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Sources of Pollution in the Oreti Freshwater Management Unit

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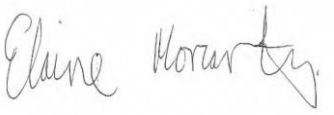

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 13 freshwater sites within the Oreti 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. Faecal source tracking (FST) tools including microbial and faecal sterol analyses were also undertaken to characterise the pollution sources for each site.

The freshwater sites sampled in this study were variable in their water quality, with *E. coli* concentrations ranging between 50 and 41,000 colony-forming units (cfu) per 100 ml of water. Forty-two percent of all water samples collected exceeded 1,000 cfu/100 ml, and seven sites had a median concentration exceeding 550 cfu/100 ml, highlighting their vulnerability to faecal contamination. 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. Some sites exhibited an increase in *E. coli* concentrations following rainfall, while others did not. A seasonal pattern was evident whereby peak microbial concentrations were observed during autumn and summer.

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

effluent discharge during base flow conditions, with additional inputs via overland flow and artificial tile drains following rainfall.

Human faecal contamination was also detected at five sites: Bog Burn downstream of Hundred Line Road, Makarewa River at Lora Gorge Road, Otepuni Creek at Nith Street, the Waihopai River upstream of Queen's Drive, and the Waikiwi Stream at North Road. Four of these sites had a human signature detected on more than one occasion, and three had a human signature under both base flow and following rainfall. Possible sources include septic tanks, stormwater, sewage discharge and urban runoff. Further investigations are strongly recommended at these sites to further identify the particular sources and transmission routes of this contamination, as human faecal sources are considered to pose the greatest risk to human health.

Campylobacter was detected in 88% of all samples, with detections at each of the 13 sites. *Campylobacter jejuni* was recovered from all *Campylobacter*-positive samples, with *C. coli* and an unspeciated thermophilic *Campylobacter* additionally detected in 8% and 12% of *Campylobacter*-positive samples, respectively. *Campylobacter* was equally present under base flow and following rainfall, although concentrations were nominally higher following rainfall. Wildfowl were determined to be the most common source of *Campylobacter*, being more than three times as prevalent as *Campylobacter* from "not wildfowl," poultry or ruminants (sheep, cattle or deer), which were all present in a similar number of samples. Six percent of samples had *Campylobacter* consistent with a human source. The prevalence of different sources varies depending on rainfall.

Molecular MBiT analysis of *Campylobacter* isolates revealed a high diversity of genotypes across the Oreti FMU, and that there was no separation of these to particular sites. Thirty-eight percent of the isolates obtained from waters in the Oreti 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. Approximately one third of these overlapping isolates were found to be of wildfowl origin, suggesting that wildfowl may be a lesser 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, 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). Further investigation is strongly recommended to determine the specific sources and routes of transmission of human contamination at five sites. Subsequent mitigation may include repair and/or replacement of infrastructure including septic tanks and sewerage pipes, and installation of improved stormwater treatment systems. Mitigation options for non-human sources 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(s), 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 multiple sources of faecal pollution (sheep and cattle). Photograph is 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 source(s) 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 Oreti FMU, Southland.

2. MATERIALS AND METHODS

2.1 SAMPLING SITES

The sampling locations selected across the Oreti Freshwater Management Unit (FMU) are listed in Table 1, and shown together with their sub-catchments (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.

Table 1. Sampling sites selected for the Oreti 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
Bog Burn, downstream of Hundred Line Road	Base-flow and rainfall	Appendix B.1
Carran Creek at Waituna Lagoon Road	Base-flow and rainfall	Appendix B.2
Makarewa River at Lora Gorge Road	Rainfall only	Appendix B.3
Makarewa River at Wallacetown	Rainfall only	Appendix B.4
Moffat Creek at Moffat Road	Rainfall only	Appendix B.5
Oreti River at Wallacetown	Rainfall only	Appendix B.6
Otapiri Stream at Otapiri Gorge	Rainfall only	Appendix B.7
Otepuni Creek at Nith Street	Base-flow and rainfall	Appendix B.8
Tussock Creek at Cooper Road	Base-flow and rainfall	Appendix B.9
Waihopai River upstream of Queen's Drive	Base-flow and rainfall	Appendix B.10
Waikiwi Stream at North Road	Base-flow and rainfall	Appendix B.11
Waituna Creek at Marshall Road	Rainfall only	Appendix B.12
Winton Stream at Lochiel	Base-flow and rainfall	Appendix B.13

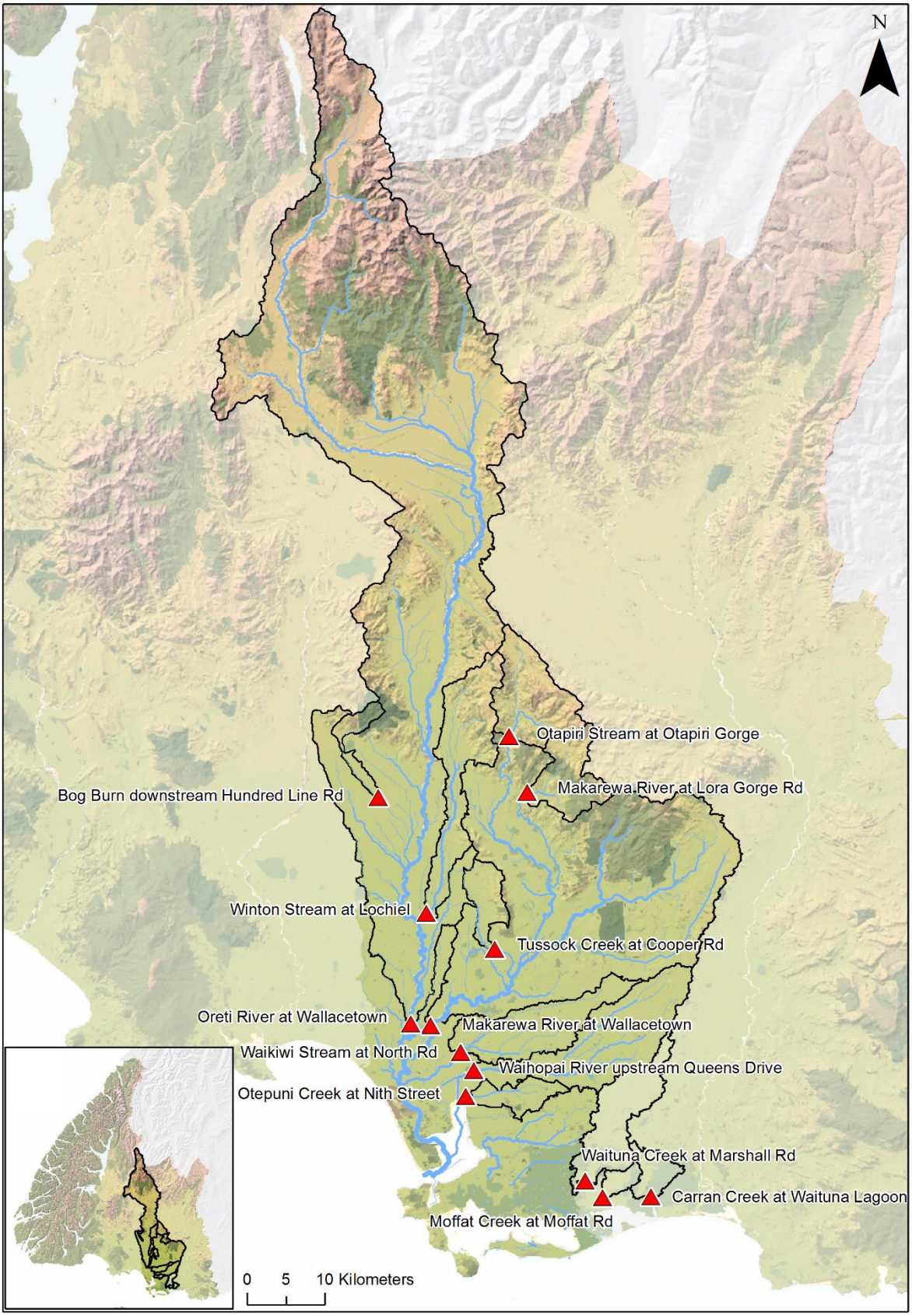

Figure 2. The Oreti FMU, with sub-catchments, sampling site locations and rivers of order 4 to 8 shown. Inset: The Oreti FMU within the wider Southland region.

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, three 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.
- Faecal sterol analysis (selected samples only).

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 44.5^{o}C (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).

2.2.5 Faecal sterol analysis

Water samples were filtered onto glass fibre filters and stored at -20^{o}C until analysis. Sterols were extracted from the filters using methods described by Gregor et al. (2002), and analysed using gas chromatography.

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 and temporal variation in microbiological water quality was observed at sites across the Oreti 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 ORETI FMU

Microbial water quality within the Oreti FMU was highly varied, with *E. coli* concentrations ranging between 50 and 41,000 cfu/100 ml. The majority of sampling locations selected within the Oreti FMU were vulnerable to very high levels of microbial contamination, with all but one site recording *E. coli* concentrations ≥1,000 cfu/100 ml (Figure 3, Figure 4).

Median *E. coli* concentrations exceeded 550 cfu/100 ml at 7 sites. In total, 42% of individual samples collected within the Oreti FMU had *E. coli* concentrations of 1,000 cfu/100 ml or more. The highest *E. coli* levels were observed at Tussock Creek at Cooper Road (41,000 cfu/100 ml, on two occasions), Bog Burn at Hundred Line Road (20,000 cfu/100 ml), Waihopai River upstream of Queen's Drive (19,000 cfu/100 ml) and the Makarewa River at Lora Gorge Road (14,000 cfu/100 ml).

There was no discernible pattern in *E. coli* or *Campylobacter* concentrations in relation to season or whether samples were collected under base flow conditions or following rainfall, however, it is noted that sites that were sampled under both conditions recorded their highest microbial concentrations following rainfall (Figures 3-5). It is also noted that the highest *E. coli* concentrations were recorded in May and December, and *Campylobacter* in May (Figure 6).

Campylobacter was isolated at all 13 sampling locations (Figure 3, Figure 4). In total, *Campylobacter* was detected in 88% of all the samples collected within the Oreti FMU, with 23% of samples having a concentration of ≥10 MPN/100 ml. The prevalence of *Campylobacter* was independent of antecedence rainfall, being detected in 86% of base-flow samples and 89% of those collected following rainfall, although concentrations were nominally higher following rainfall (Figure 7). The highest concentrations of *Campylobacter* were observed at Bog Burn at Hundred Line Road (460 MPN/100 ml), Makarewa River at Lora Gorge Road (240 MPN/100 ml), Tussock Creek at Cooper Road (110 MPN/100 ml) and the Otapiri Stream at Otapiri Gorge (93 MPN/100 ml). The highest concentrations were reported in samples collected in May (Figure 8).

All samples in which Campylobacter was detected contained *C. jejuni*. In addition, *C. coli* was identified in 8% of *Campylobacter*-positive samples, and an unspeciated thermophilic *Campylobacter* in 12% of *Campylobacter*-positive samples (Figure 9).

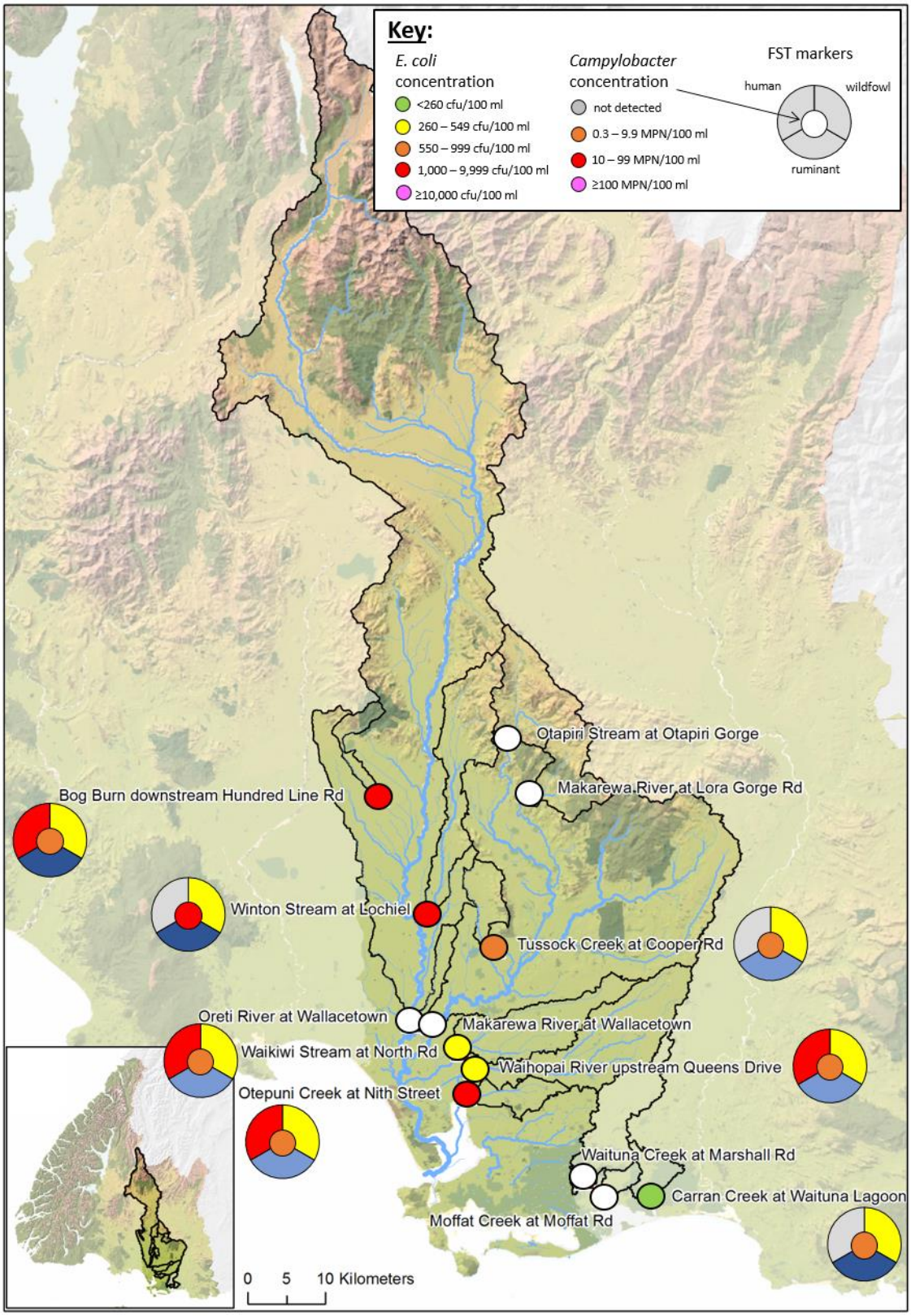

Figure 3. Overview of microbial water quality in Oreti FMU under base flow conditions. Small circles showing sampling locations on the map represent maximum *E. coli* levels for that site; white circles indicate there is no data under these conditions. Larger circles adjacent to site name represent maximum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

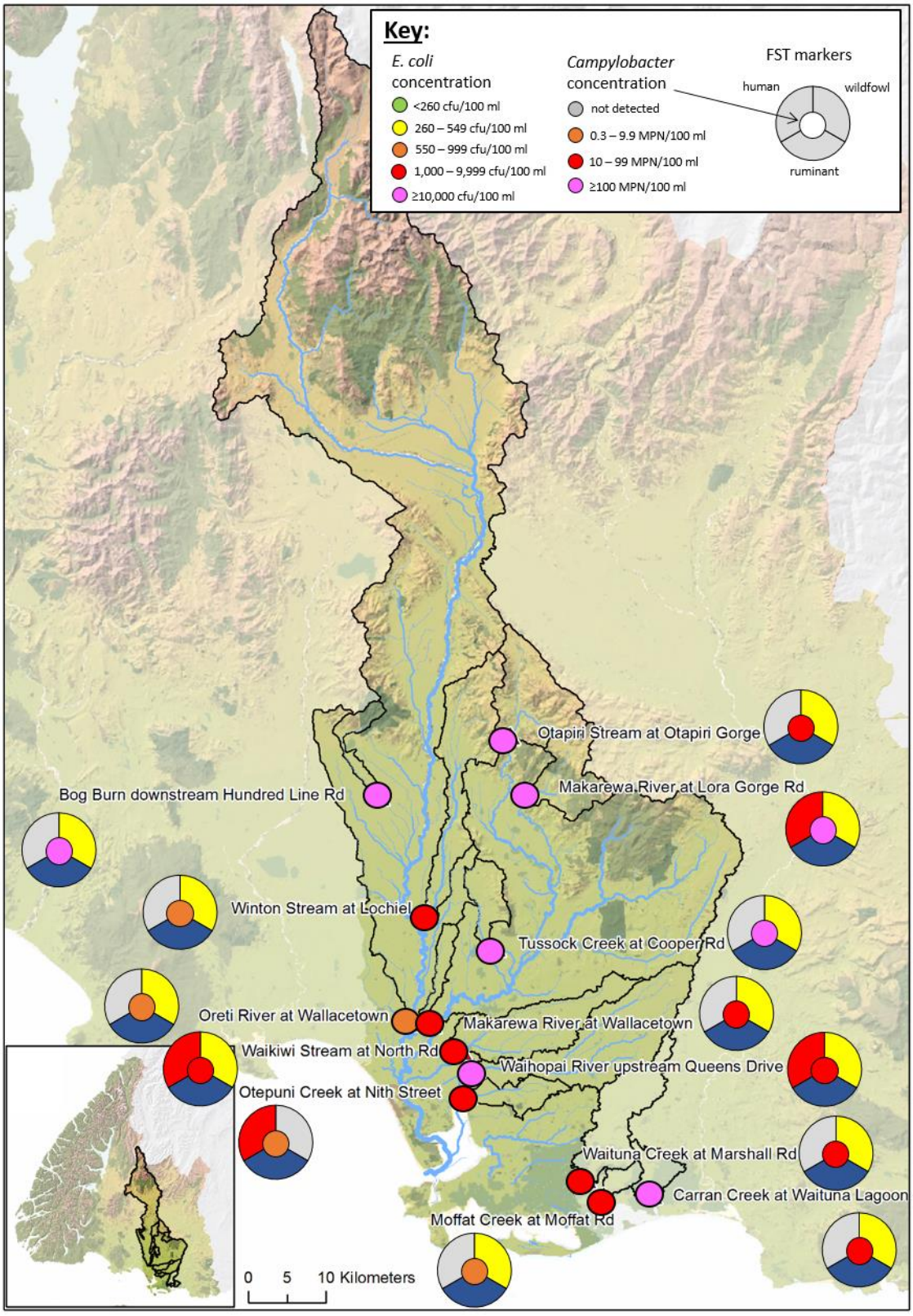

Figure 4. Overview of microbial water quality in Oreti FMU following rainfall. Small circles showing sampling locations on the map represent maximum *E. coli* levels for that site; white circles indicate there is no data under these conditions. Larger circles adjacent to site name represent maximum *Campylobacter*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

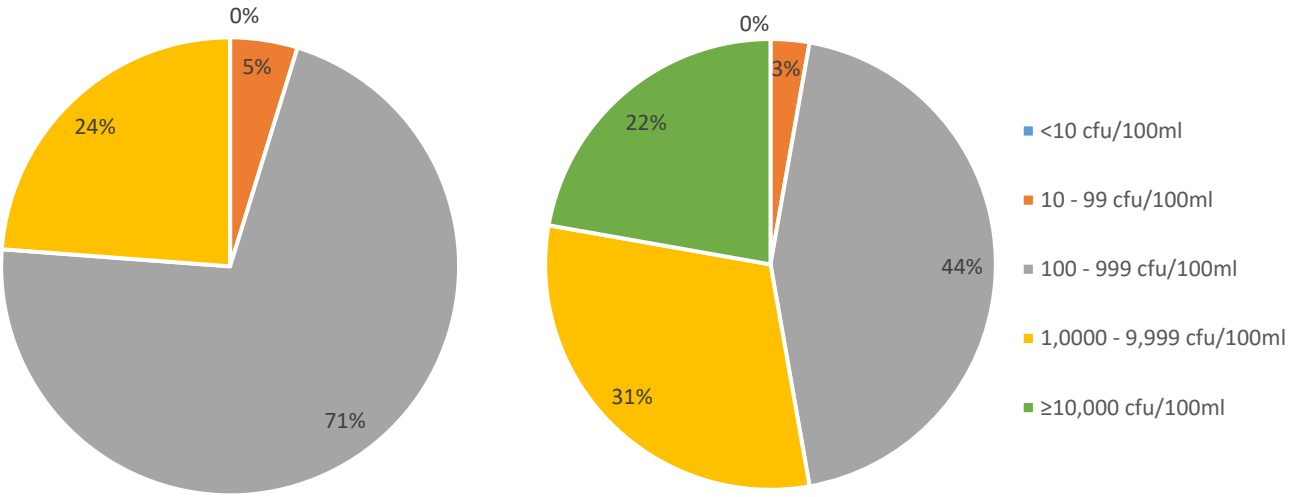

Figure 5. *E. coli* concentrations for water samples collected within the Oreti FMU under base flow conditions (left, n=21) and following rainfall (right, n=36).

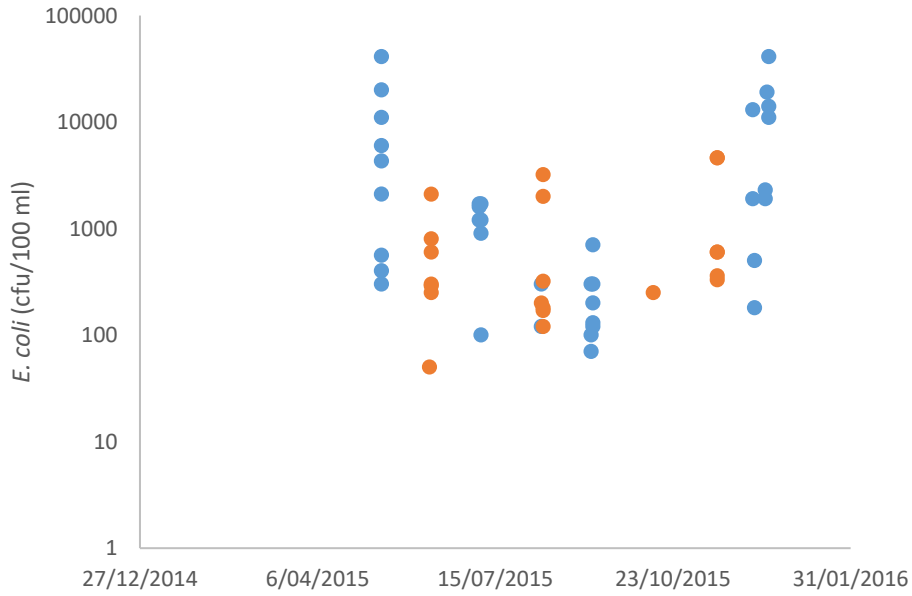

Figure 6. Concentration of *E. coli* at different sites in the Oreti FMU, across the course of the year. Samples collected following rainfall are shown in blue, and those collected under base flow conditions are in orange.

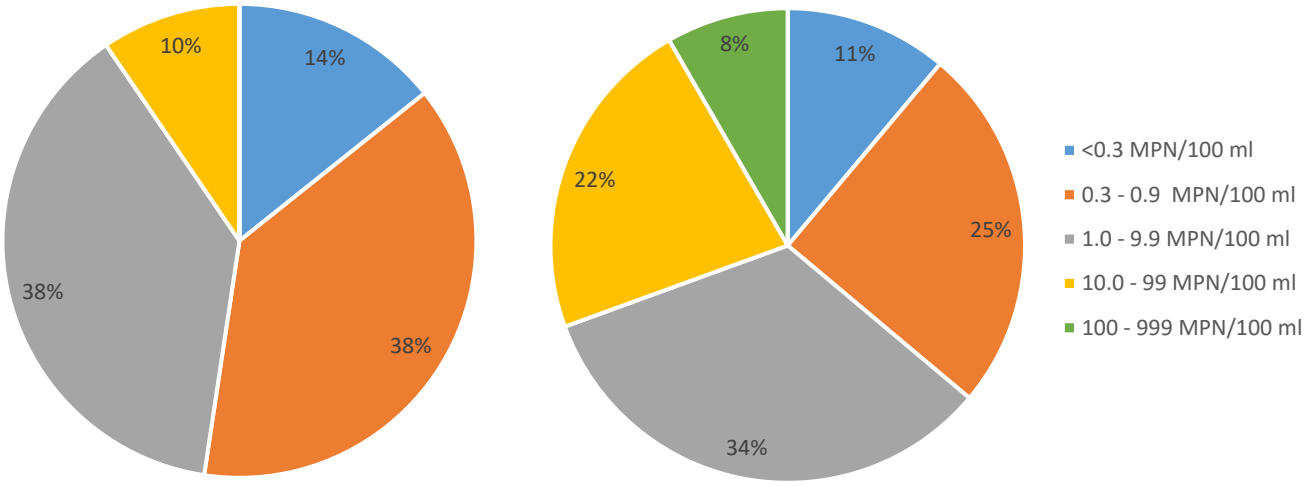

Figure 7. *Campylobacter* concentrations for water samples collected within the Oreti FMU under base flow conditions (left, n=21) and following rainfall (right, n=36).

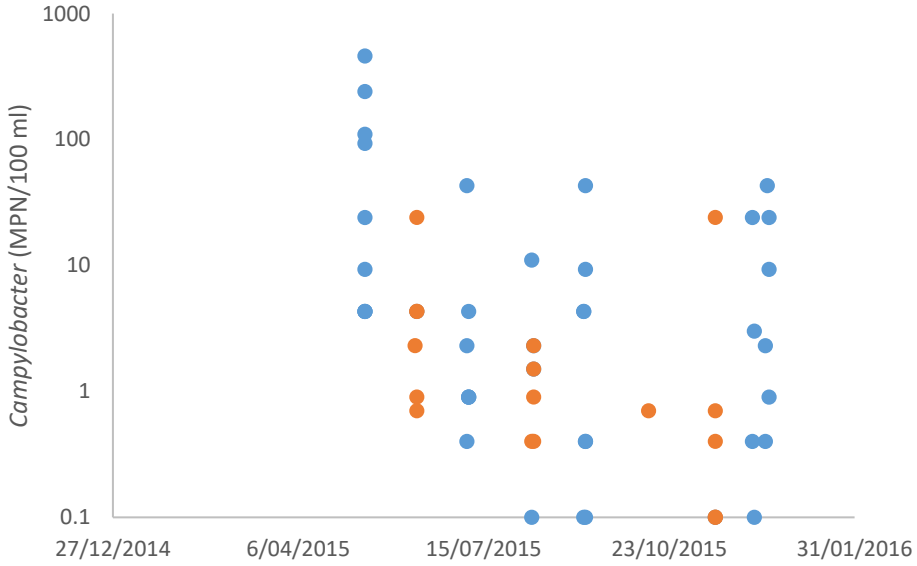

Figure 8. Concentration of *Campylobacter* at different sites in the Oreti FMU, across the course of the year. Samples collected following rainfall are shown in blue, and those collected under base flow conditions are in orange.

