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Sources of Pollution in the Mataura Freshwater Management Unit

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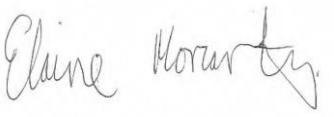

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EXECUTIVE SUMMARY

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

This report details the results of a study of faecal pollution sources at 15 freshwater sites within the Mataura 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 vulnerable to high levels of faecal contamination, with 14 sites recording *E. coli* concentrations of ≥1,000 colony-forming units (cfu)/100 ml on at least one occasion. Fifty-five percent of all water samples collected exceeded 1,000 cfu, with a maximum concentration 22,000 cfu/100 ml recorded at Mimihau Stream at Wyndham. For comparison, the current national Microbiological Guidelines for Freshwater Recreation Areas state that at *E. coli* concentrations above 550 cfu/100 ml, the local council and health authority must advise the public that the water is unsuitable for recreation, due to the elevated health risk. *E. coli* concentrations were elevated following rainfall, and a seasonal pattern was evident whereby peak microbial concentrations were observed during autumn.

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. One instance of human faecal contamination was recorded, at Oteramika Stream at Seaward Downs. As only two samples were collected from this site, further investigation is strongly recommended to determine the frequency with which human pollution is present, and to isolate its particular source.

Campylobacter was detected in 78% of all samples, representing 13 of the 15 sites. *Campylobacter jejuni* was recovered from all *Campylobacter*-positive samples, with *Campylobacter coli* and an unspeciated thermophilic *Campylobacter* each additionally recovered in 11% of samples. *Campylobacter* was more likely to be present following rainfall, and concentrations more likely to be elevated. Wildfowl were determined to be the most common source of *Campylobacter*, followed by ruminants (sheep, cattle or deer), and poultry; however, the prevalence of different sources varies depending on rainfall.

Molecular MBiT analysis of *Campylobacter* isolates revealed a high diversity of genotypes across the FMU, and that there was no separation of these to particular sites. Thirty-seven percent of the isolates obtained from waters in the Mataura 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. Only a small number of these overlapping isolates were found to be of wildfowl origin, suggesting that wildfowl may be a minor source of illness within the community compared with other sources (e.g. human or ruminant faeces), however their risk should not be discounted. Although the presence of other faecal pathogens (e.g. *E. coli* O157, *Cryptosporidium*) was not assessed, the prevalence of *Campylobacter* suggests additional pathogens may be present in the environment.

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

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

1. BACKGROUND

1.1 MICROBIAL WATER QUALITY

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

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

1.2 SOURCES OF POLLUTION AND ROUTES OF TRANSMISSION

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

1.2.1 Animal faeces

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

Cattle

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

Sheep

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

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

Other ruminants

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

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

Routes of transmission

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

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

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

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

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

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

1.2.2 Avian faeces

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

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

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

1.2.3 Human sources

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

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

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

1.3 FAECAL SOURCE TRACKING

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

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

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

1.4 *CAMPYLOBACTER*

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

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

1.5 REPORT OBJECTIVES

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

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

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

2. MATERIALS AND METHODS

2.1 SAMPLING SITES

The sampling locations selected across the Mataura Freshwater Management Unit (FMU) are listed in Table 1, and shown together with their sub-catchments in Figure 2.

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 Mataura 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
Mataura River at Gore	Rainfall only	Appendix B.1
Mataura River 200 m downstream of Mataura Bridge	Base-flow only	Appendix B.2
Mataura River at Mataura Island Bridge	Rainfall only	Appendix B.3
Waimea Stream at Mandeville	Rainfall only	Appendix B.4
Waikaia River at Waikaia	Rainfall only	Appendix B.5
Otamita Stream at Mandeville	Base-flow only	Appendix B.6
Waikaka Stream at Gore	Rainfall only	Appendix B.7
North Peak Stream at Waimea Valley Road	Rainfall only	Appendix B.8
Sandstone Stream at Kingston Crossing Road	Base-flow only	Appendix B.9
Longridge Stream at Sandstone	Rainfall only	Appendix B.10
Mimihau Stream at Wydnham	Rainfall only	Appendix B.11
Mokoreta River at Wyndham River Road	Rainfall only	Appendix B.12
Oteramika Stream at Seaward Downs	Base-flow only	Appendix B.13
Waikawa River at Progress Valley	Rainfall only	Appendix B.14
Tokanui River at Fortose Otara Road	Rainfall only	Appendix B.14

Figure 2. The Mataura FMU, with sub-catchments, sampling site locations and rivers of order 4 to 8 shown. Inset: The Mataura 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 44.5ºC (Method 9222D, APHA et al. 2012). *E. coli* was analysed by incubating faecal coliform-positive filters with media containing 4-methylumbelliferyl-ß-glucuronidase (MUG) (Method 9222G, APHA et al. 2012). Results are presented as colony-forming units (cfu).

