods such as multiplex ligation-dependent probe amplification-binary typing (MBiT) can be used to differentiate a large number of genotypes and produce phylogenetic comparisons of isolates, which can be used to attribute a host/source.

1.5 REPORT OBJECTIVES

Regional and local government have an obligation under the Resource Management Act (RMA) 1991 and the National Policy Statement for Freshwater Management (NPS-FM) 2017 to monitor and report the quality of freshwater in their region. State of the Environment (SoE) monitoring for rivers and lakes is undertaken monthly by Environment Southland (ES), and includes determination of physical, chemical and microbiological parameters. Recreational

water quality is monitored by assessing *E. coli* concentrations at freshwater swimming spots on a weekly basis over the summer bathing season (December to March), and assessing faecal coliform concentrations on a monthly basis (year-round) at popular shellfish gathering sites. This data is available to the public at websites such as Land Air Water Aotearoa (LAWA; www.lawa.org.nz) and the Environment Southland webpage (www.es.govt.nz/services/environmental-monitoring/recreational-water-quality). Recently, Hodson et al. (2017) reported on water quality state and trends in Southland between 2000 and 2016 by drawing together information collected by Environment Southland, National Institute of Water and Atmospheric Research (NIWA) and GNS Science.

Routine water quality monitoring permits the assessment of the overall state of water quality, and any trends that may be evident, However, it does not address the potential sources of contamination. The current report therefore focuses on the use of research tools – particularly faecal source tracking and MBiT source attribution of *Campylobacter* – to determine the sources of pollution that impact freshwater sites within the Waiau FMU, Southland.

2. MATERIALS AND METHODS

2.1 SAMPLING SITES

The sampling locations selected across the Waiau Freshwater Management Unit (FMU) are listed in Table 1, and shown together with their sub-catchments in Figure 2. Detailed sub-catchment information is presented for each sampling site alongside the microbiological results.

The results described in this report relate to samples collected either as a part of a monthly sampling regime by ES staff, or during targeted sampling events by both ESR and ES staff. Sampling conditions were recorded as being base-flow or post-rainfall, based on an antecedence rainfall threshold of 10 mm over 48 hours.

Table 1. Sampling sites selected for the Waiau FMU, with the conditions (i.e. base-flow or post-rainfall) each site was sampled under.

Site	Sampling conditions	Detailed sub-catchment and microbial water quality descriptions
Mararoa River at the Key	Rainfall only	Appendix B.1
Mararoa River at Weir Road	Rainfall only	Appendix B.2
Waiau River at Sunnyside	Rainfall only	Appendix B.3
Orauea River at Orawia Pukemaori Road	Base-flow and rainfall	Appendix B.4
Lill Burn at Lill Burn Monowai Road	Base-flow and rainfall	Appendix B.5

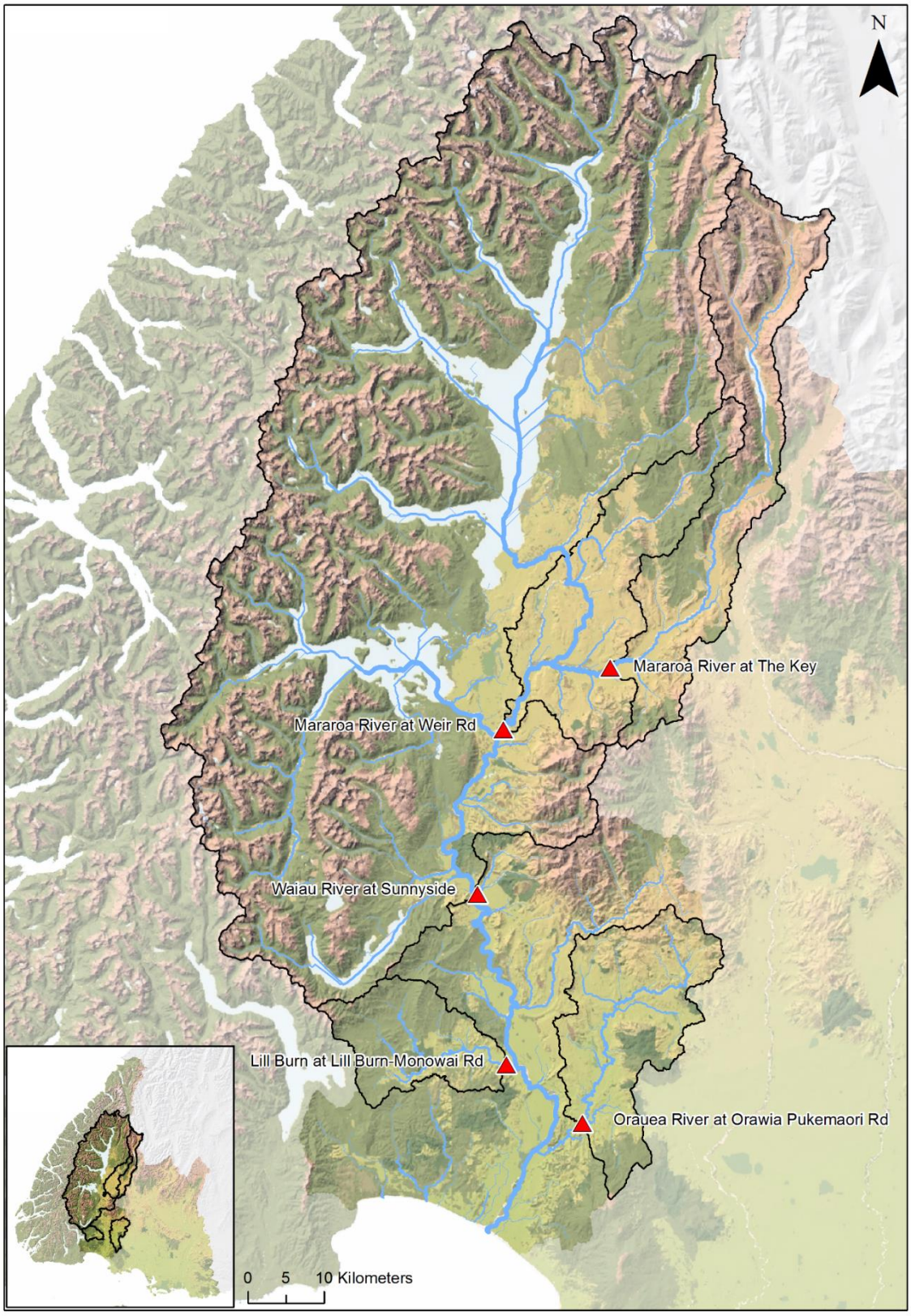

Figure 2. The Waiau FMU, with sub-catchments, sampling site locations and rivers of order 4 to 8 shown. Inset: The Waiau FMU within the wider Southland region.

2.2 MICROBIOLOGICAL ANALYSIS

Faecal coliforms and *E. coli* were measured as indicators of possible faecal contamination. *Campylobacter* spp. was measured as a pathogen of faecal origin. In addition to identifying the presence of contaminants, two methods were used to identify the possible source(s) of faecal pollution:

- Analysis of *Campylobacter* isolates by MBiT source attribution sub-typing.
- Faecal source tracking analysis for molecular (i.e. DNA) markers associated with human, ruminant, wildfowl and/or canine pollution.

A brief summary of the methodologies used for microbiological analysis is described below. Detailed information regarding these methods and the interpretation of results can be found in Appendix A.

2.2.1 Coliform and *E. coli* analysis

Faecal coliforms were analysed using membrane filtration with incubation on mFC agar for 22 hours at 44.5oC (Method 9222D, APHA et al. 2012). E. coli was analysed by incubating faecal coliform-positive filters with media containing 4-methylumbelliferyl-ß-glucuronidase (MUG) (Method 9222G, APHA et al. 2012). Results are presented as colony-forming units (cfu).

2.2.2 *Campylobacter* isolation

Campylobacter spp. were enumerated using a 3 x 5 Most Probable Number (MPN) procedure utilising Exeter broth and agar (Moriarty et al. 2008). Suspected *Campylobacter* spp. colonies were subject to confirmation based on biochemical tests (oxidase, catalase), colony morphology, Gram stains and multiplex polymerase chain reaction (PCR) (Wong et al., 2004).

2.2.3 *Campylobacter* sub-typing and source attribution

Campylobacter spp. isolates were sub-typed using multiplex ligation-dependent probe amplification-binary typing (MBiT) (Cornelius et al., 2014). This is the first time that this method has been used to characterise isolates recovered from water samples. Cluster analysis was used to assign a likely source of the isolates (e.g. poultry, wildfowl, ruminant, unknown).

2.2.4 Faecal source tracking

Water samples were filtered and DNA extracted, before real-time PCR was performed as described by Devane et al. (2007, 2013). Eight PCR markers were assayed: general (GenBac3), human (BiADO, BacH), ruminant (BacR), cow (M2), sheep (Schill), and avian (GFD, E2). Selected samples were also assayed for canine markers (DogBac).

2.3 SANITARY SURVEYS

For each site, a desktop sanitary survey was carried out to identify activities that had the potential to contribute microbial contaminants to the environment. Each survey considered:

- land use breakdown in the capture zone, including stock numbers
- consented effluent application areas
- tile drainage
- consented point source discharge (municipal or industrial wastewater)
- dwellings (i.e. septic tanks)
- other relevant activities.

This data is presented in Appendix B.

3. OVERVIEW OF MICROBIAL WATER QUALITY

A high degree of spatial or temporal variation in microbiological water quality was observed across the different sampling locations in the Waiau FMU. An overview of these findings is presented below. Detailed microbiological results for each site are presented in Appendix B.

3.1 OVERVIEW OF MICROBIAL WATER QUALITY IN THE WAIAU FMU

E. coli concentrations across the Waiau FMU were generally lower than those recorded for other FMUs in the Southland region (although sampling was more limited, with three sites sampled on only a single occasion; see reports for Mataura, Aparima and Oreti FMUs produced in parallel with this report). Thirty-three percent of individual samples from the Waiau FMU contained *E. coli* concentrations of 1,000 cfu/100 ml or more, and were collected from two of the five sampling sites (Figures 3-5). The highest *E. coli* concentrations recorded were 18,000 cfu/100 ml at the Orauea River and 7,100 cfu/100 ml at Lill Burn. The lowest concentration of *E. coli* was 20 cfu/100 ml, recorded at the Waiau River at Sunnyside.

Campylobacter was isolated from 80% of all samples, and from 4 of the 5 sites; the exception being the Mararoa River at Weir Road (Figure 3, Figure 4). *Campylobacter* was more prevalent in samples collected following rainfall than under base flow (91% of post-rain samples, 50% of base flow samples). The highest concentration of *Campylobacter* was 43 MPN/100 ml, which was detected in the sample from the Orauea River that also yielded the highest *E. coli* concentration. Most of the *Campylobacter* isolates were determined to be *C. jejnui*, which was isolated from all of the *Campylobacter*-positive samples. One sample from the Orauea River was also positive for an unspeciated thermophilic *Campylobacter*.

There was no discernible trend in *E. coli* or *Campylobacter* concentrations in relation to season or whether samples were collected under base flow conditions or following rainfall (Figures 5-8). However, it is noted that the two highest *E. coli* concentrations were recorded from samples collected in December.

An examination of the relationship between *E. coli* and *Campylobacter* revealed a significant, positive correlation of data (Spearman rank correlation, r = 0.7288, df = 13, p = 0.0021); thus, samples with high levels of *E. coli* were more likely to contain high levels of *Campylobacter* (Figure 9).

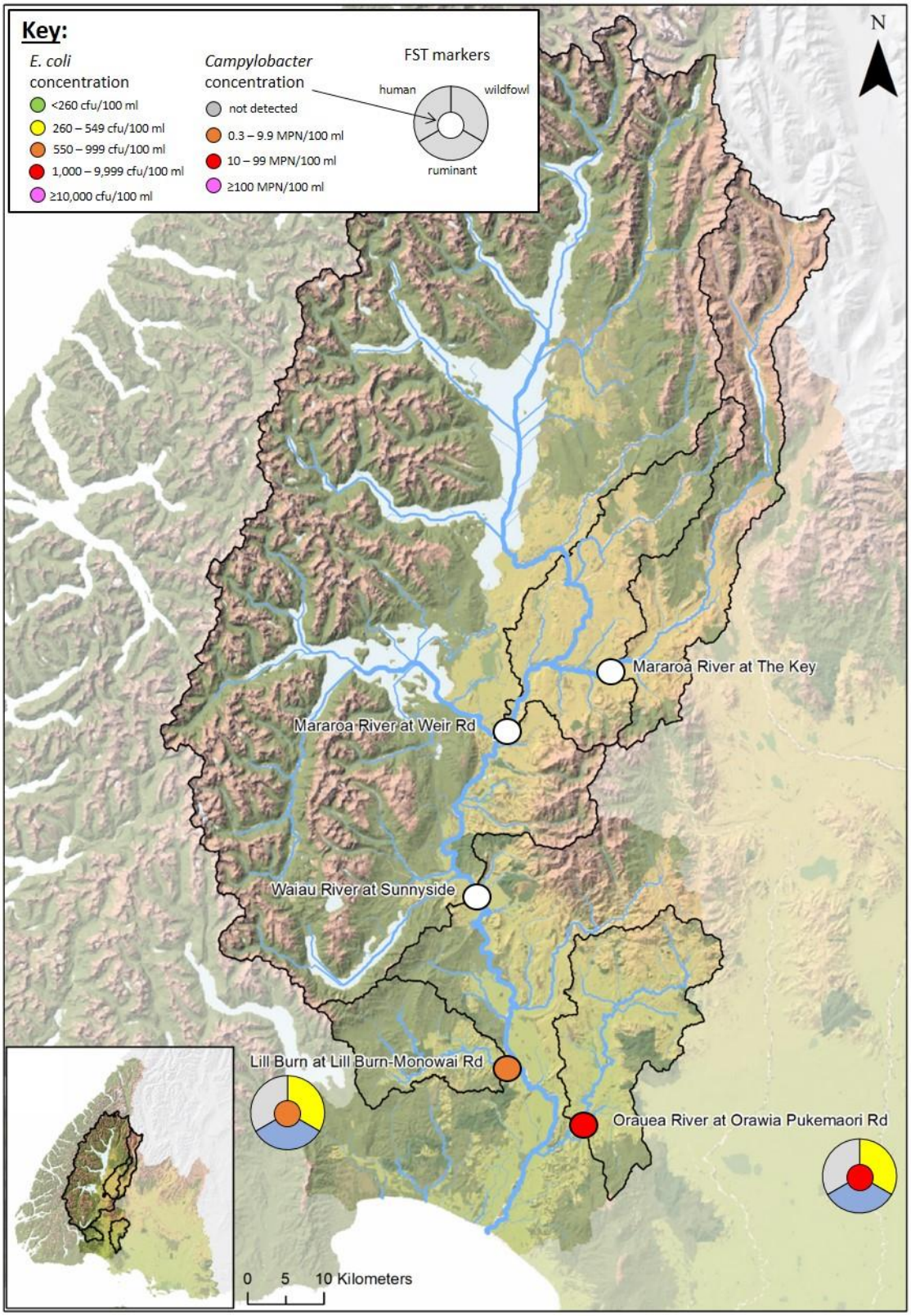

Figure 3. Overview of microbial water quality in Waiau FMU under base flow conditions. Small circles showing sampling locations on the map represent maximum *E. coli* levels for that site; white circles indicate there is no data under these conditions. Larger circles adjacent to site name represent maximum *Campylobacter* concentration and overall presence/absence of FST markers for that site.

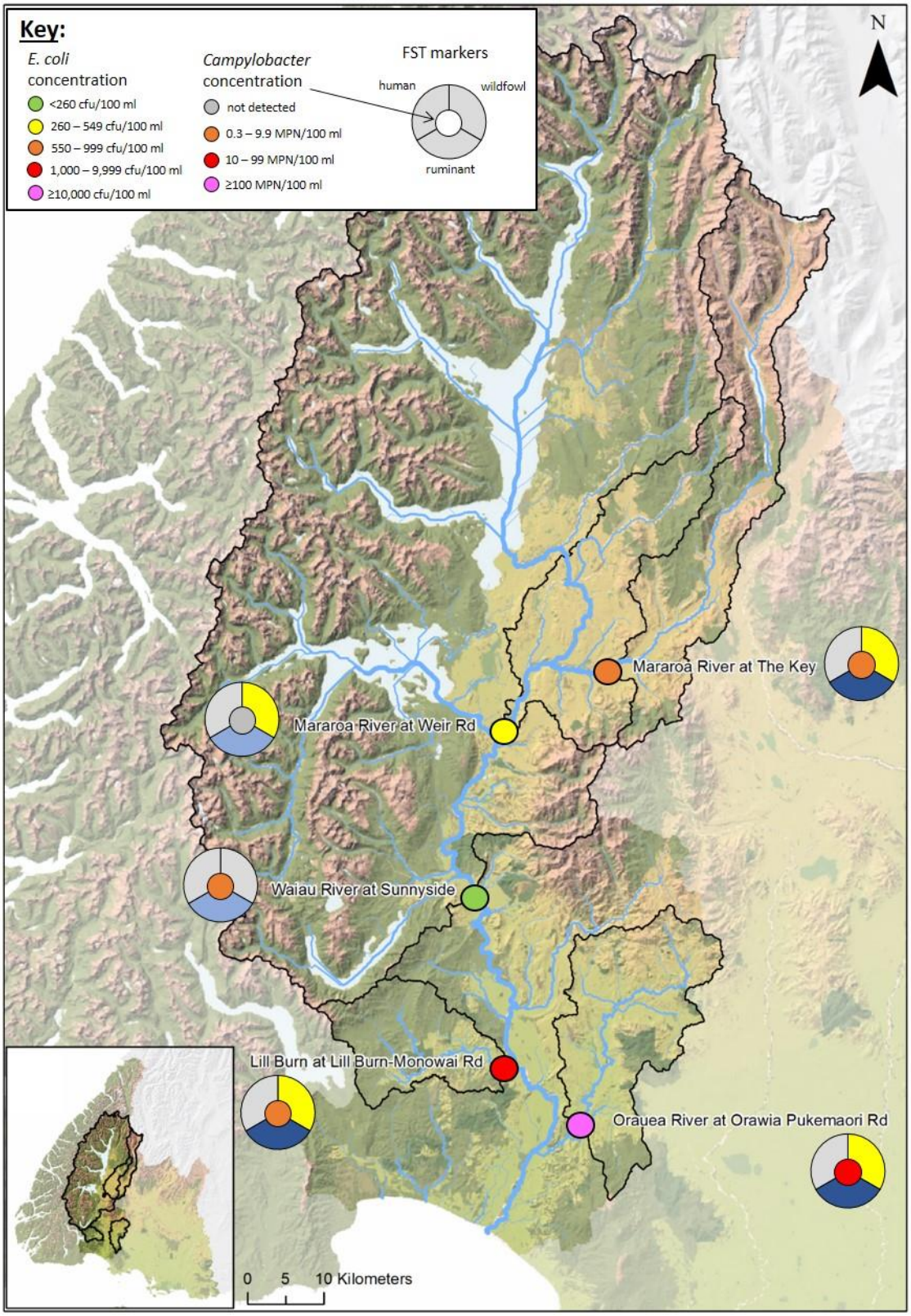

Figure 4. Overview of microbial water quality in Waiau FMU following rainfall. Small circles showing sampling locations on the map represent maximum *E. coli* levels for that site; white circles indicate there is no data under these conditions. Larger circles adjacent to site name represent maximum *Campylobacter* concentration and overall presence/absence of FST markers for that site.

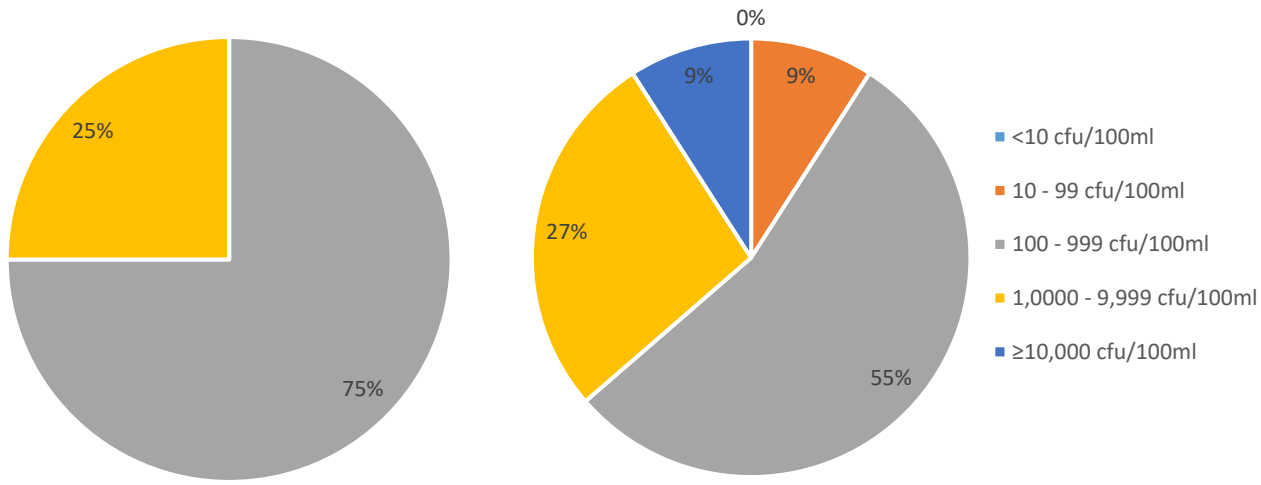

Figure 5. *E. coli* concentrations for water samples collected within the Waiau FMU under base flow conditions (left, n=4) and following rainfall (right, n=11).