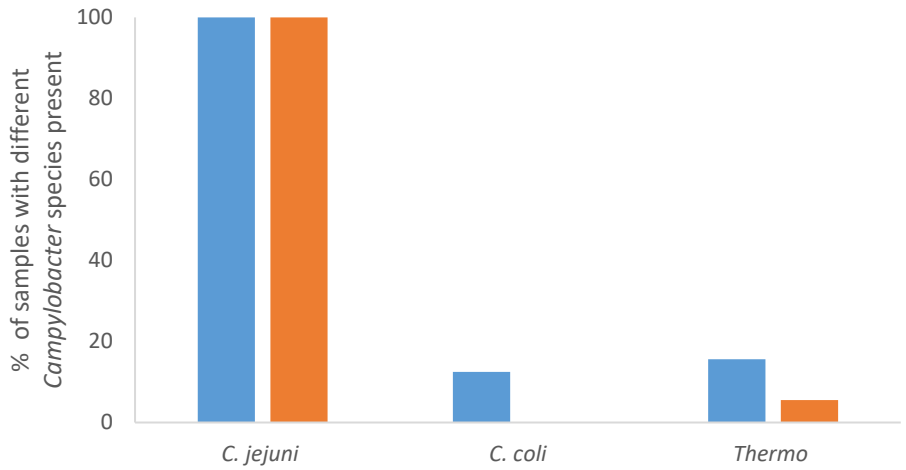

Figure 9. The prevalence of different *Campylobacter* species within *Campylobacter*-positive samples from the Oreti FMU (n=50). Blue bars represent samples collected following rainfall, and orange bars represent samples collected under base flow.

An examination of the relationship between *E. coli* and *Campylobacter* reveals a significant positive correlation of data (Spearman rank correlation, r = 0.4071 df = 55, p = 0.0017; Figure 10); thus samples with high levels of *E. coli* were more likely to contain high levels of *Campylobacter*.

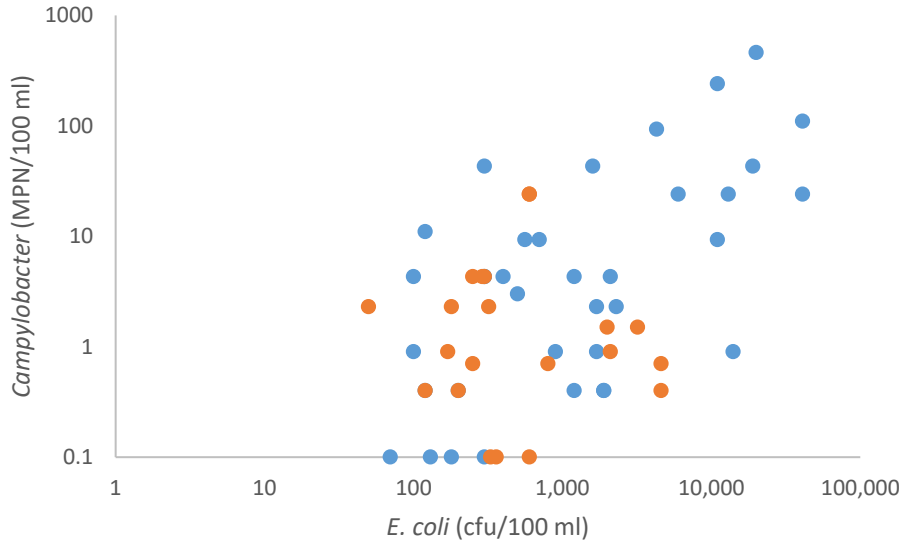

Figure 10. Relationship between *E. coli* and *Campylobacter* spp. concentrations in water samples collected within the Oreti FMU. Note that for the purposes of displaying the data on a logarithmic scale, samples in which no *Campylobacter* was detected, have been plotted as 0.1 MPN/100 ml. Samples collected 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 animal pollution was detected at all sites in the Oreti FMU (Figure 3, Figure 4), and was the dominant pollution source (i.e. accounted for 50-100% of pollution in at least half of samples) at 77% of sites. Bovine-specific FST markers were detected on at least one occasion at all 13 sites, with ovine markers detected at 12 sites.

The relative impact of ruminant sources was found to differ between samples collected under base flow and those collected following rainfall (Figure 3, Figure 4). For example, ruminant pollution accounted for up to 10% of total faecal pollution in 81% of all samples collected under base flow conditions. In contrast, ruminant pollution was the dominant pollution source in 77% of samples collected following rainfall (Figure 11). More specifically, 56% of samples collected following rainfall were positive for ovine contamination, and 50% for bovine contamination. In comparison, 29% and 24% of samples collected under base flow conditions were positive for ovine and bovine contamination, respectively (Figure 12).

Wildfowl faecal contamination was detected at all sites, although was more prevalent in samples collected under base flow (90% of samples collected) than following rainfall (61% of samples) (Figure 12).

Human faecal contamination was detected at five sites across the FMU, across a range of flow conditions. Positive samples were recorded from Bog Burn under base flow, Makarewa River at Lora Gorge Road following rainfall, and from Otepuni Creek at Nith Street, Waihopai River upstream of Queen's Drive and Waikiwi Stream at North Road, under both base flow and following rainfall (Figure 12).

Although only a small selection of samples was tested, canine-specific faecal markers were identified at Otepuni Creek at Nith Street and Tussock Creek at Cooper Road.

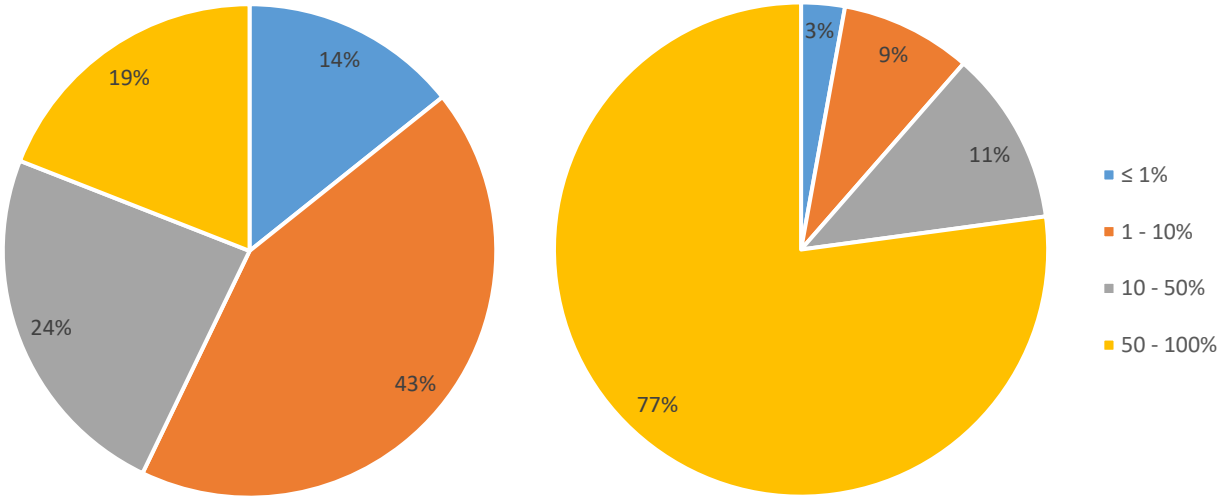

Figure 11. The proportion of samples collected under base flow conditions (left) and following rainfall (right), that were affected by different levels of ruminant faecal pollution.

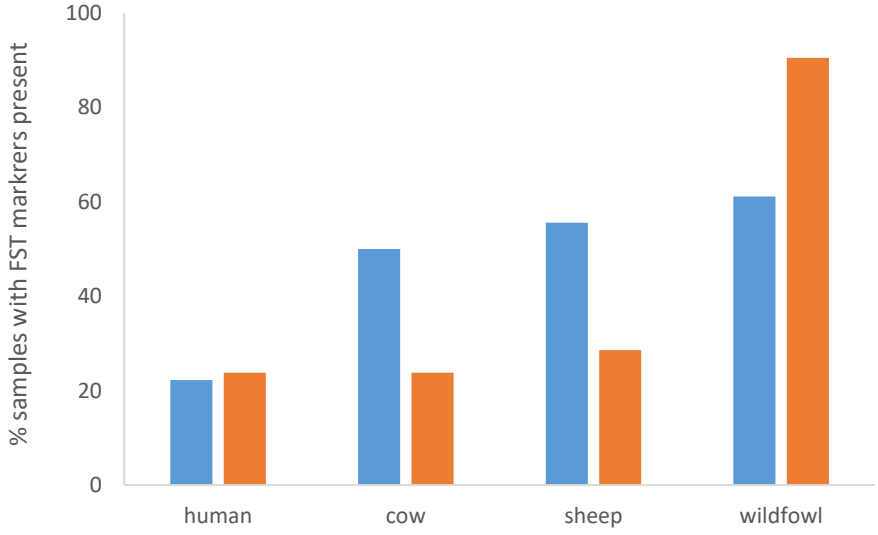

Figure 12. The percentage of samples collected from within the Oreti FMU that were positive for FST markers from different sources. Samples that were collected following rainfall are shown in blue, and those collected under base flow in orange.

3.3 CHARACTERISATION OF *CAMPYLOBACTER*

3.3.1 MBiT source attribution

MBiT source attribution analysis found that the *Campylobacter* isolated from the various sites across the Oreti FMU were of wildfowl, ruminant, poultry and human sources. Most sites were found to have *Campylobacter* from more than one source. Wildfowl were the most common source of *Campylobacter,* with 84% of *Campylobacter*-positive samples collected being positive for a wildfowl strain, followed by 'not wildfowl' (28%), poultry (26%), ovine/bovine/deer (24%) and human (6%) (Figure 13). 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 wildfowl and humans as a *Campylobacter* source did not appear to be greatly influenced by antecedence rainfall (Figure 13). In contrast, *Campylobacter* of ruminant, poultry and 'not wildfowl' origin tended to be more prevalent following rainfall than under base flow.

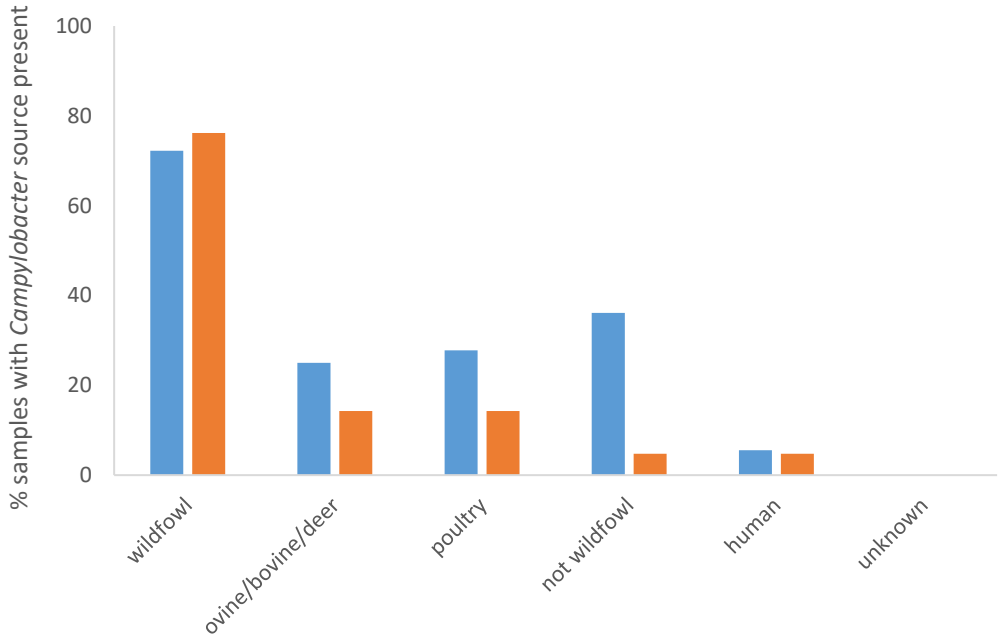

Figure 13. The percentage of *Campylobacter*-positive samples from the Oreti FMU that were identified as having different *Campylobacter* sources present (as determined by MBiT analysis). Samples that were collected following rainfall are shown in blue, and those collected under base flow in orange.

3.3.2 Genotype analysis and comparison with clinical isolates

Comparison of the MBiT genotype data for *Campylobacter* isolates from sites across the Oreti FMU (including additional isolates that were available from previous studies in the Oreti) revealed a high diversity of genotypes: of 276 isolates analysed, 112 different genotypes were identified. No clear pattern or separation of genotypes was observed based on the site from which isolates were collected (Figure 14). Comparison of the genotypes of isolates from the Oreti FMU with the isolates from the Mataura, Aparima and Waiau FMUs also shows no clear separation of genotype based on the FMU from which isolates were obtained (Figure 15).

Of the 276 individual isolates recovered from water samples in the Oreti FMU, 104 isolates (38%) representing 28 genotypes were found to 'overlap' with (i.e. were indistinguishable from) human clinical isolates from the Southland region (Figure 16). 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 104 isolates, only 32 (31%) are likely to have come from wildfowl, compared with 54% of the isolates from water samples being wildfowl-associated (Figure 17). 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 ten isolates (6%) were indistinguishable from wildfowl-associated isolates, suggesting wildfowl are a minor source of illness in the community.

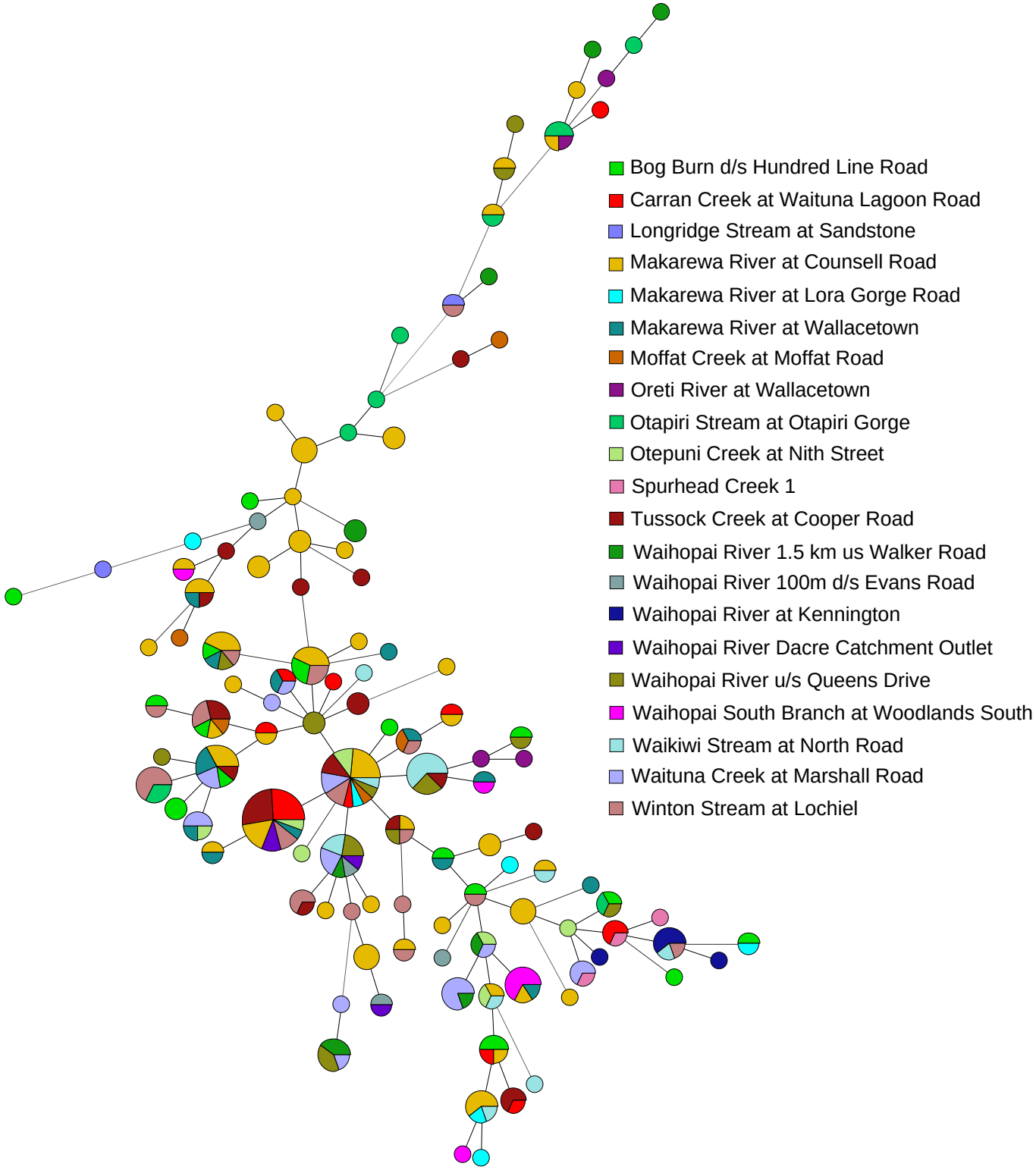

Figure 14. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from sites across the Oreti FMU, based on MBiT analysis. Each circle represents a different genotype, and each colour identifies a site. The number of circles and the spread of colours across the diagram demonstrates the diversity of genotypes within the Oreti FMU, and at individual sites.

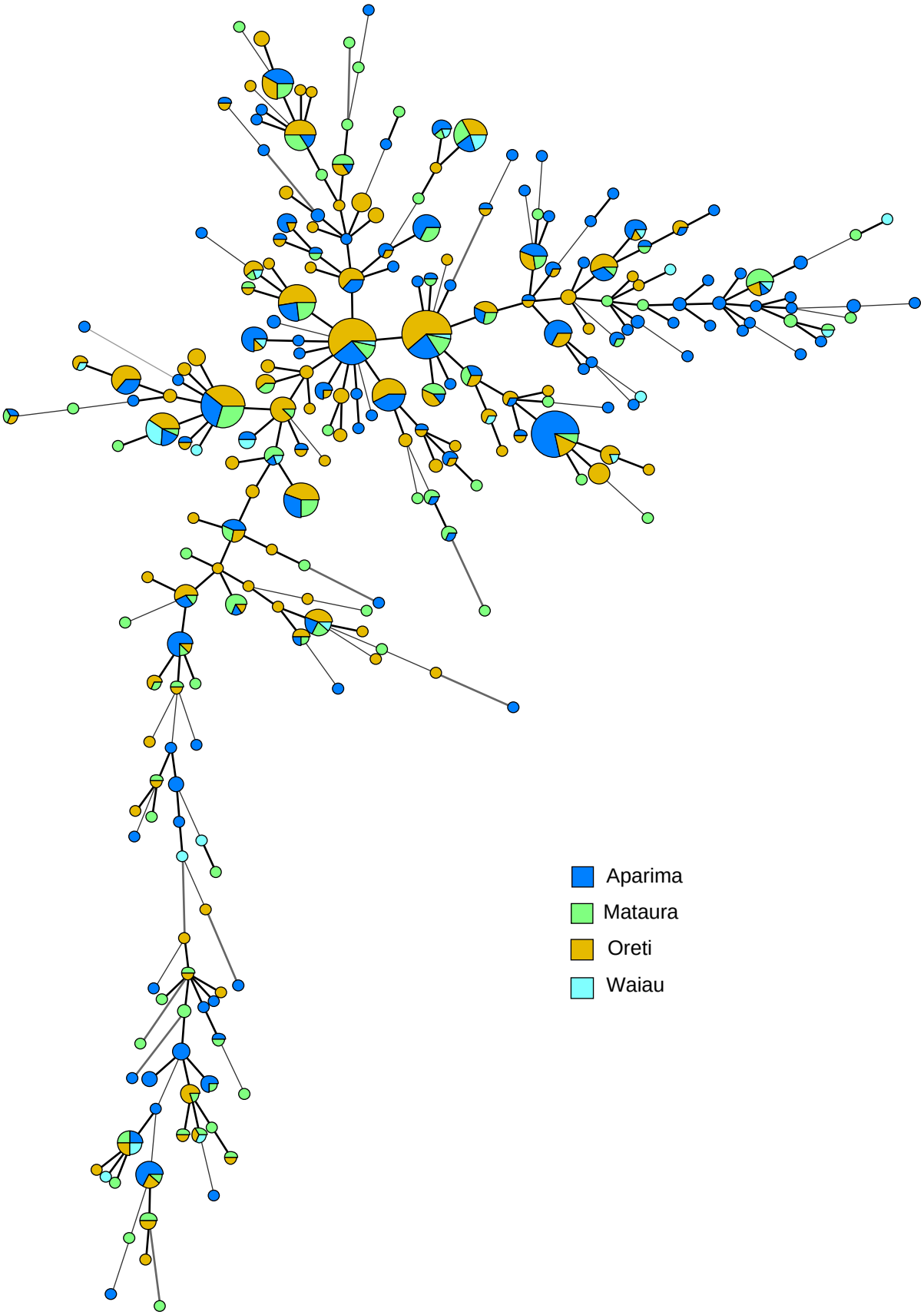

Figure 15. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from across the Southland region, based on MBiT analysis.

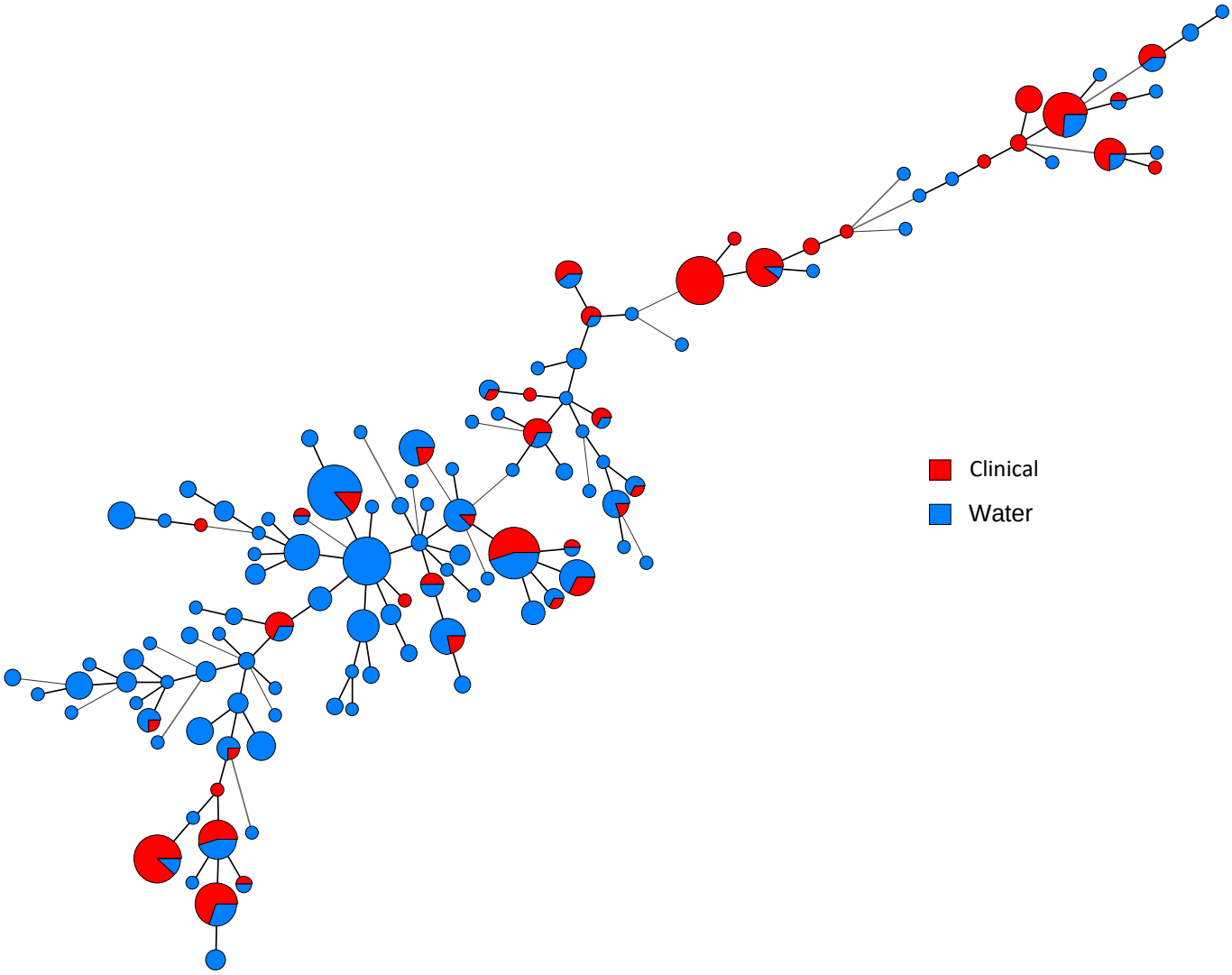

Figure 16. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from water samples from the Oreti FMU (blue) compared with human clinical isolates from the Southland region (red). Circles in which there are both blue and red segments indicate a genotype has been isolated from both the environment and clinical samples, representing the potential for human infection from waterborne sources.

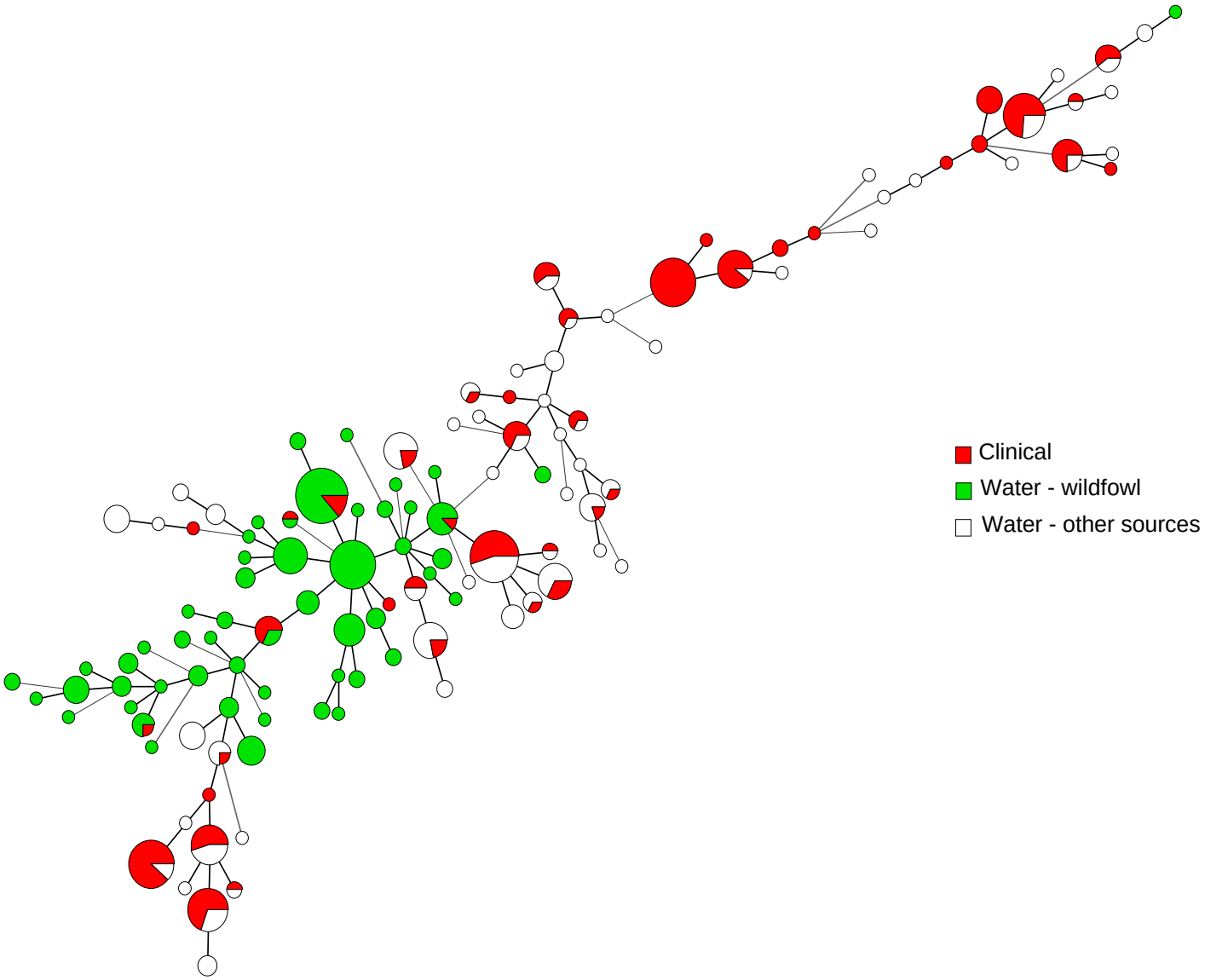

Figure 17. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from water from the Oreti FMU, highlighting those that are wildfowl-associated (green) compared with human clinical isolates (red).