2.2.2 *Campylobacter* isolation

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

2.2.3 *Campylobacter* sub-typing and source attribution

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

2.2.4 Faecal source tracking

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

2.2.5 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 across the different sampling locations the Mataura 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 MATAURA FMU

Microbial water quality within the Mataura FMU was highly varied, with *E. coli* concentrations varying between 5 and 20,000 cfu/100 ml. The majority of sampling locations selected within the Mataura FMU were vulnerable to 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 12 of the 15 sampling locations, with 55% of individual samples collected exceeding 1,000 cfu/100 ml. The highest *E. coli* levels were observed at the Mimihau Stream at Wyndham (22,000 cfu/100 ml), followed by Longridge Stream at Sandstone (19,000 cfu/100 ml), Waikaka Stream at Gore (17,000 cfu/100 ml), and the Waikawa River at Progress Valley (10,000 cfu/100 ml).

No site had samples collected under both base flow and following rainfall. The majority of sites had samples collected following rainfall, and these samples tended to have higher levels of *E. coli* than those from sites sampled under base flow conditions (Figures 3-5). Sites that were sampled following rainfall also tended to exhibit a seasonal pattern of microbial loading: *E. coli* levels were highest in autumn, with a progressive reduction in concentration during winter and spring (Figure 6; Appendix B). Mimihau Stream was the only waterway sampled post-rainfall in which peak *E. coli* levels were not associated with the samples collected in autumn. By comparison, there was no discernible seasonal pattern in *E. coli* concentration for samples collected under base flow, although far fewer samples were collected under these conditions.

Campylobacter was isolated at 13 of the 15 sampling locations – the two exceptions being Oteramika Stream at Seaward Downs and Sandstone Stream at Kingston Crossing Road, both of which were sampled only under base flow conditions. In total, *Campylobacter* was detected in 78% of water samples collected within the Mataura FMU, with 37% of samples having a concentration of 10 MPN/100 ml or greater. *Campylobacter* was more prevalent in samples that were collected following rainfall than under base flow (88% of post-rain samples, 33% base flow samples), and concentrations tended to be higher (Figures 3-4, Figure 7). The highest levels of *Campylobacter* were observed in the Waimea Stream at Mandeville (1,100 MPN/100 ml) and Mataura River at Gore (460 MPN/100 ml).

Similarly to *E. coli*, *Campylobacter* concentrations were highest in autumn, with a progressive reduction in concentration during winter and spring (Figure 8). All samples in which *Campylobacter* was detected contained *C. jejuni*. In addition, *C. coli* and an unspeciated thermophilic *Campylobacter* were each identified in 11% of *Campylobacter*-positive samples (Figure 9), all of which were collected following rainfall.