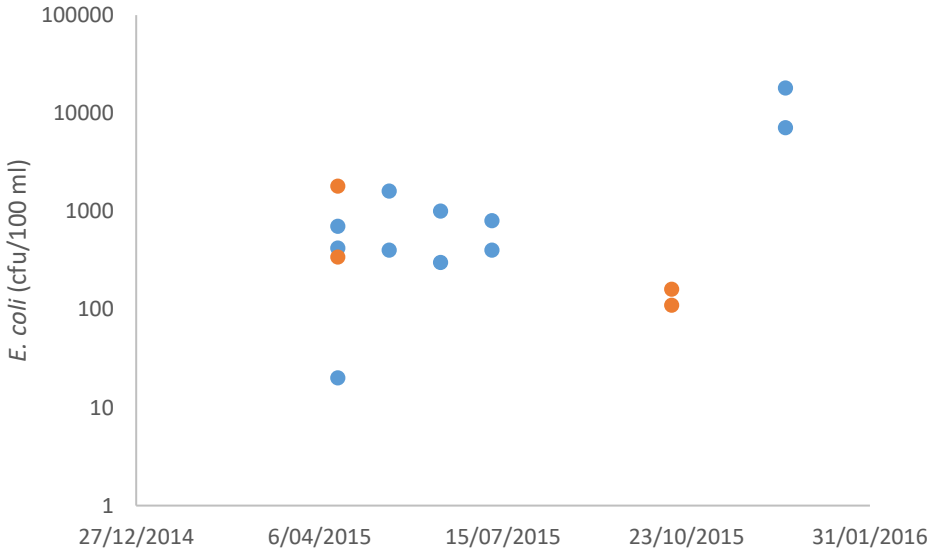

Figure 6. Concentration of *E. coli* at different sites in the Waiau FMU, across the course of the year. Samples collected following rainfall are shown in blue, and those collected under base flow conditions are in orange.

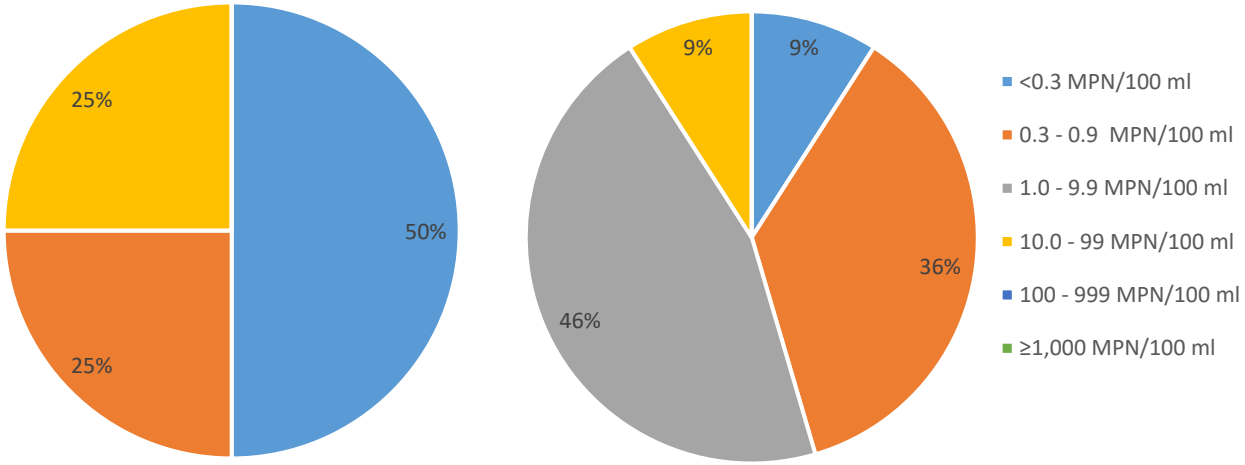

Figure 7. *Campylobacter* concentrations for water samples collected within the Waiau FMU under base flow conditions (left, n=4) and following rainfall (right, n=11)

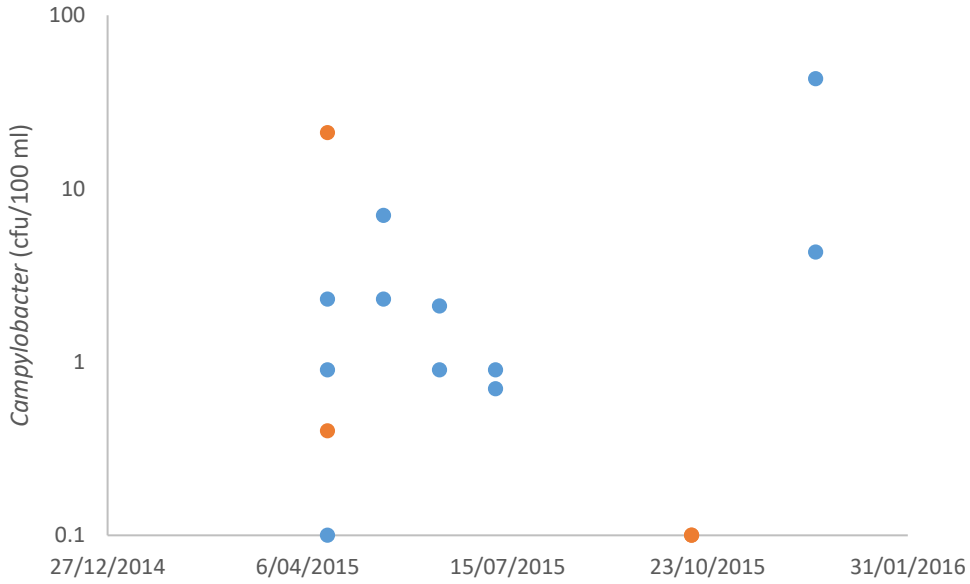

Figure 8. Concentration of *Campylobacter* at different sites in the Waiau FMU, across the course of the year. Samples collected following rainfall are shown in blue, and those collected under base flow conditions are in orange.

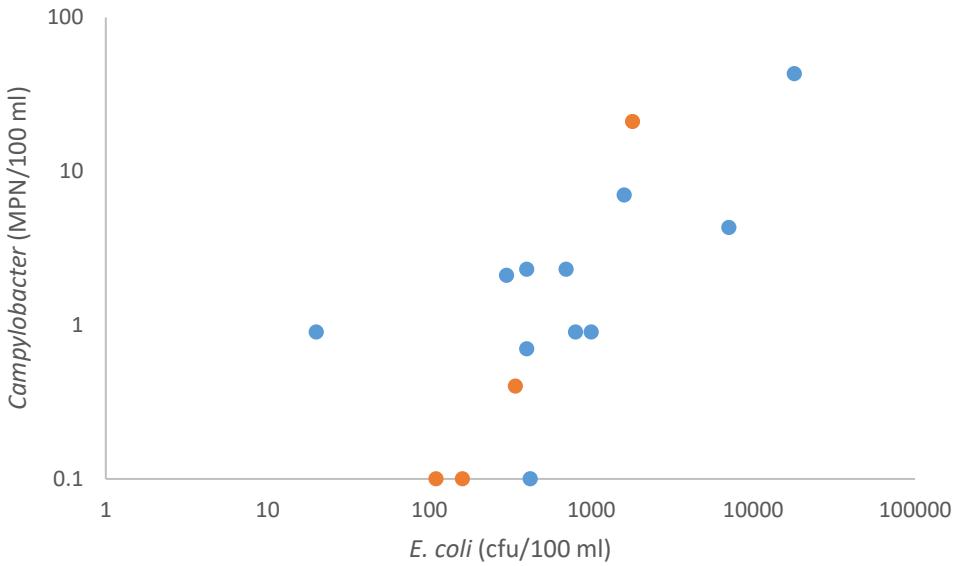

Figure 9. Relationship between *E. coli* and *Campylobacter* spp. concentrations in water samples collected within the Waiau FMU. Note that for the purposes of displaying the data on a logarithmic scale, samples in which no *Campylobacter* was detected, have been plotted as 0.1 MPN/100 ml. Samples collected following rainfall are shown in blue, and those collected under base flow conditions in orange.

3.2 POSSIBLE SOURCES OF FAECAL POLLUTION

Faecal source tracking analysis found that ruminant faecal pollution was detected at all sites within the Waiau FMU (Figure 3, Figure 4). Ruminant animals were the dominant pollution source (i.e. accounted for 50-100% of pollution in at least half of samples) at three sites, and in 53% of all samples collected. However, the relative impact of ruminant sources was found to differ between samples collected under base flow compared with those collected following rainfall (Figure 3, Figure 4). Ruminant pollution accounted for 10-50% of total faecal pollution in all of the samples collected under base flow conditions. In contrast, ruminant pollution was the dominant pollution source in 73% of samples collected following rainfall (Figure 10). More specifically, 73% of samples collected following rainfall were positive for ovine contamination, and 64% for bovine contamination. In comparison, 25% and 0% of samples collected under base flow conditions were positive for ovine and bovine contamination, respectively (Figure 11).

Wildfowl faecal contamination was detected at four of the five sites (the exception being the Waiau River at Sunnyside; Figure 3, Figure 4). The prevalence of wildfowl contamination appeared to be relatively independent of antecedence rainfall, with wildfowl-specific FST markers detected in 50% of samples collected under base flow, and 64% of samples collected following rainfall (Figure 11).

There was no human faecal pollution detected in the Waiau FMU.

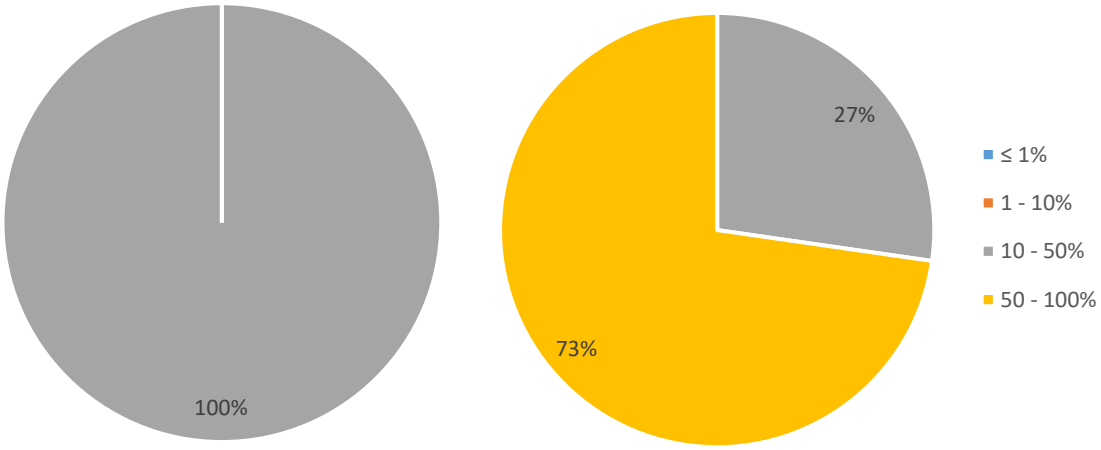

Figure 10. The percentage of samples collected under base flow (left) and following rainfall (right) that were affected by different levels of ruminant faecal pollution.

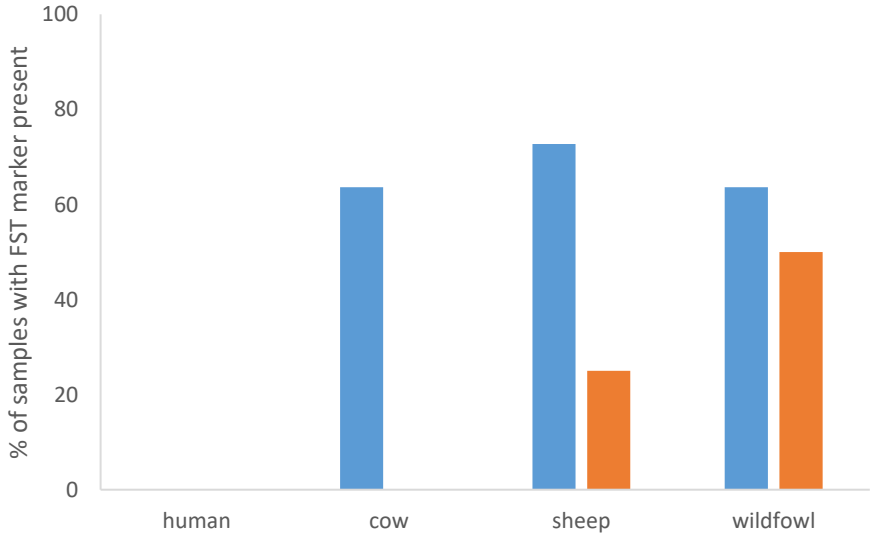

Figure 11. The percentage of samples collected from within the Waiau FMU that were positive for FST markers from different sources. Samples that were collected following rainfall are shown in blue, and those collected under base flow in orange.

3.3 CHARACTERISATION OF CAMPYLOBACTER

3.3.1 MBiT source attribution

MBiT source attribution analysis identified wildfowl, ruminant, poultry and 'not wildfowl' sources of *Campylobacter* at various sites across the Waiau FMU (Figure 12). Several samples were found to have *Campylobacter* from more than once source. Wildfowl were the most common source of *Campylobacter* (42% of all isolates), with 75% of *Campylobacter*-positive samples having a wildfowl source identified, followed by 'not wildfowl' (42%), ovine/bovine/deer (25%) and poultry (8%). Isolates identified as being from a 'not wildfowl' source are likely to be of ruminant, poultry or human origin, but could not be further resolved.

Although the prevalence of *Campylobacter* was higher in samples collected following rainfall than under base flow (as described above), the relative importance of 'not wildfowl' as a *Campylobacter* source did not appear to be greatly influenced by antecedence rainfall. In contrast, *Campylobacter* of ruminant or poultry origin was detected only following rainfall, and wildfowl was a more important source of *Campylobacter* under base flow conditions than following rainfall (Figure 12).

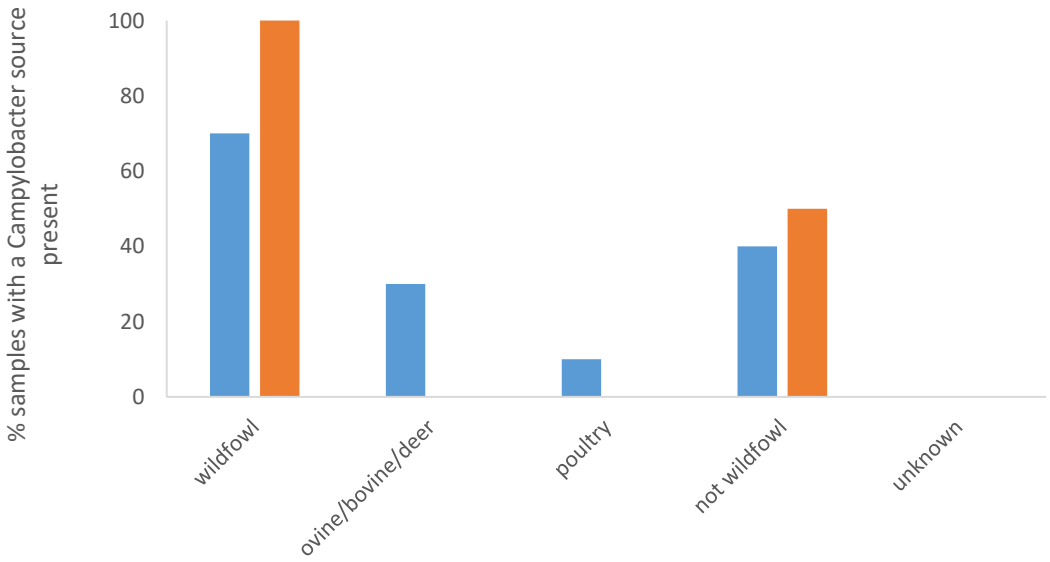

Figure 12. The percentage of *Campylobacter*-positive samples collected from within the Waiau FMU that were identified as having different *Campylobacter* sources present (as determined by MBiT analysis). Samples that were collected following rainfall are shown in blue, and those collected under base flow in orange.

3.3.2 Genotype analysis and comparison with clinical isolates

Comparison of the MBiT genotype data for *Campylobacter* isolates from sites across the Waiau FMU revealed a high diversity of genotypes: of 33 isolates analysed, 25 different genotypes were identified. No clear pattern or separation of genotypes was observed based on the site from which isolates were collected (Figure 13). Comparison of the genotypes of isolates from the Waiau FMU with the isolates from the Mataura, Oreti and Aparima FMUs also shows no clear separation of genotype based on the FMU from which isolates were obtained (Figure 14).

Of the 33 individual isolates recovered from water samples in the Waiau FMU, 14 isolates (42%) representing 9 genotypes were found to 'overlap' with (i.e. were indistinguishable from) human clinical isolates from the Southland region (Figure 15). The presence of these genotypes in clinical isolates is highly suggestive of their ability to cause disease in humans, thus their presence in the environment represents a source of waterborne infection. Of these 14 isolates, only two (14%) are likely to have come from wildfowl, compared with 42% of the isolates from water samples being wildfowl-associated (Figure 16). This suggests that *Campylobacter* from a wildfowl origin may present a lesser risk to human health than *Campylobacter* from other sources, e.g. humans or ruminants. This is also suggested by general analysis of the clinical isolates, which shows only four isolates (2%) were indistinguishable from wildfowl-associated isolates, suggesting wildfowl are a minor source of illness in the community.

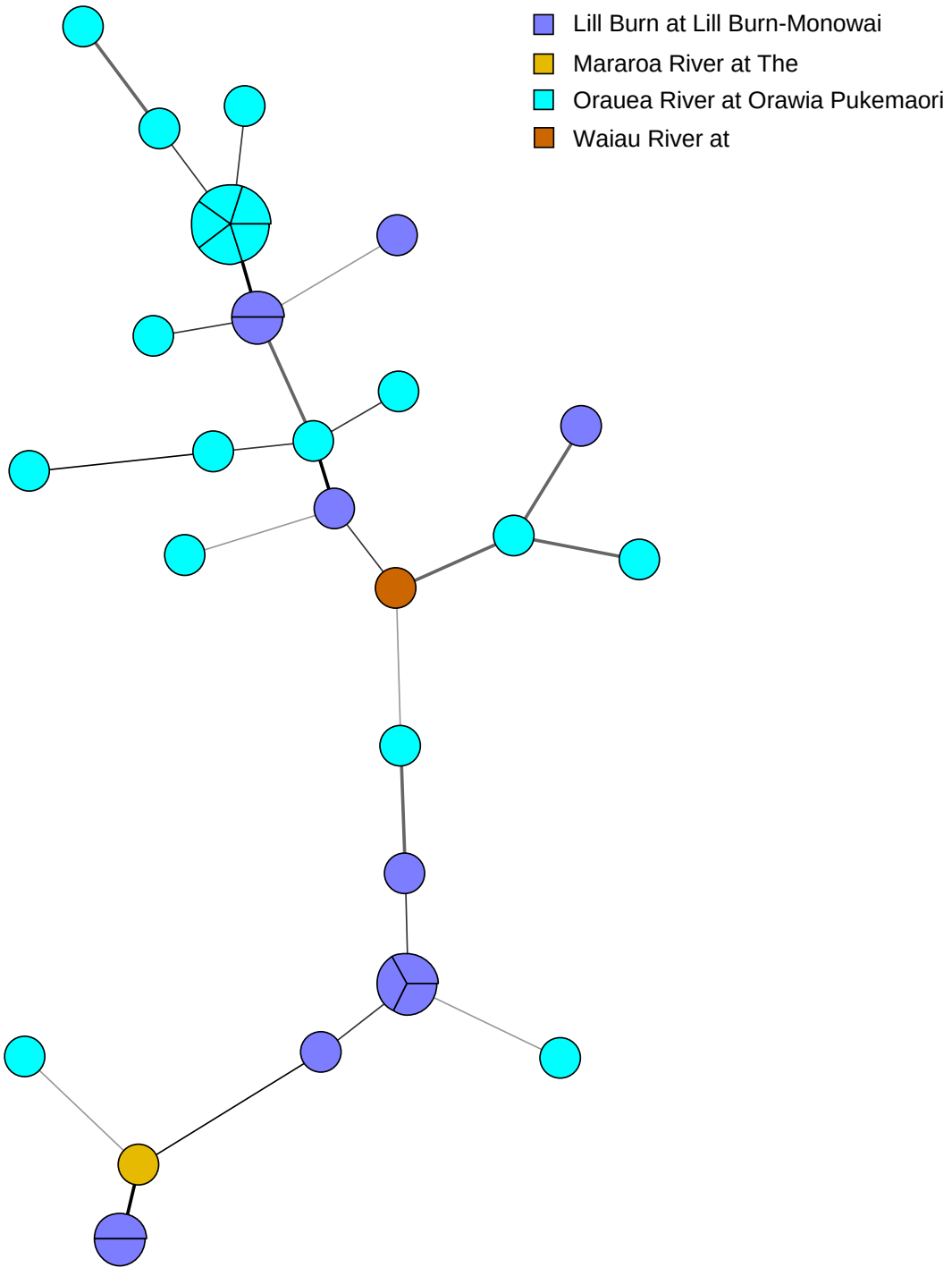

Figure 13. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from sites across the Waiau FMU, based on MBiT analysis. Each circle represents a different genotype, and each colour identifies a site. The number of circles and the spread of colours across the diagram demonstrates the diversity of genotypes within the Waiau FMU, and at individual sites.