4. DISCUSSION

4.1 MICROBIAL SOURCES AND TRANSMISSION

This study demonstrates that the microbial quality of waterways in the Oreti FMU is highly variable, with most of the sites being vulnerable to high levels of faecal contamination: *E. coli* concentrations varied between 50 and 41,000 cfu/100 ml, and 42% of all samples collected exceeding 1,000 cfu/100 ml. Seven of the thirteen sites had a median *E. coli* concentration exceeding 550 cfu/100 ml (i.e. the concentration above which the 2003 MfE/MoH Water Quality Guidelines recommend avoiding primary recreation such as swimming). High levels of variability in microbial concentration have previously been reported for waterways draining large, sparsely-populated rural catchments (e.g. Crowther et al., 2002, 2003), such as the Oreti FMU. Variables such as land use, topography and rainfall are known to influence the microbial burden of waterways (Collins et al., 2007); however, additional factors such as stocking densities, application of effluent to land, and livestock access to waterways also impact microbial water quality. This latter data is more difficult to obtain, particularly for large catchments, making it difficult to link water quality at individual sampling site to a single source, land use or management practice (Crowther et al., 2003; Monaghan et al., 2010). Further, as was the case for many of the sites sampled within the Oreti FMU, there are often multiple faecal sources, further compounding these issues (Muirhead et al., 2011). The map-based display of microbial data (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 a 'worst-case scenario' for each site, based on the data available. Given the limited amount of data and variable nature of the data collected for each site, 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 main sources of faecal pollution were wildfowl and ruminant animals: both sources were detected at all 13 sites. The combined presence of both ovine and bovine PCR markers is consistent with the large amount of agricultural activity in the catchment, which is dominated by mixed sheep and beef and dairy farming. Human contamination was also identified at a number of sites. Wildfowl, ovine, bovine and human faecal signatures were each detected under both base flow and post-rainfall across various sites.

Seven of the sites were sampled under both base- and high-flow conditions, although in some cases only a single sample was collected following rainfall. This makes interpretation of the impact of rainfall on water quality difficult, especially given the variability of the data. Some sites (e.g. Waihopai River, Waikiwi Stream) had higher microbial burdens (e.g. *E. coli*, *Campylobacter*) following rainfall, while others (e.g. Winton Stream, Otepuni Creek) were not different. At many of the sites however, there was a shift in the dominant faecal signature: ruminant pollution increased from 1-10% or 10-50% of the pollution present under base flow to 50-100% following rainfall. This suggests that rainfall-driven overland flow and/or preferential subsurface flow (e.g. via tile drains) from agricultural land are significant routes of transmission of faecal microbes to waterways in the Oreti FMU. Physiographic data for soils in the Oreti FMU show a prevalence of poorly-drained gleyed soils, bedrock/hill country that is prone to overland flow, oxidative soils with artificial drainage, and peat wetlands (Appendix B,

Hughes and Wilson, 2016). Surface runoff typically has high concentrations of faecal microbes, resulting from its interaction with faeces on the pasture. In addition, artificial drainage systems, namely mole or tile drains, are widespread across Southland, including the Oreti FMU; an estimated 76% of agricultural land within the Southland region likely has some form of artificial drainage (Monaghan, 2014; Pearson, 2015). The relative loss of faecal contaminants via runoff relative to drainage will differ between sites according to local characteristics such as soil type, land contour and density of drainage structures. The presence of ruminant pollution in waterways under base flow conditions likely results from direct deposition (e.g. stock access to unfenced waterways in pasture, passage through streams during stock movement between paddocks or to milking sheds), and/or discharge of effluents to rivers.

Seasonal patterns of agricultural contaminant loss to waterways have been demonstrated in several studies in the Southland and Otago regions, whereby rainfall and temperature are associated with the high rates of loss that are typically observed during autumn-early winter and spring (Oliver, 2005; Muirhead and Monaghan, 2012; Monaghan, 2014). A similar pattern was described in the report on faecal sources in the Mataura FMU that accompany this report. In the present study, the tendency towards peak *E. coli* concentrations in mid-to-late autumn is likely the result of faecal material accumulated on pasture over the drier summer months interacting with surface runoff during the first autumn rains. Increasing concentrations in summer may reflect greater survival of bacteria in the environment with increasing temperatures, or additional faecal contributions from lambs and calves. However, increasing the late autumn samples and summer samples were both collected following rainfall, making it difficult to apportion the effects of season and rainfall on microbial levels.

The presence of wildfowl pollution under both base flow and high flow conditions suggests that direct deposition occurs irrespective of rainfall; lower prevalence under high flow could reflect behavioural changes in the birds that reduce direct deposition during or following rainfall (e.g. if stream levels are high), or could reflect dilution.

Human faecal pollution was detected at five separate sites, with four having repeated detection of a human signature. Three sites had human pollution under both base flow and high flow conditions. Examination of the available land use and consented discharge information suggests the following potential sources for each of the contaminated sites.

- Bog Burn: Two base flow samples were positive for human contamination. At the time of sample collection there were no consents for discharge relating to human sewage – only dairy effluents to land. There is a small number of lifestyle blocks and small land holdings in the vicinity and upstream of the burn, suggesting that seepage and/or unconsented discharge from septic tanks may be entering the water.
- Makarewa River at Lora Gorge Road: One post-rain sample was positive for human contamination. Based on the available data, there is no consented discharge occurring to land or water in this sub-catchment. The catchment upstream of the sampling site is largely forested and agricultural land with a small number of residential dwellings within the vicinity of the river as it emerges from the forested upper catchment. Seepage from the septic tanks at these dwellings is a potential source of contamination.
- Otepuni Creek: All four samples from this site – three base and one high flow – were positive for human contamination. The Otepuni Creek site is in central Invercargill, and

is locally impacted by urban stormwater inputs (www.lawa.org.nz), which likely accounts for the presence of human contamination following rainfall; combined sewer overflows may also contribute. The presence of a human signal under base flow suggest that an additional route exists for transfer of contaminants – there is no noted consent to discharge sewage directly, however the potential for leaking pipes or cross-connections within the sewerage network should be considered.

- Waihopai River: One base flow and two high flow samples were positive for human contamination. There are consents for the discharge to water for treated sewage, stormwater, wastewater and floodwaters, with three of these within approximately 5 km of the sampling location. Discharge from these sources would account for the presence under both rainfall and base flow.
- Waikiwi Stream: Two base flow and one high flow sample were positive for human contamination. Within the catchment there is one consent for the discharge of stormwater to water, which may account for the human signal following rainfall. There is also a consent for the discharge of washdown effluent to water, which could represent a route for microbial transfer to the stream under base flow conditions. The nature of this 'washdown' however is not available at this time. In addition, the catchment comprises numerous small land holdings and lifestyle blocks, thus it is possible that septic tanks from these properties may be a source.

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%). It is thus unsurprising that the overall detection of *Campylobacter* is high (88%) in the rural, bird-impacted, Oreti catchment. Interestingly, although *C. jejuni* was the most commonly identified species in the national survey McBride et al. (2002), it was present in only 48% of *Campylobacter*-positive samples (compared with all positive samples in Oreti). Further, McBride et al. (2002) detected *Campylobacter lari* in 33% of positive samples from predominantly sheep-impacted sites.[1] These differences might reflect geographic differences, or differences in land use in the Oreti compared with the variety of differently impacted sites (including unimpacted and municipal) in the national survey.

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

[1] This study did not specifically look for the presence of *Campylobacter lari* - it would have been reported as an unidentified thermophilic *Campylobacter*. Unidentified thermophilic isolates were detected in 11% of *Campylobacter*-positive samples

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 is 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 ingested therefore range between 2.5 ml and 200 ml. Approximately three-quarters of the *Campylobacter*-positive samples collected across the Oreti FMU had a concentration of less than 10 MPN/100ml, meaning very large volumes of water (e.g. >8 litres) would be required to attain ID_{50}. However, a small number of samples contained high concentrations, (110-460 MPN/100 ml), such that the ingestion of 170-330 ml of water could carry a fifty-fifty chance of infection. The Guidelines define a risk of infection of 5% as being the upper limit for tolerable or acceptable risk; clearly a much smaller volume again 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., 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 by *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, a small number of wildfowl-associated isolates 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. 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. Since 38% of the *Campylobacter*-positive samples were found to contain only isolates of a wildfowl origin, the health risk in some instances might be less than that suggested by the data from Black et al. (1988), which is based on clinical isolates.

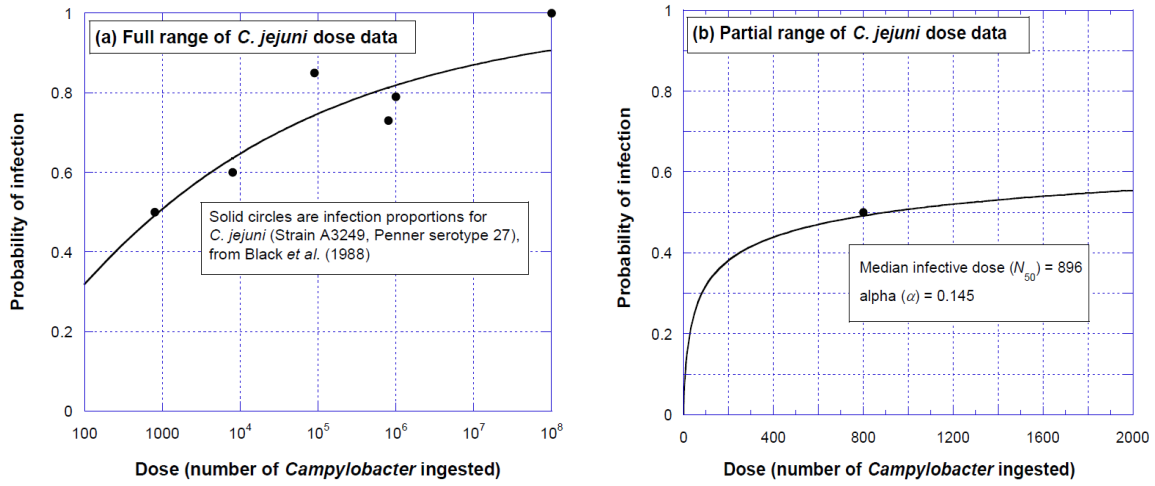

Figure 18. Dose-response curves for *Campylobacter jejuni*, estimating the probability of infection for a given dose. The lowest dose administered during the study was 800 *C. jejuni*, at which point half of the volunteers became infected. Estimating the dose response for lower concentrations requires extrapolation. From McBride et al. (2002).

Campylobacter is just one of a number of enteric pathogens that may cause human illness, and with the extent of faecal contamination present in the Oreti 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 a significant source of faecal contamination in the Oreti 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 Oreti FMU, with direct deposition, overland flow and subsurface flow via tile drains all important mechanisms for the transfer of faecal microbes to waterways. Human contamination is also a significant issue within this FMU. However, the magnitude of contamination, relative importance of different sources and routes of transmission vary slightly

between each of the 13 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 Human contamination

Human contamination could enter waterways in the Oreti FMU via seepage from septic tanks, the discharge of treated sewage, stormwater and washwater, and urban runoff. Contributions from combined sewer overflows, leaking infrastructure and/or cross connections are also possibilities. Further investigation is highly recommended to identify the specific contaminant site(s) and transmission pathway(s) that impact each site. This could include assessment of the condition of the municipal sewerage and stormwater network using cameras, checking stormwater outfalls for active discharge under dry weather conditions to highlight potential integrity issues, and the use of chemical, biological or DNA tracers to identify a point of origin for contamination (Harvey and Harms, 2002; Richards et al., 2016, 2017; Pang et al., 2017). Tracers are commonly used to model microbial transport through the subsurface – they are added at a potential point of contamination (e.g. a septic tank), and their recovery in an aquifer or waterway monitored. Chemical tracers and dyes require large inputs, which may be toxic to aquatic life. However, synthetic DNA tracers have a particular advantage in that they are environmentally safe, since they are not derived from any organisms and hence have no genetic functionality. Analysis is rapid and extremely sensitive, so that only trace amounts of DNA are required. In addition, multiple DNA tracers, each with a unique sequence, can be used to allow concurrent tracking of multiple potential sources and pathways (Pang et al., 2017). Once the specific source(s) has been identified, informed mitigation options can be explored. These may include the repair or replacement of failing septic and sewerage infrastructure, remediation of any identified cross-connections, separation of sewage and stormwater networks to avoid partially or untreated wastes being discharged to the environment, or incorporation of wetlands or other treatment options to stormwater or washwater discharge systems.

4.3.2 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).

4.3.3 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).

4.3.4 Wildfowl

It can be difficult to manage contamination of waterways caused by wildfowl, particularly in large rural 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 excluded 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).

4.3.5 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.

Since faecal pollution of waters by humans is considered the greatest risk to human health, these sources should be addressed first. Additional monitoring and site assessment at the five sites at which human contamination was present should be undertaken to identify the particular source(s) of the human signature (e.g. combined sewer overflows, particular septic tanks etc). However, faecal 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 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.

5. CONCLUSIONS

Waterways in the Oreti FMU are vulnerable to high levels of faecal contamination. Overall microbial levels appear less prone to the influence of rainfall than do sites within other management areas in the Southland region (e.g. Mataura FMU), with a nominal increase in *E. coli* and *Campylobacter* at some sites, but not at others. 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 or wash in from dairy crossings, and/or discharge of farm effluents to the water. Following rainfall, ruminant animals are the dominant pollution source, with both overland flow/surface runoff and subsurface flow through tile drains being important routes of transmission of faecal materials to waterways. Human faecal contamination was identified at five sites, with repeated detection at four of these. Potential sources vary between sites, but include failing septic tanks, sewage discharge, stormwater and urban runoff or cross-connections between the sewage and stormwater networks. Human faecal contamination is considered to pose the greatest risk to human health, and further investigations at these sites should be undertaken to identify the specific source(s) and transmission routes. This could include the inspection of sewerage infrastructure and the use of tracers such as synthetic DNA.

Campylobacter was isolated from 88% of samples, occasionally at quite high concentrations. Wildfowl, ruminants, poultry and humans 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 is 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.

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 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. Management of human contamination may involve repair or replacement of septic tank and sewerage infrastructure, or installation of stormwater and washwater treatment systems.

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	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 19). 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

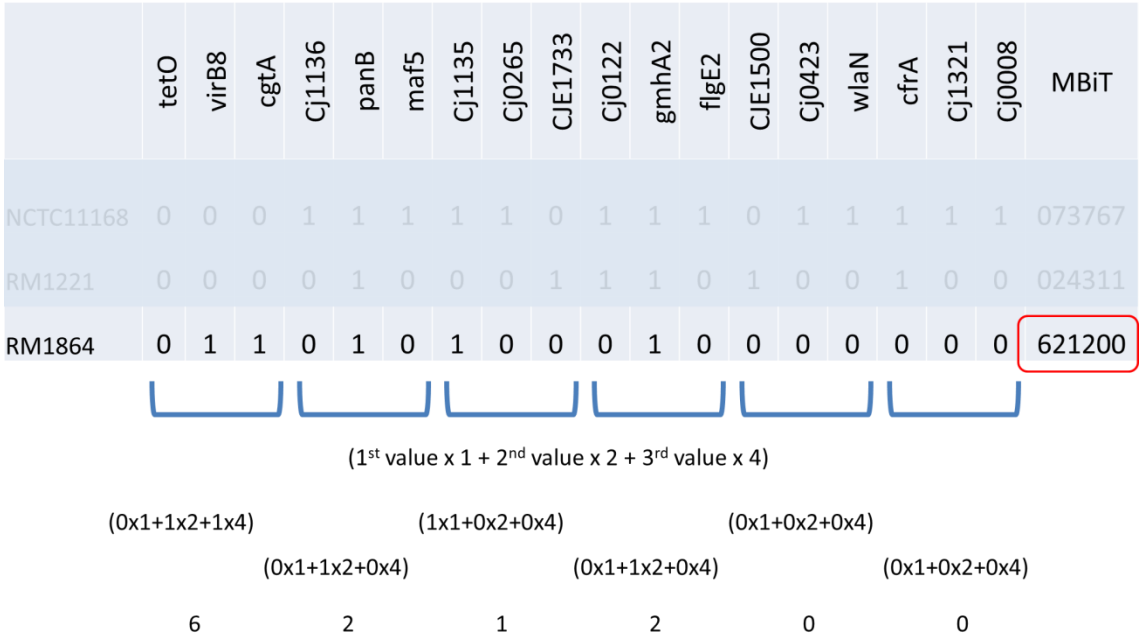

Figure 19. Example of an MBiT pattern naming.

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.

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

A.5 FAECAL STEROL ANALYSIS

Sterols are lipids that have important biological functions in plants and animals, including maintenance of cell wall structure. The sub-group of “faecal sterols” is a group of C27-, C28- and C29-cholestane-based sterols that is found mainly in animal faeces. The sterol profile of faeces can be distinctive between species, and depends on the interaction of three factors. Firstly, the animal’s diet determines the relative quantities of sterol precursors (cholesterol, 24-ethylcholesterol, 24-methylcholesterol, and/or stigmasterol) entering the digestive system. Secondly, animals differ in their endogenous biosynthesis of sterols (for example, humans on a low cholesterol diet synthesise cholesterol). Thirdly, and perhaps most importantly, is that the anaerobic bacteria in the animal gut biohydrogenate sterols to stanols of various isomeric configurations.

The sterol cholesterol can be hydrogenated to one or more of four possible stanols. In human beings, cholesterol is preferentially reduced to coprostanol, whereas in the environment cholesterol is predominately reduced to cholestanol. Similarly, plant-derived 24-ethylcholesterol is reduced to 24-ethylcoprostanol and 24-ethylepicoprostanol in the gut of herbivores, whereas in the environment it is primarily reduced to 24-ethylcholestanol.

Initial use of faecal sterols used the presence of coprostanol, which is the principal human biomarker, as in indicator of human faecal pollution. High relative amounts can indicate fresh human faecal material. Coprostanol constitutes 60% of the total sterols found in human faeces, while dogs and birds typically have either no coprostanol or only trace amounts, present in their faeces. However, herbivores and other animals can have considerable amounts of coprostanol in their faeces, although at lower levels than the amount of 24-ethylcoprostanol.

Therefore the ratios of one sterol to another are a better approach to assigning sources of pollution.

Table 4 lists the key ratios used by ESR, which are evaluated using a decision tree approach. Fresh faecal material is relatively simple to evaluate, but when faecal sources are mixed, and when plant sterols and other environmental sources are added, the interpretation can become more complex. A holistic expert evaluation is undertaken, with assignment of sources made where the sterols support such an interpretation.

Faecal sterol analysis was performed by filtering 1–4 litres of river water onto glass fibre filters. Filters were stored frozen until they were analysed using the extraction procedure described by Gregor et al. (2002). Faecal sterol analysis using stored filters was undertaken only for selected samples. Interpretation guidelines for faecal sterol ratios are provided in

Table 4.

A.6 PRESENTATION OF RESULTS IN THIS REPORT

Tables 5, 6 and 7 provide a key for interpretation of results, which can be used to assist with reviewing results for each site.

Table 4. Faecal sterol ratios indicative of faecal pollution.

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

Table 5. 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 6. 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				

Table 7. Explanation of faecal sterol results and interpretation.

Total Sterols	Total sterols expressed in ng/l			
Coprostanol	Level of coprostanol expressed as ng/l			
Faecal	If ratio F1 (coprostanol/cholestanol) or ratio F2 (24-ethylcoprostanol/24-ethylcholestanol) are greater than 0.5 it suggests human or animal faecal material. F1 tends to dominate human faeces, F2 in herbivore faeces.			
	Result in brackets indicates that close to reaching threshold			
	F1 + F2	F1	F2	No
Human	Human sources of faecal contamination are indicated when: Ratio H1 (%coprostanol/total sterols) is > 5-6% Ratio H2 (5β/(5β+5α stanols)) is > 0.7 Ratio H3 (coprostanol/24-ethylcoprostanol) is ≥ 1.0			
	H1, H2 and H3 meet thresholds	2 of 3 ratios meet thresholds	H3 meets threshold	None meet threshold
	Yes (3)	Yes (2)	>1	No
Ruminant	Herbivore sources of faecal material are indicated when: Ratio R1 (24-ethylcoprostanol/total sterols) is >5-6% Ratio R2 (coprostanol/coprostanol+24-ethylcoprostanol) is <30% Ratio R3 (24-ethylcholesterol/24-ethylcoprostanol) is <1.0			
	R1, R2 and R3 meet thresholds	2 of 3 ratios meet thresholds	R2 meets threshold	None meet threshold
	Yes (3)	Yes (2)	<30	No
Wildfowl	Wildfowl sources of faecal material are indicated when: %coprostanol:total sterols is <4% 24-ethylcoprostanol:total sterols is <4% %of alpha stanols:cholestanol, 24-ethylcholestanol is >2% 24-ethylcholesterol/24-ethylcoprostanol is >7% 24-ethylcholestanol/(24-ethylcholestanol+24-ethylcoprostanol+24-ethylepicoprostanol) is >0.4 cholestanol/(cholestanol+coprostanol+epicoprostanol) is >0.5			
	Meets all criteria	Almost meets criteria		
	Yes	(Yes)	No	
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 BOG BURN DOWNSTREAM OF HUNDRED LINE ROAD

Bog Burn was sampled downstream of Hundred Line Road on five occasions between May and December 2015. Samples were collected in June, August and November under base flow conditions, whilst sampling events in May and December were preceded by rainfall (Table 8).

Under base flow conditions, *E. coli* levels were highly varied, ranging from 170 cfu/100 ml in August to 4,300 cfu/100 ml in November. Low levels of *Campylobacter* (0.7-0.9 MPN/100 ml were detected in all three base flow samples, and which were subsequently identified as *C. jejuni*. MBiT source attribution analysis determined that the *Campylobacter* isolated in June and August was of wildfowl origin, while strains of both poultry and ruminant sources were present in November. Faecal source tracking identified faecal pollution as coming from a number of sources, including ruminants, wildfowl and humans. Ruminant animals accounted for up to half of the faecal pollution present in June and August (10-50%), and the majority of the contamination in November (50-100%). Analysis for ovine and bovine-specific markers yielded bovine markers in August; this might suggest that other ruminant animals (i.e. deer) were present, or alternatively, that the pollution was aged, such that specific ruminant FST markers were undetected in the other samples, particularly from November. Wildfowl markers were present in all base-flow samples. Human FST markers were identified in the base-flow samples collected in June and August, although no faecal sterol signature for human contamination was detected. This difference may reflect the differing methodologies and detection limits associated with chemical versus DNA pollution signatures.

E. coli levels were elevated following rainfall, with especially high levels detected in the May sample (20,000 cfu/100 ml). Coincident with the highest levels of *E. coli*, the highest levels of *Campylobacter* were also detected in May (460 MPN/100ml), while the December sample contained low levels of *Campylobacter* similar to those observed under base flow conditions (0.4 MPN/100 ml). *C. jejuni* was isolated from both samples, with *C. coli* also isolated from the highly contaminated May sample. MBiT source analysis identified the *Campylobacter* present in the May sample as being a mix of wildfowl and ruminant origin, with the *Campylobacter* present in December originating from a poultry source. Faecal source tracking of the two post-rainfall samples determined that ruminant pollution dominated the May sample, with both bovine and ovine-specific makers were identified. In contrast, the December sample contained negligible ruminant contamination. Wildfowl markers were identified in both post-rainfall samples.

Land use in the Bog Burn at Hundred Line Road sub-catchment shows an almost equal distribution between sheep and beef, dairy, and plantation forestry (Figure 20, Figure 21). Review of land use and consent information does not suggest an obvious source of human contamination, and this should be further investigated (Figure 20, Table 9).

Table 8. Results for microbial and FST analysis of water samples collected from Bog Burn, downstream of Hundred Line Road.

Site		Bog Burn downstream of Hundred Line Road				
Sample #		CMB150808	CMB151380	CMB152078	CMB150481	CMB152245
Client #		20152090	20152906	20153989	20151843	20154513
Date Sampled		09/06/2015	11/08/2015	17/11/2015	12/05/2015	14/12/2015
Rainfall		No	No	No	Yes	Yes
		Microbial Properties				
Faecal coliforms		900	220	4,600	22,000	2,100
E. coli		800	170	4,300	20,000	1,900
Campylobacter		0.7	0.9	0.7	460	0.4
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni & C. coli*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	2	2		1	
	Ovine/Bovine/ Deer			1	2	
	Poultry			1		1
	Not Wildfowl					
	Unknown					
		Faecal Source Tracking				
General - GenBac3		++++	++++	++++	++++	++++
Ruminant		10-50%	10-50%	50-100%	50-100%	≤1%
Human - BacH		+	+	-	-	+
Human - BiADO		+	+	-	-	-
Cow		-	+	-	+	-
Sheep		-	-	-	+	-
Wildfowl - GFD		-	+	-	-	+
Wildfowl - E2		+	+	+	+	+
Canine		nt	nt	-	nt	nt
		Sterol Properties				
Total Sterols		1059	1402	nt	nt	nt
Coprostanol		23	53			
Faecal		-	F1+F2			
Human		-	No			
Ruminant		-	Yes (2)			
Wildfowl		-	No			

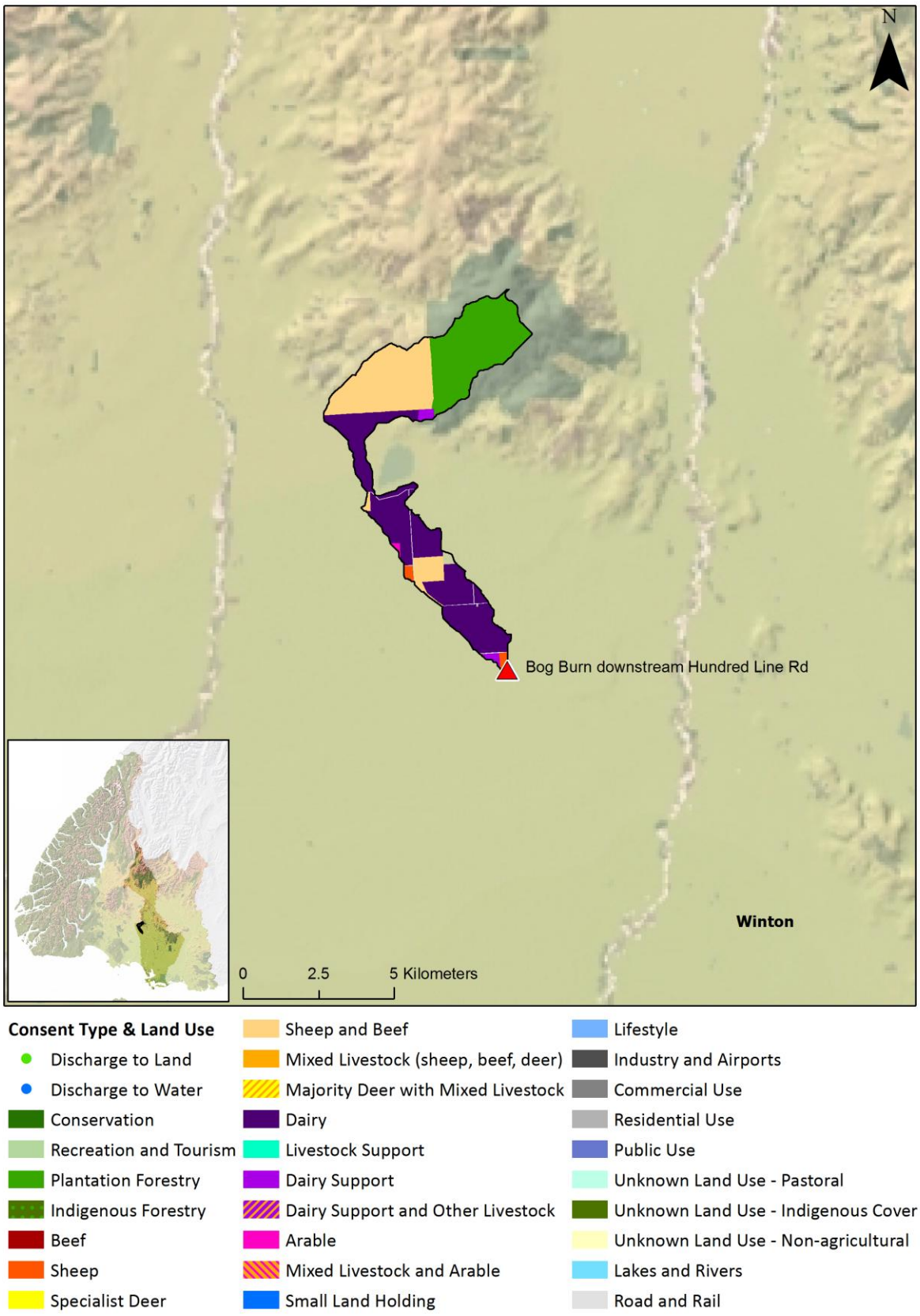

Figure 20. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Bog Burn downstream of Hundred Line Road sampling site.