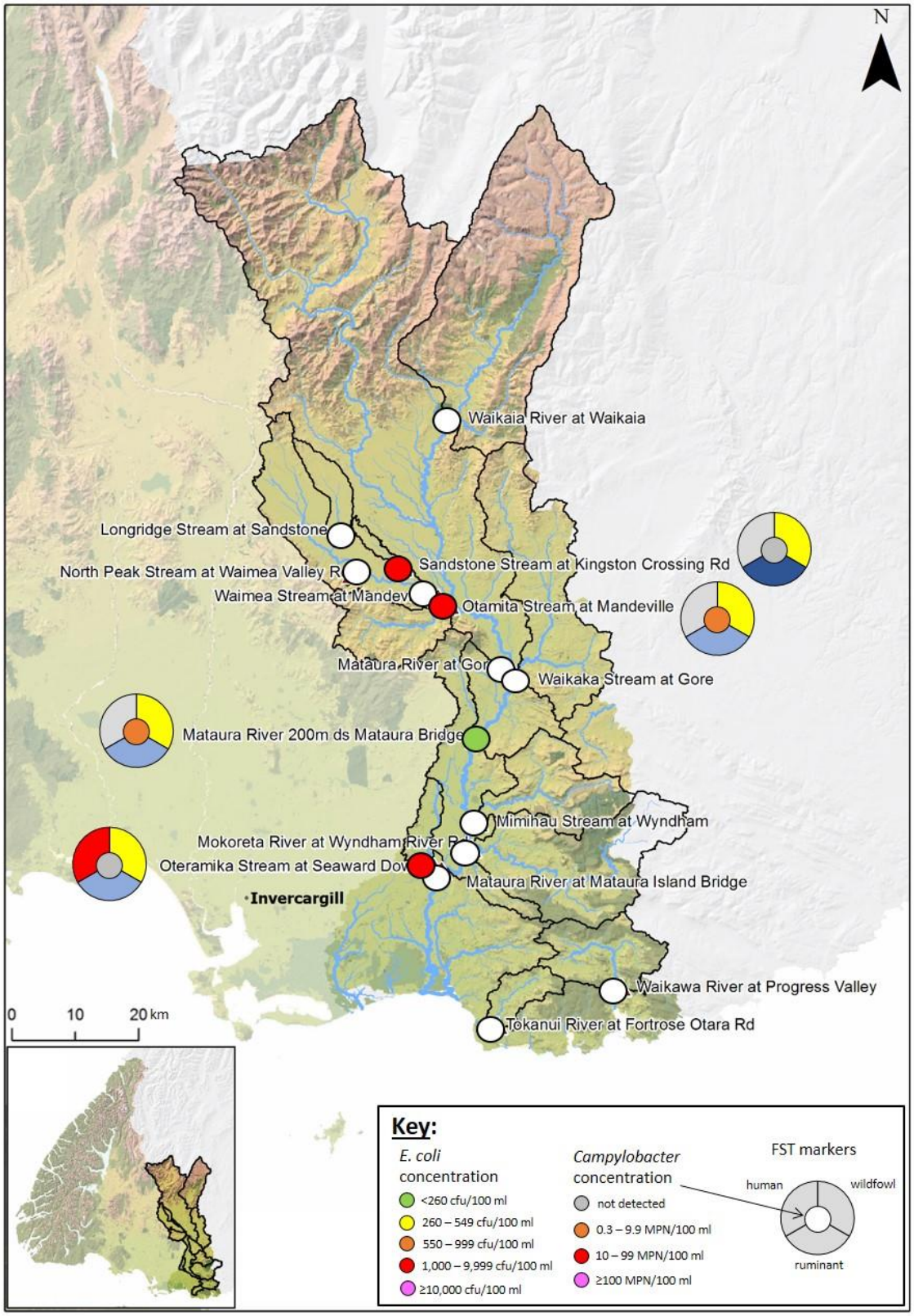

Figure 3. Overview of microbial water quality in Mataura FMU under base flow conditions. Small circles showing sampling locations on the map represent maximum *E. coli* levels for that site; white circles indicate there is no data under these conditions. Larger circles adjacent to the site name represent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