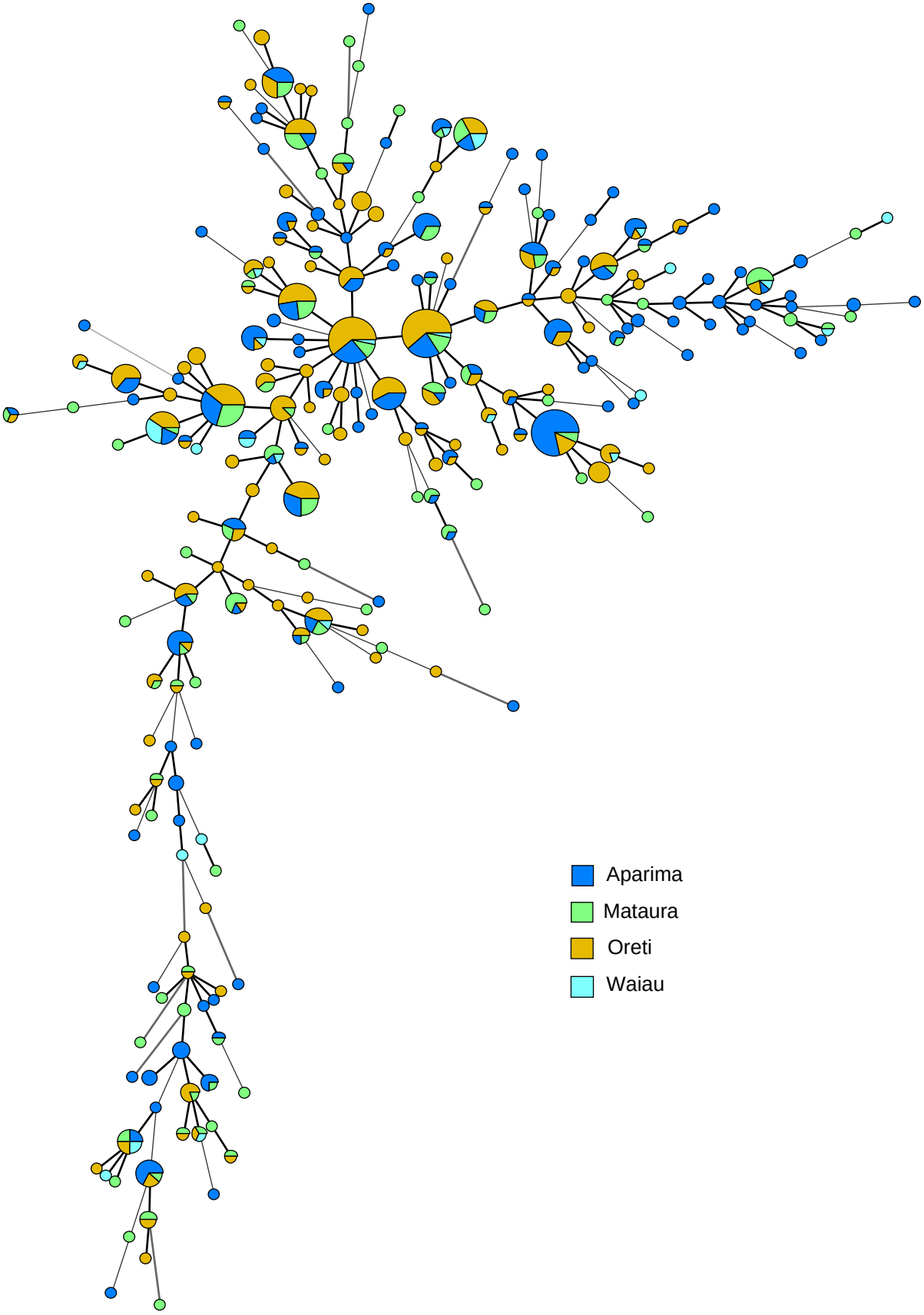

Figure 14. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from across the Southland region, based on MBiT analysis. A total of 713 isolates from the four FMUs were analysed

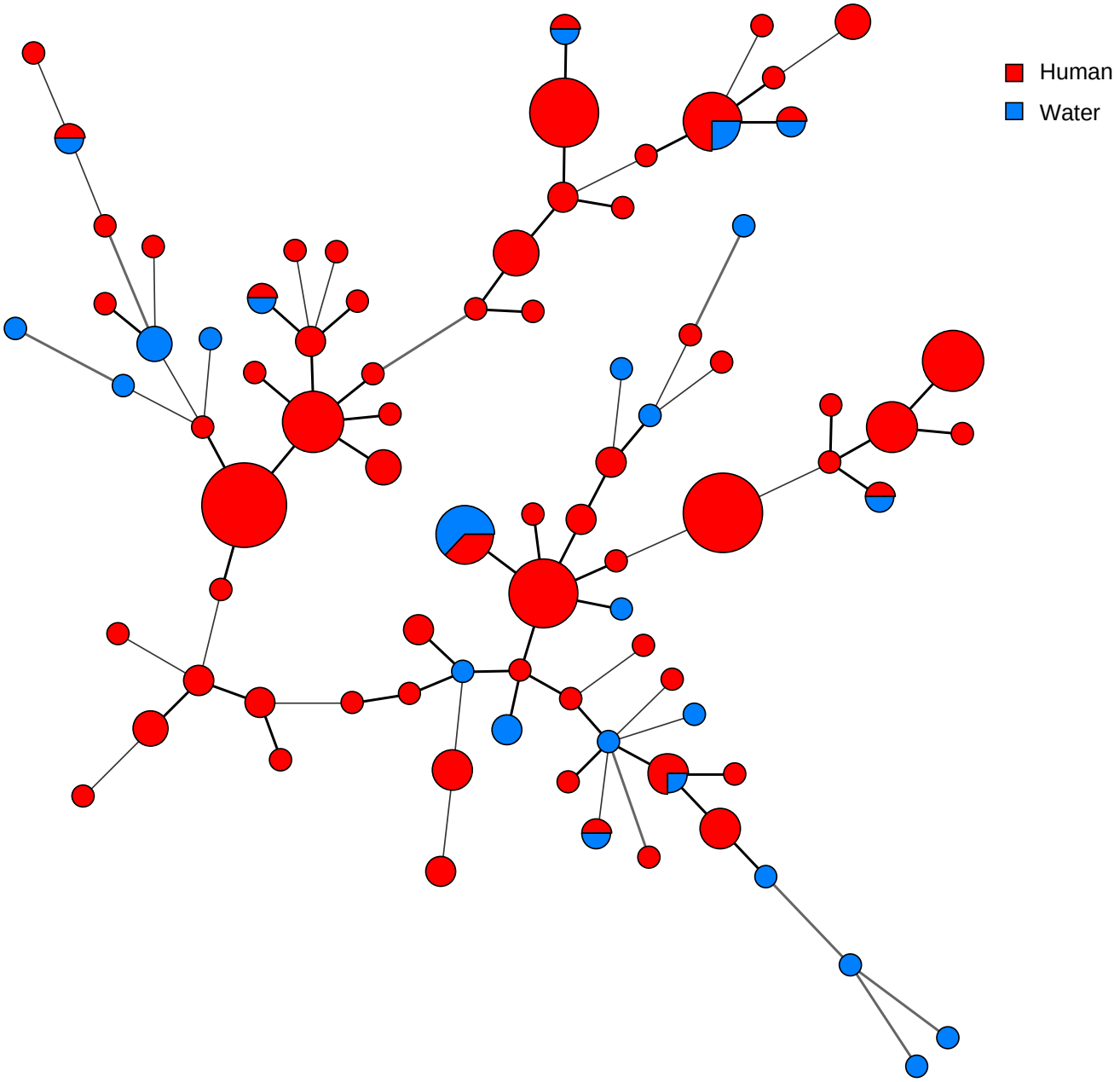

Figure 15. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from water samples from the Waiau FMU (blue) compared with human clinical isolates from the Southland region (red). Circles in which there are both blue and red segments indicate a genotype has been isolated from both the environment and clinical samples, representing the potential for human infection from waterborne sources.

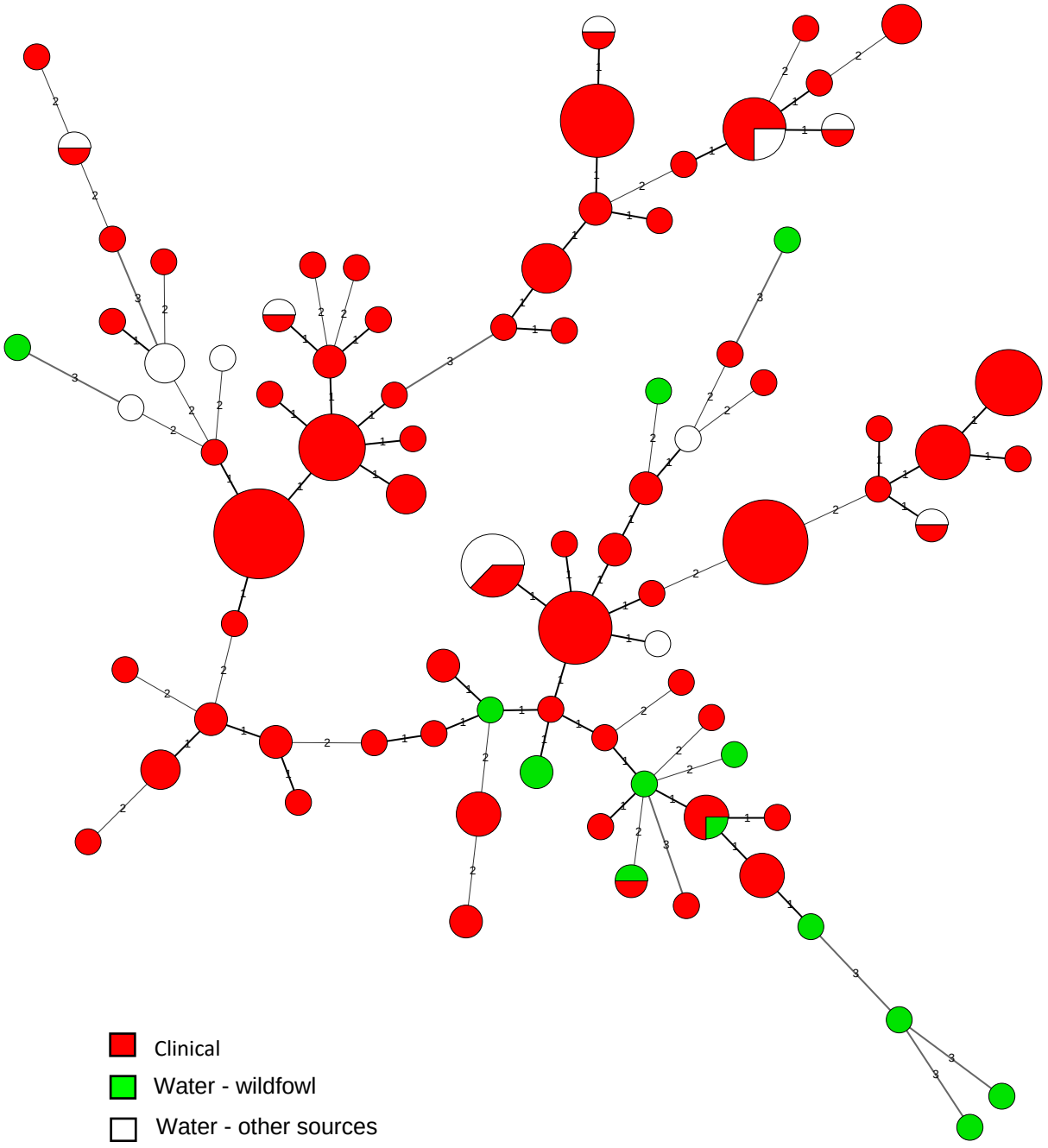

Figure 16. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from the Waiau FMU, highlighting the overlap between clinical isolates with environmental (i.e. water) isolates that are wildfowl associated (green), and those that are associated with a different source (white).

4. DISCUSSION

4.1 MICROBIAL SOURCES AND TRANSMISSION

The map-based display of microbial data (i.e. Figure 3, Figure 4) shows the peak *E. coli* and *Campylobacter* concentrations recorded for each site, with the overall presence or absence of FST markers also shown. In essence, these figures demonstrate something of a 'worst-case scenario' for each site, based on the data available. Given the limited amount of data collected for each site – for some sites a single sample was collected – this was considered to be the most informative way to represent the public health risk that could be associated with contact with these waterways, and the possible sources of that risk. The data showed that the microbial quality of waterways within the Waiau FMU was variable, with two sites in particular (Orauea River and Lill Burn) being vulnerable to high levels of faecal contamination. However, the pattern of contamination at these two sites differed: at the Orauea River, five of six samples exceeded 550 cfu/100 ml *E. coli* (i.e. the concentration above which the Microbiological Water Quality Guidelines for Freshwater Recreation Areas (MfE and MoH, 2003) recommend avoiding primary recreation such as swimming). At Lill Burn, five of six samples were within this guideline (although exceeded the 'alert value' of 260 cfu/100 ml which requires further sampling and investigation be undertaken), with a single sample exceeding 7,000 cfu/100 ml highlighting vulnerability to substantial contamination. Nonetheless, overall levels of microbial contamination were lower than for other sites in the Southland region (refer to the reports for the Mataura, Aparima and Oreti FMUs produced in parallel with this report). This could result in part from fewer samples being collected, but also likely reflects land use in the Waiau FMU, with the sub-catchments for 4 of the 5 sites dominated by non-agricultural use – particularly forestry and conservation land. Other variables, including land topography, soil physiographics, stock type and density, and irrigation and effluent management are known to influence the microbial burden of waterways (Crowther et al., 2002; Collins et al., 2007; Monaghan et al., 2010).

The main sources of faecal pollution were wildfowl and ruminant animals, with both sources present under both base flow and following rainfall. The combined presence of both ovine and bovine markers is consistent with agricultural activity in the FMU being dominated by mixed sheep and beef farming. Although only two of the sites were sampled under both high flow and base flow, *E. coli* concentrations were not greatly different under different flow conditions. However, there was a shift to the dominance of ruminant pollution following rainfall, increasing from 10-50% of the faecal signature under base flow to 50-100% in all but one sample. The presence of ruminant pollution under base flow likely results from direct deposition (e.g. stock access to unfenced waterways in pasture, passage through streams during stock movement between paddocks), or discharge of effluents to rivers. No consented discharge to water is noted in council data, however unconsented activity is a possibility. Following rainfall, overland and/or subsurface flow (e.g. via tile drains) appear to become significant routes for the transmission of faecal microbes to waterways in the Waiau FMU. Physiographic data shows that soils in the Waiau FMU feature large areas of bedrock/hill country that are prone to overland flow (Appendix B; Hughes and Wilson, 2016). In addition, artificial drainage systems are widespread across the Southland region, with an estimated 76% of agricultural land thought to have some form of artificial drainage (Monaghan, 2014; Pearson, 2015). Although the density of these systems in the Waiau FMU is thought to be lower than for other

catchments, although there are some localised areas of moderate to high density drainage where subsurface flow is a significant route of transmission. The nominal increase in microbial concentrations following rainfall – particularly compared with the large increases observed in other areas of Southland – likely reflects the relatively low agricultural land use in the Waiau compared with other FMUs. The sub-catchment with the greatest proportion of agriculture – for the Orauea River – had the greatest increase in post-rain E. coli.

The prevalence of wildfowl pollution was similar under both high flow and low flow conditions, suggesting direct faecal deposition into or in the immediate vicinity of the waterways occurs irrespective of antecedence rainfall. No human pollution was detected in the Waiau FMU.

4.2 HEALTH RISK

A high prevalence of *Campylobacter* in New Zealand's waterways has previously been reported (55-60%; Savill et al., 2011; McBride et al., 2002; Devane et al., 2005), and is attributable to its high prevalence in animal groups and our rural landscape, rather than environmental persistence of the bacteria (McBride et al., 2011). Prevalence appears to vary in accordance with the faecal sources present; McBride et al. (2002) reported *Campylobacter* was more commonly detected at sites that were predominantly impacted by birds (72%) and sheep (66%) than municipal wastes (49%). In the current study, *Campylobacter* was identified as coming from a mix of sources, predominantly wildfowl and ruminants. Thus, while it might seem surprising that *Campylobacter* is so prevalent (80% of samples) given the high percentage of conservation and forest land in the FMU, this highlights the risk that wildfowl-associated *Campylobacter* may be present even in the absence of significant agricultural activity. It is also worth noting that within sub-catchments, sampling locations are in close proximity to agricultural activity that was present.

Exposure to *Campylobacter* will result in some people becoming infected, and some of those people becoming ill. Most of the people that develop illness (i.e. campylobacteriosis) will experience mild gastrointestinal illness. However, in a minority of cases, there is a small possibility of severe health effects, such as Guillain-Barre syndrome or reactive arthritis. Exposure is a function of the concentration of *Campylobacter* in the water, and the volume ingested (i.e. the dose). If it is assumed that all of the *Campylobacter* isolated from these waterways are capable of causing disease, then dose response curves could be used to estimate the health risk to water users. Figure 18 illustrates a dose response curve for *C. jejnui,* which accounts for ~90% of all human cases of campylobacteriosis (Lee and Newell, 2006). It shows that the ingestion of 800 *C. jejuni* is associated with a 50% probability of infection (ID_{50}) (Medema et al., 1996; McBride et al., 2002). Ingestion rates for primary recreation have been estimated at between 10 and 100 ml per hour, with average exposure between 0.25 and 2 hours (McBride, 2012); estimates of water volumes ingested therefore range between 2.5 ml and 200 ml. Considering that most of the *Campylobacter* concentrations determined in the Waiau FMU were less than 10 MPN/100 ml, very large volumes of water (e.g. >8 litres) would need to be ingested to attain ID_{50}. However, the Guidelines define a risk of infection of 5% as being the upper limit for tolerable or acceptable risk; clearly a much smaller volume will be required to meet this risk. Further, the dose response for *Campylobacter* was derived from a feeding study involving adult volunteers (Black et al.,

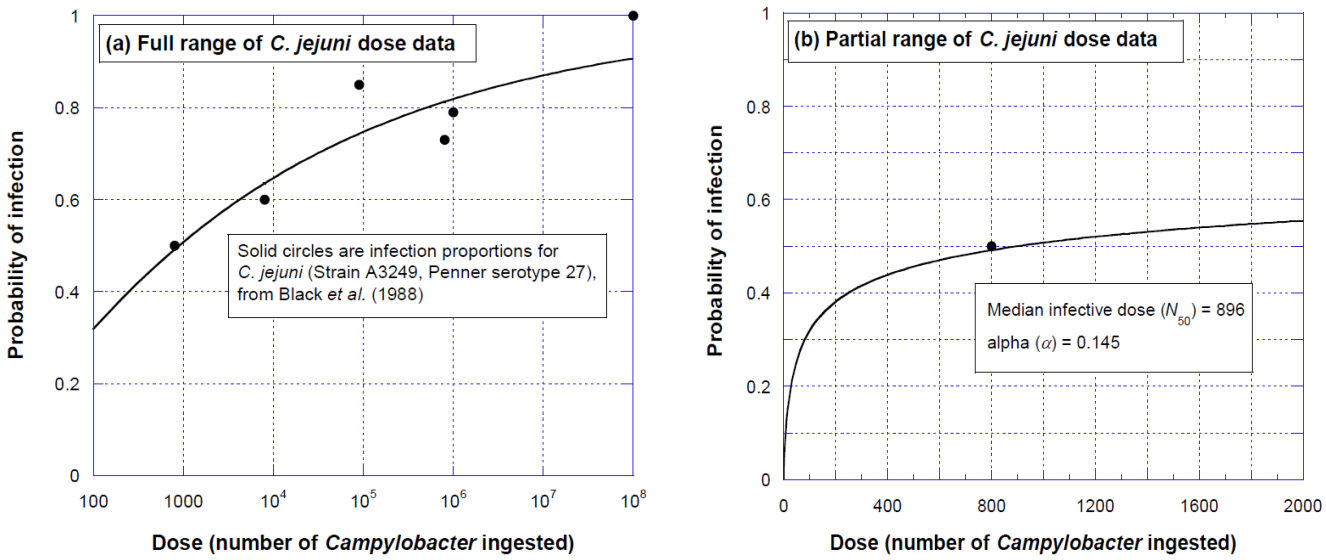

Figure 17. Dose-response curves for *Campylobacter jejuni*, estimating the probability of infection for a given dose. The lowest dose administered during the study was 800 *C. jejuni*, at which point half of the volunteers became infected. Estimating the dose response for lower concentrations requires extrapolation. From McBride et al. (2002).

1988), and more recent studies suggest that the infective dose may be much lower, particularly for susceptible population subgroups, such as children or people who are immunocompromised (Teunis et al., 2005). If this is so, the exposure required for infection (e.g. volume of water ingested) will be lower than suggested above. Despite the significance of campylobacteriosis to public health, dose response information on *Campylobacter* infection is scarce, and confounded by limited exposure doses. In particular, the risk associated with exposure to low doses of *Campylobacter* is not well known, although its success as a parasite (i.e. one of the most common in the western world), suggests high infectivity (Teunis et al., 2005). The probability of illness resulting from *Campylobacter* infection is also not well known (Teunis et al., 2005); one estimate suggests 28% of infections result in illness (Soller et al., 2010).

There are further uncertainties around the risks of infection and illness from *Campylobacter*. Although not conclusive, there is some epidemiological evidence, which is supported by animal models and cell culture, that some strains of *Campylobacter* may be host-specific, and that these different strains have different rates of human infectivity (McBride et al., 2011).

Campylobacter from avian sources are suggested to pose a limited threat to human health (McBride et al., 2011), although they remain implicated in cases of human disease (French et al., 2009; Mohan et al. 2013). Indeed, two wildfowl-associated *Campylobacter* genotypes isolated from water samples in the Waiau FMU were found to be indistinguishable from human clinical isolates, suggesting that those wildfowl types are capable of causing illness in humans. Analysis of all clinical isolates from the Southland region also shows little overlap with wildfowl-associated genotypes, suggesting wildfowl are a minor source of illness in the community. Since 33% of the *Campylobacter*-positive samples were found to contain only isolates of wildfowl origin, the health risk from these samples might be less than that suggested by the

data from Black et al. (1988), which is based on clinical isolates. However, we cannot say with certainty whether the low level of overlap between wildfowl-associated and clinical isolates results from a lower exposure rate (i.e. the public are simply not exposed to *Campylobacter* of wildfowl origin), or a lower infectivity or virulence in wildfowl-associated strains.

Campylobacter is just one of a number of enteric pathogens that may cause human illness, and with the demonstration that faecal contamination is present in the Waiau FMU, it is likely that other pathogens are also present. Pathogen type, prevalence and concentration differs between faecal sources. Human faeces is considered to pose the greatest risk to human health, even when it is only a minor component of the overall pollution, due to the risk that human-specific pathogens, especially viruses, are present (Devane and Gilpin, 2015). Keeping human wastes out of waterways must therefore be prioritised. The risk posed by treated human sewage should be evaluated on a case by case basis, as different treatment processes differentially inactivate pathogens and alter the correlation between indicator bacteria and pathogens. However, risk modelling studies have suggested that fresh bovine faeces are associated with the same level of risk as human wastes, due to the prevalence of STEC, *Campylobacter* and *Cryptosporidium* (Soller et al., 2010, 2014; Devane and Gilpin, 2015). Wildfowl are considered to pose the lowest relative risk to human health. However, these international studies do not include information on the health risk posed by sheep, which are also a significant source of faecal contamination in the Waiau FMU and the wider New Zealand landscape.