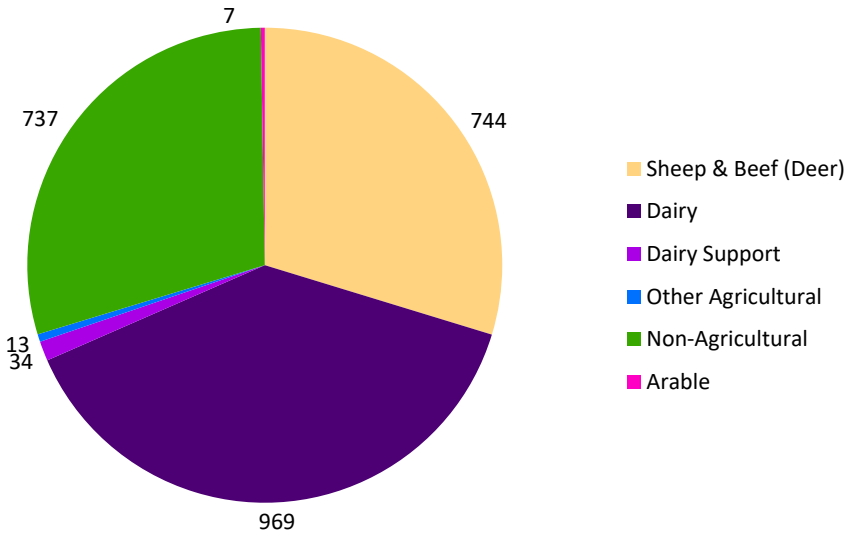

Figure 21. Land use (in hectares) in the catchment for the Bog Burn downstream of Hundred Line Road sampling site.

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

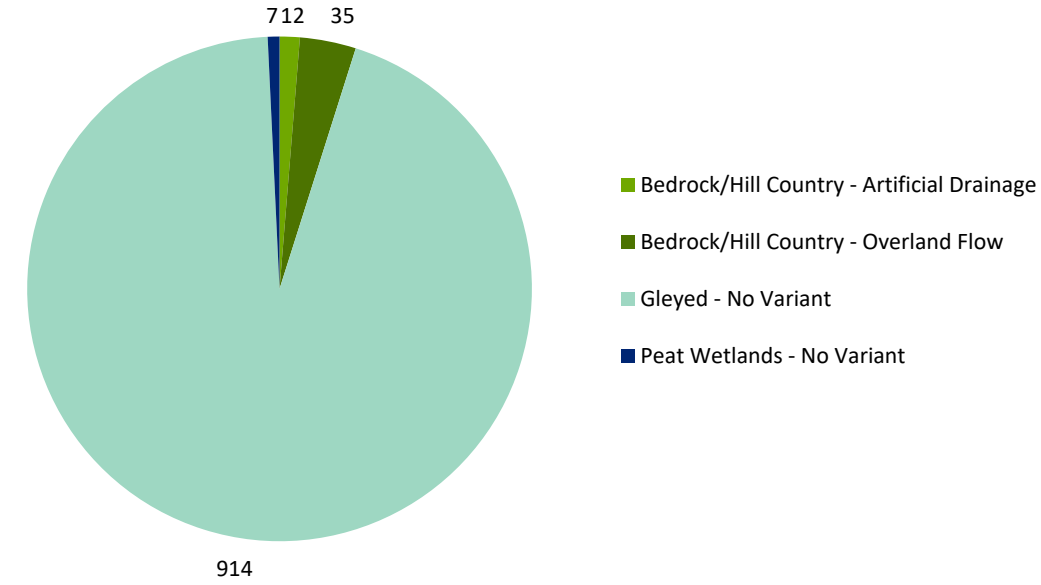

Figure 22. Dairying land (in hectares) in the catchment for Bog Burn downstream of Hundred Line Road sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 9. Number of consented catchment discharges to land and water in the catchment for the Bog Burn downstream of Hundred Line Road sampling site.

Bog Burn downstream Hundred Line Road		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	6
To Land Total		6
Grand Total		6

Note: Consent information accurate as of April 2017

B.2 CARRAN CREEK AT WAITUNA LAGOON ROAD

Water quality was analysed for Carran Creek at Waituna Lagoon Road. Samples were taken on six occasions, with three occurring under base flow conditions (June, August and October), and three occurring following rainfall (July, September and December) (Table 10).

Samples collected under base flow conditions contained a relatively low microbial burden. *E. coli* levels varied between 50 and 250 cfu/100ml. *Campylobacter* was detected in all base-flow samples (0.4-2.3 MPN/100 ml), and was determined to be *C. jejuni* and of wildfowl origin. *C. jejuni* from a poultry source was also identified in the June sample. Faecal source tracking showed that the relative impact of ruminant pollution was highly varied under base flow conditions: ruminant pollution accounted for 10-50% of overall pollution at the site in June, increasing to 50-100% in August, and falling below detection limits in October. Bovine-specific markers were identified the June samples, with ovine markers present in August. Wildfowl PCR markers were present in both June and August.

Samples collected following rainfall recorded much higher and more variable levels of *E. coli* than those seen under base-flow conditions (1700, 70 and 13,500 cfu/100 ml for July, September and December, respectively). *Campylobacter* levels were low or undetected in these first two samples (2.3, <0.3 MPN/100 ml), but increased to 24 MPN/100 ml in the December sample. *Campylobacter* was determined to be *C. jejuni*, with wildfowl and ruminant sources. FST analysis suggests that following rainfall, ruminant pollution represents a dominant source of faecal pollution, accounting for up to 100% of pollution in July and December, and up to 50% in September. However, whilst ovine, bovine and wildfowl markers were all identified for the July sample, no other markers were detected in the subsequent samples. This suggests that some other ruminant, for example deer, may account for the significant faecal contamination present during the spring and summer (e.g. October and December).

Review of the land use in the sub-catchment shows a mixture of dairy, sheep and beef, and conservation activities (Figure 23, Figure 24).

Table 10. Results for microbial and FST analysis of water samples collected from Carran Creek at Waituna Lagoon Road.

Site		Carran Creek at Waituna Lagoon Road					
Sample #		CMB150792	CMB151377	CMB151766	CMB150952	CMB151540	CMB152218
Client #		20152059	20152896	20153284	20152642	20153074	20154421
Date Sampled		08/06/2014	10/08/2015	12/10/2015	06/07/2015	07/09/2015	7/12/2015
Rainfall		No	No	No	Yes	Yes	Yes
		Microbial Properties					
Faecal coliforms		50	200	250	1,700	70	15,000
E. coli		50	200	250	1,700	70	13,000
Campylobacter		2.3	0.4	0.7	2.3	<0.3	24
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	nt	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	2	1	2	1		5
	Ovine/Bovine/ Deer				1		1
	Poultry	1					
	Not Wildfowl				1		
	Unknown						
		Faecal Source Tracking					
General - GenBac3		++++	++++	++	++++	++++	+++
Ruminant		10-50%	50-100%	<1%	50-100%	10-50%	50-100%
Human - BacH		+	+	-	+	+	-
Human - BiADO		-	-	-	-	-	-
Cow		+	-	-	+	-	-
Sheep		-	+	-	+	-	-
Wildfowl - GFD		+	+	-	+	-	-
Wildfowl - E2		+	-	-	-	-	-

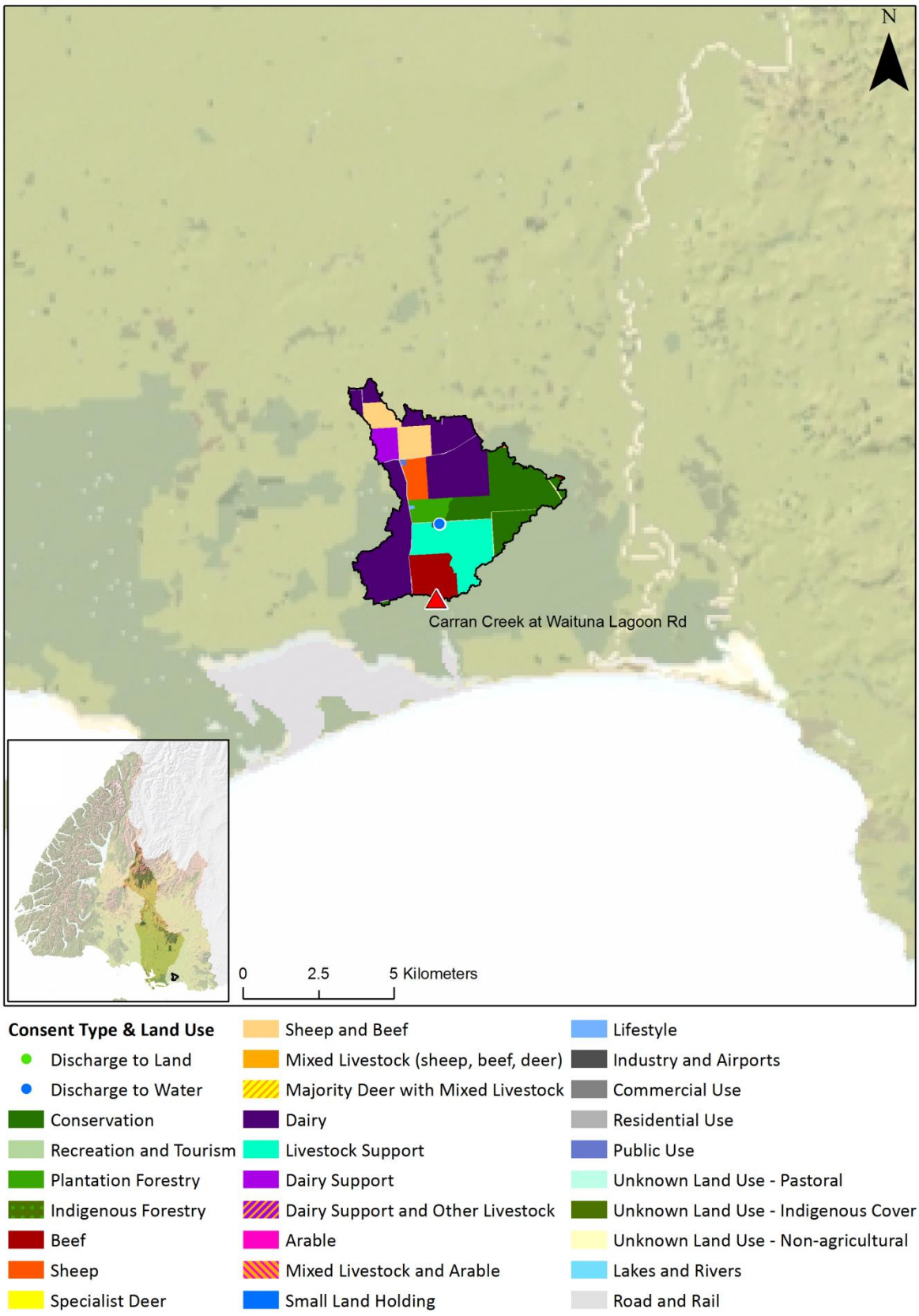

Figure 23. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Carran Creek at Waituna Lagoon Road sampling site.

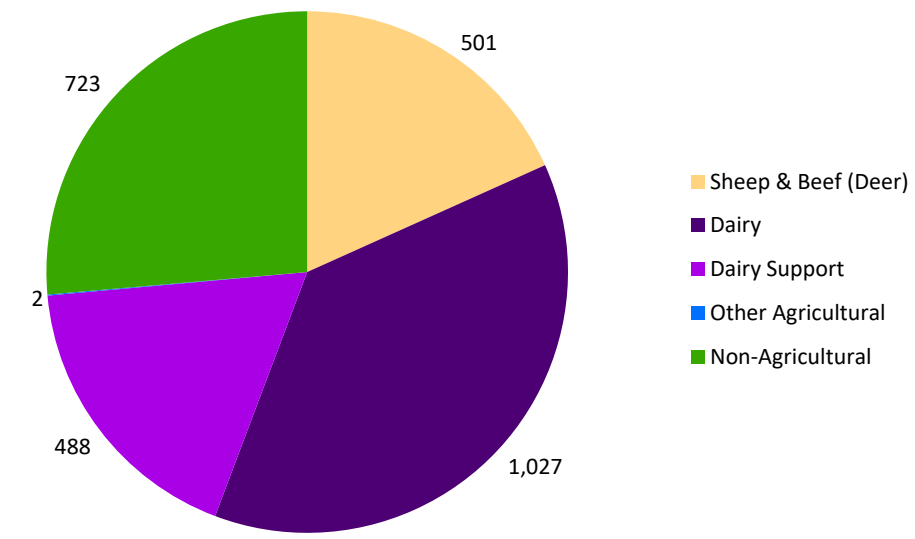

Figure 24. Land use (in hectares) in the catchment for the Carran Creek at Waituna Lagoon Road sampling site.

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

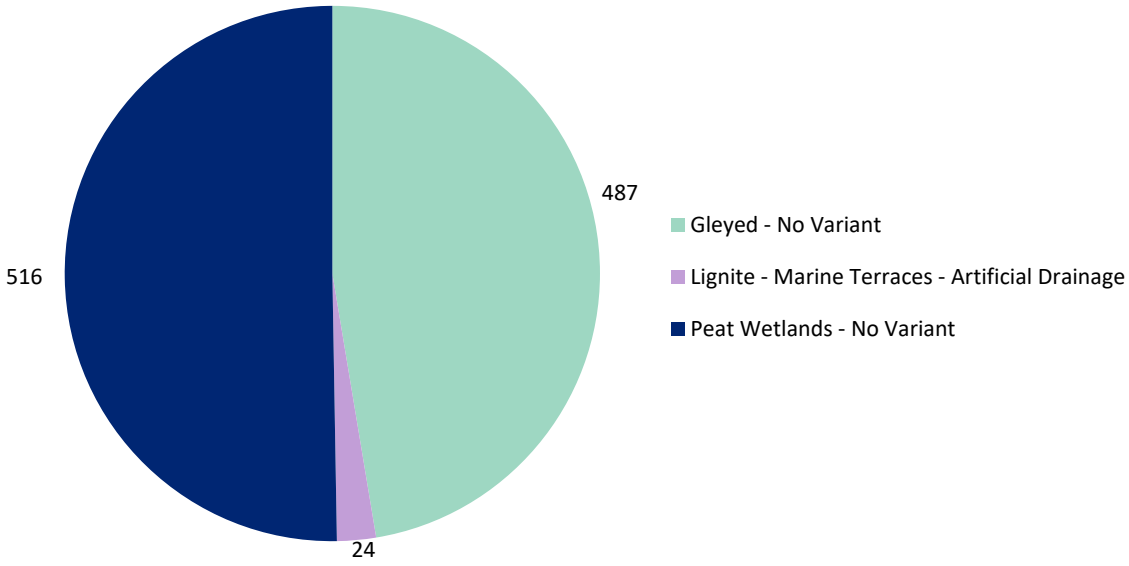

Figure 25. Dairying land (in hectares) in the catchment for the Carran Creek at Waituna Lagoon Road sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016.

Table 11. Number of consented catchment discharges to land and water in the catchment for the Carran Creek at Waituna Lagoon Road sampling site.

Carran Creek at Waituna Lagoon Road		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	4
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
	Wintering Pad/Feedlot Effluent (land)	1
To Land Total		6
To Water	Mine water, Stormwater	1
To Water Total		1
Grand Total		7

Note: Consent information accurate as of April 2017

B.3 MAKAREWA RIVER AT LORA GORGE ROAD

Water samples were collected from the Makarewa River at Lora Gorge Road on three occasions between autumn and summer 2015. Each sampling event was preceded by rainfall (Table 12).

E. coli levels were highly variable at this site, with concentrations of 11,000 and 14,000 cfu/100 ml in May and December, and 120 cfu/100 ml in September. *Campylobacter* was detected in all three samples, with elevated levels present in the May sample (240 MPN/100 ml) and low levels in the two subsequent samples (0.4 and 0.9 MPN). Genotype analysis determined that *C. jejuni* was present in all three samples, with *C. coli* also present in the May sample. MBiT analysis found the *Campylobacter* in the May sample to be of ruminant origin, while those in the September and December samples were of wildfowl origin.

FST analysis suggests that ruminant pollution was the dominant pollution type at this site (50-100% in May and September, 10-50% in December). Ovine-specific markers were identified in all three samples, with bovine markers also present in the December sample. Human and wildfowl markers were identified in the September water sample.

Faecal sterol analysis of the September sample identified chemical signatures for ruminant and wildfowl pollution, but did not detect human contamination. The lack of detection of a human sterol signature might be the result of different methodologies and detection limits between microbial- and chemical-based analyse, or the complexities of interpreting sterol profiles from samples with multiple faecal sources.

A review of the land use in the sub-catchment shows an almost equal split of sheep and beef farming (including some sheep-only and some mixed use with deer) and non-agricultural use (conservation and plantation forestry) (Figure 26, Figure 27)

Table 12. Results for microbial and FST analysis of water samples collected the Makarewa River at Lora Gorge Road.

Site		Makarewa River at Lora Gorge Road		
Sample #		CMB150473	CMB151551	CMB152261
Client #		20151820	20153091	20154551
Date Sampled		12/05/2015	08/09/2015	16/12/2015
Rainfall		Yes	Yes	Yes
		Microbial Properties		
Faecal coliforms		14,000	140	14,000
E. coli		11,000	120	14,000
Campylobacter		240	0.4	0.9
***Campylobacter* Species**		*C. jejuni & C. coli*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**		1	2
	Ovine/Bovine/ Deer	2		
	Poultry			
	Not Wildfowl			
	Unknown			
		Faecal Source Tracking		
General - GenBac3		++++	++++	++++
Ruminant		50-100%	50-100%	10-50%
Human - BacH		-	+	-
Human - BiADO		-	+	-
Cow		-	-	+
Sheep		+	+	+
Wildfowl - GFD		-	+	-
Wildfowl - E2		-	-	-
		Sterol Properties		
Total Sterols		nt	1930	nt
Coprostanol			35	
Faecal			F1+F2	
Human			No	
Ruminant			Yes (2)	
Wildfowl			Yes	

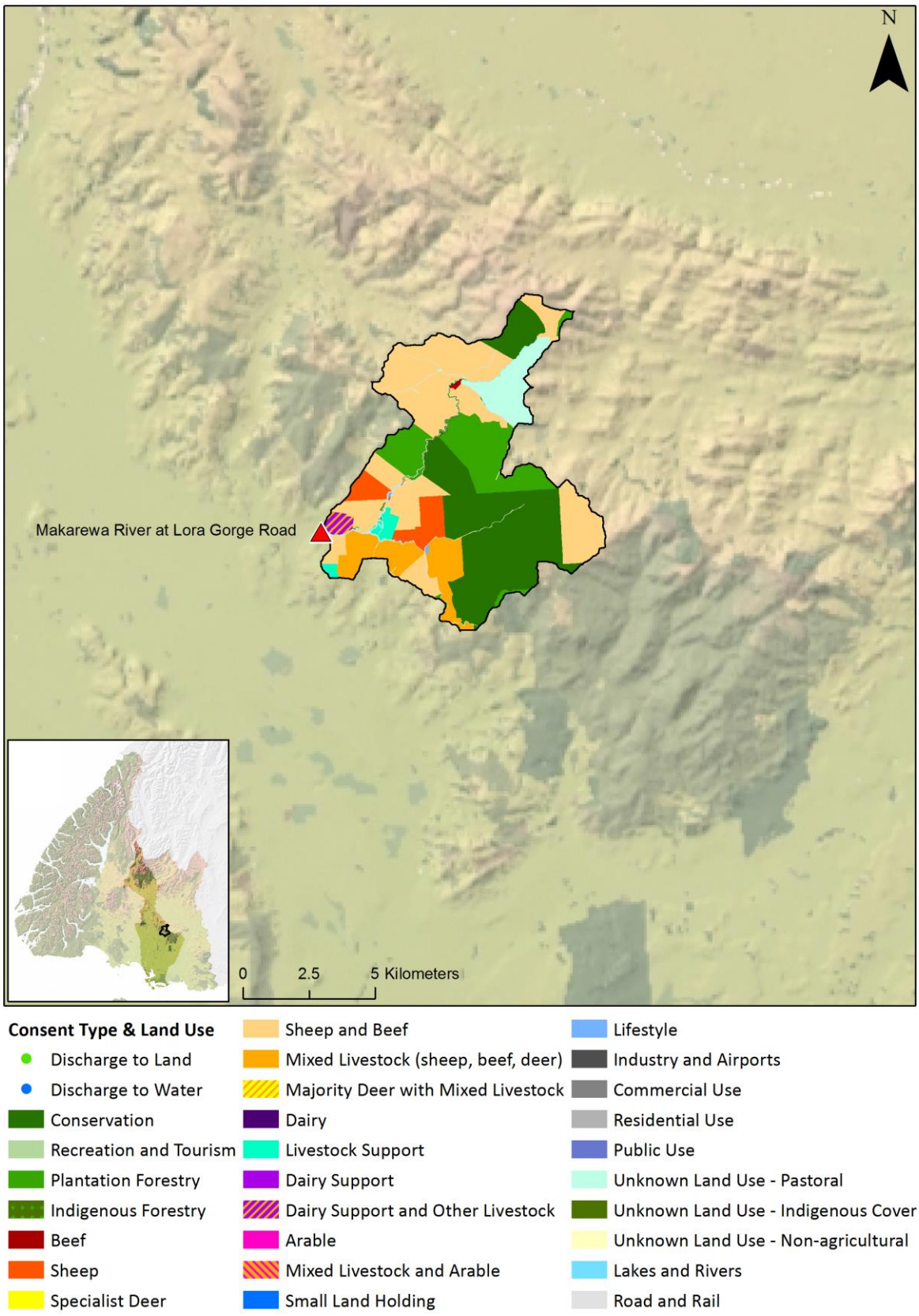

Figure 26. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Makarewa River at Lora Gorge Road sampling site.

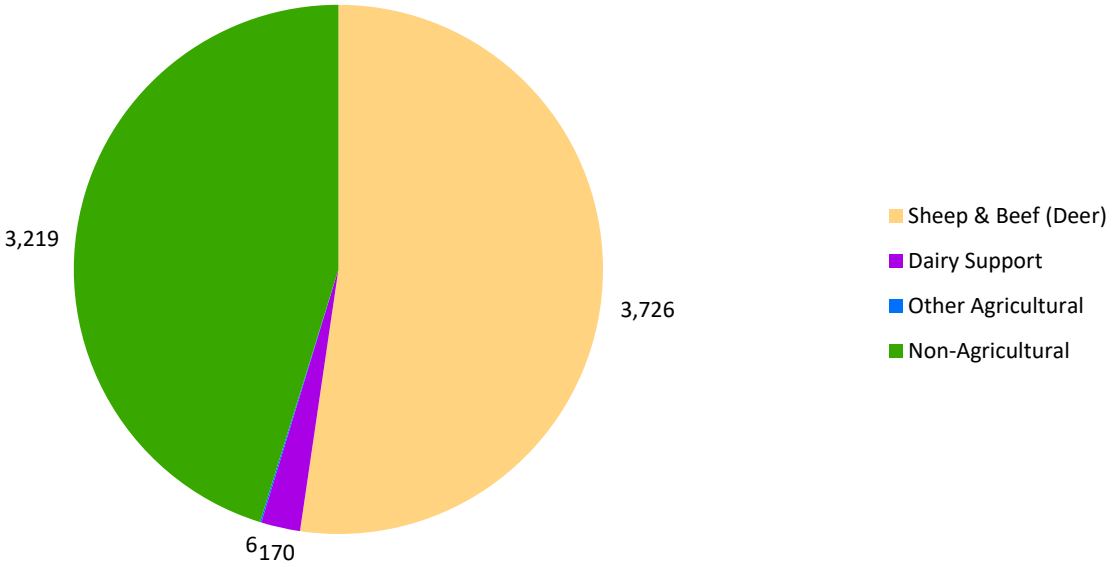

Figure 27. Land use (in hectares) in the catchment for the Makarewa River at Lora Gorge Road sampling site.

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 activity, nor any consents, for the Makarewa River at Lora Gorge Road sub-catchment.

B.4 MAKAREWA RIVER AT WALLACETOWN

The Makarewa River was also sampled at Wallacetown. Sampling took place on three occasions (May, July and September 2015) following rainfall (Table 13).

E. coli levels at this site were highest in the July sample (1,200 cfu/100 ml) with both May and September samples containing 300 cfu/100 ml. *Campylobacter* was identified in all three samples, with 43 MPN/100 ml in May and 4.3/100 ml in the other two samples. All isolates were determined to be *C. jejuni*. MBiT analysis revealed the *Campylobacter* to be from a number of sources, namely wildfowl, with poultry and human sources present in the September sample.

FST analysis determined that ruminant pollution was the dominant pollution source in all three samples (50-100%). Ovine, bovine and wildfowl markers were each detected in the July and September samples; no markers were detected in the May sample.

Land use in the Makarewa River at Wallacetown sub-catchment is a mixture of beef and sheep (46%), dairy (28%, including support activities), and non-agricultural land (21%; conservation and forestry) (Figure 28, Figure 29).

Table 13. Results for microbial and FST analysis of water samples collected from the Makarewa River at Wallacetown.