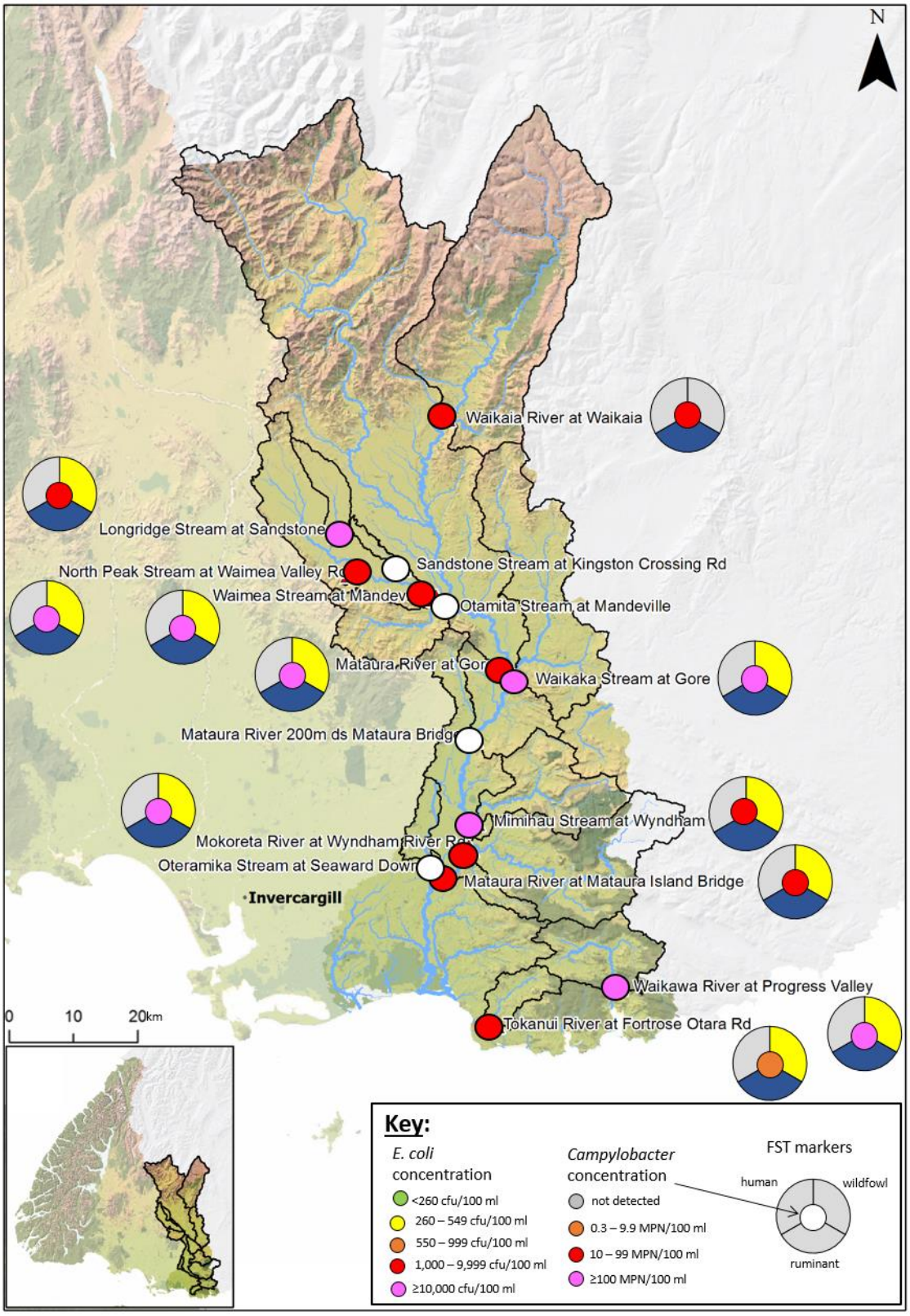

Figure 4. Overview of microbial water quality in Mataura FMU following rainfall. Small circles showing sampling locations on the map represent maximum *E. coli* levels for that site; white circles indicate there is no data under these conditions. Larger circles adjacent to the site name represent maximum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

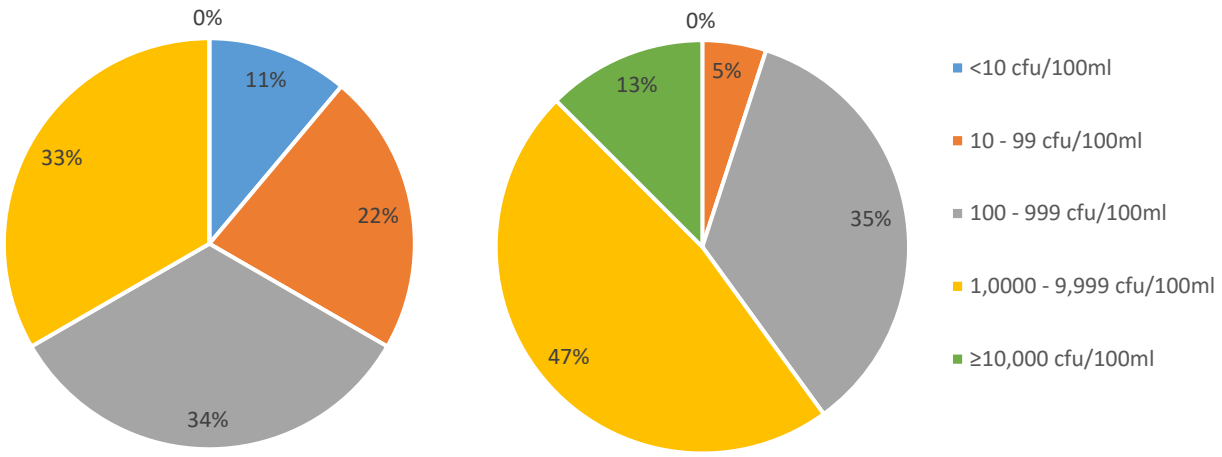

Figure 5. *E. coli* concentrations for water samples collected within the Mataura FMU under base flow conditions (left, n=9) and following rainfall (right, n=40).