4.3 MANAGEMENT AND MITIGATION

The identification of faecal contamination source(s) and transmission route(s) is essential to implementing targeted mitigation strategies. Wildfowl and ruminant animals are significant faecal sources within the Waiau FMU, with direct deposition, overland flow and subsurface flow via tile drains all likely to be important mechanisms for the transfer of faecal microbes to waterways. However, the magnitude of contamination, relative importance of different sources and routes of transmission vary slightly between each of the 5 sites surveyed. Because of the complex interaction of faecal source, land topography, soil type, and climatic factors, one solution will not be suited to or effective for all sites. A site-specific solution that considers these various factors and targets the flow conditions or seasons where contamination is greatest, will yield the greatest benefit for water quality. Visual inspections of the site are highly recommended in providing as much detail as possible on which informed decisions can be made.

4.3.1 Direct deposition

Direct deposition by ruminant animals can be reduced by fencing streams and wetlands to exclude stock, removing the direct source. Fencing also allows for the creation of a riparian buffer strip (RBS), ideally vegetated, that reduces the momentum of surface runoff, aiding in infiltration and promoting the retention of faecal microbes within the soil (Collins et al., 2007). The effectiveness of RBS in attenuating faecal microbes is influenced by the slope of the land, width of the buffer, soil type, amount of runoff and the degree to which microbes are attached

to soil particles. Quantitative design guidelines for RBS are described by Collins et al. (2005), based on microbial attenuation modelling. The use of bridges at stream crossing for dairy cattle has also been shown to reduce direct faecal inputs and improve water quality (Collins et al., 2007). Stock exclusion strategies may yield greater benefits where cattle are farmed (i.e. beef or dairy) rather than sheep, since sheep tend to be less attracted to waterways than cattle. A literature review by Muirhead (2011) reported finding no publications on the effectiveness of fencing sheep in reducing *E. coli* concentrations in streams. Deer are also attracted to water, and fencing to exclude deer from wallowing areas that are connected to streams has been shown to reduce contaminant loading to the stream (McDowell, 2008). However, deer have been observed to pace the fenceline and/or create new wallows, undermining the longevity of the water quality benefits. The creation of a new 'safe' wallow (not connected to the stream) in combination with the fencing of any connected wallows is recommended as an approach to reducing water contamination associated with deer (McDowell, 2009).

4.3.2 Indirect sources

Strategies that can be used to reduce ruminant contamination associated with overland and/or subsurface flow will depend on characteristics of the land and farm management practices. Identifying locations that are associated with a high risk of microbial transfer to waterways is a key step in adjusting agricultural practices to improve water quality. For example, the ability of soils to attenuate faecal microbes depends on soil type and slope. Poorly drained soils, soils with low infiltration rates, soils with high preferential flow (macropores or cracking), land with artificial drainage, or hilly terrain, have a high risk of transferring microbes to waterways. High intensity grazing should be avoided on such land. During periods of wet weather, grazing rotation and exclusion of stock from paddocks adjacent to waterways, or that are prone to saturation and/or pugging, can help reduce runoff and wash-in of faeces following rainfall.

Irrigation management can also be useful in reducing contaminant loss. Land application of effluent should be limited to areas with a low risk of runoff or preferential flow, or areas of higher risk that are remote from waterways, to maximise the opportunity for microbial attenuation in the soil. Irrigation (of effluent or water) should be avoided where soils are at or near saturation, to reduce runoff; however, this may require storage of effluents for deferred irrigation, particularly in winter when soil moisture deficits are small, if any. Alternatively, where soil or climate conditions are unsuited to effluent irrigation, microbial treatment of effluent prior to discharge may be of benefit (e.g. upgrade a conventional 2-stage stabilisation pond to an Advanced Pond System (APS), or the installation of constructed wetlands). Finally, irrigator type and operation can influence runoff, with higher ground speed applying a more uniform pattern of application, and spray irrigation resulting in less bypass flow than border strip irrigation (Collins et al., 2007).

4.3.3 Wildfowl

It can be difficult to manage contamination of waterways caused by wildfowl, particularly in large rural and/or remote catchments. Since wildfowl pollution typically enters waterways via direct deposition, physically separating birds from the water would be expected to be effective in reducing their impact. Unlike livestock, birds cannot be contained by fencing, and so

strategies for reducing wildfowl inputs tend to focus on managing population size, or disturbing the birds to discourage settling beside vulnerable waterways.

The primary method for controlling wildfowl populations is hunting, although recreational hunting of some species (e.g. Canada geese, paradise ducks) is insufficient and may be supplemented by culling operations. To a lesser extent, population control may also be aided through nest disturbance, oiling of eggs or 'egg-pricking' (injecting eggs with formalin) to prevent hatching (Spurr and Coleman, 2005; MfE, 2018). Non-lethal methods to deter the presence of wildfowl include 'physical scaring', such as the use of plastic tapes and streamers, installation of bird spikes to prevent roosting, horns and sirens, or scarecrows. However, these approaches are effective at only a local scale, and simply move birds on to another area rather than address the underlying problem; thus, whilst used to some effect in protecting agricultural crop damage caused by wildfowl, they are likely to be less effective in reducing wildfowl defecation into waterways (Spurr and Coleman, 2005; MfE, 2018).

4.3.4 Prioritising mitigations

The benefits of these various mitigation strategies need to be balanced against the cost that will inevitably be associated with their implementation, such as material and labour costs for fencing and planting riparian zones, upgrades to effluent treatment systems or reduced productivity associated with reduced stock densities. Mitigations should be prioritised based on risk assessments that identify priority areas for improvement, whilst also considering which particular strategies provide the 'greatest return for investment' (i.e. greatest reduction in microbial contamination). Catchment water quality models such as CLUES (Catchment Land Use for Environmental Sustainability model, ftp://ftp.niwa.co.nz/clues) allow users to assess the effects of changes in land use and farm practice (e.g. stocking rates, fencing), and can help in ranking various mitigation scenarios. The protection of public health must be at the forefront of this decision-making. Discussions around mitigation options should also be held in consultation with landowners and the public.

Although no human faecal pollution was detected, the pollution of waters by livestock or wildfowl represent a real human health risk that should not be diminished or dismissed. Population control through hunting is likely the most cost-effective means to reduce wildfowl contamination of waterways, but may be unappealing to some within the community. Strategies to reduce ruminant contamination could include fencing for stock exclusion, riparian planting, stock management (intensity, grazing rotation), irrigation management and wastewater treatment.

5. CONCLUSIONS

The microbial quality of waterways in the Waiau FMU is variable – although overall levels of microbial contamination are lower than for other FMUs in the Southland region, several sites remain vulnerable to high levels of faecal contamination. Under base flow conditions, wildfowl appear to be the dominant source of pollution, likely due to direct defecation into the water and along banks and verges. Ruminant signatures are also commonly detected under base flow, suggesting direct deposition by livestock either as a result of free access to the stream and/or discharge of farm effluents to the water. Following rainfall, ruminant animals are the dominant pollution source, with overland flow/surface runoff and possibly subsurface flow through tile drains being significant routes of transmission of faecal materials to waterways. Human waste does not appear to be a significant faecal source in the Waiau FMU, with no human contamination detected.

Campylobacter was isolated from 80% of samples, with concentrations generally less than 10 MPN/100 ml. Wildfowl, ruminants, and poultry were all identified as being sources of *Campylobacter*. *Campylobacter* genotypes that were indistinguishable from human clinical cases in the Southland region were identified. Although there is little data available on the probabilities of infection and/or illness at lower *Campylobacter* concentrations, these finding suggests that there may be a health risk associated with contact with these waterways. Although the presence of other faecal pathogens (e.g. *E. coli* O157, *Cryptosporidium*) was not assessed, the prevalence of *Campylobacter* suggests this is also a possibility.

Within the Waiau FMU, the Orauea River at Orawia Pukemaori Road and Lill Burn at Lill Burn Monowai Road appear most vulnerable to faecal contamination. Because of the interaction between faecal source, soil type, land contour, artificial drainage and climate factors in determining contaminant transfer to waterways, and the variation in these between sites, a single mitigation strategy will not be effective for all sites. Risk assessments should be used in conjunction with water quality models to prioritise approaches to mitigate the greatest health risks and that afford the greatest improvements to water quality for a given investment. Population control through hunting is likely the most cost-effective means to reduce wildfowl contamination of waterways, but may be unacceptable to some members of the community. Strategies to reduce ruminant contamination could include fencing for stock exclusion, riparian planting, stock management (intensity, grazing rotation), irrigation management and wastewater treatment.

ABBREVIATIONS

APHA	American Public Health Association
Cp	cyclic threshold
CSO	combined sewer overflow
DNA	deoxyribosenucleic acid
ES	Environment Southland
ESR	Institute of Environmental Science and Research
FMU	Freshwater Management Unit
FST	faecal source tracking
ID_{50}	pathogen dose associated with a 50% probability of infection
MBiT	multiplex ligation-dependent probe amplification-binary typing
MLST	multilocus sequence typing
MPLA	multiplex ligation-dependent probe amplification
MPN	Most Probable Number
MST	Minimum spanning tree
MUG	4-methyl-umbelliferyl-β-D-glucuronide
NTC	non-template control
OD	optical density
ONPG	hydrolyse otho-nitrophenyl-β-D-galactopyranoside
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
RBS	riparian buffer zone
STEC	shiga toxin-producing *E. coli*
Thermo	thermophilic (with particular reference to *Campylobacter*)
T_m	melt temperature
UPGMA	unweighted pair group method with arithmetic method
WWTP	wastewater treatment plant

GLOSSARY

attenuation	the reduction of contaminant concentrations in the environment
base flow	the portion of stream flow that is sustained between rainfall events; stream flow during fair weather
bovine	relating to cattle
colony-forming units	method of estimating the concentration of bacteria in a water sample, based on the number of distinguishable colonies that grown in a culture plate
enteric pathogen	microorganisms that live in the intestine and can cause illness
faecal indicator organism	a microorganism that is associated with the gut or faeces of an animal and whose presence in environmental waters can be used to indicate faecal contamination
faecal source tracking	a 'toolbox' of methods that can be used to determine the source of faecal contamination (e.g. whether it is of human, ruminant, wildfowl etc origin)
genotypes	the genetic makeup or DNA sequence of an organism
illness	sickness that results from infection, with symptoms commonly including vomiting, diarrhoea and fever
infection	where a microorganism becomes established in the body and is able to multiply. Infection may cause illness or be asymptomatic (without symptoms).
isolates	bacteria that have been recovered from an environmental or clinical sample (e.g. water). They represent an individual colony from a culture plate, which is then sub-cultured, to ensure a pure culture (e.g. bacteria are the same).
Most Probable Number	probabilistic method to estimate the concentration of bacteria in a water sample, based on dilution series and the pattern of positive tubes
ovine	relating to sheep
pathogen	an organism, particularly bacteria, viruses or protozoa that cause disease
pathogenicity	qualitative term to describe the ability of an infectious agent to cause disease in a host (i.e. an organism is pathogenic or not)
polymerase chain reaction	a method used in molecular biology to make multiple copies of a DNA sequence

phylogenetic	the evolutionary development and diversification of a species or group of organisms, or of a particular feature of an organism
riparian zone	the interface between land and a river or stream
strain	a genetic variant or sub-type of a species of microorganism
thermophilic	thrives at high temperatures; synonymous with thermotolerant
thermotolerant	able to survive higher temperatures. As relates to *Campylobacter*, includes *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* that can grow at 42 ^{o}C and account for >90% of human campylobacteriosis.
virulence	a pathogens ability to cause infection or disease in a host. Similar to pathogenicity, but is quantitative, describing the degree of pathology.
zoonotic	a pathogen or disease that can be transmitted from animals to humans

APPENDIX A: MICROBIOLOGICAL METHODS AND REPORTING

The following sections provide detailed descriptions of the microbiological methods used during this study, and which are described briefly in Section 2. Commentary is also provided for some methods to aid in interpretation of results.

A.1 COLIFORM AND *E. COLI* ANALYSIS

Water samples were analysed for faecal coliforms and *E. coli* using membrane filtration (APHA et al., 2012). Analysis of thermotolerant (i.e. faecal) coliforms by membrane filtration uses an enriched lactose medium and an incubation temperature of 44.5±0.2ºC for selectivity. Differentiation of *E. coli* is achieved by incubating coliform-positive filters with media containing 4-methyl-umbelliferyl-β-D-glucuronide (MUG); *E. coli* possess the enzyme glucuronidase, which hydrolyses MUG to produce a fluorescent product when viewed under UV light (365nm).

Faecal coliform and *E. coli* analyses were performed by Hill Laboratories, with all results reported via ES to ESR.

A.2 *CAMPYLOBACTER* SPP. ISOLATION

Campylobacter spp. were enumerated using a 3 x 5 MPN procedure in 30 ml volumes of m-Exeter Broth (Moriarty et al. 2008). Following inoculation, tubes were incubated at 42°C for 48 h under microaerophilic conditions (in the presence of 10% CO_2). MPN tubes were plated onto m-Exeter agar (Fort Richards, Auckland, New Zealand) and incubated at 37°C for a minimum of 4 h under microaerophilic conditions (10% CO_2), followed by transfer to an incubator for the remainder of a 48 h total incubation period. Suspected *Campylobacter* spp. colonies were confirmed using biochemical tests (oxidase, catalase), colony morphology, Gram stains, and a multiplex polymerase chain reaction (PCR), as described by Wong et al. (2004). This PCR procedure allows for isolates to be classified as *Campylobacter jejuni*, *Campylobacter coli*, or thermotolerant *Campylobacter* spp.

A.3 *CAMPYLOBACTER* SUB-TYPING AND SOURCE ATTRIBUTION BY MBiT

ESR has developed a multiplex ligation-dependent probe amplification-binary typing (MBiT) assay for the sub-typing and source attribution of the *Campylobacter* species *C. jejuni* and *C. coli*. This assay targets 18 pathogenicity- or survival-associated genes (Table 2) and allows the analysis of an isolate in a single reaction (Cornelius et al., 2014). A simple heat-lysis preparation is used to release DNA from the bacterial cells, with multiplex ligation-dependent

probe amplification (MLPA) detection of gene targets occurring via a hybridisation-ligation-PCR process. The result of the analysis is a profile for each isolate with the presence or absence of each gene target. A six-digit nomenclature is then used to describe each gene pattern (Figure 18). Isolates with the same pattern of gene targets are described as indistinguishable. It is then possible to use the pattern of gene products to produce phylogenetic comparisons of isolates. Source attribution is possible on the basis that *Campylobacter* from different sources tend to cluster separately from one another. There is of course some overlap, and genotypes may cluster separately from isolates from known sources. The effectiveness of the attribution depends on the size of the source library of known isolates, which ideally has temporal and spatial overlap with the isolates of interest.

Campylobacter spp. isolates to be analysed by MBiT were purified, and then a single colony picked into 250 µl of 2% Chelex buffer. The tube was heated for 5 min at 98°C to denature the DNA then cooled, before the MLPA reaction was performed as described in Cornelius et al. (2014). At the conclusion of the PCR step, the sample was diluted 1:10, LIZ500 size standard added, and products separated by capillary electrophoresis on an ABI 3700 DNA Analyser. Analysis of electropherograms, and subsequent band assignment, cluster analysis and burst diagram production was performed using BioNumerics 7.5 (Applied Maths).

Peak detection used thresholds of 5% of the OD range and 5% of the curve range with correction for peak intensity profile. Filtering by relative peak height was also performed using minimum relative height of 15% and maximum distance of 30%. Bands were then assigned to 18 band classes using position tolerance of 0.75%. Manual adjustment of bands was made where necessary.

Cluster analysis used categorical value similarity matrix with unweighted pair group method with arithmetic method (UPGMA) cluster analysis. Burst diagrams were created using minimum spanning tree (MST) analysis for categorical data. The size of each circle in a burst diagram represents the number of isolates with that MBiT profile. The branches in a burst diagram represent the number of difference in loci: branches are thick bold if only one locus is different; a thinner solid line if there are two or three differences in loci; a dashed line for four differences; and a dotted line if there are more than four differences in loci.

Up to six *Campylobacter* isolates from each water sample were analysed and assigned to a source cluster by comparison of each isolate with those from known sources. Sources were assigned depending on the number of isolates in each cluster from a particular source.

A.4 PCR MARKERS FOR FAECAL SOURCE TRACKING (FST)

There is a wide range of microorganisms other than the traditional faecal indicators (i.e. coliforms, *E. coli* and enterococci), that may be present in animal faeces. Some of these microorganisms are specific to certain animal hosts, and as such, are useful in faecal source identification. Using molecular methods, it is possible to extract the total DNA from a water sample, and to examine this sample for the presence genetic “markers” from these source-specific organisms. The presence of a target marker is suggestive that its host animal is a source of faecal pollution. However, each marker has a degree of non-specificity; they are

Table 2. Summary of MBiT gene targets and their methodologies.

Probe	Size	Probe	Methodology	Reference
tetO	124	survival	tetracycline resistance, normally plasmid-borne	Taylor 2005, Schmidt-Ott 2005
virB8	142	survival	type IV secretion/competence protein, inner membrane protein, pVir borne	Bacon 2002
cgtA	160	cell surface	polysugar synthesis, β-1,4-N-acetylgalactosaminyl-transferase	Bereswill 2003, Nachamkin 2002, Gilbert 2000
Cj1136	178	cell surface	putative galactosyltransferase	Parkhill 2000
panB	196	survival	3-methyl-2-oxobutanoate hydroxymethyltransferase, pantothenate biosynthesis, selective metabolic advantage under certain conditions	Parkhill 2000
maf5	214	mobility	hypothetical protein Cj1337, motility accessory factor, PseE protein	Parkhill 2000, Karlyshev 2002, Jagannathan 2005
Cj1135	232	cell surface	putative two-domain glycosyltransferase	Parkhill 2000
Cj0265	250	survival	putative cytochrome C-type haem-binding periplasmic protein	Parkhill 2000
CJE1733	268	survival	arsenical-resistance protein, putative	Fouts 2005
Cj0122	286	unknown	hypothetical protein Cj0122	Parkhill 2000
gmhA2	311	cell surface	putative phosphoheptose isomerase, polysaccharide synthetic region (capsule)	Parkhill 2000
flgE2	338	mobility	flagellar hook subunit protein, variable sequence and antigenicity, might be under selective pressure from immune system of colonised host	Parkhill 2000
CJE1500	365	cell surface	polysaccharide deacetylase family protein	Fouts 2005
Cj0423	391	unknown	putative integral membrane protein	Parkhill 2000
wlaN_4	418	cell surface	putative galactosyltransferase, LOS outer core biosynthesis	Dorrell 2005, Parker 2005, Kordinas 2005
cfrA	445	survival	putative iron uptake protein	Parkhill 2000
Cj1321	473	mobility	putative transferase, within flagellin glycosylation locus, characteristic of livestock clade, acetyl transferase	Parkhill 2000
Cj0008	503	unknown	hypothetical protein Cj0008	Parkhill 2000

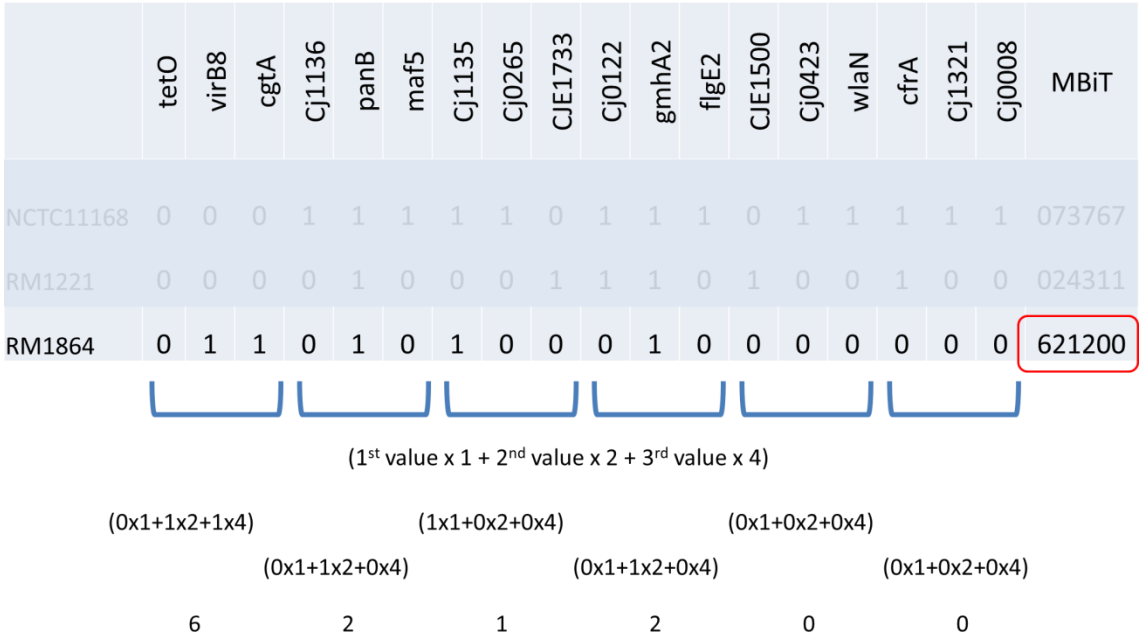

Figure 18. Example of an MBiT pattern naming.

strongly associated with, but not exclusive to, their host animal. Assays for different markers also differ in their sensitivity (Table 3).

Water samples (150 ml) were filtered and DNA extracted, then real-time PCR was performed using the qPCR reagent and cycling conditions outlined in Devane et al. (2007; 2013). The PCR assays applied to water samples are listed in Table 3. Each qPCR assay run included a non-template control (NTC), and an extraction blank of purified water to monitor for DNA contamination and standard concentrations of each target. The standard curve was generated from 10-fold serial dilutions as outlined in Devane et al. (2013). SYBR™ green assays were subjected to melting curve analysis, and amplicons checked that they were within 0.3°C of the melting temperature (T_m) of positive controls on each LightCycler 480® run. All samples and controls were analysed in duplicate. Samples that registered a cyclic threshold (Cp) value above 40 were considered to be below the detection limit.