Site		Makarewa River at Wallacetown		
Sample #		CMB150470	CMB150977	CMB151549
Client #		20151816	20152646	20153087
Date Sampled		12/05/2015	07/07/2015	08/09/2015
Rainfall		Yes	Yes	Yes
		Microbial Properties		
Faecal coliforms		330	1,500	300
E. coli		300	1,200	300
Campylobacter		4.3	4.3	43
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	3	3	2
	Ovine/Bovine/ Deer			
	Poultry			2
	Not Wildfowl		1	1
	Human			2
	Unknown			
		Faecal Source Tracking		
General - GenBac3		++++	++++	++++
Ruminant		50-100%	50-100%	50-100%
Human - BacH		+	+	+
Human - BiADO		-	-	-
Cow		-	+	+
Sheep		-	+	+
Wildfowl - GFD		-	+	+
Wildfowl - E2		-	-	-

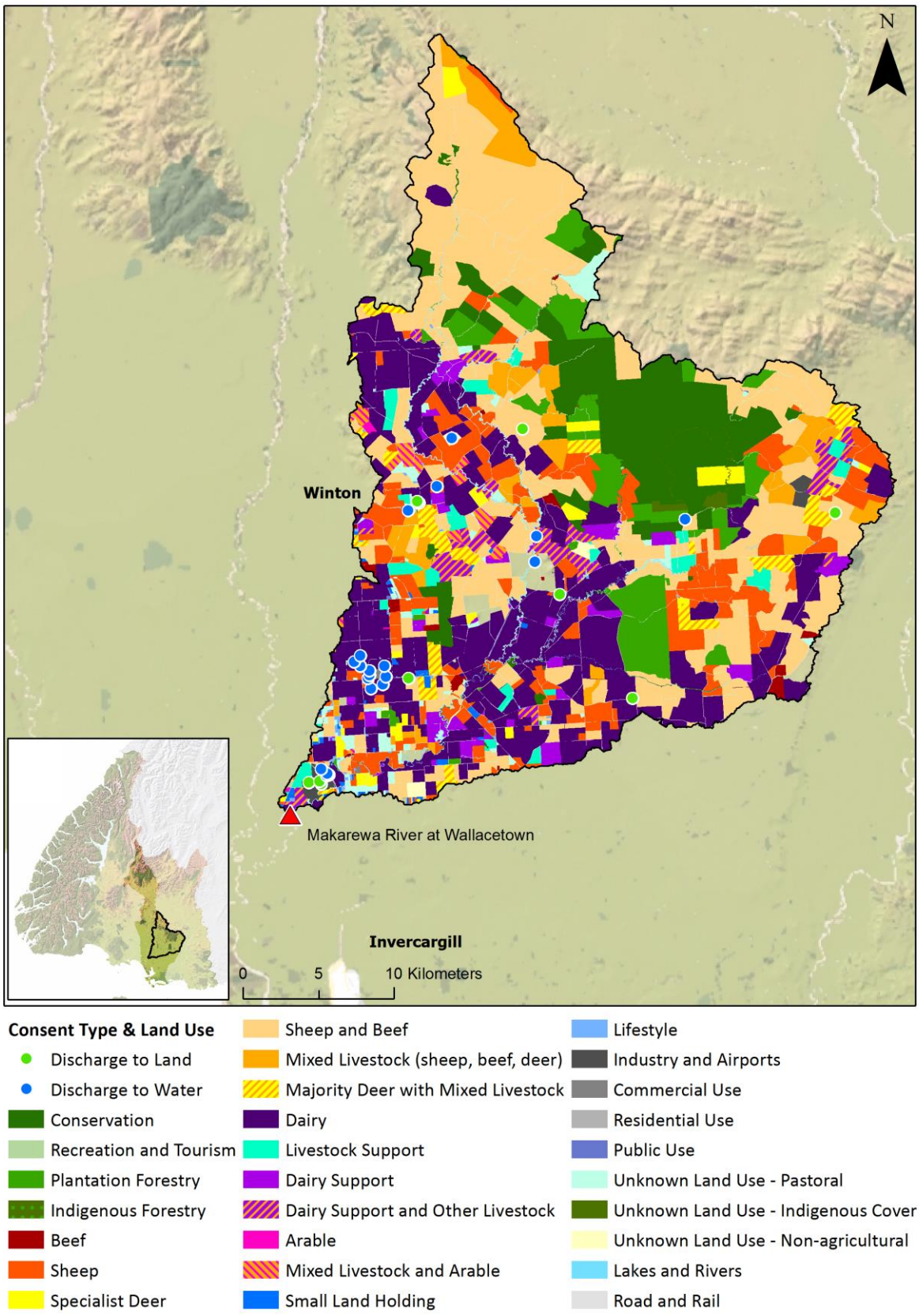

Figure 28. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Makarewa River at Wallacetown sampling site.

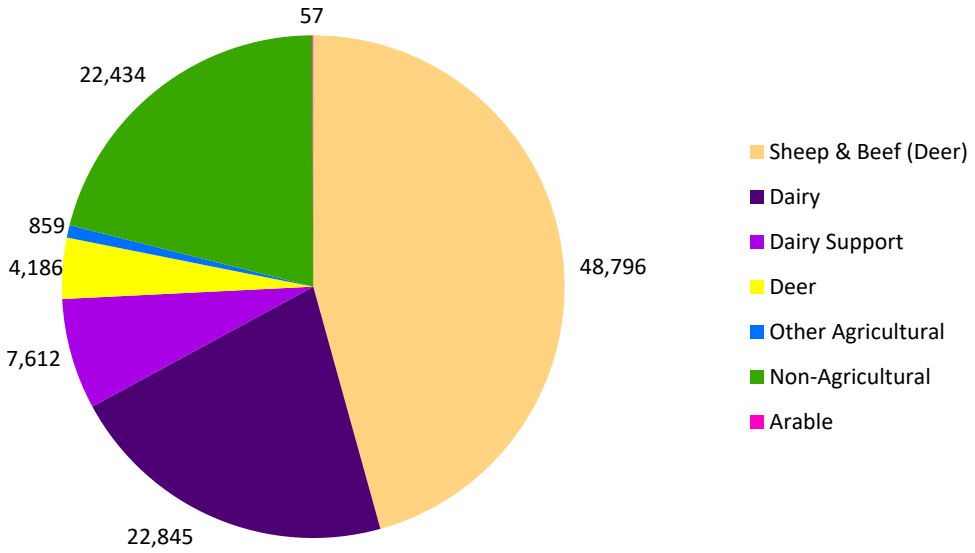

Figure 29. Land use (in hectares) in the catchment for the Makarewa River at Wallacetown sampling site.

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

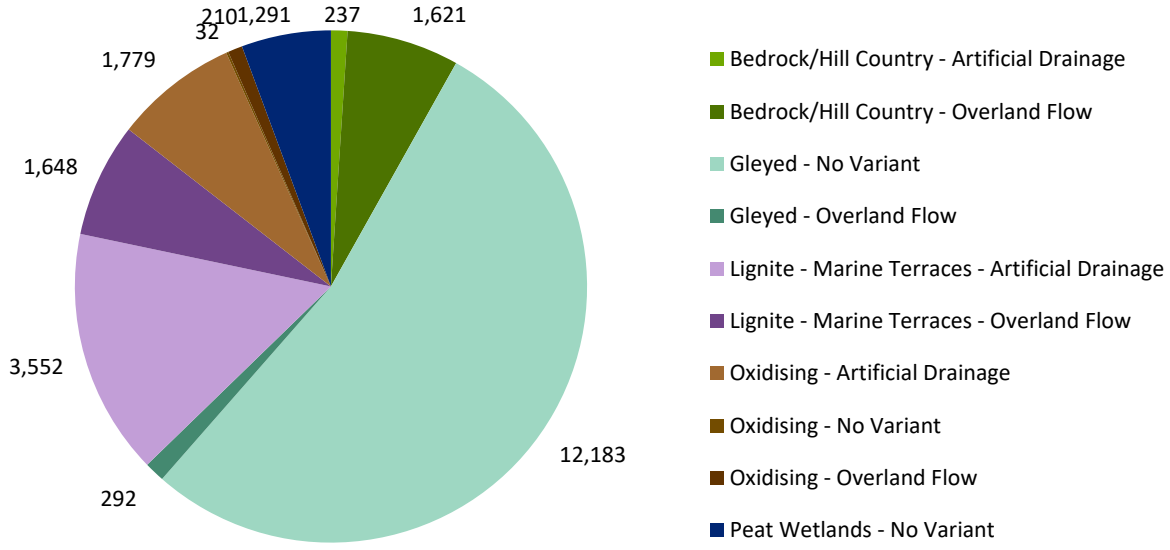

Figure 30. Dairying land (in hectares) in the catchment for the Makarewa River at Wallacetown sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 14. Number of consented catchment discharges to land and water in the catchment for the Makarewa River at Wallacetown sampling site.

Makarewa River at Wallacetown		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	1
	Ash	2
	Ash, Dairy Factory Effluent, Wash Water, Waste Water	1
	Cereal bait	1
	Dairy Shed Effluent (land)	78
	Dairy Shed Effluent (land), Leachate	1
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	22
	Industrial Effluent	1
	Industrial Effluent, Meat Works Effluent, Wash Water	1
	Meat Works Effluent, Offal, Tannery Effluent, Wool Scour Effluent	1
	Meat Works Effluent, Waste Water	1
	Mine water, Stormwater	1
	Oil/Grease	1
	Refuse - Commercial	3
	Sewage (Treated), Sewage Package Plant	1
	Stormwater	1
	Waste Water	1
	Wintering Pad/Feedlot Effluent (land)	2
To Land Total		120
To Water	Ground water	1
	Ground water, Mine water, Stormwater	1
	Ground water, Stormwater	2
	Meat Works Effluent, Waste Water	2
	Mine water	1
	Oxidation Pond Effluent, Sewage (Treated), Sewage Package Plant, Waste Water	1
	Pumped Drainage	1
	Sludge, Wash Water	1
	Stormwater	13
	Wash Water, Waste Water	1
To Water Total		24
Grand Total		144

Note: Consent information accurate as of April 2017

B.5 MOFFAT CREEK AT MOFFAT ROAD

Water samples were collected from Moffat Creek at Moffat Creek Road in July, August and September 2015. Each sample collection followed a rainfall event (Table 15).

The highest *E. coli* levels at this site (1200 cfu/100ml) were observed in July. *E. coli* was present at 300 cfu/100 ml in both August and September samples. Low levels of *Campylobacter* were detected in July (0.4 MPN/100 ml) and September (4.3 MPN/100ml), with isolates identified as *C. jejuni*. MBiT source analysis determined the *Campylobacter* was of wildfowl origin, with poultry and 'not wildfowl' (i.e. ruminant, poultry or human) sources also present in the September sample.

FST analysis determined that ruminant pollution was the dominant pollution type in and September (50-100%), although it accounted for only 10-50% of overall faecal pollution in August. Bovine-specific PCR markers were detected in both the July and September samples, with wildfowl makers also present in July. No specific markers were identified from the August sample. The finding of bovine markers is consistent with 98% of land in the sub-catchment being used for dairy and dairy-support activities (Figure 31, Figure 32).

Table 15. Results for microbial and FST analysis of water samples collected from Moffat Creek at Moffat Road.

Site		Moffat Creek at Moffat Road		
Sample #		CMB150953	CMB151378	CMB151541
Client #		20152643	20152897	20153075
Date Sampled		06/07/2015	10/08/2015	07/09/2015
Rainfall		Yes	Yes	Yes
		Microbial Properties		
Faecal coliforms		1,200	300	300
E. coli		1,200	300	300
Campylobacter		0.4	<0.3	4.3
***Campylobacter* Species**		*C. jejuni*	nt	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	1		1
	Ovine/Bovine/ Deer			
	Poultry			1
	Not Wildfowl			2
	Unknown			
		Faecal Source Tracking		
General - GenBac3		++++	+++	++++
Ruminant		50-100%	10-50%	50-100%
Human - BacH		+	-	+
Human - BiADO		-	-	-
Cow		+	-	+
Sheep		-	-	-
Wildfowl - GFD		+	-	-
Wildfowl - E2		-	-	-

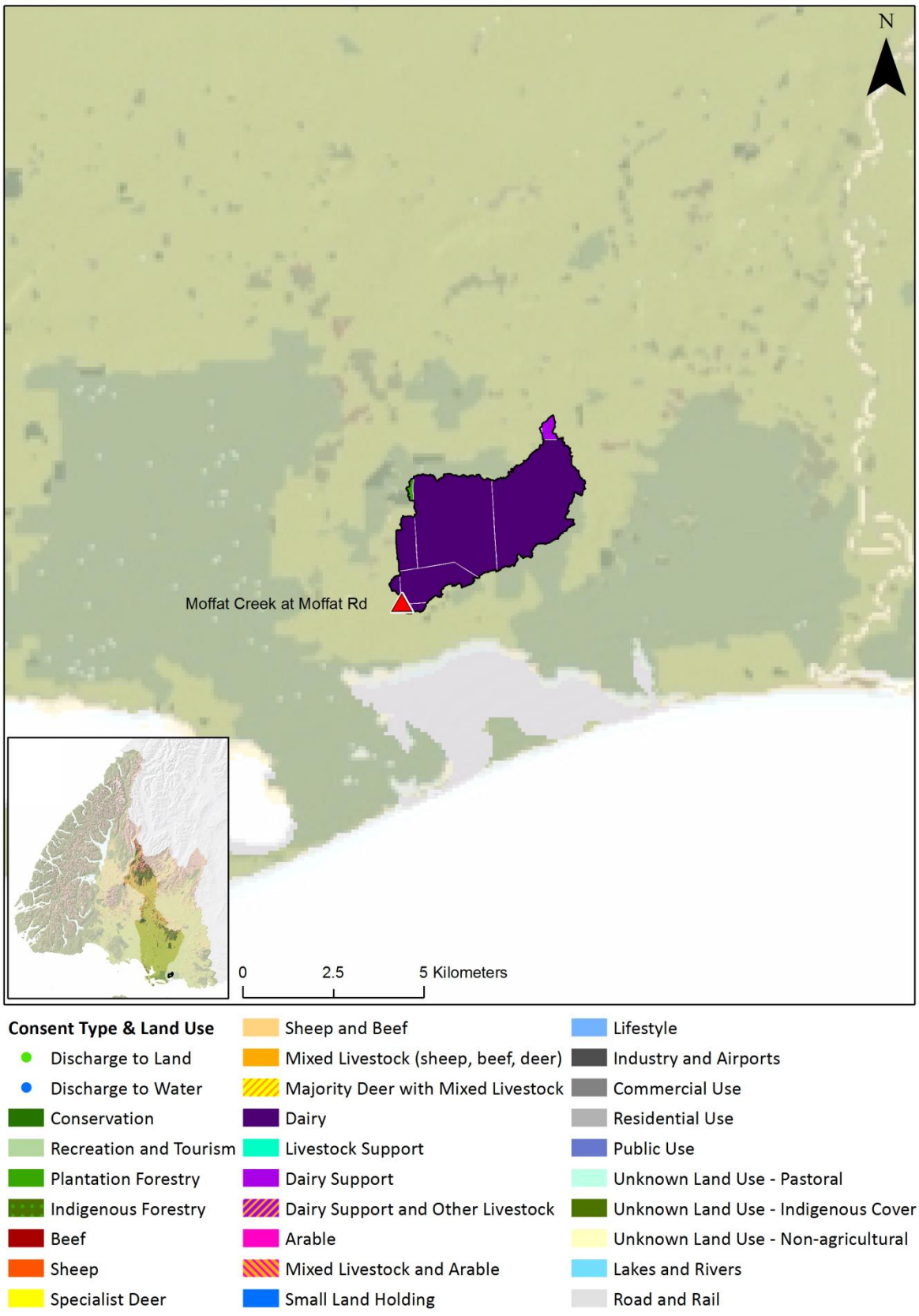

Figure 31. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Moffat Creek at Moffat Road sampling site.

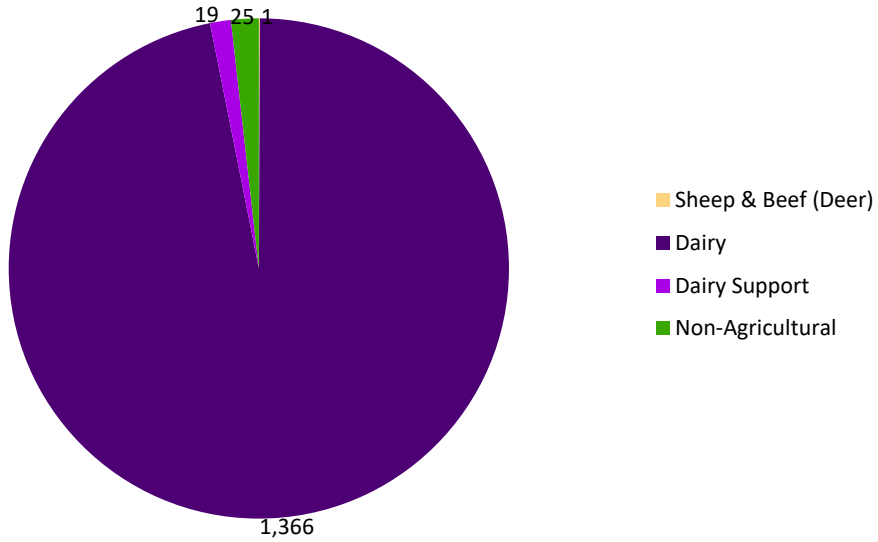

Figure 32. Land use (in hectares) in the catchment for the Moffat Creek at Moffat Road.

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

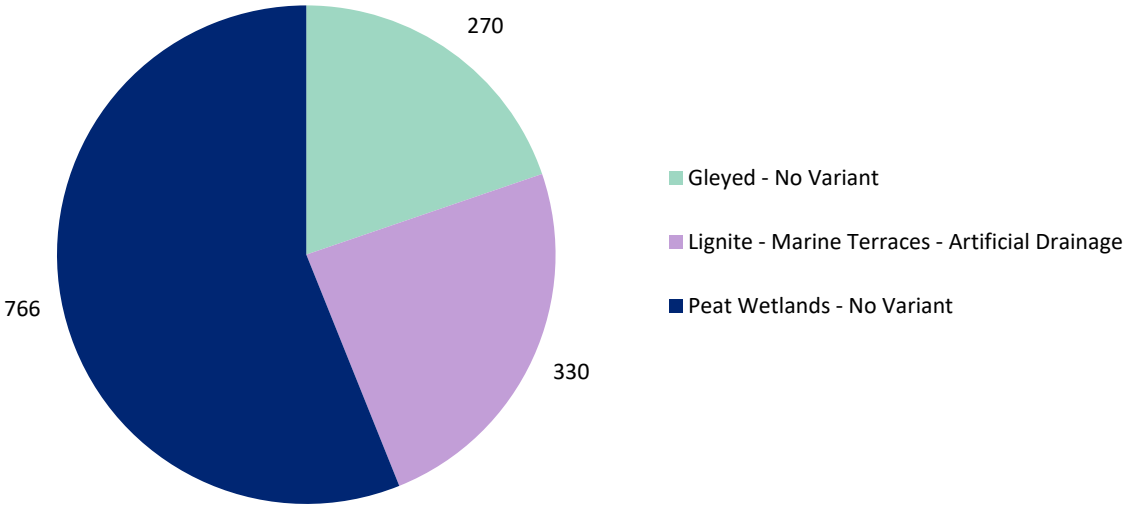

Figure 33. Dairying land (in hectares) in the catchment for the Moffat Creek at Moffat Road sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 16. Number of consented catchment discharges to land and water in the catchment for the Moffat Creek at Moffat Road sampling site.

Moffat Creek at Moffat Road		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	2
To Land Total		2
Grand Total		2

Note: Consent information accurate as of April 2017

B.6 ORETI RIVER AT WALLACETOWN

The Oreti River was sampled at Wallacetown on three occasions between autumn and spring 2015, with each sampling event being preceded by rainfall (Table 17).

E. coli levels were highest in the May sample (560 cfu/100 ml; 100-130 cfu/100 ml in July and September). *Campylobacter* was detected in the May (9.3 MPN/100 ml) and July (0.9 MPN/100 ml) samples, with isolates identified as *C. jejuni*; an unspeciated thermophylic *Campylobacter* also present in the July sample. MBiT analysis showed the *Campylobacter* in the July sample to be of wildfowl origin, while the origin of that in the May sample was 'not wildfowl' (i.e. likely to be ruminant, poultry or human, but unable to be further resolved).

Faecal source tracking analysis suggests that ruminant pollution is the dominant pollution source at this site, accounting for up to 100% of pollution across the three sampling events. Ovine-specific markers were detected in May and July, with bovine markers present in May. Wildfowl PCR markers were also present in May and September.

Review of land use in the sub-catchment shows a mixture of land use types, including sheep and beef (42%), dairy (17%), a small amount of specialist deer (2%) and conservation and forestry (together 34%) (Figure 34, Figure 35).

Table 17. Results for microbial and FST analysis of water samples collected from Oreti River at Wallacetown.

Site		Oreti River at Wallacetown		
Sample #		CMB150469	CMB150980	CMB151548
Client #		20151845	20152677	20153086
Date Sampled		12/05/2015	07/07/2015	08/09/2015
Rainfall		Yes	Yes	Yes
		Microbial Properties		
Faecal coliforms		630	100	210
E. coli		560	100	130
Campylobacter		9.3	0.9	<0.3
***Campylobacter* Species**		*C. jejuni*	*C. jejuni & Thermo*	nt
MBiT *Campylobacter* Source	**Wildfowl**		2	
	Ovine/Bovine/ Deer			
	Poultry			
	Not Wildfowl	2		
	Unknown			
		Faecal Source Tracking		
General – GenBac3		++++	+++	++++
Ruminant		50-100%	50-100%	50-100%
Human - BacH		+	-	+
Human - BiADO		-	-	-
Cow		+	-	-
Sheep		+	+	-
Wildfowl - GFD		+	-	+
Wildfowl - E2		+	-	-

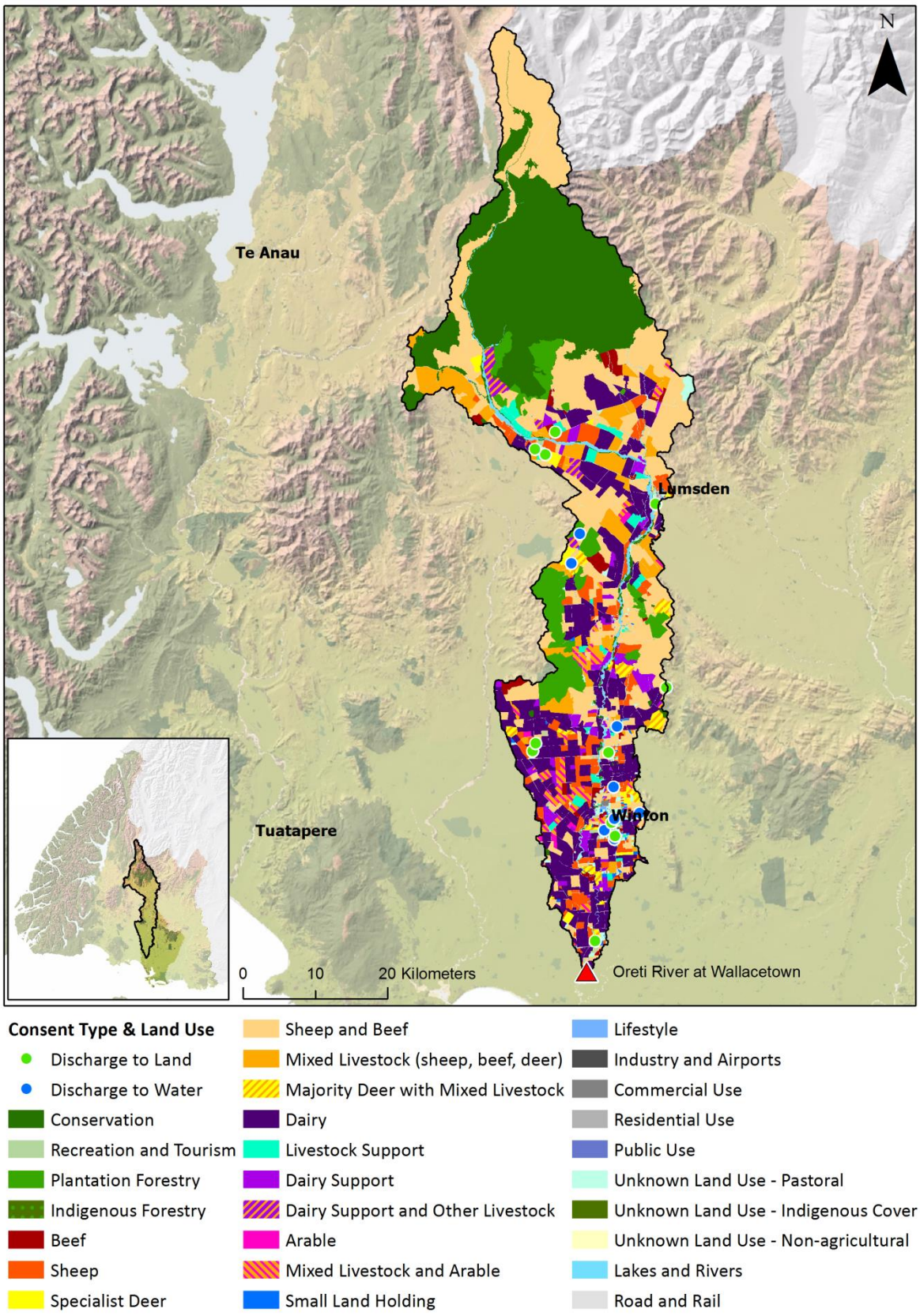

Figure 34. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Oreti River at Wallacetown sampling site.

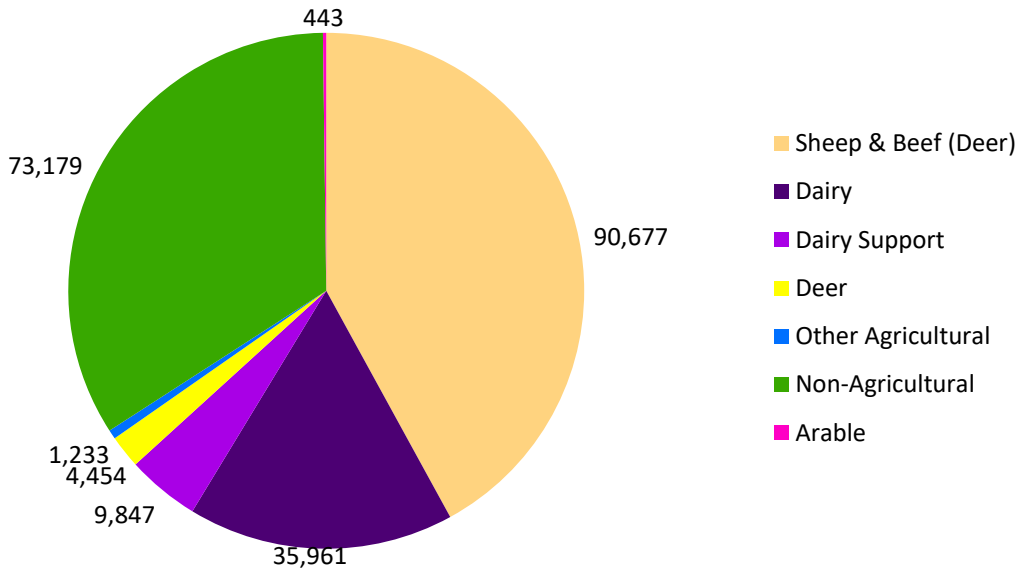

Figure 35. Land use (in hectares) in the catchment for the Oreti River at Wallacetown sampling site.

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

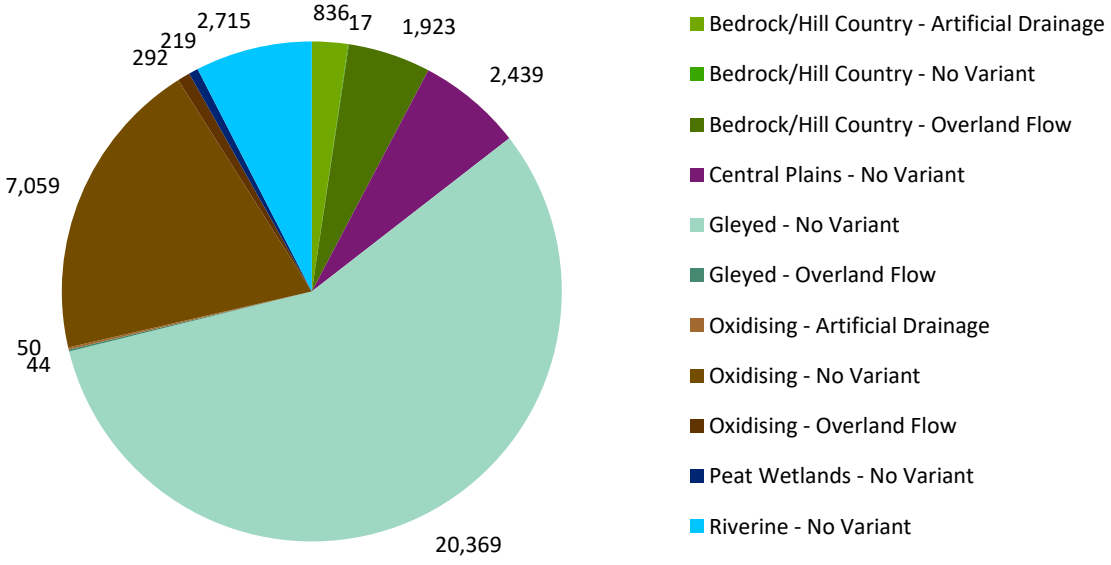

Figure 36. Dairying land (in hectares) in the catchment for the Oreti River at Wallacetown sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 18. Number of consented catchment discharges to land and water in the catchment for the Oreti River at Wallacetown sampling site.