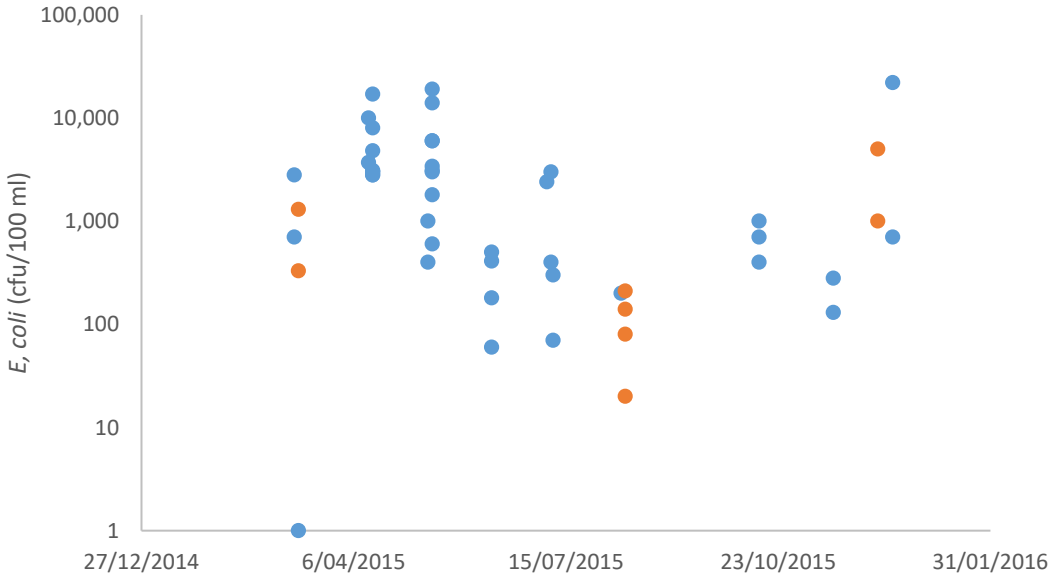

Figure 6. Concentration of *E. coli* at different sites in the Mataura FMU, across the course of the year. Samples collected following rainfall are shown in blue, and those collected under base flow conditions are in orange.

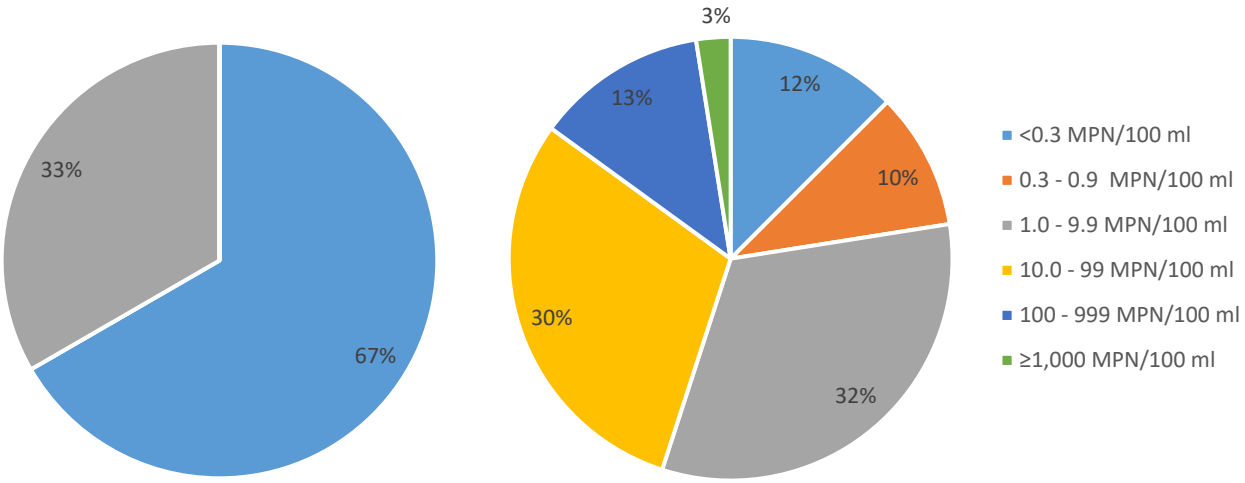

Figure 7. *Campylobacter* concentrations for water samples collected within the Mataura FMU under base flow conditions (left, n=9) and following rainfall (right, n=40).