The General marker (GenBac3) is reported on a semi-quantitative scale of + (weakly positive) to ++++ (very strongly positive), or not detected (-). Samples that return a + or ++ result for GenBac3 may not have sufficient levels of contamination to permit the detection of more specific markers.

The Ruminant-specific marker (BacR) is reported using a percentage value. These percentage values are based on the levels of this marker relative to the level of general GenBac3 indicator that has been reported for fresh ruminant faeces.

- Samples reported as up to 100% ruminant are consistent with all of the general faecal marker having come from a ruminant source.
- Lower levels (10-50%) may be a consequence of the presence of other sources of pollution. However, it is also possible that ruminant sources may account for all of the pollution, but that this includes aged faecal material, as the relative levels of the ruminant marker decline more rapidly than the general indicator.
- Levels of less than 10% indicate that ruminant pollution was only a minor contributor.

All other marker assays are reported as presence/absence (i.e. + or -). In assessing the presence of human faecal contamination, at least two markers must be assayed; contamination is supported when two or more human markers are detected.

A.5 PRESENTATION OF RESULTS IN THIS REPORT

Tables 4 and 5 provide a key for interpretation of results, which can be used to assist with reviewing results for each site.

Table 3. Summary of PCR markers used in this study, including microbial targets, sensitivity and specificity.

Assay (marker)	Target	Sensitivity	Detected in faeces from:	Negative in faeces from:
General (GenBac3)	Bacteroidales 16S rRNA	High	Human, cow, sheep, deer, goat, pig, rabbit, possum, cat, dog, horse, duck, swan, seagull, geese, chicken	(can be low in seagull and geese faeces)
Human (BacH)	Bacteroidales 16S rRNA	Medium[1]	Human, cat, dog, rabbit, possum, chicken, goat	Cow, sheep, deer, horse, duck
Human (BiADO)	*Bifidobacterium adolescentis* 16S rDNA	Medium[2]	Human, seagulls	Cow, sheep, deer, horse, goat, pig, rabbit, geese, chicken, cat
Ruminant (BacR)	Bacteroidales 16S rRNA	High	Cow, sheep, deer, goat	Human (individuals), horse, pig, rabbit, duck, swan, seagull, chicken, dog
Cow (M2)	Bovine-specific faecal genetic markers	Low	Cow, deer	Sheep, goat, horse, pig, human (individuals), ducks, swan, geese, seagulls, cat, dog, possum, rabbit
Sheep (Schill)	Cytochrome b of mitochondrial DNA	Medium	Sheep	Cow, deer, human (individuals), swan, geese, seagull, chicken, horse, cat, pig, possum, rabbit
Avian (GFD)	Avian-specific faecal 16S rRNA	Medium	Duck, swan, seagull, geese, chicken	Human, cow, sheep, deer, horse, goat, pig, rabbit, possum, cat, dog
Avian (E2)	*Desulfovibrio*-like species 16S rRNA	Low	Duck	Human, cow, sheep, deer, horse, goat, rabbit, possum, cat, dog
Canine (DogBac)	Bacteroidales 16S rRNA	High	Dog	Human (individuals), cow, sheep, deer, goat, horse, pig, rabbit, possum, duck, swan, seagull, geese, chicken, cat

[1]. Most sensitive human assay
[2]. Less sensitive than BacH

Table 4. Guideline for general data, microbial results and MBiT interpretation

Site	Site name				
Sample #	ESR Sample Number				
Client #	Environment Southland Sample Number				
Date Sampled	Date sampled				
Rainfall	Yes/No				
Faecal coliforms	Membrane filtration-based count of faecal coliforms colony forming units (cfu)/100 ml)				
E. coli	Membrane filtration-based count of E*. coli* colony forming units (cfu)/100 ml)				
Campylobacter	MPN count of *Campylobacter*/100 ml				
Species	Determined by PCR as either *C. jejuni, C. coli* or other thermotolerant *Campylobacter* (Thermo)				
MBiT Typing	MBiT patterns of analysed isolates. Colours reflect source attribution. The "not wildfowl" means sources is ovine/bovine/deer or poultry. These could also be human sewage source, as these genotypes cause disease in humans.				
	Wildfowl	Ovine/ Bovine/Deer	Poultry	Not Wildfowl	Unknown

Table 5. Explanation of PCR-based markers

General (GenBac3)	Indicator of possible faecal pollution. Scale indicates level detected, with samples with Positive or greater levels generally valid for examination of other markers				
Full name	Very Strong Positive	Strong Positive	Positive	Low Levels	Not Detected
Abbreviation	++++	+++	++	+	-
Ruminant	Percentage of herbivore faecal pollution relative to the GenBac3 marker				
	50-100%	10-50%	1-10%	Less than 1%	Not Detected
Human - BacH	These markers are typically reported as presence/absence (+/-). Where a very high level is detected, this is shown as ++. Presence at this level (++) suggests the presence of a major source. The presence of markers at lower levels does not definitively rule out the chances of a significant source being present.				
Human - BiADO					
Cow					
Sheep					
Wildfowl - GFD					
Wildfowl - E2					
Canine					
nt	Not tested				

APPENDIX B: SUBCATCHMENT-SPECIFIC INFORMATION AND MICROBIAL WATER QUALITY

The following sections document the microbial and FST analysis results for water samples collected from the various sampling locations, together with an overview of land use and consented discharge activities within the sub-catchment.

B.1 MARAROA RIVER AT THE KEY

A single water sample was collected from the Mararoa River at The Key in April 2015 (Table 6). The sample was collected following rainfall. *E. coli* was present at a concentration of 700 cfu/100 ml. *Campylobacter* was also present at low levels (2.3 MPN/100 ml); further analysis determined it to be *C. jejuni* from a ruminant source.

Faecal source tracking analysis identified ruminant animals as being the dominant pollution source, accounting for up to 100% of faecal pollution. Neither ovine- nor bovine-specific markers were detected, suggesting that the other ruminant animals such as deer may have been the primary source. Alternatively, it may suggest that the pollution level is sufficiently low or is aged such that the more specific source markers could not be identified. FST also identified wildfowl-specific markers.

A review of the land use for the Mararoa River at The Key sub-catchment shows that primary land use is non-agricultural (conservation and recreation), followed by mixed livestock (beef, sheep and deer) (Figure 19, Figure 20).

Table 6. Results for microbial and FST analysis of the water sample collected from the Mararoa River at The Key.

Site		Mararoa River at The Key
Sample #		CMB150395
Client #		20151650
Date Sampled		16/04/2015
Rainfall		Yes
		Microbial Properties
Faecal coliforms		700
E. coli		700
Campylobacter		2.3
Campylobacter Species		*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl	
	Ovine/Bovine/Deer	1
	Poultry	
	Not Wildfowl	
	Unknown	
		Faecal Source Tracking
General - GenBac3		++++
Ruminant		50-100%
Human - BacH		-
Human - BiADO		-
Cow		-
Sheep		-
Wildfowl - GFD		+
Wildfowl - E2		-

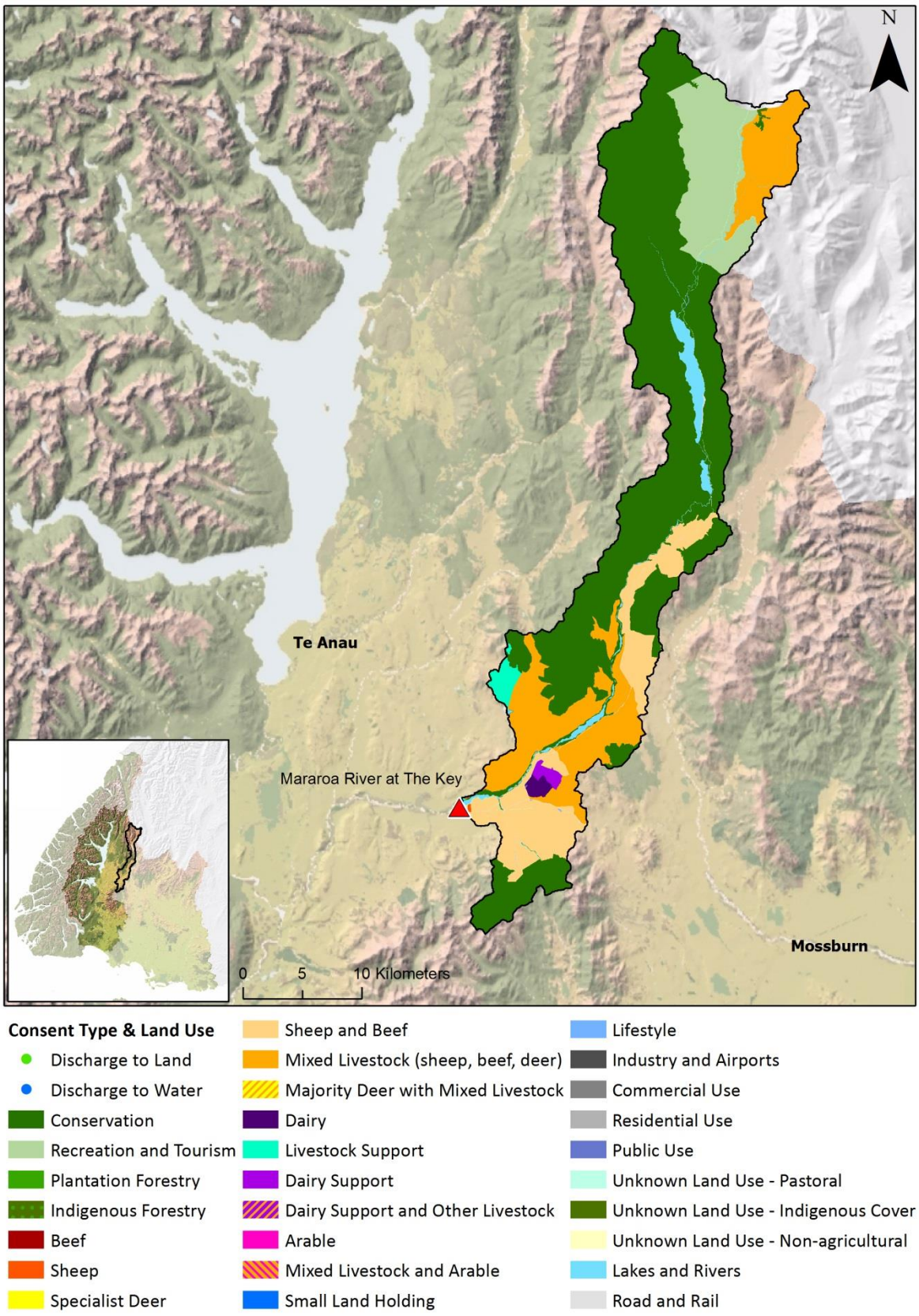

Figure 19. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Mararoa River at The Key sampling site.

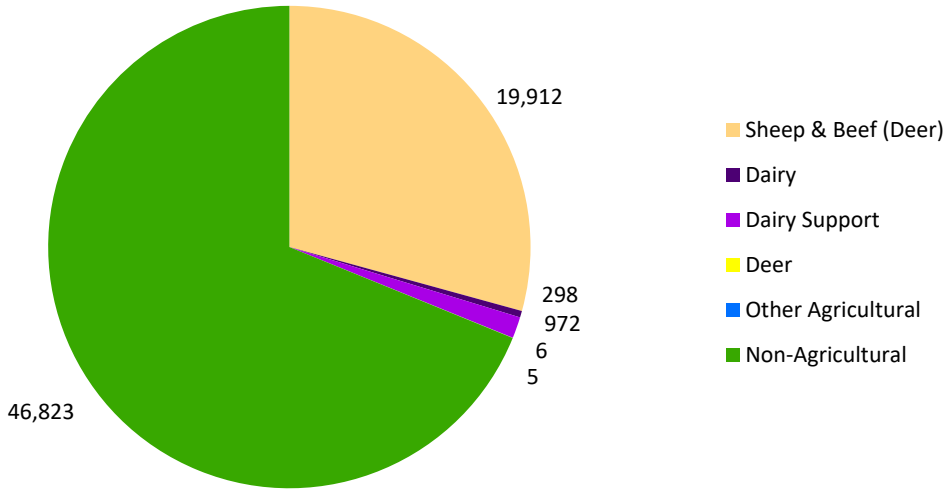

Figure 20. Land use (in hectares) in the catchment for the Mararoa River at The Key sampling site.

Sheep & Beef (Deer) (Sheep, Beef, Sheep and Beef, Mixed Livestock, Unknown Land Use – Pastoral, Mixed Livestock and Arable), **Dairy** (Dairy), **Dairy Support** (Dairy Support, Dairy Support and Other Livestock, Livestock Support), **Deer** (Specialist Deer, Majority Deer with Mixed Livestock), **Other Agricultural** (Small Land Holding, Lifestyle, Other Animals, Flower & Bulb Growers, Nurseries and Orchards, Horticulture), **Non-agricultural** (Commercial, Conservation, Indigenous Forestry, Plantation Forestry, Public Use, Recreation and Tourism, Residential Use, Road and Rail, Unknown Land Use - Indigenous Cover, Unknown Land Use - Non-agricultural, Lakes and Rivers, Industry and Airports), **Arable** (Arable). Based on 2015 Southland Land Use Information

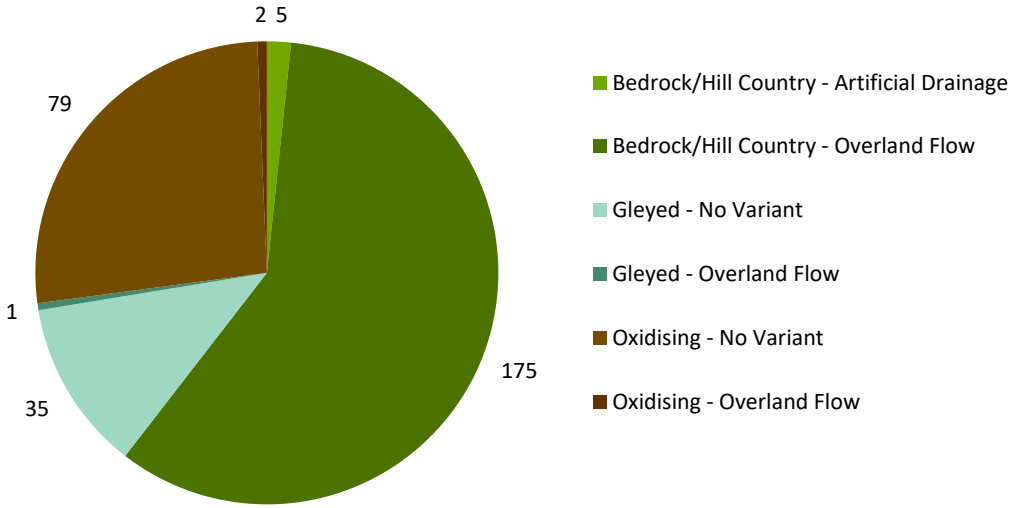

Figure 21. Dairying land (in hectares) in the catchment for the Mararoa River at The Key sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 7. Number of consented catchment discharges to land and water in the catchment for the Mararoa River at The Key sampling site.

Mararoa River at The Key		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	1
To Land Total		1
Grand Total		1

Note: Consent information accurate as of April 2017

B.2 MARAROA RIVER AT WEIR ROAD

A single, post-rainfall sample was also collected from the Mararoa River at Weir Road (Table 8). *E. coli* levels here were lower than they were upstream at the Key, at 420 cfu/100 ml. *Campylobacter* was not detected at the Weir Road site.

Faecal source tracking identified ruminant pollution as accounting for up to 50% of pollution in the sample, with bovine-specific PCR markers detected. Wildfowl-specific FST markers were also identified.

Land use in the Mararoa River at Weir Road sub-catchment includes significant areas of conservation and recreation land, with mixed livestock (beef, sheep and deer) and associated livestock support. Approximately 6% of the land is used for dairy and dairy support (Figure 22, Figure 23).

Table 8. Results for microbial and FST analysis of the water sample collected from the Mararoa River at Weir Road.

Site		Mararoa River at Weir Road
Sample #		CMB150396
Client #		20151653
Date Sampled		16/04/2015
Rainfall		Yes
		Microbial Properties
Faecal coliforms		530
E. coli		420
Campylobacter		<0.3
Campylobacter Species		nt
MBiT *Campylobacter* Source	Wildfowl	
	Ovine/Bovine/Deer	
	Poultry	
	Not Wildfowl	
	Unknown	
		Faecal Source Tracking
General - GenBac3		++++
Ruminant		10-50%
Human - BacH		-
Human - BiADO		-
Cow		+
Sheep		-
Wildfowl - GFD		+
Wildfowl - E2		-

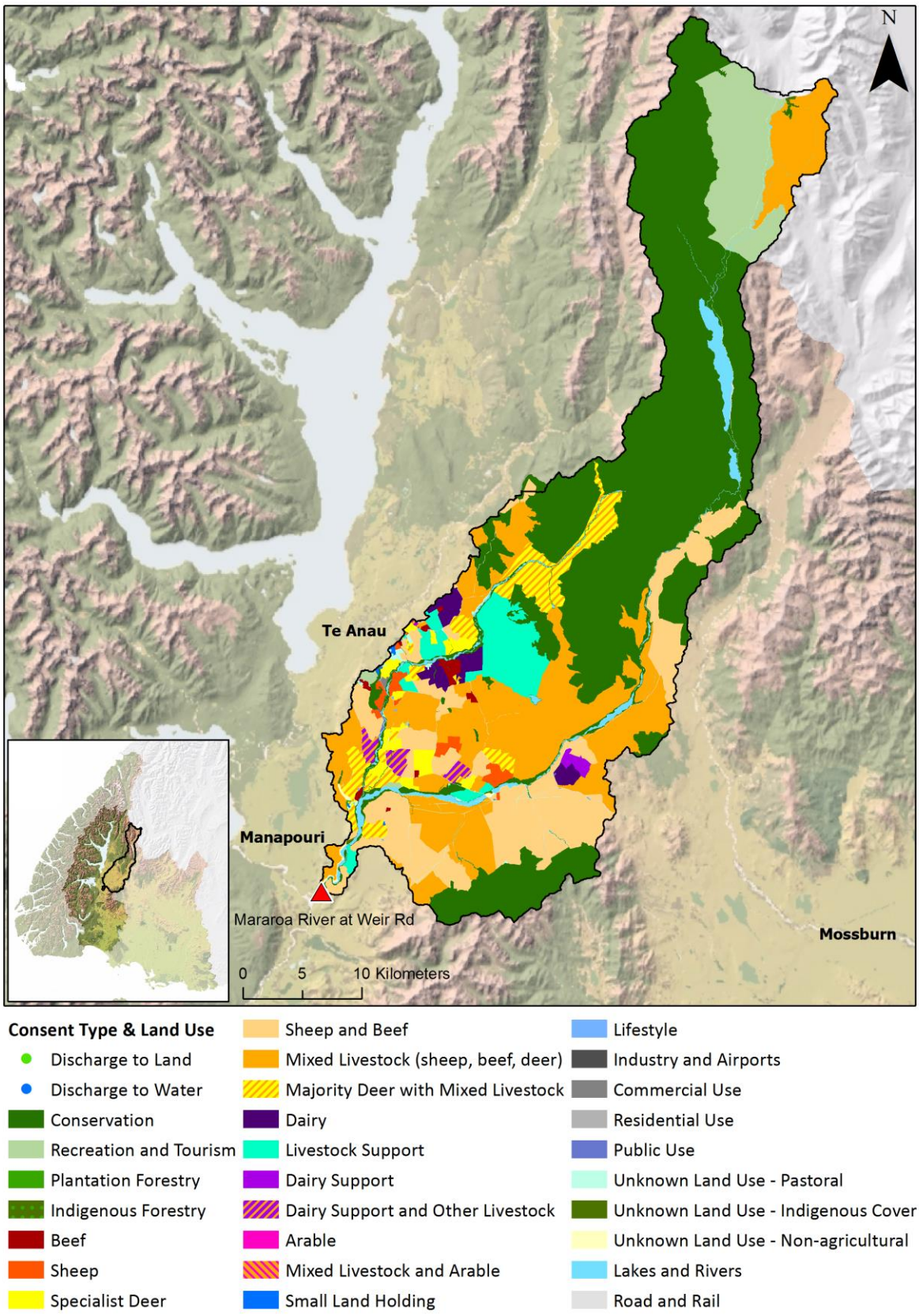

Figure 22. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Mararoa River at Weir Road sampling site.

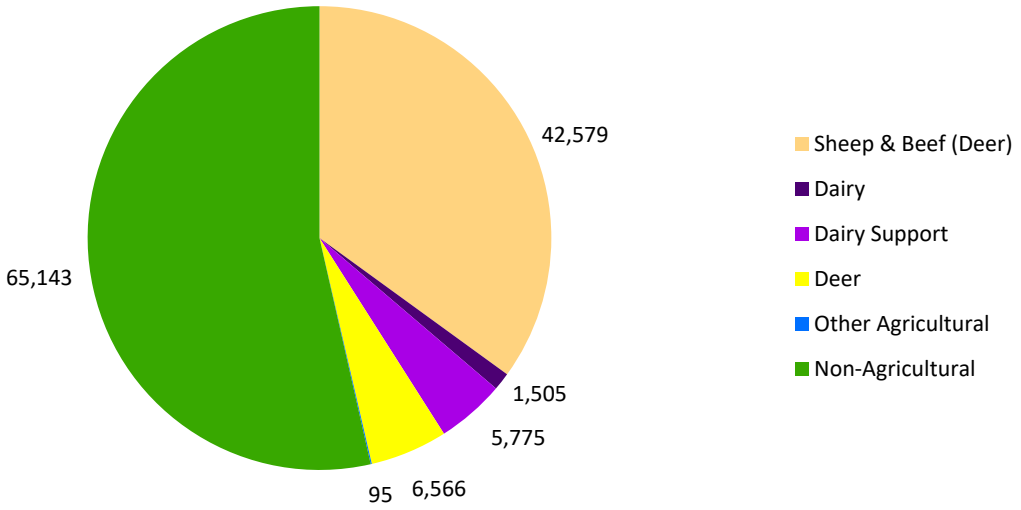

Figure 23. Land use (in hectares) in the catchment for the Mararoa River at Weir Road sampling site.