Oreti River at Wallacetown		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	3
	1080	2
	Blood	2
	Calcium Magnesium Acetate	1
	Clean Fill	3
	Dairy Shed Effluent (land)	124
	Dairy Shed Effluent (land), Leachate	1
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	34
	Filter Backwash	1
	Meat Works Effluent, Sludge	1
	Meat Works Effluent, Waste Water	1
	Oil/Grease	2
	Oxidation Pond Effluent, Sewage (Treated), Waste Water	1
	Refuse - Commercial, Refuse - Domestic, Refuse - Industrial	1
	Septic Tank Effluent	1
	Wash Down Effluent	2
	Wash Down Effluent, Wash Water	2
	Wash Down Effluent, Wash Water, Waste Water	1
	Wash Water	6
	Wash Water, Waste Water	1
To Land Total		190
To Water	Other (sediment laden water)	1
	Filter Backwash	2
	Ground water, Stormwater	1
	Industrial Effluent, Wash Water	1
	Sewage (Treated), Waste Water	1
	Stormwater	5
To Water Total		11
Grand Total		201

Note: Consent information accurate as of April 2017

B.7 OTAPIRI STREAM AT OTAPIRI GORGE

Water samples were collected from the Otapiri Stream at Otapiri Gorge in May, September and December 2015. Each sampling event was preceded by rainfall (Table 19).

E. coli levels at this site were highly variable, with a peak concentration of 11,000 cfu/100 ml observed in December, compared with 4,300 and 200 cfu/100 ml in May and September, respectively.

Campylobacter was detected in all three samples, although the pattern of abundance differed from that for *E. coli*. The highest levels of *Campylobacter* (93 MPN/100 ml) were observed in May, with a significant reduction in subsequent samples (0.4 and 9.3 MPN/100 ml in September and December samples). *Campylobacter* isolates were determined to be *C. jejuni*, with *C. coli* also present in the May sample. The source of the *Campylobacter* in both the May and September samples remains unclear (i.e. 'not wildfowl'). The December sample contained *Campylobacter* from a combination of wildfowl, ruminant and poultry sources.

FST analysis found ruminant pollution to be the dominant pollution source at this site (50-100% in all samples). Ovine-specific markers were identified in all three samples, with bovine specific markers also present in the May sample. Wildfowl markers were identified in the September sample. These findings are consistent with the patterns of land-use within the sub-catchment: 90% of land is used for sheep and beef farming (including sheep-only and mixed sheep/beef/deer). Small portions of land are used for dairy, specialist deer, and plantation forestry (Figure 37, Figure 38).

Table 19. Results for microbial and FST analysis of water samples collected from Otapiri Stream at Otapiri Gorge.

Site		Otapiri Stream at Otapiri Gorge		
Sample #		CMB150472	CMB151550	CMB152262
Client #		20151819	20153090	20154552
Date Sampled		12/05/2015	08/09/2015	16/12/2015
Rainfall		Yes	Yes	Yes
		Microbial Properties		
Faecal coliforms		5,300	200	16,000
E. coli		4,300	200	11,000
Campylobacter		93	0.4	9.3
***Campylobacter* Species**		*C. jejuni & C. coli*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**			1
	Ovine/Bovine/ Deer			1
	Poultry			2
	Not Wildfowl	4	1	
	Unknown			
		Faecal Source Tracking		
General - GenBac3		++++	++++	++++
Ruminant		50-100%	50-100%	50-100%
Human - BacH		+	+	+
Human - BiADO		-	-	-
Cow		+	-	-
Sheep		+	+	+
Wildfowl - GFD		-	+	-
Wildfowl - E2		-	-	-

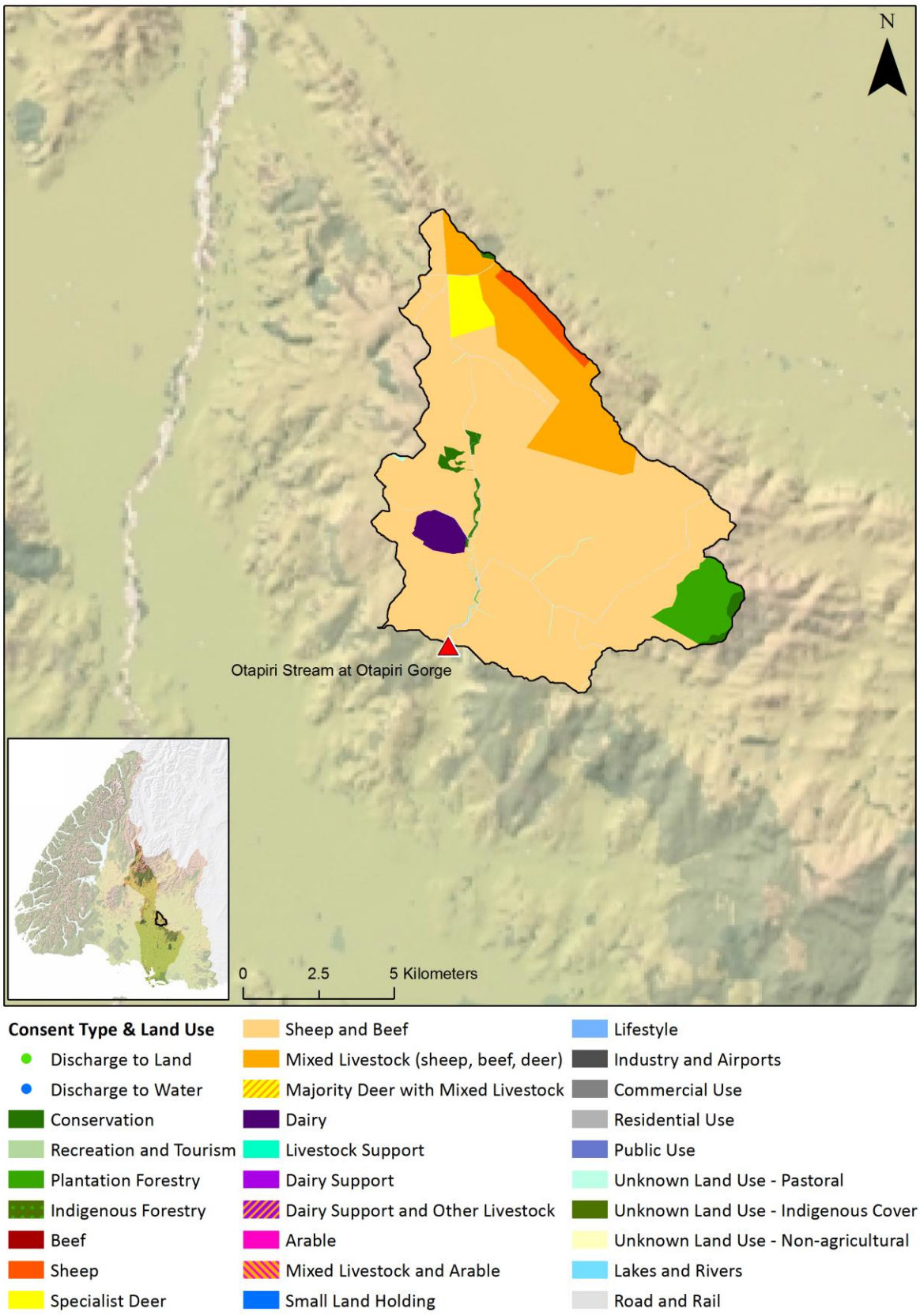

Figure 37. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Otapiri Stream at Otapiri Gorge sampling site.

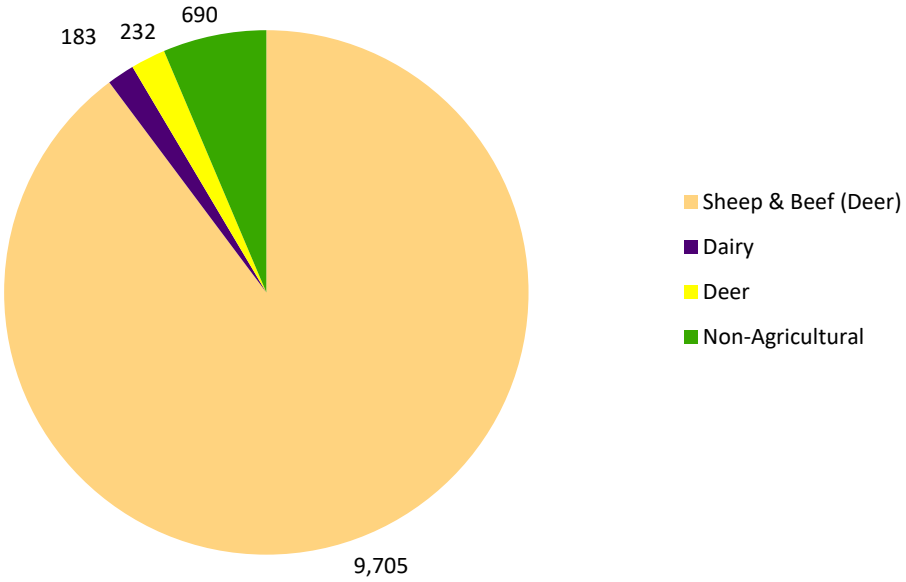

Figure 38. Land use (in hectares) in the catchment for the Otapiri Stream at Otapiri Gorge sampling site.

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

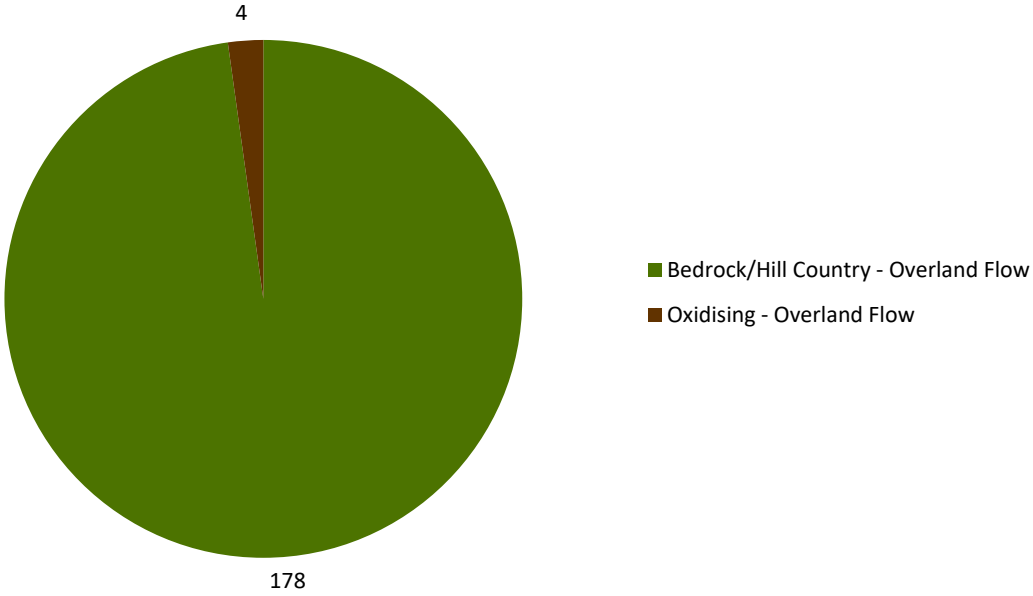

Figure 39. Dairying land (in hectares) in the catchment for the Otapiri Stream at Otapiri Gorge sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 20. Number of consented catchment discharges to land and water in the catchment for the Otapiri Stream at Otapiri Gorge sampling site.

Otapiri Stream at Otapiri Gorge		
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.8 OTEPUNI CREEK AT NITH STREET

Water samples were collected from the Otepuni Creek at Nith Street on four occasions – three times under base-flow conditions (June, August and November 2015) and once following rainfall (July 2015) (Table 21).

Under base flow conditions, *E. coli* was elevated (2,100-4,600 cfu/100 ml), and *Campylobacter* was present (0.4-1.5 MPN/100ml) in all samples. *Campylobacter jejuni* was isolated from all three samples. Isolates from June and August were determined to be of wildfowl origin, while those from November were of human origin. Faecal source tracking identified a range of pollution sources; ruminant animals were not a significant source of pollution under base flow, accounting for 10% of pollution at most. Source-specific markers for sheep were identified in the June and August samples, and for cattle in August only. Wildfowl and human-specific markers were detected in all three base-flow samples. A canine marker was also detected in the November sample (the only sample for which it was tested).

Following rainfall, the microbial burden of the water did not alter significantly, although the faecal sources and their relative dominance did. Concentrations of *E. coli* and *Campylobacter* were 1,700 cfu/100 ml and 0.9 MPN/100 ml, respectively. The *Campylobacter* was identified as *C. jejuni*, with an unspeciated isolate also present. Isolates were determined to be of wildfowl and ruminant origin. Faecal source tracking found that in contrast to base flow conditions, ruminant pollution was dominant following rainfall (50-100%). Human, bovine and ovine FST markers were all identified.

Faecal sterol analysis also identified human pollution as being present in all four samples (base flow and post-rainfall). Ruminant signatures were also identified in all four samples, while no wildfowl signatures were detected.

Land use in the sub-catchment is a mixture of sheep and beef, dairy, small land holdings and non-agricultural use (Figure 40, Figure 41). Much of the non-agricultural use comprises residential areas, which may be the source of the human contamination. Review of consented discharges in the area show one consent each for stormwater to land and to water; there are no (consented) discharges of municipal sewage or other wastewaters (Table 22). The consistent finding of human faecal contamination should be further investigated to identify its source, as it presents a significant public health risk.

Table 21. Results for microbial and FST analysis of water samples collected from Otepuni Creek at Nith Road.

Site		Otepuni Creek at Nith Street			
Sample #		CMB150804	CMB151385	CMB152076	CMB150979
Client #		20152073	20152919	20153981	20152656
Date Sampled		09/06/2015	11/08/2015	17/11/2015	07/07/2015
Rainfall		No	No	No	Yes
		Microbial Properties			
Faecal coliforms		2,200	3,800	4,600	1,700
E. coli		2,100	3,200	4,600	1,700
Campylobacter		0.9	1.5	0.4	0.9
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni & Thermo*
MBiT *Campylobacter* Source	**Wildfowl**	2	3		1
	Ovine/Bovine/ Deer				1
	Poultry				
	Not Wildfowl				
	Human			1	
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	++++	++++
Ruminant		≤1%	1-10%	≤1%	50-100%
Human - BacH		+	+	+	+
Human - BiADO		+	+	+	+
Cow		-	+	-	+
Sheep		+	+	-	+
Wildfowl - GFD		+	+	-	-
Wildfowl - E2		+	+	+	-
Canine		nt	nt	+	nt
		Sterol Properties			
Total Sterols		1773	2508	4939	6281
Coprostanol		172	257	1940	435
Faecal		F1+F2	F1+F2	F1+F2	F1+F2
Human		Yes (3)	Yes (2)	Yes (3)	Yes (2)
Ruminant		Yes (1)	Yes (1)	Yes (3)	Yes (3)
Wildfowl		No	No	No	No

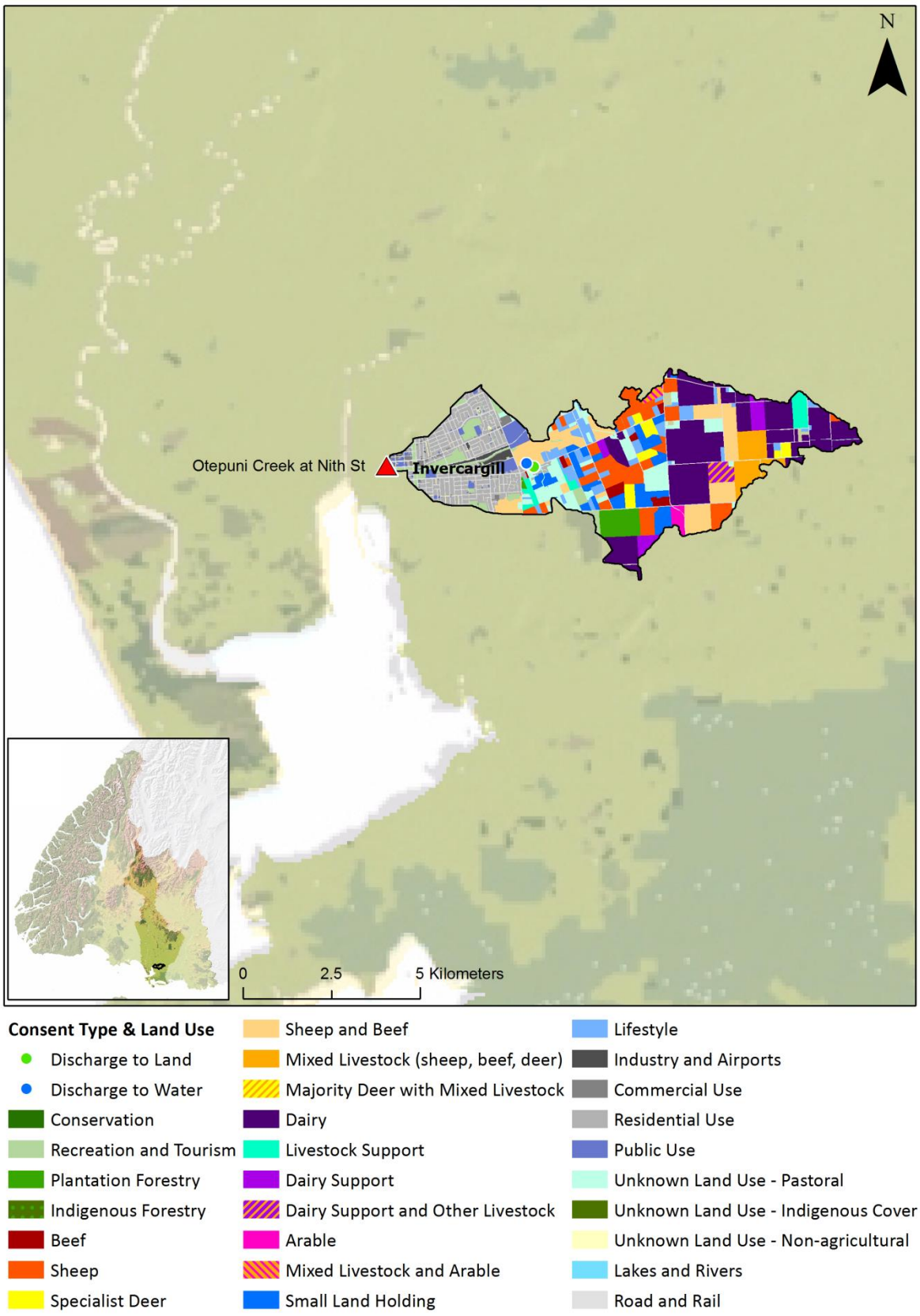

Figure 40. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Otepuni Creek at Nith Street sampling site.

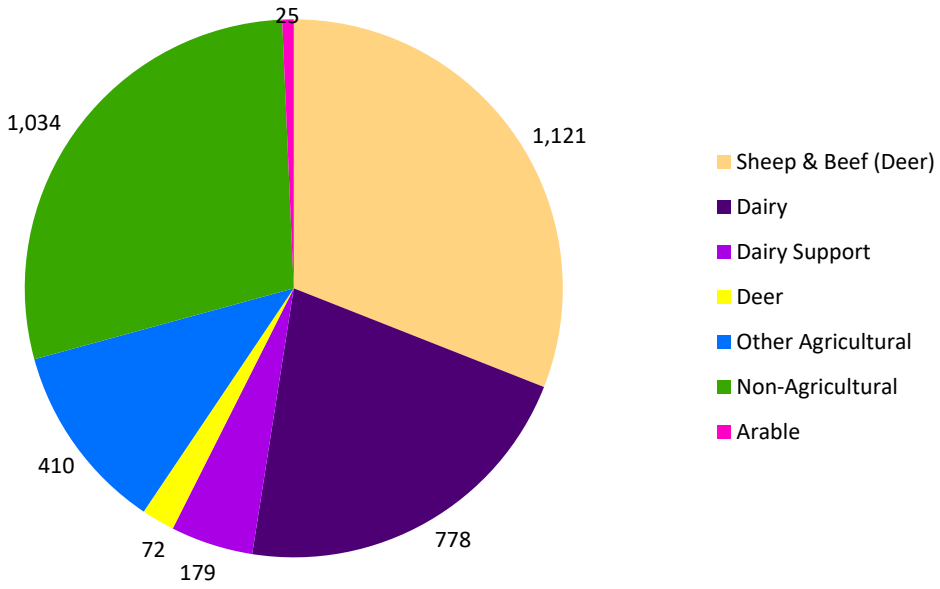

Figure 41. Land use (in hectares) in the catchment for the Otepuni Creek at Nith Street sampling site.

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

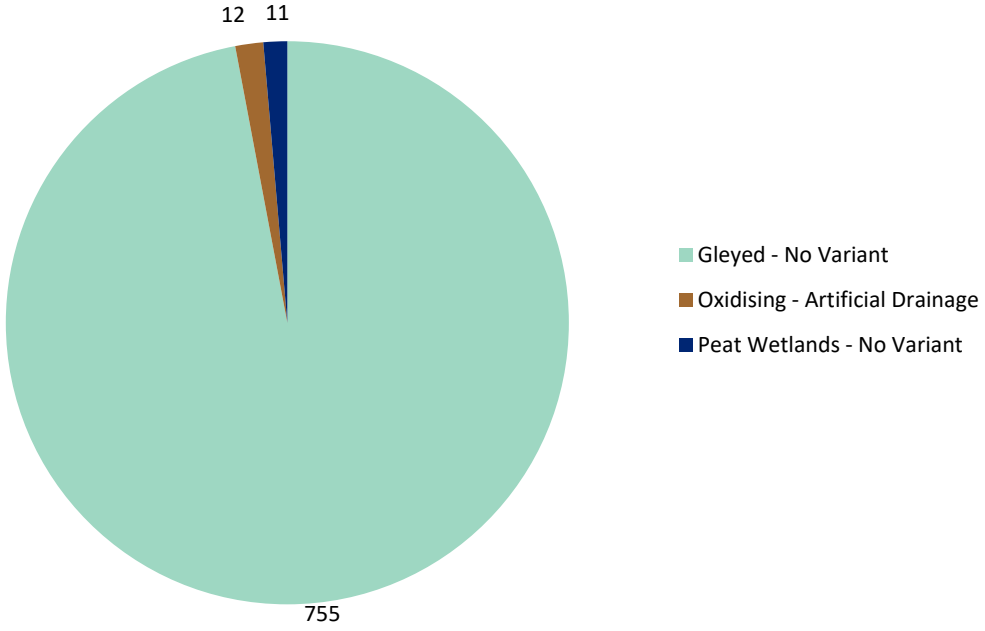

Figure 42. Dairying land (in hectares) in the catchment for the Otepuni Creek at Nith Street sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 22. Number of consented catchment discharges to land and water in the catchment for the Otepuni Creek at Nith Street sampling site.

Otepuni Creek at Nith Street		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	1
	Dairy Shed Effluent (land)	4
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
	Stormwater	1
To Land Total		7
To Water	Stormwater	1
To Water Total		1
Grand Total		8

Note: Consent information accurate as of April 2017

B.9 TUSSOCK CREEK AT COOPER ROAD

Water samples were collected from Tussock Creek at Copper Road on six occasions, between May and December 2015. Samples collected in June, August and November represent base flow conditions, while May, September and December were collected following rainfall (Table 23).

Samples collected under base flow conditions had *E. coli* levels of 250, 180 and 600 cfu/100ml in June, August and November, respectively. *Campylobacter* was present in the June and August samples (2.3-4.3 MPN/100 ml). Genotype analysis identified the *Campylobacter* as *C. jejuni*, with MBiT analysis confirming a wildfowl source. Faecal source tracking found that under base flow conditions, ruminant contamination accounted for less than half (but typically 1-10%) of overall faecal pollution. Wildfowl-specific markers were detected in all three base flow samples, with canine-specific markers also identified in November (the only sample for which canine markers were tested).

Microbial contamination was significantly higher in those samples collected following rainfall. *E. coli* levels of 41,000 cfu/100 ml were reported in both May and December samples, although much lower levels of 700 cfu/100 ml were reported for September. *Campylobacter* was present in all three samples, at higher levels than observed under base flow conditions; the highest concentration of 110 MPN/100ml was observed in May (9.3 and 24 MPM/100ml in August and December). *C. jejuni* was identified as being present in all samples, with *C. coli* also present in the May sample. MBiT analysis determined each sample contained *Campylobacter* of wildfowl and poultry origins, with a ruminant source also identified in the December sample.

In contrast to the relatively low dominance of ruminant pollution under base flow conditions, ruminant contamination accounted for 50-100% of faecal pollution present following rainfall. Faecal source tracking identified bovine-specific markers in all three post-rain samples. Ovine-specific markers were also present in May and December, and wildfowl markers in September and December. Faecal sterol analysis detected a ruminant signature only.

Land use in the sub-catchment is dominated by sheep and mixed livestock (sheep, beef and deer) agriculture (together 63%), with dairy, specialist deer and conservation use also present (Figure 43, Figure 44).

Table 23. Results for microbial and FST analysis of water samples collected from Tussock Creek at Cooper Road.