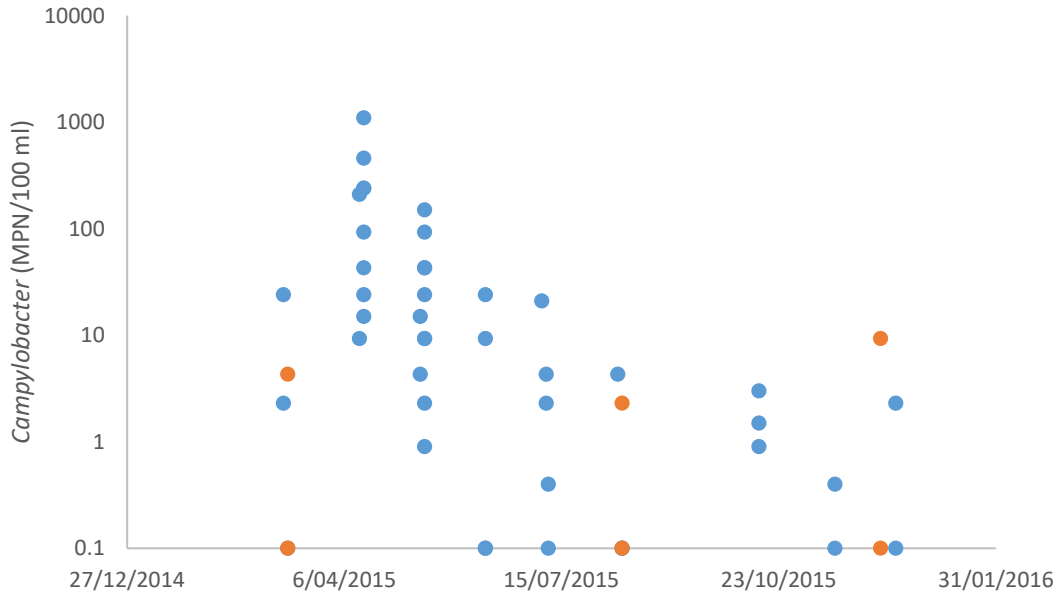

Figure 8. Concentration of *Campylobacter* at different sites in the Mataura FMU, across the course of the year. Samples collected following rainfall are shown in blue, and those collected under base flow conditions are in orange.

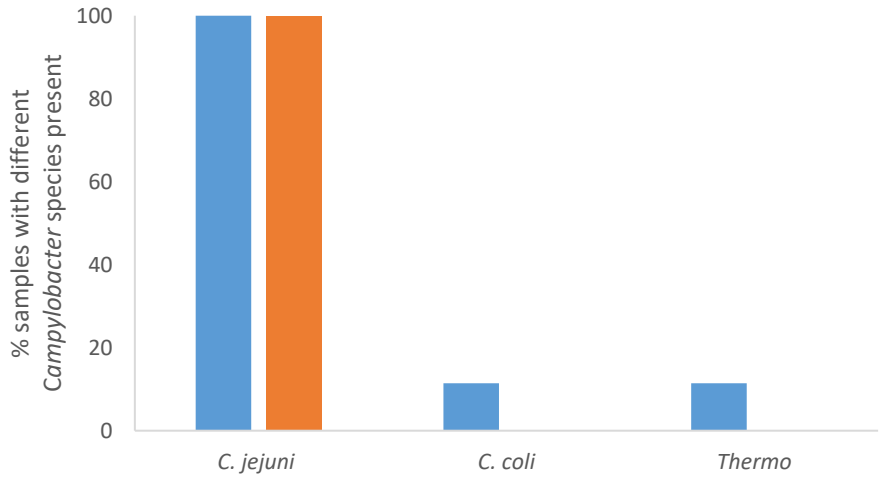

Figure 9. The prevalence of different *Campylobacter* species within *Campylobacter*-positive samples from the Mataura FMU (n=38). Blue bars represent samples collected following rainfall, and orange bars represent samples collected under base flow.

An examination of the relationship between *E. coli* and *Campylobacter* reveals a significant positive correlation of data (Spearman rank correlation, r = 0.6858, df = 47, p <0.0001; Figure 10); thus samples with high levels of *E. coli* were more likely to contain high levels of *Campylobacter*.