Sheep & Beef (Deer) (Sheep, Beef, Sheep and Beef, Mixed Livestock, Unknown Land Use – Pastoral, Mixed Livestock and Arable), **Dairy** (Dairy), **Dairy Support** (Dairy Support, Dairy Support and Other Livestock, Livestock Support), **Deer** (Specialist Deer, Majority Deer with Mixed Livestock), **Other Agricultural** (Small Land Holding, Lifestyle, Other Animals, Flower & Bulb Growers, Nurseries and Orchards, Horticulture), **Non-agricultural** (Commercial, Conservation, Indigenous Forestry, Plantation Forestry, Public Use, Recreation and Tourism, Residential Use, Road and Rail, Unknown Land Use - Indigenous Cover, Unknown Land Use - Non-agricultural, Lakes and Rivers, Industry and Airports), **Arable** (Arable). Based on 2015 Southland Land Use Information

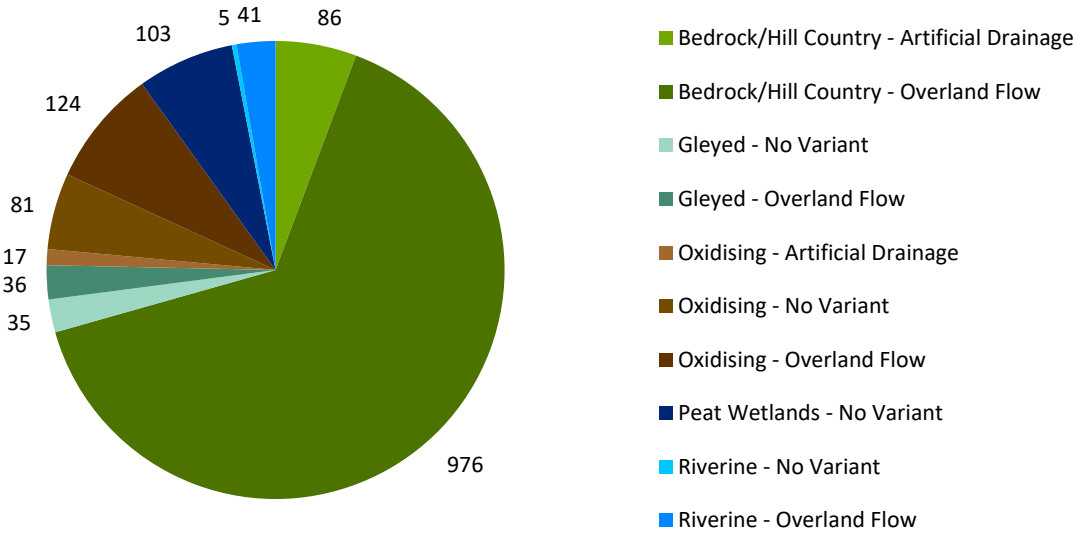

Figure 24. Dairying land (in hectares) in the catchment for the Mararoa River at Weir Road sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 9. Number of consented catchment discharges to land and water in the catchment for the Mararoa River at Weir Road sampling site.

Mararoa River at Weir Road		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	3
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
	Oil/Grease	2
	Silt	1
	Wintering Pad/Feedlot Effluent (land)	1
To Land Total		8
To Water	Suspended Sediment	1
To Water Total		1
Grand Total		9

Note: Consent information accurate as of April 2017

B.3 WAIAU RIVER AT SUNNYSIDE

The Waiau River was sampled at Sunnyside in April 2015, following rainfall (Table 10). Low levels of *E. coli* (20 MPN/100 ml) and *Campylobacter* (0.9 MPN/100 ml) were detected. Analysis of *Campylobacter* isolates determined that they were *C. jejuni*, and that they had come from a wildfowl source.

Faecal source tracking analysis determined that ruminant animals were responsible for up to 50% of overall contamination at this site, although no specific PCR markers were detected. This is likely a result of the low levels of pollution present at this site, such that any source-specific markers present were below limits of detection.

Land in the sub-catchment for the Waiau River at Sunnyside is predominantly conservation land (Figure 25), with approximately 5% of land used for agriculture, mostly sheep, beef and/or deer (Figure 26).

Table 10. Results for microbial and FST analysis of the water sample collected from the Waiau River at Sunnyside.

Site		Waiau River at Sunnyside
Sample #		CMB150397
Client #		20151654
Date Sampled		16/04/2015
Rainfall		Yes
		Microbial Properties
Faecal coliforms		20
E. coli		20
Campylobacter		0.9
Campylobacter Species		*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl	1
	Ovine/Bovine/Deer	
	Poultry	
	Not Wildfowl	
	Unknown	
		Faecal Source Tracking
General - GenBac3		+++
Ruminant		10-50%
Human - BacH		+
Human - BiADO		-
Cow		-
Sheep		-
Wildfowl - GFD		-
Wildfowl - E2		-

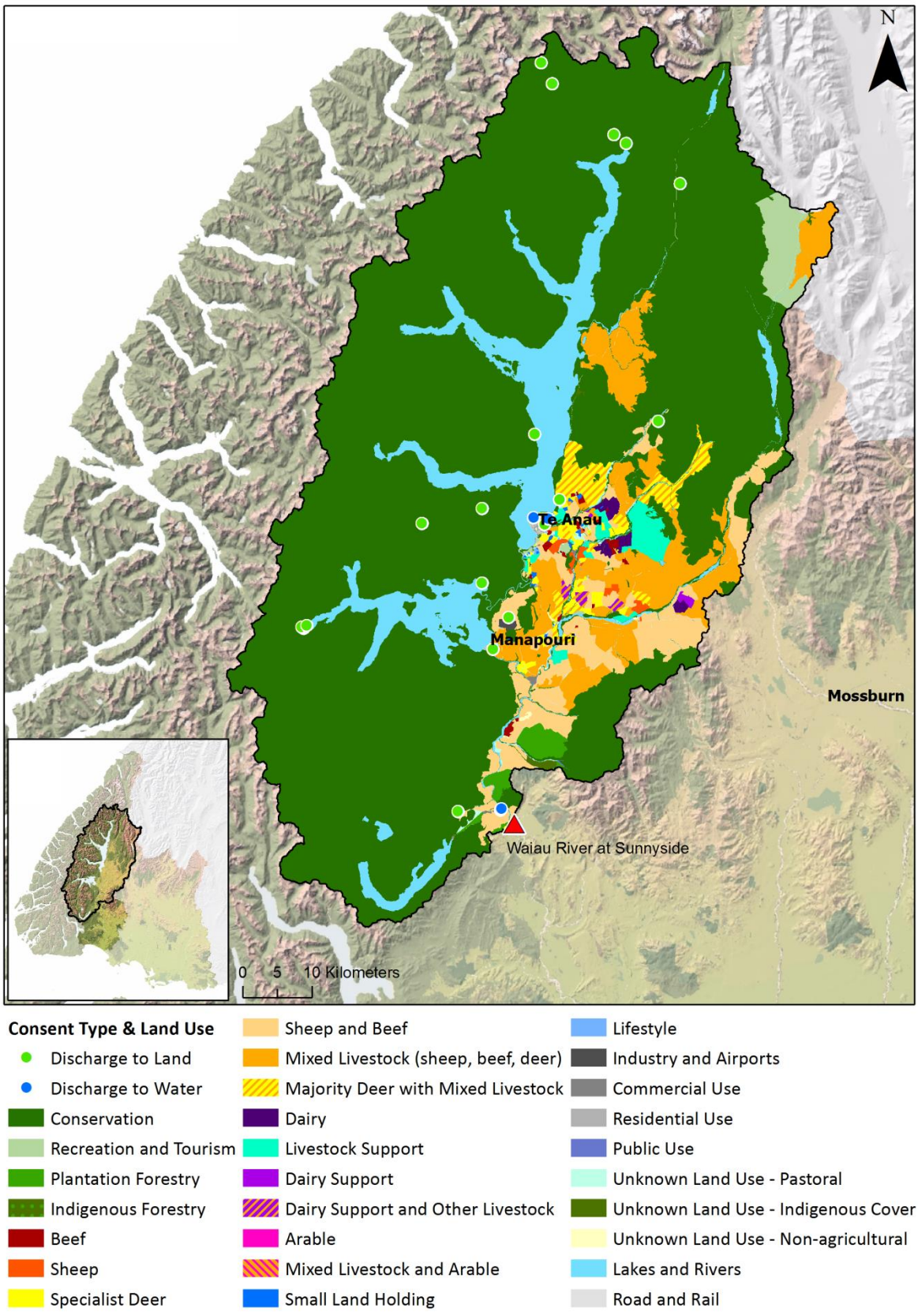

Figure 25. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Waiau River at Sunnyside sampling site.

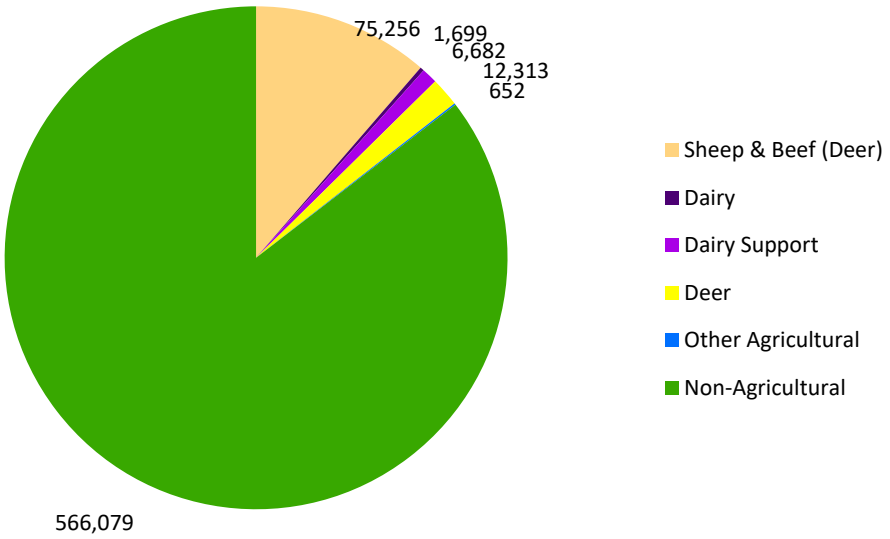

Figure 26. Land use (in hectares) in the catchment for the Waiau River at Sunnyside sampling site.

Sheep & Beef (Deer) (Sheep, Beef, Sheep and Beef, Mixed Livestock, Unknown Land Use – Pastoral, Mixed Livestock and Arable), **Dairy** (Dairy), **Dairy Support** (Dairy Support, Dairy Support and Other Livestock, Livestock Support), **Deer** (Specialist Deer, Majority Deer with Mixed Livestock), **Other Agricultural** (Small Land Holding, Lifestyle, Other Animals, Flower & Bulb Growers, Nurseries and Orchards, Horticulture), **Non-agricultural** (Commercial, Conservation, Indigenous Forestry, Plantation Forestry, Public Use, Recreation and Tourism, Residential Use, Road and Rail, Unknown Land Use - Indigenous Cover, Unknown Land Use - Non-agricultural, Lakes and Rivers, Industry and Airports), **Arable** (Arable). Based on 2015 Southland Land Use Information

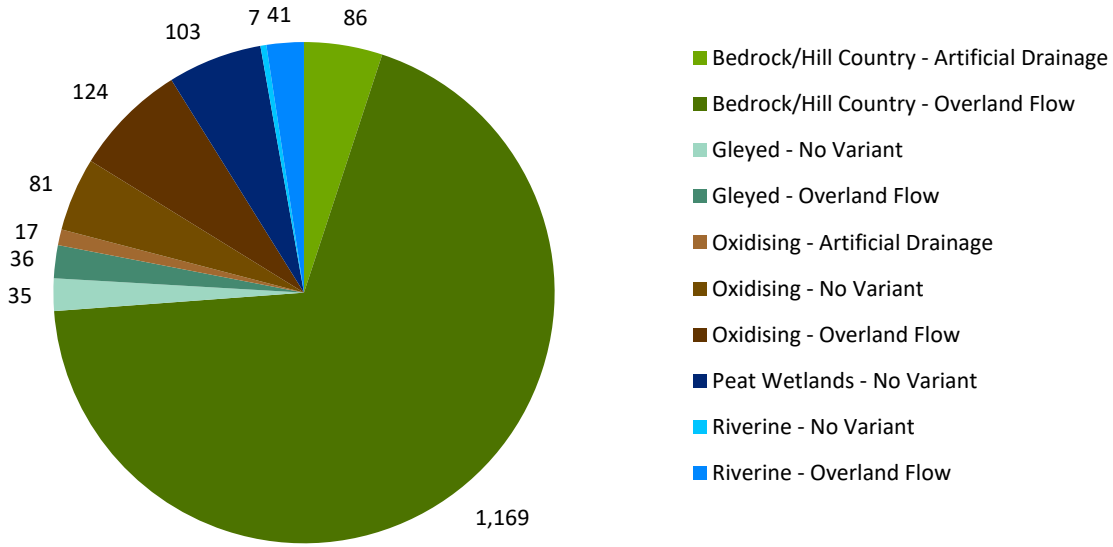

Figure 27. Dairying land (in hectares) in the catchment for the Waiau River at Sunnyside sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 11. Number of consented catchment discharges to land and water in the catchment for the Waiau River at Sunnyside sampling site.

Waiau River at Sunnyside		
Subtype	Contaminant	Total
To Land	1080	11
	1080, Hazardous Substances	1
	Clean Fill	4
	Dairy Shed Effluent (land)	4
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
	Hazardous Substances	1
	Oil/Grease	2
	Oxidation Pond Effluent, Septic Tank Effluent, Sewage (Treated), Sewage Sludge, Waste Water,	1
	Pesticides/Herbicides	2
	Septic Tank Effluent	1
	Septic Tank Effluent, Sewage (Treated)	2
	Septic Tank Effluent, Sewage (Treated), Waste Water	1
	Sewage (Treated)	10
	Sewage (Treated), Stormwater, Waste Water	1
	Sewage (Treated), Waste Water	2
	Silt	1
	Wash Water	1
	Wintering Pad/Feedlot Effluent (land)	1
To Land Total		47
To Water	Aquaculture Waste Water	1
	Cooling Water	1
	Floodwaters, Water (Hydro)	3
	Hydro electric power generation sundry contaminant	2
	Hydro electric power generation sundry contaminant, Water (Hydro)	1
	Mine Waste	1
	Mine water, Suspended Sediment, Wash Water	2
	Oil/Grease	1
	Oxidation Pond Effluent, Sewage (Treated), Waste Water	1
	Sewage (Treated), Waste Water	1
	Stormwater	2
	Suspended Sediment	4
	Suspended Sediment, Wash Water	1
	Wash Water	1
	Water (Hydro)	4
To Water Total		26
Grand Total		73

Note: Consent information accurate as of April 2017

B.4 ORAUEA RIVER AT ORAWIA PUKEMAORI ROAD

Water samples were collected from the Orauea River at Orawai Pukemaori Road between April and December 2015 (Table 12). Two samples (April and October) were collected under base flow conditions, while the remainder (May, June, July and December) were collected following rainfall.

The two samples collected under base flow conditions were quite different to each other– the April sample had high *E. coli* (1,800 cfu/100 ml), with *Campylobacter* also detected (21 MPN/100 ml). The *Campylobacter* was identified as *C. jejuni*, with both wildfowl and 'not wildfowl' (i.e. ruminant, poultry or human) sources implicated. Faecal source tracking analysis identified ruminants as contributing up to 50% of the overall pollution, with ovine- and wildfowl-specific PCR markers identified. In contrast, the October base-flow sample had low levels of *E. coli* (160 cfu/100 ml), with no *Campylobacter* detected. Faecal source tracking again suggested ≤50% of the faecal pollution was of ruminant origin, although no specific markers were detected, possibly due to the low overall pollution level in the sample.

Following rainfall, *E. coli* concentrations varied between 800 and 1,600 cfu/100 ml, with the exception of the December sample which contained 18,000 cfu/100 ml. *Campylobacter* was present in all four post-rain samples, with the highest concentrations (43 and 7 MPN/100 ml) observed in those samples with the highest *E. coli* levels. The *Campylobacter* was determined to be *C. jejuni*, with a thermophilic *Campylobacter* also present in July. All samples contained *Campylobacter* from a wildfowl source, with the July and December samples also containing isolates from a ruminant and poultry source, respectively. Faecal source tracking analysis identified ruminant pollution as being the dominant pollution source (50-100%) in all four samples. Ovine markers were detected in all four samples, and was the only source-specific marker detected in the heavily contaminated December sample. Both bovine and wildfowl markers were also detected in the May, June and July samples.

A review of land use in the sub-catchment shows predominantly agricultural use (approximately 79%), with a mix of sheep, dairy, deer, and mixed livestock regimes (Figure 28, Figure 29).

Table 12. Results for microbial and FST analysis of water samples collected from the Orauea River at Orawia Pukemaori Road.

Site		Orauea River at Orawia Pukemaori Road					
Sample #		CMB150399	CMB151776	CMB150565	CMB150816	CMB151037	CMB152266
Client #		20151658	20153347	20151872	20152119	20152705	20154556
Date Sampled		16/04/2015	15/10/2015	14/05/2015	11/06/2015	9/07/2015	16/12/2015
Rainfall		No	No	Yes	Yes	Yes	Yes
		Microbial Properties					
Faecal coliforms		1,800	200	1,600	1,100	800	20,000
E. coli		1,800	160	1,600	1,000	800	18,000
Campylobacter		21	<0.3	7	0.9	0.9	43
Campylobacter Species		*C. jejuni*	nt	*C. jejuni*	*C. jejuni*	*C. jejuni & Thermo*	*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl	2		3	2	1	1
	Ovine/Bovine/ Deer					1	
	Poultry						6
	Not Wildfowl	1		2			
	Unknown						
		Faecal Source Tracking					
General - GenBac3		++++	+++	++++	++++	++++	++++
Ruminant		10-50%	10-50%	50-100%	50-100%	50-100%	50-100%
Human - BacH		-	-	-	-	+	-
Human - BiADO		-	-	+	-	-	-
Cow		-	-	+	+	+	-
Sheep		+	-	+	+	+	+
Wildfowl - GFD		+	-	-	-	+	-
Wildfowl - E2		+	-	+	-	+	-

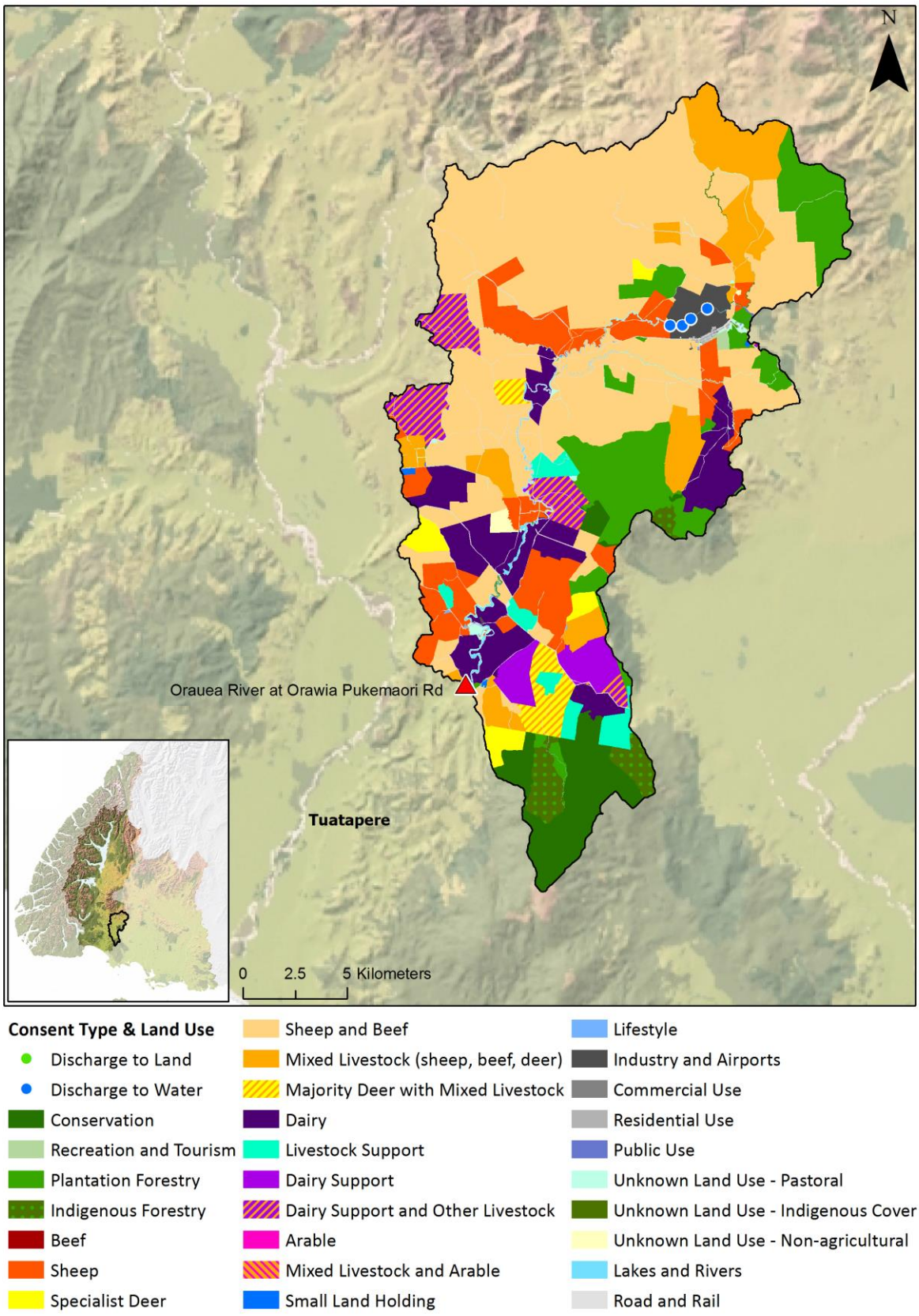

Figure 28. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Orauea River at Orawia Pukemaori Road sampling site.