Site		Tussock Creek at Cooper Road					
Sample #		CMB150801	CMB151382	CMB152073	CMB150474	CMB151552	CMB152263
Client #		20152070	20152916	20153978	20151823	20153094	20154553
Date Sampled		09/06/2015	11/08/2015	17/11/2015	12/05/2015	08/09/2015	16/12/2015
Rainfall		No	No	No	Yes	Yes	Yes
		Microbial Properties					
Faecal coliforms		270	200	600	53,000	900	41,000
E. coli		250	180	600	41,000	700	41,000
Campylobacter		4.3	2.3	<0.3	110	9.3	24
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	nt	*C. jejuni & C. coli*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	4	3		1	3	2
	Ovine/Bovine/ Deer						2
	Poultry				1	2	1
	Not Wildfowl				1		1
	Unknown						
		Faecal Source Tracking					
General - GenBac3		++++	++++	++++	++++	++++	++++
Ruminant		10-50%	1-10%	1-10%	50-100%	50-100%	50-100%
Human - BacH		-	+	-	+	+	+
Human - BiADO		-	-	-	-	-	-
Cow		-	-	-	+	+	+
Sheep		-	-	-	+	-	+
Wildfowl - GFD		+	+	-	-	+	+
Wildfowl - E2		+	+	+	-	+	+
Canine		nt	nt	+	nt	nt	nt
		Sterol Properties					
Total Sterols		nt	nt	nt	nt	4222	nt
Coprostanol						192	
Faecal						F1+F2	
Human						No	
Ruminant						Yes (3)	
Wildfowl						No	

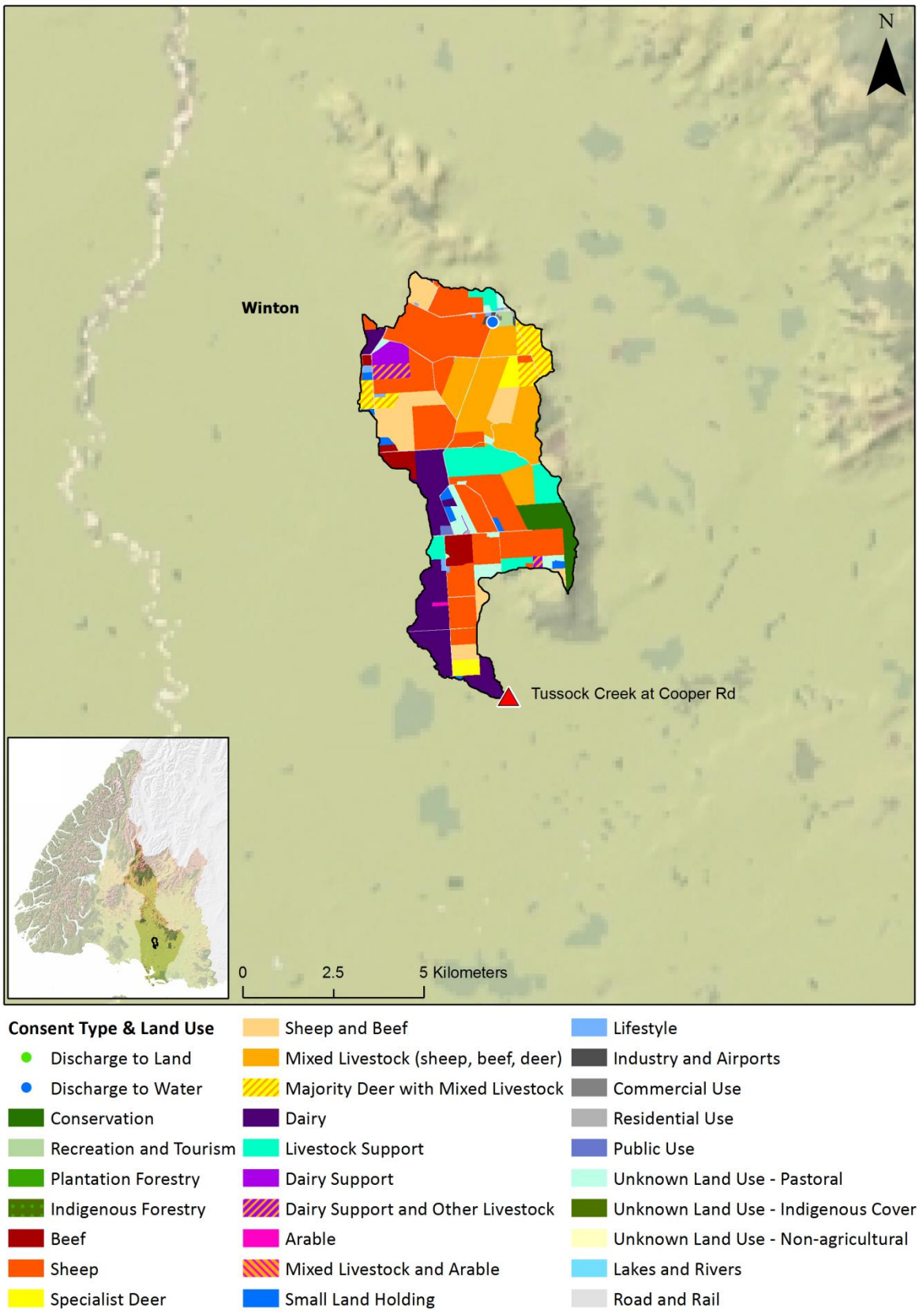

Figure 43. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Tussock Creek at Cooper Road sampling site.

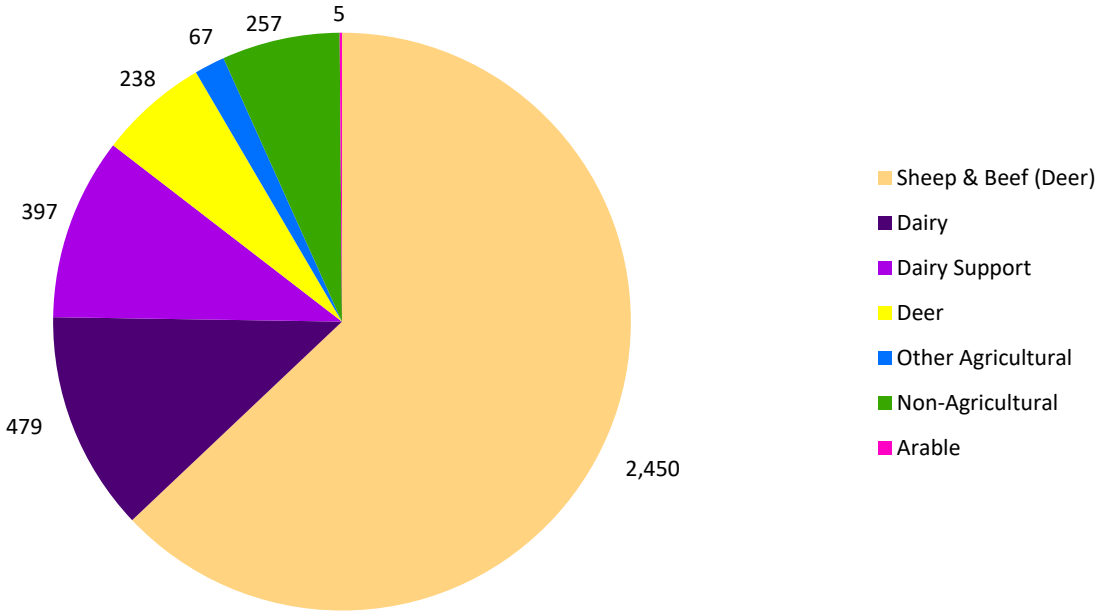

Figure 44. Land use (in hectares) in the catchment for the Tussock Creek at Cooper Road sampling site.

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

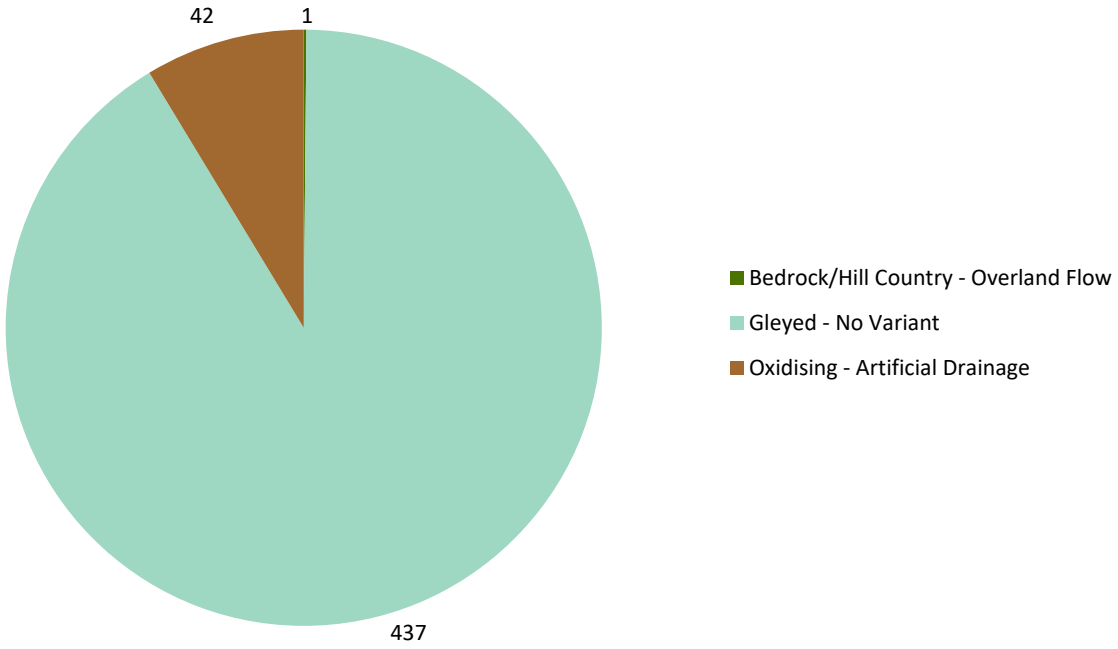

Figure 45. Dairying land (in hectares) in the catchment for the Tussock Creek at Cooper Road sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 24. Number of consented catchment discharges to land and water in the catchment for the Tussock Creek at Cooper Road sampling site.

Tussock Creek at Cooper Road		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	1
	Oil/Grease	1
To Land Total		2
To Water	Oxidation Pond Effluent, Sewage (Treated), Sewage Package Plant, Waste Water	1
To Water Total		1
Grand Total		3

Note: Consent information accurate as of April 2017

B.10 WAIHOPAI RIVER UPSTREAM OF QUEEN'S DRIVE

The Waihopai River was sampled upstream of Queen's Drive. Water samples were collected on six occasions between May and December 2015. Three sampling events occurred during base flow conditions (June, August and November), while the other three took place following rainfall (May, early December and mid-December) (Table 25).

E. coli levels for the samples collected under base flow conditions were between 120-330 cfu/100 ml. *Campylobacter* was detected in two of the samples (June 4.3 MPN/100 ml and August 0.4 MPN/100 ml). Isolates from both *Campylobacter*-positive samples were determined to be *C. jejuni* of a wildfowl origin. A 'not-wildfowl' source was also identified in the June sample. Faecal source tracking revealed that under base flow conditions, ruminant pollution accounts for less than half (but typically less than 10%) of the faecal pollution present. Ovine PCR markers were present in June and August, and bovine markers in August. Wildfowl markers were present in all three base flow samples. Human faecal contamination was detected in the June sample.

Microbial burden in samples collected following rainfall was highly varied: *E. coli* levels in May and early December were 400 and 180 cfu/100 ml respectively, whilst the sample collected in mid-December contained 19,000 cfu/100 ml. *Campylobacter* was also highest in mid-December (43 MPN/100 ml), compared with 4.3 MPN/100 ml in May and none detected in the early December sample. Both *Campylobacter*-positive samples were found to contain *C. jejuni*. MBiT source attribution analysis identified a wildfowl source in both *Campylobacter*-positive samples, with poultry and 'not wildfowl' sources present in the contaminated December sample. Faecal source tracking determined that ruminants were the dominant pollution source in May and mid-December (50-100%), but only accounted for 1-10% in the early December sample. Ovine PCR markers were identified in May, bovine markers in the mid-December, and wildfowl markers in all three samples. Human faecal contamination was also identified in the May and mid-December samples.

Sterol analysis was carried out for the three samples where human contamination was identified. This analysis identified a human contamination signature in the June, base-flow sample, but not the two post-rainfall samples. Sterol analysis also identified ruminant, but not wildfowl signatures. The differences in the contamination sources identified by sterol analysis highlights the differences in sensitivities between chemical and microbial analysis.

Land use in the sub-catchment for this site is dominated by dairy and associated support activities (together 53%), with other agricultural activity mostly sheep or mixed sheep and beef. Residential land and small lifestyle blocks each account for less than 5% of land use in the catchment (Figure 46, Figure 47). Consented discharges in the sub-catchment include septic tank effluent to land, and treated sewage, stormwater and wastewater to water (Table 26). Although the scale or volume of discharge is not available, these discharges could represent the source of the human contamination of the Waihopai River upstream of Queen's Drive.

Table 25. Results for microbial and FST analysis of water samples collected from the Waihopai River, upstream of Queen's Drive.

Site		Waihopai River upstream of Queen's Drive					
Sample #		CMB150803	CMB151384	CMB152075	CMB150476	CMB152235	CMB152256
Client #		20152072	20152918	20153980	20151825	20154437	20154515
Date Sampled		09/06/2015	11/08/2015	17/11/2015	12/05/2015	08/12/2015	15/12/2015
Rainfall		No	No	No	Yes	Yes	Yes
		Microbial Properties					
Faecal coliforms		290	150	330	520	200	20,000
E. coli		290	120	330	400	180	19,000
Campylobacter		4.3	0.4	<0.3	4.3	<0.3	43
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	nt	*C. jejuni & Thermo*	nt	*C. jejuni & Thermo*
MBiT *Campylobacter* Source	**Wildfowl**	1	1		3		5
	Ovine/Bovine/ Deer						
	Poultry						1
	Not Wildfowl	3					1
	Unknown						
		Faecal Source Tracking					
General - GenBac3		++++	++++	++++	++++	++++	++++
Ruminant		10-50%	1-10%	1-10%	50-100%	1-10%	50-100%
Human - BacH		+	+	+	+	+	+
Human - BiADO		+	-	-	+	-	+
Cow		-	+	-	-	-	+
Sheep		+	+	-	+	-	-
Wildfowl - GFD		+	+	-	+	+	+
Wildfowl - E2		+	+	+	+	-	-
Canine		nt	nt	-	nt	nt	nt
		Sterol Properties					
Total Sterols		1485	nt	nt	13,574	nt	19,008
Coprostanol		88			534		545
Faecal		F1 + F2			F1+F2		F1+F2
Human		Yes (1)			No		No
Ruminant		Yes (2)			Yes (2)		Yes (2)
Wildfowl		No			No		No

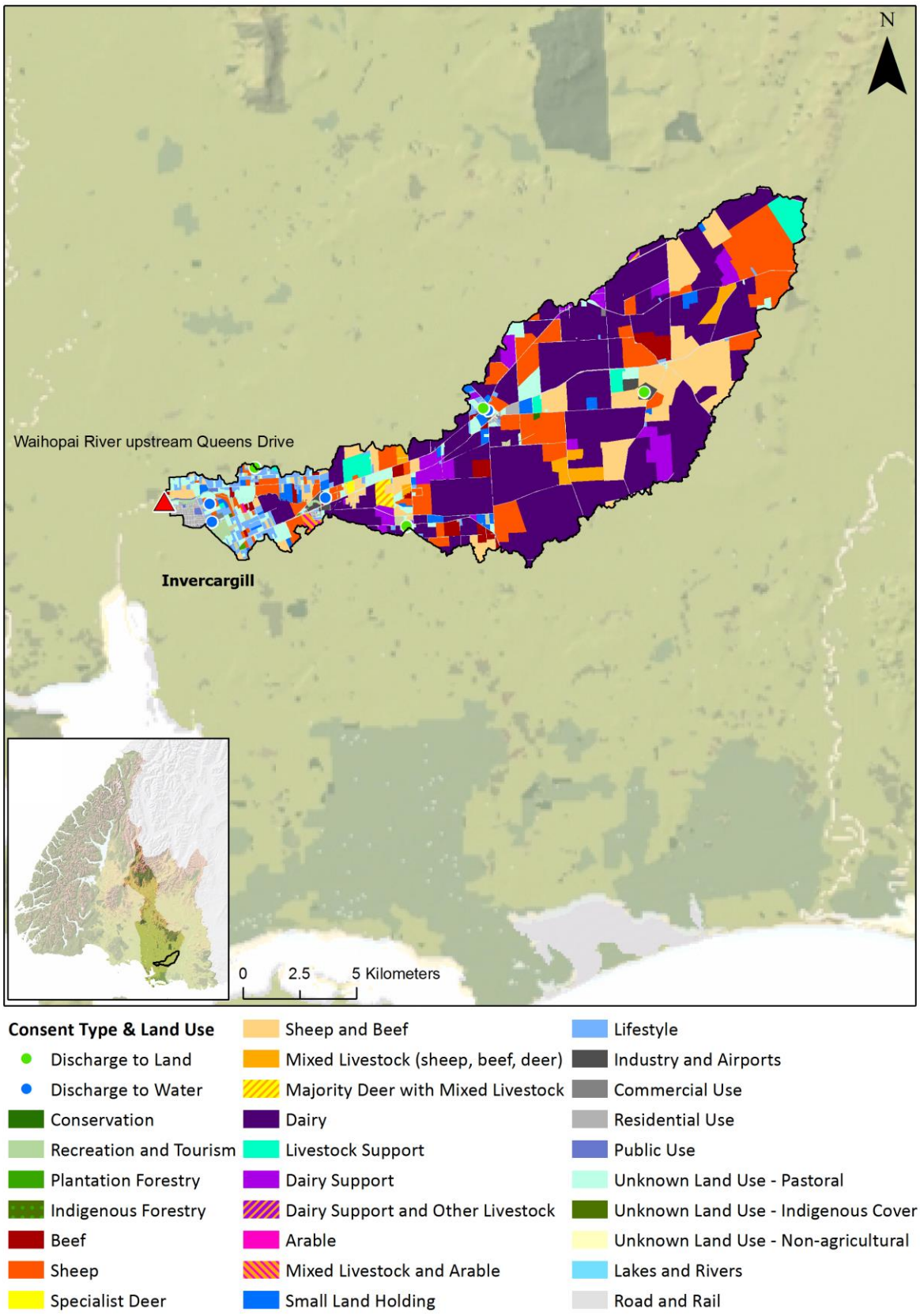

Figure 46. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Waihopai River upstream of Queen's Drive sampling site.

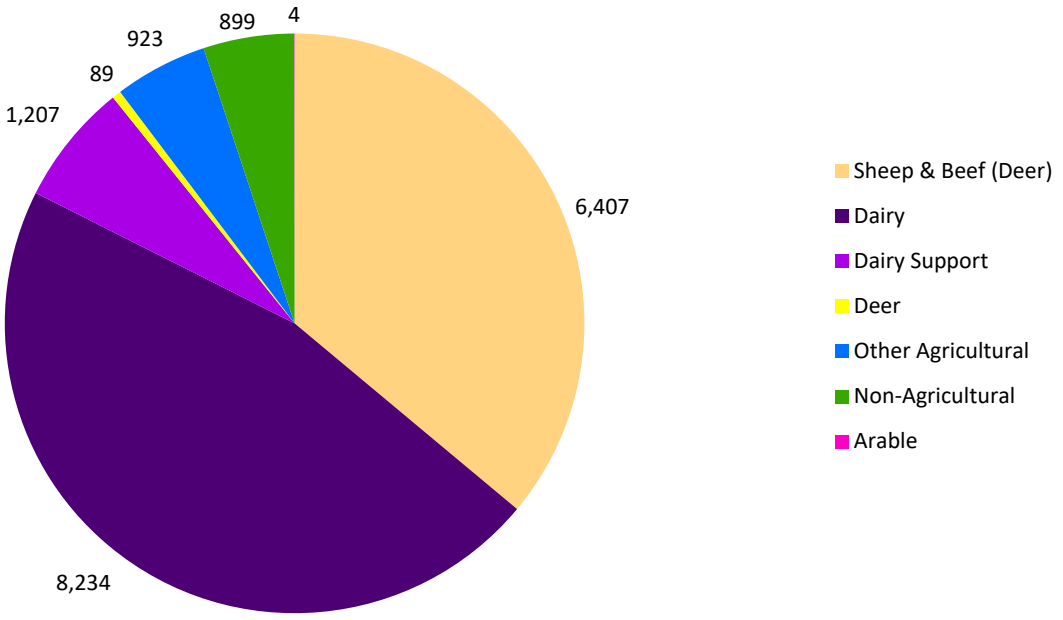

Figure 47. Land use (in hectares) in the catchment for the Waihopai River upstream of Queens's Drive sampling site.

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

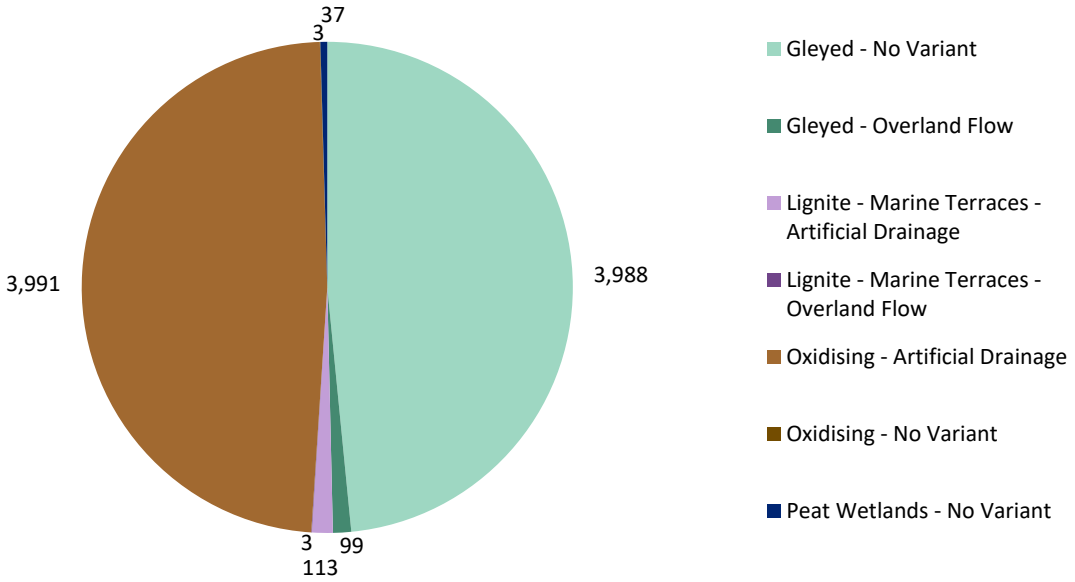

Figure 48. Dairying land (in hectares) in the catchment for the Waihopai River upstream of Queen's Drive sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 26. Number of consented catchment discharges to land and water in the catchment for the Waihopai River upstream of Queen's Drive sampling site.

Waihopai River upstream Queens Drive		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	3
	Blood, Meat Works Effluent, Wash Water, Waste Water	1
	Blood, Wash Down Effluent	1
	Dairy Shed Effluent (land)	41
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	7
	Offal	1
	Oil/Grease	1
	Septic Tank Effluent, Sewage (Treated), Waste Water	1
	Septic Tank Effluent, Waste Water	1
	Waste Water	1
	Wintering Pad/Feedlot Effluent (land)	1
To Land Total		59
To Water	Floodwaters	1
	Sewage (Treated)	1
	Stormwater	2
	Waste Water	1
To Water Total		5
Grand Total		64

Note: Consent information accurate as of April 2017

B.11 WAIKIWI STREAM AT NORTH ROAD

Water samples were collected from Waikiwi Stream at North Road. Three samples were collected under base flow conditions (June, August and November), and three following rainfall (May, July and December) (Table 27).

E. coli levels present under base flow conditions were consistently between 300 and 360 cfu/100 ml. Low levels of *Campylobacter* – determined to be *C. jejuni* of wildfowl origin – were detected in June and August. Faecal source tracking determined that the impact of ruminant pollution under base flow conditions was low (1-10%), and neither ovine nor bovine PCR markers were detected. Wildfowl markers were present in all three base flow samples. Human contamination markers were detected in June and August. Faecal sterol analysis appeared to contradict the FST findings, finding ruminant faecal signatures but not those of human or wildfowl.

E. coli levels were elevated following rainfall, with the highest levels recorded in May (6,000 cfu/100ml; 900 and 500 cfu/100ml in July and December). Elevated levels of *Campylobacter* were also observed in May (24 MPN/100 ml), with other sample containing similar levels to those observed under base flow (0.9-3.0 MPN/100 ml). Genotype analysis identified the *Campylobacter* isolates as *C. jejuni.* MBiT source analysis identified a wildfowl *Campylobacter* source in all three post-rainfall samples, as well as a ruminant source in May and July. Faecal source tracking analysis determined that ruminant animal pollution was the dominant pollution type at this site in May and July (50-100%), but was less prevalent in December (10-50%). Both ovine and bovine markers were detected in May and July, but neither marker was detected in December. Wildfowl markers were present in each post-rainfall sample. Human markers were also detected in July. Faecal sterol analysis identified human and ruminant animal signatures.

Land use in the Waikiwi Stream at North Road sub-catchment is almost exclusively agricultural (96%), including sheep and/or sheep and beef, dairy and associated support, small land holdings and lifestyle blocks, and deer (Figure 49, Figure 50). Based on the available information, there are no obvious sources of the human contamination (e.g. no consented discharges of sewage or septic tank effluent, very little residential land); however, that the three positive samples were collected consecutively suggests a common source or event.

Table 27. Results for microbial and FST analysis of water samples collected from Waikiwi at North Road.

Site		Waikiwi Stream at North Road					
Sample #		CMB150802	CMB151383	CMB152074	CMB150475	CMB150978	CMB152234
Client #		20152071	20152917	20153979	20151824	20152654	20154436
Date Sampled		09/06/2015	11/08/2015	17/11/2015	12/05/2015	07/07/2015	08/12/2015
Rainfall		No	No	No	Yes	Yes	Yes
		Microbial Properties					
Faecal coliforms		330	330	370	6,000	1,000	900
E. coli		300	320	360	6,000	900	500
Campylobacter		4.3	2.3	<0.3	24	0.9	3
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	nt	*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	3	3		2	1	3
	Ovine/Bovine/ Deer				1	1	
	Poultry						
	Not Wildfowl						
	Unknown						
		Faecal Source Tracking					
General - GenBac3		++++	++++	++++	++++	++++	++++
Ruminant		1-10%	1-10%	1-10%	50-100%	50-100%	10-50%
Human - BacH		+	+	+	+	+	+
Human - BiADO		+	+	-	-	+	-
Cow		-	-	-	+	+	-
Sheep		-	-	-	+	+	-
Wildfowl - GFD		+	+	+	+	+	+
Wildfowl - E2		+	+	+	-	+	-
Canine		nt	nt	-	nt	nt	nt
		Sterol Properties					
Total Sterols		1330	1840	nt	nt	4960	nt
Coprostanol		51	86			253	
Faecal		F1+F2	F1+F2			F1+F2	
Human		No	No			Yes 1)	
Ruminant		Yes (2)	Yes (1)			Yes (2)	
Wildfowl		No	No			No	

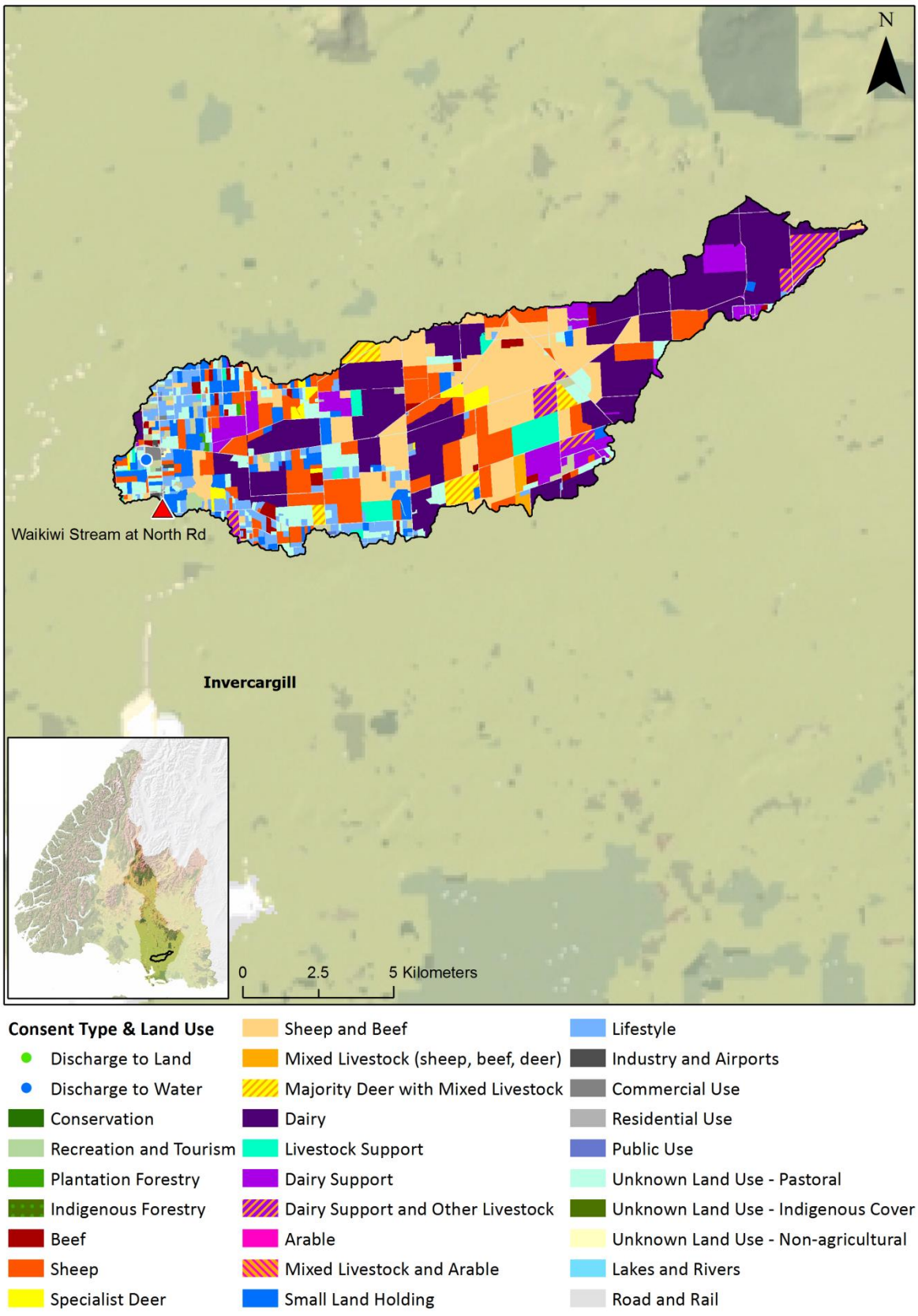

Figure 49. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Waikiwi Stream at North Road sampling site.