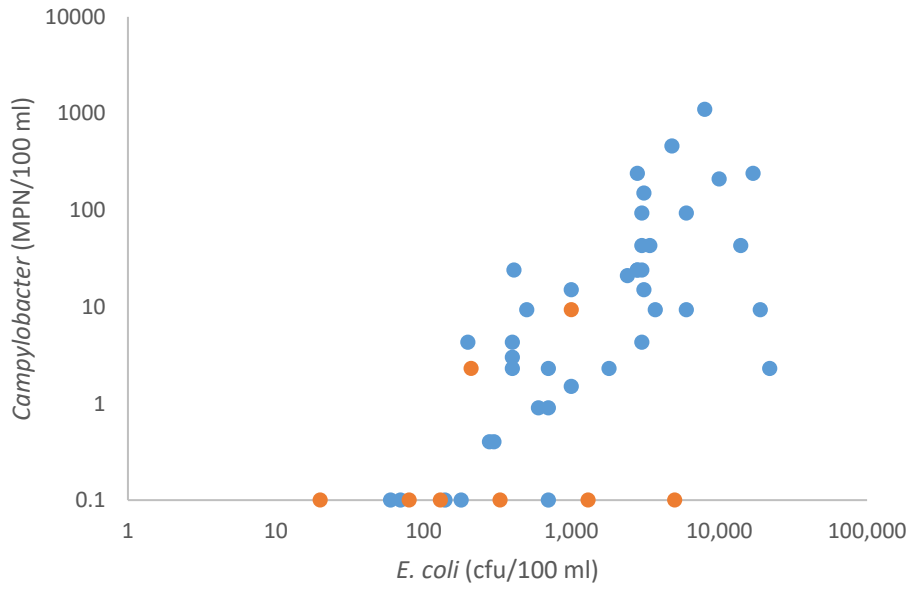

Figure 10. Relationship between *E. coli* and *Campylobacter* spp. concentrations in water samples collected within the Mataura FMU. Note that for the purposes of displaying the data on a logarithmic scale, samples in which no *Campylobacter* was detected, have been plotted as 0.1 MPN/100 ml. Also for display purposes, samples collected following rainfall are shown in blue, and those collected under base flow conditions in orange.

3.2 SOURCES OF FAECAL POLLUTION

Faecal source tracking analysis found that ruminant animal pollution was detected at all sites in the Mataura FMU (Figure 3, Figure 4). Bovine-specific FST markers were detected on at least one occasion at 13 sites, with ovine markers detected at 12 sites. There was no correlation between the degree of faecal contamination (i.e. as determined *E. coli* concentrations) and the size of the site's sub-catchment, nor the amount of agricultural activity (total agriculture, sheep and beef, dairying, by total area or percentage of land use) occurring in the sub-catchment (Spearman correlation, p value for all comparisons >0.05).

The relative impact of ruminant sources was found to increase following rainfall (Figure 3, Figure 4). For example, ruminant pollution accounted for ≤10% of total faecal pollution in more than half of all samples collected under base flow conditions. In contrast, ruminant pollution was the dominant pollution source (i.e. 50-100% of pollution) in 70% of samples collected following rainfall (Figure 11). Eighty-six percent of samples collected following rainfall were positive for ovine contamination, and 54% for bovine contamination. In comparison, 11% and 22% of samples collected under base flow conditions were positive for ovine and bovine contamination, respectively (Figure 12).

At some sites that were sampled following rainfall, the impact of ruminant pollution was observed to vary through the year. Samples collected during April or May tended to be dominated by ruminant pollution, whilst those sampled earlier or later in the year were less impacted by ruminant sources. For example, at Longridge Stream at Sandstone, ruminant sources accounted for 50-100% of faecal pollution in May, 10-50% in July, and 1-10% in December. At the Waikaka River at Gore, ruminant pollution was dominant in April and May, but reduced to 10-50% of the faecal pollution present in October and November. Other sites, including the Waimea Stream at Mandeville, Waikaia River at Waikaia and Mimihau Stream at Wyndham, were dominated by a ruminant faecal source throughout the study period.

Wildfowl faecal contamination was detected at all but one site (Waikaia River at Waikaia; Figure 3, Figure 4). The prevalence of wildfowl-specific markers was less dependent on rainfall than was ruminant contamination, being detected in 65% of samples collected following rainfall and 78% of samples collected under base flow (Figure 12).

One single instance of human faecal contamination was detected, at Oteramika Stream at Seaward Downs, under base flow conditions (Figure 3, Figure 12).