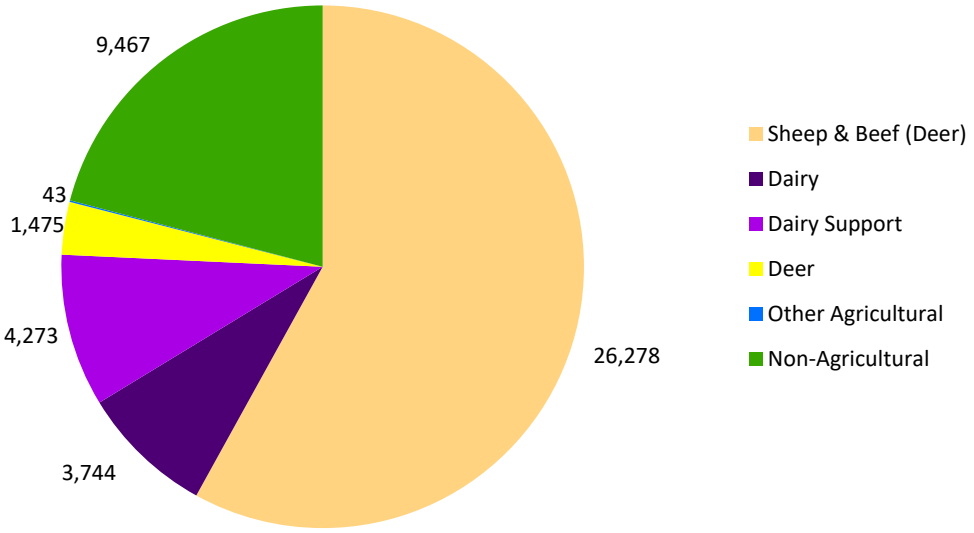

Figure 29. Land use (in hectares) in the catchment for the Orauea River at Orawia Pukemaori Road sampling site.

Sheep & Beef (Deer) (Sheep, Beef, Sheep and Beef, Mixed Livestock, Unknown Land Use – Pastoral, Mixed Livestock and Arable), **Dairy** (Dairy), **Dairy Support** (Dairy Support, Dairy Support and Other Livestock, Livestock Support), **Deer** (Specialist Deer, Majority Deer with Mixed Livestock), **Other Agricultural** (Small Land Holding, Lifestyle, Other Animals, Flower & Bulb Growers, Nurseries and Orchards, Horticulture), **Non-agricultural** (Commercial, Conservation, Indigenous Forestry, Plantation Forestry, Public Use, Recreation and Tourism, Residential Use, Road and Rail, Unknown Land Use - Indigenous Cover, Unknown Land Use - Non-agricultural, Lakes and Rivers, Industry and Airports), **Arable** (Arable). Based on 2015 Southland Land Use Information

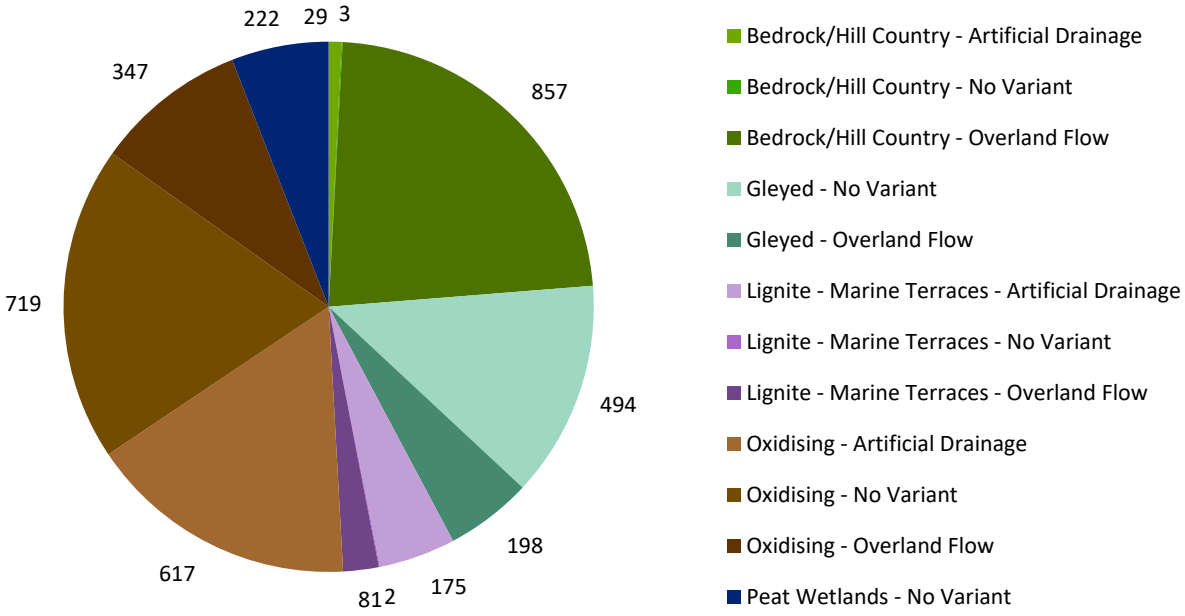

Figure 30. Dairying land (in hectares) in the catchment for the Orauea River at Orawia Pukemaori Road sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 13. Number of consented catchment discharges to land and water in the catchment for the Orauea River at Orawia Pukemaori Road sampling site.

Orauea River at Orawia Pukemaori Road		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	11
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
	Filter Backwash	1
	Mine Waste, Sludge	1
	Pesticides/Herbicides	2
To Land Total		16
To Water	Ground water, Mine Waste	1
	Hydro electric power generation sundry contaminant, Water (Hydro)	1
	Mine water	1
	Mine water, Stormwater	1
	Stormwater	3
To Water Total		7
Grand Total		23

Note: Consent information accurate as of April 2017

B.5 LILL BURN AT LILL BURN MONOWAI ROAD

Water samples were collected from Lill Burn at Lill Burn-Monowai Road on six occasions, between April and December 2015 (Table 14). Two samples (April and October) were collected under base flow conditions, while the remainder (May, June, July and December) were collected following rainfall. Microbial burden appeared reasonably constant across the samples, other than December, although the sources of contamination were variable.

Under base flow conditions, *E. coli* levels were 110 and 340 cfu/100 ml, with low levels of *Campylobacter* (0.4 MPN/100 ml; *C. jejuni* from a wildfowl source) detected in the April sample. Faecal source tracking analysis found that under base flow conditions, ruminant pollution accounted for 10-50% of the faecal pollution present at this site. The only FST markers detected in these samples was the wildfowl GDF marker present in April.

Following rainfall, *E. coli* levels were recorded at 300-400 cfu/100ml between May and July, with much higher levels present in December (7,100 cfu/100ml). Low levels of *Campylobacter* were isolated from all four post-rain samples, and were identified as *C. jejuni*. MBiT source analysis identified a ruminant source in the June sample, while wildfowl sources were identified in July and December. Three of the samples also had a 'not wildfowl' (i.e. likely ruminant or human) source identified. Faecal source tracking analysis found that in contrast to base flow conditions, ruminant pollution dominated the site following rainfall, accounting for up to 100% of faecal pollution in three of the four samples. Ovine markers were present in each sample, and bovine markers also present in three samples (June, July and December). Wildfowl markers were also present in these same three samples.

Land use in the Lill Burn sub-catchment is predominantly non-agricultural (approximately 83%; conservation, indigenous forestry and plantation forestry), with some mixed livestock (beef, sheep and deer) and livestock support activity (Figure 31, Figure 32).

Table 14. Results for microbial and FST analysis of water samples collected from Lill Burn at Lill Burn Monowai Road.

Site		Lill Burn at Lill Burn Monowai Road					
Sample #		CMB150398	CMB151775	CMB150564	CMB150815	CMB151036	CMB152267
Client #		20151656	20153345	20151870	20152117	20152703	20154557
Date Sampled		16/04/2015	15/10/2015	14/05/2015	11/06/2015	9/07/2015	16/12/2015
Rainfall		No	No	Yes	Yes	Yes	Yes
		Microbial Properties					
Faecal coliforms		340	120	400	400	400	7,500
E. coli		340	110	400	300	400	7,100
Campylobacter		0.4	<0.3	2.3	2.1	0.7	4.3
Campylobacter Species		*C. jejuni*	nt	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl	1				2	1
	Ovine/Bovine/ Deer				2		
	Poultry						
	Not Wildfowl			1	2		3
	Unknown						
		Faecal Source Tracking					
General - GenBac3		++++	+++	++++	++++	++++	++++
Ruminant		10-50%	10-50%	10-50%	50-100%	50-100%	50-100%
Human - BacH		+	-	-	+	+	-
Human - BiADO		-	-	-	-	-	-
Cow		-	-	-	+	+	+
Sheep		-	-	+	+	+	+
Wildfowl - GFD		+	-	-	+	+	+
Wildfowl - E2		-	-	-	+	-	-

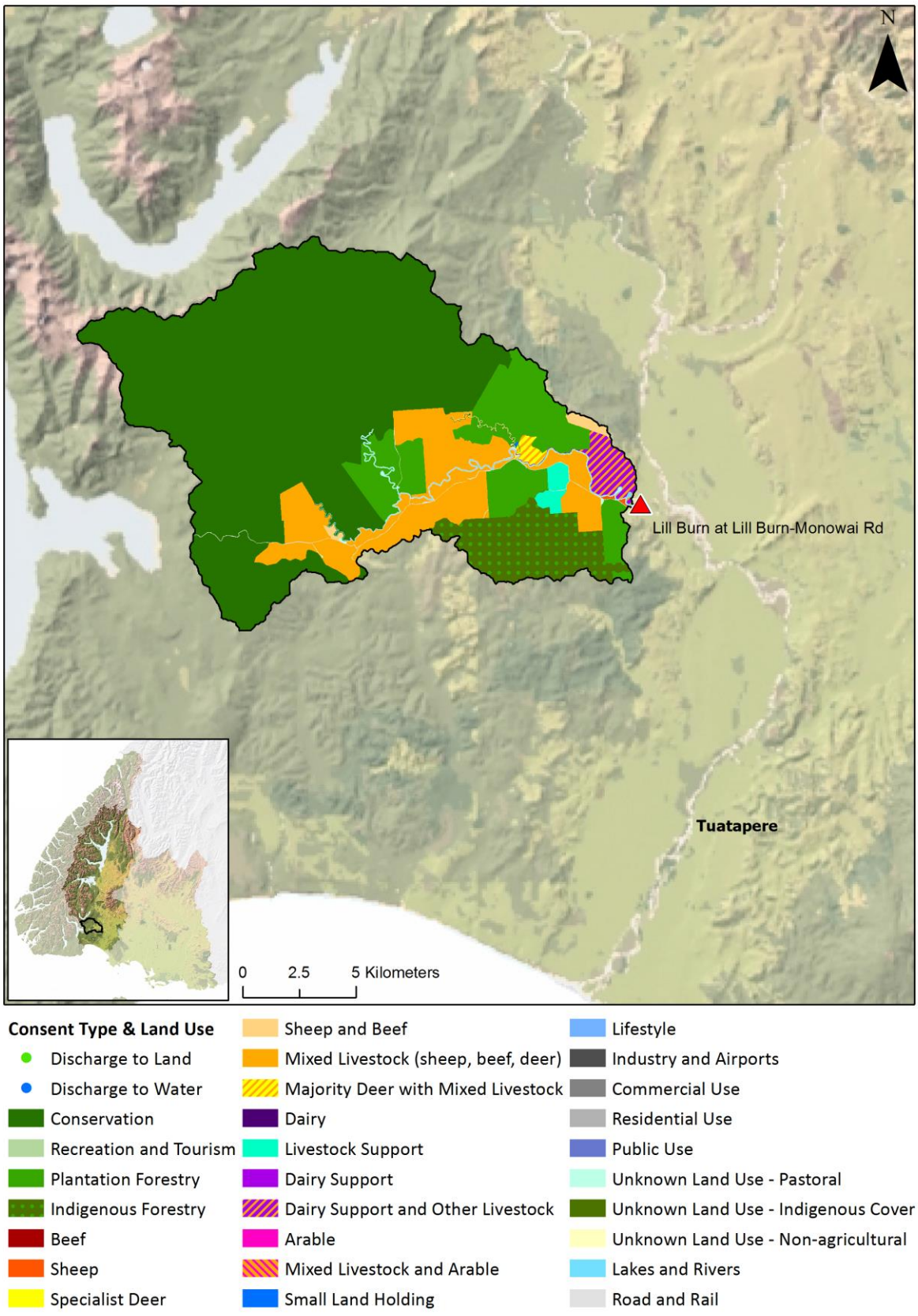

Figure 31. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Lill Burn at Lill Burn-Monowai Road sampling site.

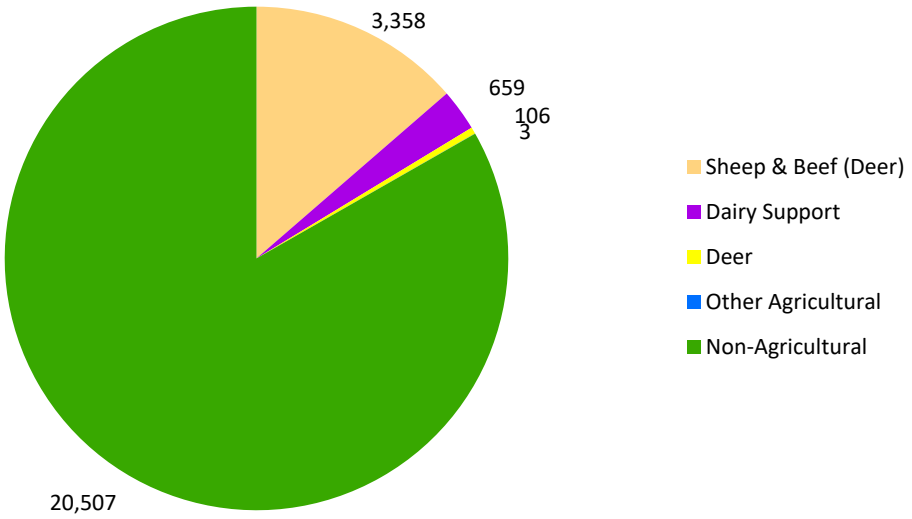

Figure 32. Land use (in hectares) in the catchment for the Lill Burn at Lill Burn-Monowai Road sampling site.

Sheep & Beef (Deer) (Sheep, Beef, Sheep and Beef, Mixed Livestock, Unknown Land Use – Pastoral, Mixed Livestock and Arable), **Dairy** (Dairy), **Dairy Support** (Dairy Support, Dairy Support and Other Livestock, Livestock Support), **Deer** (Specialist Deer, Majority Deer with Mixed Livestock), **Other Agricultural** (Small Land Holding, Lifestyle, Other Animals, Flower & Bulb Growers, Nurseries and Orchards, Horticulture), **Non-agricultural** (Commercial, Conservation, Indigenous Forestry, Plantation Forestry, Public Use, Recreation and Tourism, Residential Use, Road and Rail, Unknown Land Use - Indigenous Cover, Unknown Land Use - Non-agricultural, Lakes and Rivers, Industry and Airports), **Arable** (Arable). Based on 2015 Southland Land Use Information

There is no dairying in the Lill Burn at Lill Burn-Monowai Road sub-catchment.

Table 15. Number of consented catchment discharges to land and water in the catchment for the Lill Burn at Lill Burn-Monowai Road sampling site.

Lill Burn at Lill Burn-Monowai Road		
Subtype	Contaminant	Total
To Water	Mine water, Wash Water	1
To Water Total		1
Grand Total		1

REFERENCES

Adhikari B, Connolly JH, Madie P, Davies PR. 2004. Prevalence and clonal diversity of *Campylobacter jejuni* from dairy farms and urban sources. *New Zealand Veterinary Journal* 52, 378-383.

Ahmed F. 1999. *Animal Sources of Human Campylobacteriosis*. MSc thesis. Massey University, Palmerston North, New Zealand.

Atherholt TB, LeChevallier MW, Norton WD, Rosen JS. 1998. Effect of rainfall on *Giardia* and *Cryptosporidium*. *Journal of American Water Works Association*, 90, 66-80

Atwill ER, Li X, Grace D, Gannon V. 2012. Zoonotic waterborne pathogen loads in livestock, p 115-56. *In* Dufour A, Bartram J, Bos R, V G (ed), *Animal Waste, Water Quality and Human Health*. Published on behalf of WHO by IWA Publishing, Glasgow.

Bagshaw, C.S. (2002). *Factors influencing direct deposition of cattle faecal material in riparian zones*. MAF Technical Paper No. 2002/19. Ministry of Agriculture and Forestry, Wellington. 25p.

Bailey GD, Vanselow BA, Hornitzky MA, Hum SI, Eamens GJ, Gill PA, Walker KH, Cronin JP. 2003. A study of the foodborne pathogens: *Campylobacter*, *Listeria* and *Yersinia*, in faeces from slaughter-age cattle and sheep in Australia. *Communicable Diseases Intelligence* 27, 249-257.

Baker MG, Sneyd E, Wilson NA. 2007. Is the major increase in notified campylobacteriosis in New Zealand real? *Epidemiology and Infection* 135, 163-170.

Ball A,Till D. 1998. *Review of Potential Waterborne Human Pathogens in New Zealand.* Prepared for the Ministry for the Environment. ESR Client Report CSC97/22. Institute of Environmental Science and Research, Christchurch, New Zealand.

Black RE, Levine MM, Clements ML, Highes, TP, Blaser MJ. 1988. Experimental *Campylobacter jejuni* infection in humans. *Journal of Infectious Diseases* 157, 472–479.

Bolton DJ, O'Neill CJ, Fanning S. 2012. A preliminary study of *Salmonella*, verocytotoxigenic *Escherichia coli/Escherichia coli* O157 and *Campylobacter* on four mixed farms. *Zoonoses and Public Health* 59, 217-228.

Browning GF, Chalmers RM, Snodgrass DR, Batt RM, Hart CA, Ormarod SE, Leadon D, Stoneham SJ, Rossdale PD. 1991. The prevalence of enteric pathogens in diarrhoeic thoroughbred foals in Britain and Ireland. *Equine Veterinary Journal* 23, 405-409.

Bunic S, Avery SM. 1997. *Escherichia coli* O157LH7 in health dairy cows*. New Zealand Veterinary Journal* 45, 45-48.

Callaway TR, Keen JE, Edrington TS, Bumgard LH, Spicer L, Fonda ES, Griswold KE, Overton TR, van Amburgh ME, Anderson RC, Genovese KJ, Pool TK, Harvey RB, Nisbet DJ. 2005. Faecal prevalence and diversity of *Salmonella* species in lactating dairy cattle in four states. *Journal of Dairy Science* 88, 3603-3608.

Castro-Hermida JA, Almeida A, Gonzalez-Warleta M, Correia da Costa JM, Rumbo-Lorenzo C, Mezo M. 2007. Occurrence of Cryptosporidium parvum and Giardia duodenalis in health adult domestic ruminants. *Parasitology Research* 101, 1443-1448.

Close M, Dann R, Ball A, Pirie R, Savill M, Smith Z. 2008. Microbial groundwater quality and its health implications for a border-strip irrigated dairy farm catchment, South Island, New Zealand. *Journal of Water and Health* 6, 83-98.

Collins R. 2004. Faecal contamination of pastoral wetlands. *Journal of Environmental Quality* 33, 1912-1918.

Collins R, Ross C, Donnison A, McLeod M. 2003. *Riparian Attenuation of Feacal Microbes*. MAF Technical Paper No 2002/16. Ministry of Agriculture and Forestry, Wellington, New Zealand. 16p.

Collins R, McLeod M, Donnison A, Ross C. 2005. Surface runoff and riparian management III. Objective 9 of the Pathogen Transmission Routes Research Programme. NIWA Client Report HAM2005-054. Prepared for the Ministry of Agriculture and Forestry. 12p.

Collins R, McLeod M, Hedley M, Donnison A, Close M, Hanly J, Horne D, Ross C, Davies-Colley R, Bagshaw C, Matthews L. 2007. Best management practises to mitigate faecal contamination by livestock of New Zealand waters. *New Zealand Journal of Agricultural Research* 50, 267-278.

Cookson AL. Croucher D, Pope C, Bennet J, Thomson-Carter F, Attwood GT. 2006. Isolation, characterisation and epidemiological assessment of Shiga toxin-producing *Escherichia coli* O84 isolates from New Zealand. *Journal of Clinical Microbiology* 44, 1863-1866.

Cornelisen CD, Gillespie PA, Kirs M, Young RG, Forrest RW, Barter PJ, Knight BR, Harwood VJ. 2011. Motueka River plume facilitates transport of ruminant faecal contaminants into shellfish growing waters, Tasman Bay, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 45 (3), 477-495.

Davies RH, Dalziel R, Gibbens JC, Wilesmith JW, Ryan JMB, Evans SJ, Byrne C, Paiba GA, Pascoe SJS, Teale CJ. 2004. National survey for *Salmonella* in pigs, cattle and sheep and slaughter in Great Britain (1999-2000). *Journal of Applied Microbiology* 96, 750-760.

Davies-Colley RJ, Nagels JW, Smith RA, Young RG, Phillips CJ. 2004. Water quality impact of a dairy cow herd crossing a stream. *New Zealand Journal of Marine and Freshwater Research* 38, 569-576.

Devane ML, Gilpin BJ. 2015. Human Health Risks of Faecal Pollution from Different Sources. A Review of the Literature. Report No. CSC 15019. Prepared for Environment Canterbury, Community and Public Health, Christchurch City Council, and the Ministry of Health. Institute of Environmental and Science Research, Christchurch, New Zealand.

Devane ML, Nicol C, Ball A, Klena JD, Scholes P, Hudson JA, Baker MG, Gilpin BJ. 2005. The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. *Journal of Applied Microbiology* 98, 980-990.

Devane ML, Robson B, Nourozi F, Wood D, Gilpin BJ. 2013. Distinguishing human and possum faeces using PCR markers. *Journal of Water and Health* 11, 397-409.

Edwards AC, Kay D, McDonald AT, Francis C, Watkins J, Wilkinson JR, Wyer MD. 2008. Farmyards: an overlooked source for highly contaminated runoff. *Journal of Environmental Management* 87, 51-59.

ESR. 2007. *Notifiable and other Diseases in New Zealand. Annual Report* 2006. Prepared for the Ministry of Health. Institute of Environmental and Science Research (ESR), Porirua, New Zealand. 60p.

ESR. 2017. *Notified Diseases in New Zealand: Annual Report 2016*. Prepared for the Ministry of Health. Institute of Environmental and Science Research (ESR), Porirua, New Zealand. 63p.