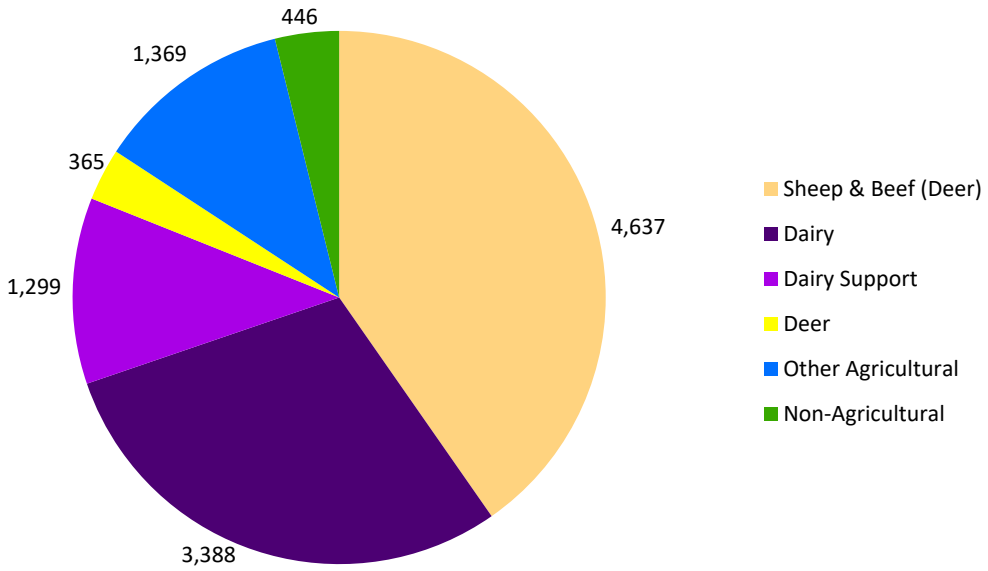

Figure 50. Land use (in hectares) in the catchment for the Waikiwi Stream at North Road sampling site.

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

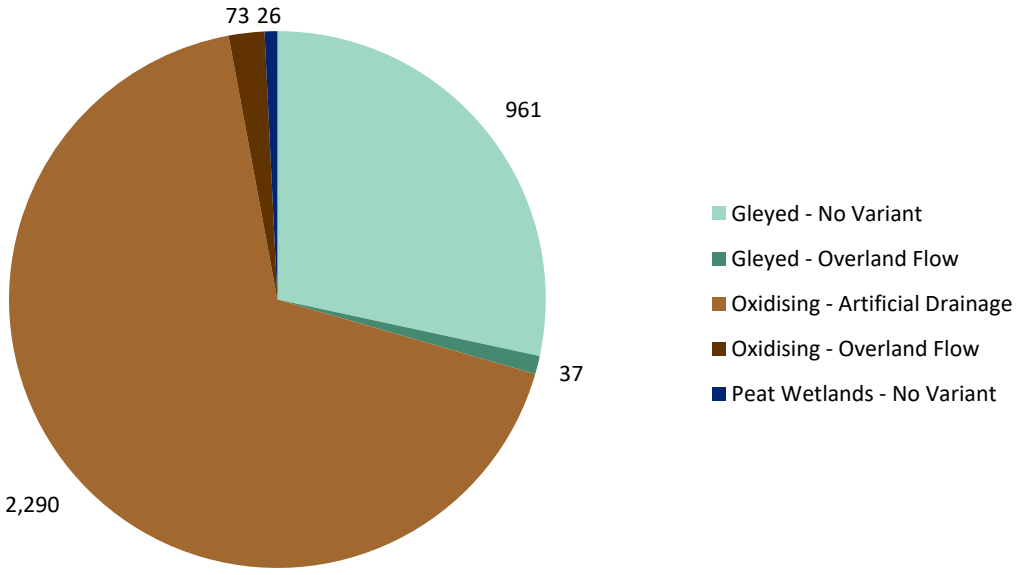

Figure 51. Dairying land (in hectares) in the catchment for the Waikiwi Stream at North Road sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 28. Number of consented catchment discharges to land and water in the catchment for the Waikiwi Stream at North Road sampling site.

Waikiwi Stream at North Road		
Subtype	Contaminant	Total
To Land	Oil	1
	Dairy Shed Effluent (land)	13
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	4
	Hazardous Substances, Wash Water	1
To Land Total		19
To Water	Stormwater, Wash Down Effluent	1
To Water Total		1
Grand Total		20

Note: Consent information accurate as of April 2017

B.12 WAITUNA CREEK AT MARSHALL ROAD

Water samples were collected from Waituna Creek at Marshall Road on four occasions between winter and summer 2015. Each sampling event followed rainfall (Table 29).

E. coli levels were determined to be 1,600 cfu/100ml in the July sample, with low levels in August and September (100-120 cfu/100 ml), and elevated levels again present in December (1,900 cfu/100 ml).

Campylobacter was detected in all four samples. Concentrations were highest in the July sample (43 MPN/100 ml), and were reduced in each subsequent sample (11, 4.3 and 0.4 MPN/100 ml). Genotype analysis determined that *C. jejuni* was present in all samples, with an unspeciated thermophylic *Campylobacter* also present in the July sample. MBiT source analysis identified a wildfowl source present for all four samples, with a 'not wildfowl' source also present in July and August, and poultry and human sources in September.

Faecal source tracking results suggest that ruminant pollution was dominant at this site in July and September (50-100%), but was minimal in December (1-10%). Ovine and bovine markers were both present in July, but not in subsequent samples. Wildfowl markers were present in July and September. No source-specific PCR markers were identified from the December sample.

A review of land use in the sub-catchment shows significant dairy, beef and sheep farming. Non-agricultural use is largely attributable to conservation land and plantation forestry (together 8%) (Figure 52, Figure 53).

Table 29. Results for microbial and FST analysis of water samples collected from Waituna Creek at Marshall Road.

Site		Waituna Creek at Marshall Road			
Sample #		CMB150954	CMB151379	CMB151542	CMB152219
Client #		20152644	20152898	20153076	20154423
Date Sampled		06/07/2015	10/08/2015	07/09/2015	07/12/2015
Rainfall		Yes	Yes	Yes	Yes
		Microbial Properties			
Faecal coliforms		2,300	300	100	2,000
E. coli		1,600	120	100	1,900
Campylobacter		43	11	4.3	0.4
Campylobacter Species		*C. jejuni* & Thermo	*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl	5	5	2	1
	Ovine/Bovine/ Deer				
	Poultry			2	
	Not Wildfowl	1	1		
	Human			2	
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	No PCR sample supplied for analysis	++++	+++
Ruminant		50-100%		50-100%	1-10%
Human - BacH		+		+	-
Human - BiADO		-		-	-
Cow		+		-	-
Sheep		+		-	-
Wildfowl - GFD		+		+	-
Wildfowl - E2		-		-	-

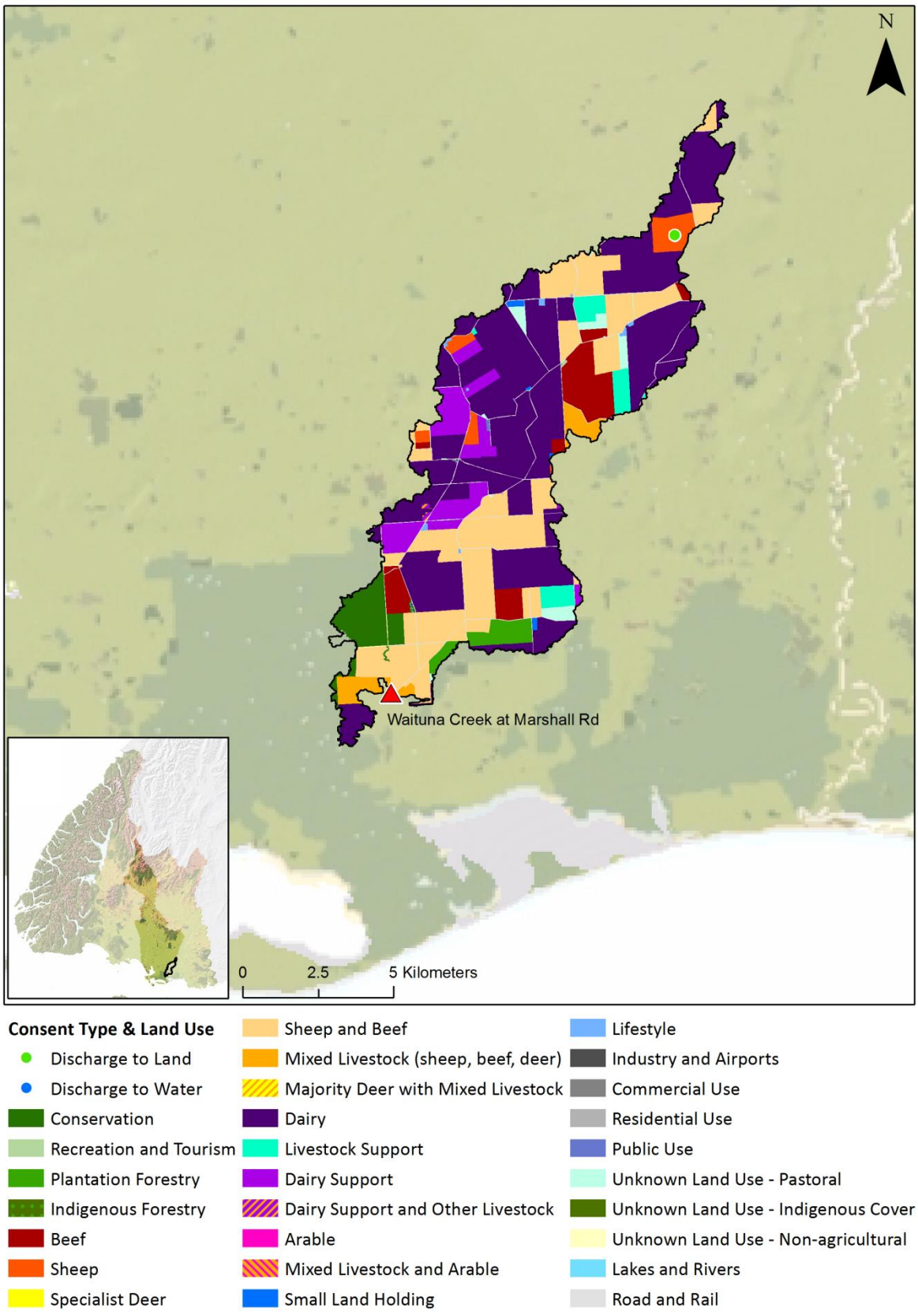

Figure 52. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Waituna Creek at Marshall Road sampling site.

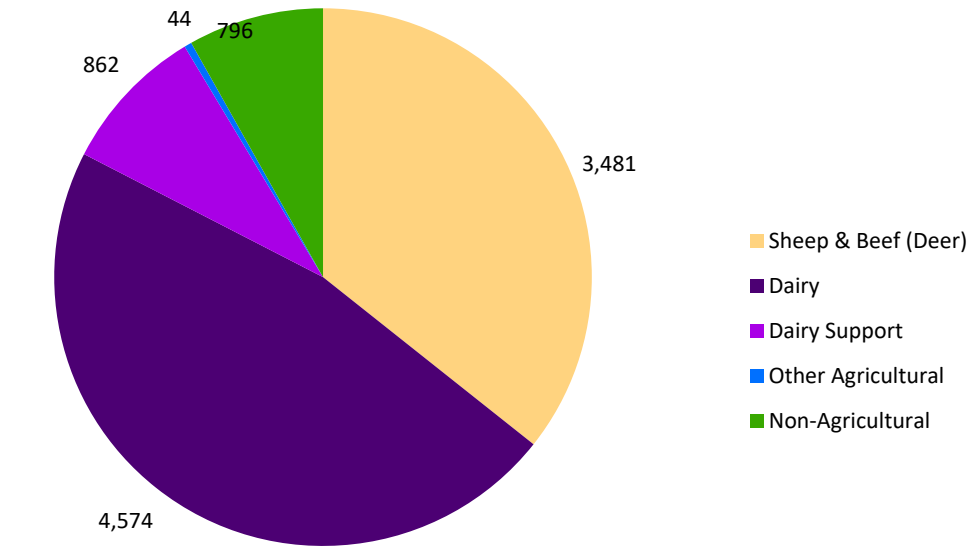

Figure 53. Land use (in hectares) in the catchment for the Waituna Creek at Marshall Road sampling site.

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

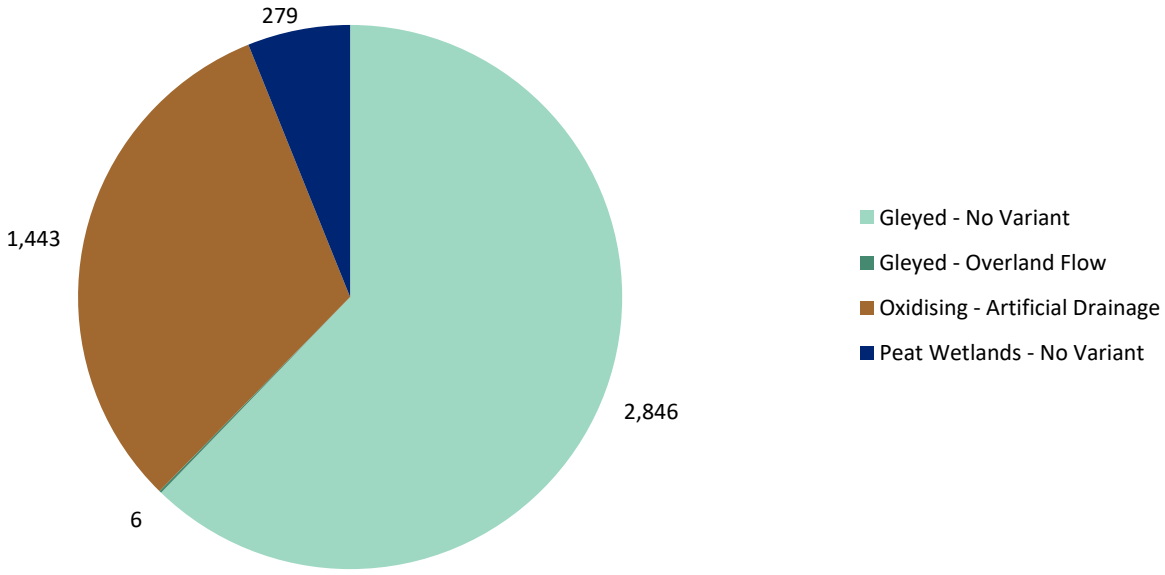

Figure 54. Dairying land (in hectares) in the catchment for the Waituna Creek at Marshall Road sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 30. Number of consented catchment discharges to land and water in the catchment for the Waituna Creek at Marshall Road sampling site.

Waituna Creek at Marshall Road		
Subtype	Contaminant	Total
	Dairy Factory Effluent	1
	Dairy Shed Effluent (land)	22
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	7
To Land Total		30
Grand Total		30

Note: Consent information accurate as of April 2017

B.13 WINTON STREAM AT LOCHIEL

Winton Stream was sampled at Lochiel on five occasions: three under base flow conditions (June, August and November 2015), and two following rainfall (May and December 2015) (Table 31).

Microbial content of the stream was variable under base flow conditions. *E. coli* levels were 600 cfu/100 ml in both June and November, rising to 2,000 cfu/100 ml in August. *Campylobacter* was detected in all three samples, with the highest concentration (24 MPN/100 ml) present in the two samples with the lower *E. coli* counts. *Campylobacter* isolates were determined to be *C. jejuni*, with an unspeciated *Campylobacter* also present in November. MBiT source analysis identified wildfowl and ruminant sources, with a poultry source also present in November. Faecal source tracking found ruminant pollution to account for more than half of the pollution at this site in the June and August samples, and 1-10% in November. The more contaminated August sample was found to contain ovine-, bovine- and wildfowl-specific markers, while the June sample had wildfowl markers and no markers were detected in the November sample.

Following rainfall, *E. coli* levels were high (2,100-2,300 cfu/100 ml), with *C. jejuni* present in both samples (2.3-4.3 MPN/100 ml). Source attribution identified a wildfowl *Campylobacter* source in May, and poultry and 'no wildfowl' sources in December. Faecal source tracking also highlighted differences between these two samples: ruminant pollution accounted for 50-100% of the pollution present in May, and 1-10% of the pollution present in December. Further, ovine, bovine and wildfowl markers were all detected in the May sample, whilst only wildfowl markers were present in December.

Land use in the Winton Stream at Lochiel sub-catchment is a mixture of sheep, sheep and beef, dairy and majoriy/specialist deer. There is also small land holding and residential use in and around Winton (Figure 55, Figure 56). Consented discharges include dairy shed effluent and meatworks effluent to land, and treated sewage and stormwater to water (Table 32).

Table 31. Results for microbial and FST analysis of water samples collected from Winton Stream at Lochiel.

Site		Winton Stream at Lochiel				
Sample #		CMB150800	CMB151381	CMB152072	CMB150471	CMB152246
Client #		20152065	20152911	20153973	20151818	20154514
Date Sampled		09/06/2015	11/08/2015	17/11/2015	12/05/2015	14/12/2015
Rainfall		No	No	No	Yes	Yes
		Microbial Properties				
Faecal coliforms		630	2,000	600	3,100	3,600
E. coli		600	2,000	600	2,100	2,300
Campylobacter		24	1.5	24	4.3	2.3
Campylobacter Species		*C. jejuni*	*C. jejuni*	*C. jejuni* & *Thermo*	*C. jejuni*	*C. jejuni*
MBiT Campylobacter Source	**Wildfowl**	5	3	5	2	
	Ovine/Bovine/ Deer	1	1			
	Poultry			2		2
	Not Wildfowl					1
	Unknown					
		Faecal Source Tracking				
General - GenBac3		++++	++++	++++	++++	++++
Ruminant		50-100%	50-100%	1-10%	50-100%	1-10%
Human - BacH		+	-	-	+	-
Human - BiADO		-	+	-	-	-
Cow		-	+	-	+	-
Sheep		-	+	-	+	-
Wildfowl - GFD		+	+	-	+	+
Wildfowl - E2		+	+	+	+	-
Canine		nt	nt	-	nt	nt

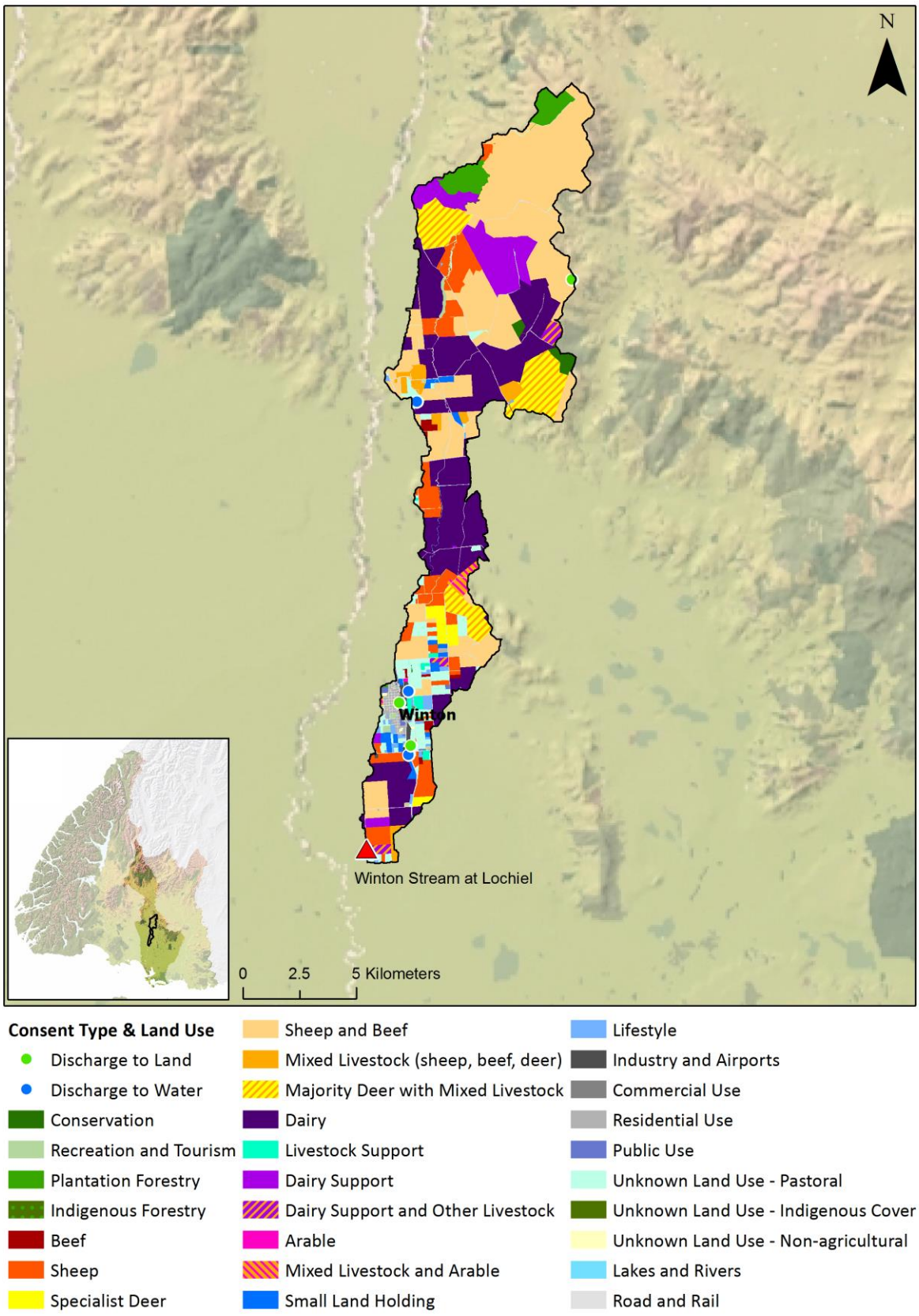

Figure 55. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Winton Stream at Lochiel sampling site.

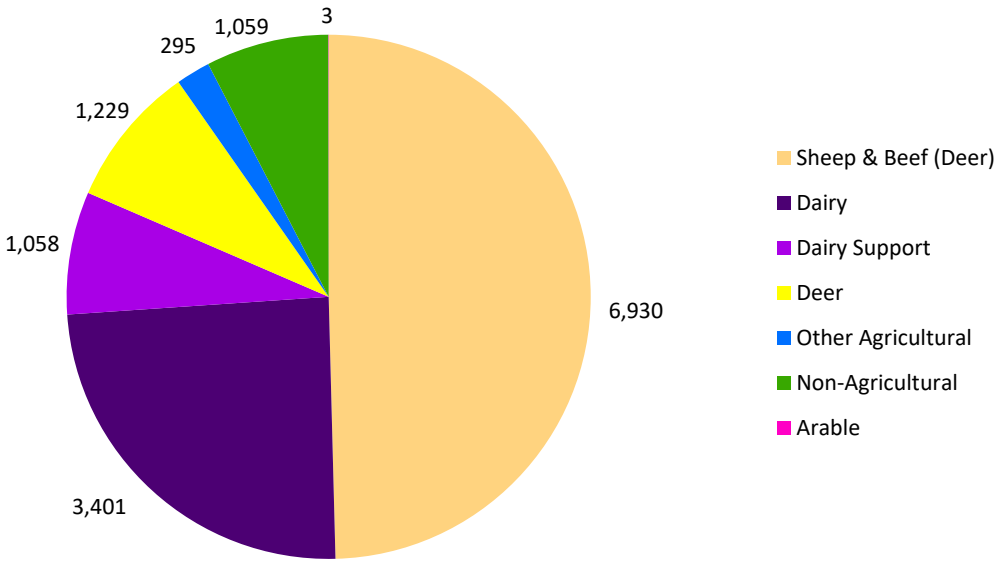

Figure 56. Land use (in hectares) in the catchment for the Winton Stream at Lochiel sampling site.

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

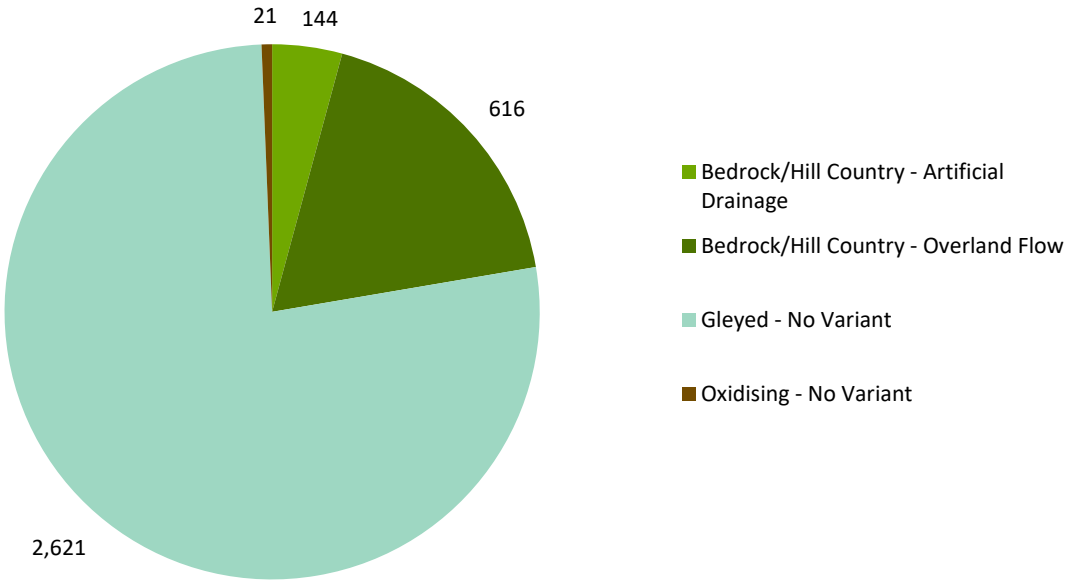

Figure 57. Dairying land (in hectares) in the catchment for the Winton Stream at Lochiel sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 32. Number of consented catchment discharges to land and water in the catchment for the Winton Stream at Lochiel sampling site.

Winton Stream at Lochiel		
Subtype	Contaminant	Total
To Land	Oil	1
	Dairy Shed Effluent (land)	8
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	3
	Meat Works Effluent, Sludge	1
	Wash Down Effluent, Wash Water	1
	Wash Water	1
To Land Total		15
To Water	Sewage (Treated), Waste Water	1
	Stormwater	2
To Water Total		3
Grand Total		18

Note: Consent information accurate as of April 2017

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