Eyles R, Niyogi D, Weinstein P, Townsend T, Brooks H, Trott A. 2002. Ecosystem Change and *Campylobacter* in Freshwaters: A New Zealand Perspective. In: *Society for Applied Microbiology Summer Conference: Pathogens in the Environment and Changing Ecosystems, Nottingham, UK*. Society for Applied Microbiology, London, UK.

Fakir JD. 1986*. A study of thermophilc Campylobacter in cattle, sheep, and laboratory animals*. MPhil thesis, Massey University, Palmerston North, New Zealand.

Field K, Samadpour M. 2007. Faecal source tracking, the indictor paradigm, and managing water quality. *Water Research* 41, 3517-3538.

Fong TT, Lipp EK. 2005. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiology and Molecular Biology Reviews* 69, 357-371.

Fogarty LR, Haack SK, Wolcott MJ, Whitman RL. 2003. Abundance and characteristics of the recreational water quality indicator bacteria *Escherichia coli* and enterococci in gull faeces. *Journal of Applied Microbiology* 94, 865-878.

French NP, Midwinter A, Holland B, Collins-Emerson J, Pattison R, Colles F, Carter P. 2009. Molecular epidemiology of Campylobacter jejuni isolates from wild-bird faecal material in children's playgrounds. *Applied and Environmental Microbiology* 75, 779-783.

Gilpin B, Devane M, Nourozi F, Wood D. 2007. *Faecal Indicators in Scats from Black Swans* (*Cygnus atratus*). Prepared for Envirolink, Report TSD30. Institute of Environmental Science, Christchurch, New Zealand. 19p.

Gilpin BJ, Thorrold B, Scholes P, Longhurst RD, Devane M, Nicol C, Walker S, Robson B, Savill M. 2008. Comparison of *Campylobacter jejuni* genotypes from dairy cattle and human sources from the Matamata-Piako district of New Zealand. *Journal of Applied Microbiology* 105, 1354-1360.

Gourmelon M, Caprais MP, Mieszkin S, Marti R, Wéry N, Jardé E, Derrien M, Jadas-Hécart A, Communal PY, Jaffrezic A, Pourcher AM. 2010. Development of microbial and chemical MST tools to identify the origins of the faecal pollution in bathing and shellfish harvesting waters in France. *Water Research* 44, 4812-4824.

Greening GE, Lewis GD. 2010. Aquaculture and Mariculture. In: *Water Health. Volume II.* Encyclopaedia of Life Support Systems (EOLSS) Publishers Co. Ltd, UNESCO, London. Pp. 196-212.

Grinberg A, Pomroy WE, Weston JF, Ayanegui-Alcerreca A, Knight D. 2005. The occurrence of *Cryptosporidium parvum*, *Campylobacter* and *Salmonella* in newborn dairy calves in the Manawatu region of New Zealand. *New Zealand Veterinary Journal* 53, 315-320.

Grinberg A, Pomroy WE, Carslake HB, Shi Y, Gibson IR, Drayton BM. 2009. A study of neonatal cryptosporidiosis of foals in New Zealand. *New Zealand Veterinary Journal* 57, 284-289.

Harvey RA, Harms H. 2002. Tracers in Groundwater: Use of Microorganisms and Microspheres. In Bitton G (ed). *Encyclopaedia of Environmental Microbiology.* Volume 6. pp. 3494-3202.

Harwood VJ, Staley C, Badgley BD, Borges K, Korajkic A. 2013. Microbial source tracking markers for detection of faecal contamination in environmental waters: relationships between pathogens and human health outcomes. *FEMS Microbiology Reviews* 38, 1-40.

Hatch JJ. 1996. Threats to public health from gulls (*Laridae*). *International Journal of Environmental Health and Research* 6, 5-16.

Heather B, Robertson H. 2005. *The Field Guide to the Birds of New Zealand*. Viking Books, Auckland. 432p.

Hedley M, Hanly J, Horne D, Midwinter A, Whelan N, Lonas G, Collins R, Donnison A, Ross C. 2004. Pathogen movement from dairy pastures through runoff and artificial drainage systems. Massey University, National Institute of Water and Atmospheric Research (NIWA) AgResearch,

Hewitt J, Williamson W. 2014*. Evaluation of faecal source tracking methods as an indicator for human faecal contamination in shellfish growing areas. Part 1: Background, literature and capability review*. Prepared for the Ministry of Primary Industries. ESR Client Report No. FW14026. Institute of Environmental Science and Research (ESR), Wellington, New Zealand. 64p.

Higgan Q, Holmes J, Clark G. 2001. Salmonella Brandenburg – an epidemiological study of animal and human cases using AgriBase and EpiSurv. In *SIRC 2001 – The 13th Annual Colloqium of the Spatial Information Research Centre*, University of Otago, Dunedin, New Zealand.

Hodson R, Dare, J, Merg ML, Couldrey M. 2017. *Water Quality in Southland: Current State and Trends. Technical Report.* Publication No. 2017-04. Environment Southland, Invercargill, New Zealand.

Hughes B, Wilson K. 2016. *Guide for using the Southland physiographic zones technical sheets*. Environment Southland Publication No. 2016/12. 39p.

Hurcombe SD, Fox JG, Kohn CW. 2009. Isolation of *Campylobacter fetus* subspecies fetus in a two-year old quarterhorse with chronic diarrhoea of an undetermined etiology. *Journal of Veterinary Diagnostic Investigation* 21, 266-269.

Jay-Russell MT, Madigan JE, Bengson Y, Madigan S, Hake AF, Foley JE, Byrne BA. 2014. *Salmonella oranienburg* isolated from horses, wild turkeys and an edible home garden fertilised with raw horse manure. *Zoonoses and Public Health* 61, 64-71.

Jellison KL, Distel DL, Hemond HF, Schauer DB. 2004. Phylogenetic analysis of the hypervariable region of the 18S rRNA gene of *Cryptosporidium* oocysts in the faeces of Canada geese (*Branta canadensis*): evidence for five novel genotypes. *Applied and Environmental Microbiology* 70, 452-458.

Jones K, Howard S, Wallace JS. 1999. Intermittent shedding of thermophilic campylobacters by sheep at pasture. *Journal of Applied Microbiology* 86, 531-536.

Kassa H, Harrington BJ, Bisesi MS. 2004. Cryptosporidiosis: a brief literature review and update regarding *Cryptosporidium* in feces of Canada geese (*Branta canadensis*). *Journal of Environmental Health* 66, 34-40.

Kay D, Crowther J, Stapleton CM, Wyer MD, Fewtrell L, Edwards A, Francis CA, McDonald AT, Watkins J, Wilkinson J. 2008. Faecal indicator organism concentrations in sewage and treated effluents. *Water Research* 42, 442-454.

Kudva IT, Blanch K, Hode CJ. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology* 64, 3166-3174.

Kunze DJ, Loneragan GH, Platt TM, Miller MF, Besser TE, Koomaraie M, Stephens T, Brashears MM. 2008. Salmonella enterica burden in harvest-ready cattle populations from the southern high plains of the United States. *Applied and Environmental Microbiology* 74, 345-351.

Learmonth JJ, Ionas G, Pita AB, Cowie RS. 2003. Identification and genetic characterisation of Giardia and Cryptosporidium strains in humans and dairy cattle in the Waikato region of New Zealand. *Water Science and Technology* 47, 21-26

Lee MD, Newell DG. 2006. Campylobacter in poultry: filling an ecological niche. *Avian Diseases* 50, 1-9.

McBride GB. 2012. Issues in Setting Secondary Contact Recreation Guidelines for New Zealand Freshwaters. National Institute for Water and Atmospheric Research, Hamilton, NZ.

McBride GB, Till D, Ryan T, Ball A, Lewis G, Palmer S, Weinstein P. 2002 *Freshwater Microbiology Research Programme. Pathogen Occurrence and Human Health Risk Assessment Analysis*. Ministry for the Environment Technical Publication (http://mfe.govt.nz/publications/water/freshwatermicrobiology-nov02/)

McBride GB, Ball A, French N, Harper S, Horn B, Lake R, Elliot S, Marshall J, van der Logt P. 2011. *Campylobacter in Food and the Environment: Examining the Link with Public Health*. MAF Technical Paper No: 2011/61. 21p.

McDowell RW. 2006. Contaminant losses in overland flow from cattle, deer and sheep dung. *Water, Air and Soil Pollution* 174, 211-222.

McDowell RW. 2008. Water quality of a stream recently fenced off from deer. *New Zealand Journal of Agricultural Research* 51, 291-298.

McDowell RW. 2009. The use of safe wallows to improve water quality in deer farmed catchments. *New Zealand Journal of Agricultural Research* 52, 81-90

McDowell RW, Paton RJ. 2004. Water and soil quality in an Otago deer farm. *Proceedings of the New Zealand Grasslands Association* 66, 187–193.

McDowell R, Wilcock R. 2008. Water quality and the effects of different pastoral animals. *New Zealand Veterinary Journal* 56, 289-96.

Meanger JD and Marshall RB. 1989. Seasonal prevalence of thermophilic Campylobacter infections in dairy cattle and a study of infection in sheep. *New Zealand Veterinary Journal* 37, 18-20.

MfE. 2008. *Proposed National Environmental Standard for On-Site Wastewater Systems. Discussion Document*. MfE Publication Number ME 890. Ministry for the Environment, Wellington.

MfE. 2018. Regional Information for Setting Draft Targets for Swimmable Lakes and Rivers. Published by the Ministry for the Environment on behalf of a joint taskforce of central and local government representatives. Ministry for the Environment (MfE), Wellington, New Zealand.

MfE, MoH. 2003. *Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas*. Ministry for the Environment (MfE) and Ministry of Health (MoH), Wellington, New Zealand.

Milnes AS, Stewart I, Clifton-Hadley FA, Davies RH, Newell DG, Sayers AR, Cheasty T, Cassar C, Ridley A, Cook AJC, Evans SJ, Teale CJ, Smith RP, McNally A, Toszeghy M, Futter R, Kay A, Paiba GA. 2008. Intestinal carriage of verocytotoxic *Escherichia coli* O157, thermophilic *Campylobacter* and *Yersinia enterolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. *Epidemiology and Infection* 136, 739-751

Mohan V, Steenson M, Marshall J, Fearnhead P, Holland BR, Hotter G, French NP. 2013. *Campylobacter jejuni* colonisation and population structure in populations of ducks and starlings in New Zealand. *Microbiology Open* 2, 659-673.

Monaghan RM. 2014. *The influence of land use and soil properties on contaminant accumulation and loss from farming systems*. Prepared for Environment Southland. RE500/2014/16. AgResearch, Lincoln, New Zealand. 52p.

Monaghan RM, de Klein CA, Muirhead RW. 2008. Prioritisation of farm scale remediation efforts for reducing losses of nutrients and faecal indicator organisms to waterways: a case study of New Zealand dairy farming. *Journal of Environmental Management* 87, 609-22.

Moore JE, Gilpin D, Crothers E, Canney A, Kaneko A, Matsuda M. 2002. Occurrence of *Campylobacter* spp., *Cryptosporidium* spp. in gulls (*Larus* spp.). *Vector Borne and Zoonotic Diseases 2*, 111-114.

Moriarty EM, Sinton LW, MacKenzie ML, Karki N, Wood DR. 2008. A survey of enteric bacteria and protozoans in fresh bovine faeces on New Zealand dairy farms. *Journal of Applied Microbiology* 105, 2015-2025.

Moriarty EM, Karki, N, MacKenzie M, Sinton LW, Wood DR, Gilpin BJ. 2011a. Faecal indicators and pathogens in selected New Zealand waterfowl. *New Zealand Journal of Marine and Freshwater Research* 45, 679-688.

Moriarty EM, MacKenzie ML, Karki N, Sinton LW. 2011b. Survival of *Escherichia coli*, enterococci and *Campylobacter* spp. in sheep faeces on pastures. *Applied and Environmental Microbiology* 77, 1797-1803.

Moriarty EM, McEwan N, MacKenzie ML, Karki N, Sinton LW, Wood DR. 2011c. Incidence and prevalence of microbial indicators and pathogens in ovine faeces in New Zealand. *New Zealand Journal of Agricultural Research* 54, 71-81.

Moriarty EM, Weaver L, Sinton LW, Gilpin BJ. 2012. Survival of *Escherichia coli*, enterococci and *Campylobacter jejuni* in Canada goose faeces on pasture. *Zoonoses and Public Health* 59, 490-497.

Moriarty EM, Downing M, Bellamy J, Gilpin BJ. 2015. Concentrations of faecal coliforms, *Escherichia coli*, enterococci and *Campylobacter* spp. in equine faeces. *New Zealand Veterinary Journal,* DOI 10.1080/00480169.2014.952789

Muirhead RW, Monaghan RM. 2012. A two reservoir model to predict *Escherichia coli* losses to water from pastures grazed by dairy cows. *Environment International* 40:8-14.

Muithead RW, Elliot AH, Monaghan RM. 2011. A model framework to assess the effect of fairy farms and wild fowl on microbial water quality during base flow conditions. *Water Research* 45, 2863-2874.

Murphy J, Devane ML, Robson B, Gilpin BJ. 2005. Genotypic characterisation of bacteria cultured from duck faeces. *Journal of Applied Microbiology* 99, 301-309.

Pang L, Robson B, Farkas K, McGill E, Varsani , Lea Gillot, Li J, Abraham P. 2017. Tracking effluent discharges in undisturbed stony soil and alluvial gravel aquifer using synthetic DNA tracers. *Science of the Total Environment* 592, 144-152.

Pantos O. 2017. *Recreational Shellfish Safety Monitoring: A Review of the Current Monitoring Programme and Methods*. Prepared for the Gisborne District Council. ESR Report No. CSC 17007. Institute for Environmental Science and Research, Christchurch.

Pattis, I. 2017. *Characterisation of microbial transport pathways and mechanisms into waterways*. Client Report No. CSC 17015. Prepared for Environment Southland. Institute of Environmental and Science Research, Christchurch, New Zealand.

Pattis I, Moriarty E, Billington C, Gilpin B, Hodson R, Ward N. 2017. Concentrations of *Campylobacter* spp., *Escherichia coli*, enterococci and *Yersinia* spp. in the faeces of farmed red deer in New Zealand. *Journal of Environmental Quality*, DOI 10.2134/jeq2017.01.0002

Pearson L. 2015. *Artificial subsurface drainage in Southland*. Technical Report. Publication No. 2015-07. 20p.

Perrucci S, Buggiani C, Sgorbini M, Cerchiai I, Otranto D, Traversa D. 2011. *Cryptosporidium parvum* infection in a mare and her foal with foal heat diarrhoea*. Veterinary Parasitology* 182, 333-336.

Pichner R, Sander A, Steinruck H, Gaeris M. 2005. Occurrence of *Salmonella* spp. and shigatoxin-producing *Escherichia coli* (STEC) in horse faeces and horse meat products. *Berl. Munch Tieraztl Wochenschr* 118, 321-325.

Pritchard GC, Smith R, Ellis Iverson J, CheatsyJ, Willshaw GA. 2009. Verocytotoxic *Escherichia coli* O157 in animals on public amenity premises in England and Wales, 1997 to 2007. *Veterinary Record* 164, 545-549

Oliver DM, Heathwaite AL, Haygarth PM, Clegg CD. 2005. Transfer of *Escherichia coli* to water from drained and undrained grassland after grazing. *Journal of Environmental Quality* 34, 918-924.

Oporto B, Esteban JI, Aduriz G, Juste RA, Hurtado A. 2007. Prevalence and strain diversity of thermophilic campylobacters in cattle, sheep and swim farms. *Journal of Applied Microbiology* 103, 977-984.

Quilez J, Torres E, Chalmers RM, Hadfield SJ, del Cacho E, Sanchez-Acedo C. 2008. Cryptosporidium genotypes and subtypes in lambs and goat kids in Spain. *Applied and Environmental Microbiology* 74, 6026-6031

Richards PL, David M, Georgakakis C, DeRose N, Rodgers MD. 2016. *Two New Techniques for Evaluating Connectivity of Septic Fields to Great Lake Watersheds and Embayments.* Technical Report 145. The College at Brockport, State University of New Work. 36p.

Richards S, Withers PJA, Paterson E, McRoberts CW, Stutter M. 2017. Potential tracers for tracking septic tank effluent discharges in watercourses. *Environmental Pollution* 228, 245-255.

Ritter L, Solomon K, Sibley P et al. 2002. Sources, pathways, and relative risks of contaminants in surface water and groundwater: A perspective prepared for the walkerton inquiry. *Journal of Toxicology and Environmental Health A* 65, 1-142

Robson J, Muirhead R, Laurenson S. 2015. *Direct faecal inputs to streams by sheep - is it a problem for water quality?* AgResearch Ltd, Invermay Agricultural Centre, Mosgiel, New Zealand.

Rohela M, Lim YA, Jamaiah I, Khadijah PY, Laang ST, Nazri MH, Murulhuda Z. 2005. Occurrence of *Cryptosporidium* oocysts in wrinkled hornbill and other birds in Kuala Lumpur National Zoo. *Southeast Asian Journal of Tropical Medicine and Public Health* 36, Supplement 4, 34-40.

Ryan UM, Bath C, Robertson I, Read C, Elliot A, McInnes L, Traub R, Besier B. 2005. Sheep may not be an important zoonotic reservoir for *Cryptosporidium* and *Giardia* parasites. *Applied and Environmental Microbiology* 71, 4992-4997.

Santin M, Trout JM, Fayer R. 2007. Prevalence and molecular characterisation of Cryptosporidium and Giardia species and genotypes in sheep in Maryland. *Veterinary Parasitology* 146, 17-24.

Scott TM, Rose JB, Jenkins TM, Farrah SR, Lukasik J. 2002. Microbial source tracfking: current methodology and future directions. *Applied and Environmental Microbiology* 68, 5796-5803.

Shepard ML, Swecker WS, Jensen RV, Ponder MA. 2012. Characterisation of the fecal bacteria communities of forage-fed horses by pyrosequencing of 16S rRNA V4 gene amplicons. *FEMS Microbiology Letters* 326, 62-68.

Sinton LW, Braithwaite RR, Hall, CH, MacKenzi ML. 2007. Survival of indicator and pathogenic bacteria in bovine faeces. *Applied and Environmental Microbiology* 73, 7917-7925.

Smith RP, Chalmers RM, Miller-Doblies D, Clifton-Hadley FA, Elwin K, Watkins J, Paiba GA, Hadfield SJ, Giles M. 2010. Investigation of farms linked to human patients with cryptosporidiosis in England and Wales. *Preventative Veterinary Medicine* 94, 9-17.

Soller JA, Schoen ME, Bartrand T, Ravenscroft JE, Ashbolt NJ. 2010. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. *Water Research* 44, 4674-4691.

Soller JA, Schoen ME, Varghese A, Ichida AM, Boehm AB, Eftim S, Ashbolt NJ, Ravenscroft JE. 2014. Human health risk implications on multiple sources of faecal indicator bacteria in a recreational waterbody. *Water Research* 66, 254-264.

Spurr EB, Coleman JD. 2005. *Review of Canada Goose Population Trends, Damage and Control in New Zealand*. Landcare Research Science Series No. 30. Manaaki Whenua Press, Lincoln, New Zealand.

Teunis P, van den Brandhof, Nauta M, Wagenaar J, van den Kerkhof H, van Pelt W. 2005. A reconsideration of the Campylobacter dose-response relation. *Epidemiology and Infection* 133, 583-592.

Traversa D, Otranto D, Milillo P, Latrofa MS, Giangaspero A, Di Cesare A, Paoletti B. 2012. *Giardia duodenalis* sub-Assemblage of animal and human origin in horses. *Infection, Genetics and Evolution* 12, 1642-1646.

USEPA. 2006. Chapter 17. Bacterial Indicators of Potential Pathogens. In *Voluntary Estuary Monitoring Manual*. Washington DC: United States Environmental Protection Agency (USEPA). EPA 842-B-06-003.

Wahlstrom H, Tysen E, Olsson Engvall E, Brandstrom B, Eriksson E, Morner T, Vagsholm I. 2003. Survey of *Campylobacter* species, VTEC O157 and *Salmonella* species in Swedish wildlife. *Veterinary Record* 153, 74-80.

Weaver RW, Entry JA, Graves A. 2005. Number of faecal streptococci and Escherichia coli in fresh and dry cattle, horse and sheep manure. *Canadian Journal of Microbiology* 51, 847-851.

WHO. 2011. *Guidelines for Drinking Water Quality*. 4th edition. Geneva: World Health Organisation (WHO).

Wilcock, RJ. 2006. *Assessing the Relative Importance of Faecal Pollution in Rural Catchments*. Prepared for Environment Waikato. NIWA Client Report HAM2006-104. National Institute of Water and Atmospheric Research, Hamilton, New Zealand.

Wilcock RJ, Monaghan RM, Quinn JM, Campbell AM, Thorrold BS, Duncan MJ, McGowan AW, Betteridge K. 2006. Land-use impacts and water quality targets in the intensive

dairying catchment of the Toenepi stram, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 40,123-140.

Wilcock RJ, Monaghan R, Thorrold B et al. 2007. Land-water interactions in five contrasting dairying catchments: Issues and solutions *Land Use and Water Resources Research* 7, 2.1-2.10

Wittum TE, Mollenkopf DF, Erdman MM. 2012. Detection of *Salmonella enterica* isolates producing CTX-M cephalosporinase in US livestock populations. *Applied and Environmental Microbiology* 78, 7487-7491.

Wood SA, Banks J, Hewitt J, Moriarty EM, Gilpin BJ. 2016. Advances in water and human health. In: *Advances in New Zealand Freshwater Science*. Jellyman PG, Davie, TJA, Pearson CP, Harding JS. (eds). New Zealand Freshwater Sciences Society and New Zealand Hydrological Society. pp 595-612.

Wu PY. 2001. *A longitudinal study of Campylobacter spp. on a New Zealand dairy farm*. MSc thesis, Massey University, Palmerston North, New Zealand.

Zhou L, Kassa H, Tischler ML, Xiao L. 2004. Host-adapted *Cryptosporidium* spp. in Canada geese (*Branta canadensis*). *Applied Environmental Microbiology* 70, 4211-4215.

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