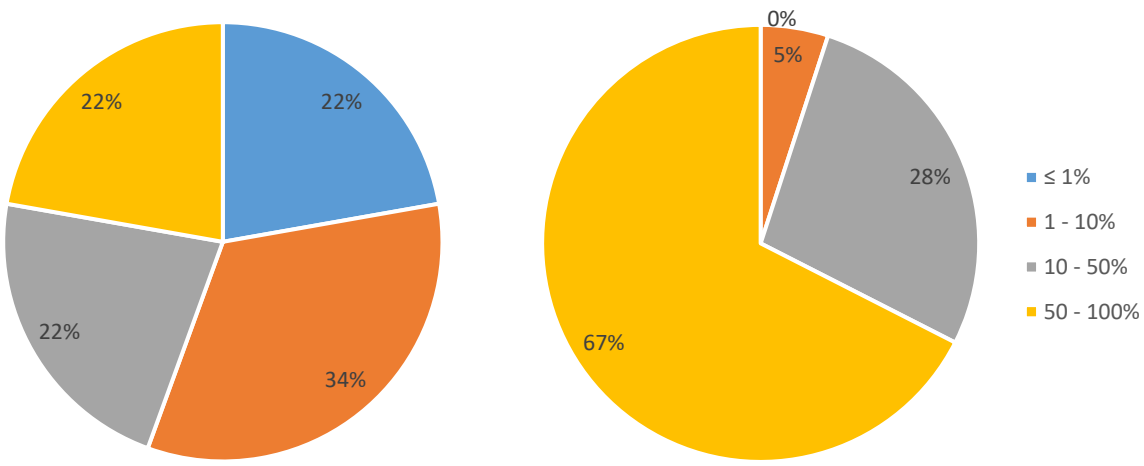

Figure 11. The proportion of samples collected under base flow conditions (left) and following rainfall (right), that were affected by different levels of ruminant faecal pollution.

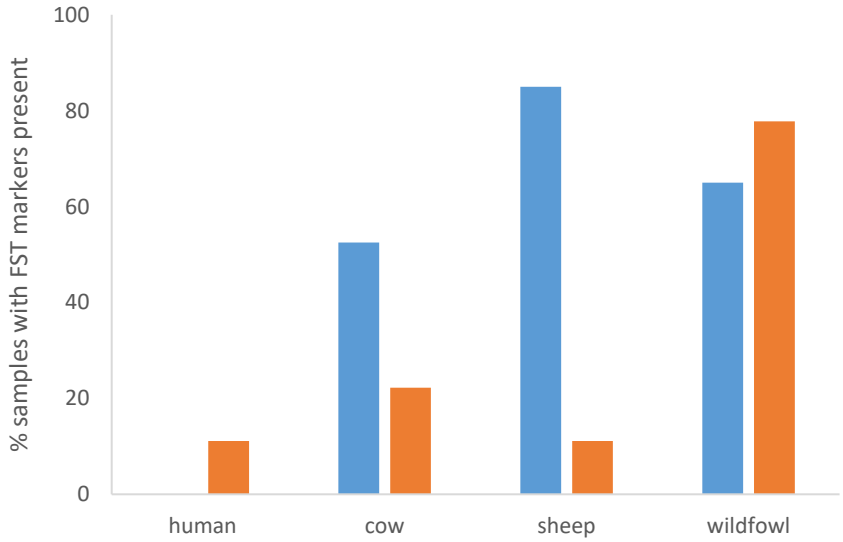

Figure 12. The percentage of samples collected from within the Mataura FMU that were positive for FST markers from different sources. Samples that were collected following rainfall are shown in blue, and those collected under base flow in orange.

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 Mataura FMU were of wildfowl, ruminant and poultry sources. Most sites were found to have *Campylobacter* from more than one source. Wildfowl were the most common source of *Campylobacter* (45% of all isolates), with 66% of *Campylobacter*-positive samples collected being positive for a wildfowl strain, followed by 'not wildfowl' (47%), ovine/bovine/deer (29%) and poultry (18%). 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 overall prevalence of *Campylobacter* was higher in samples collected following rainfall than under base flow (as described in Section 3.1), the relative importance of wildfowl and 'not wildfowl' as a *Campylobacter* source did not appear to be greatly influenced by antecedence rainfall. In contrast, *Campylobacter* of ruminant origin was detected only following rainfall, and poultry was a more common source of *Campylobacter* under base flow conditions than following rainfall (Figure 13).

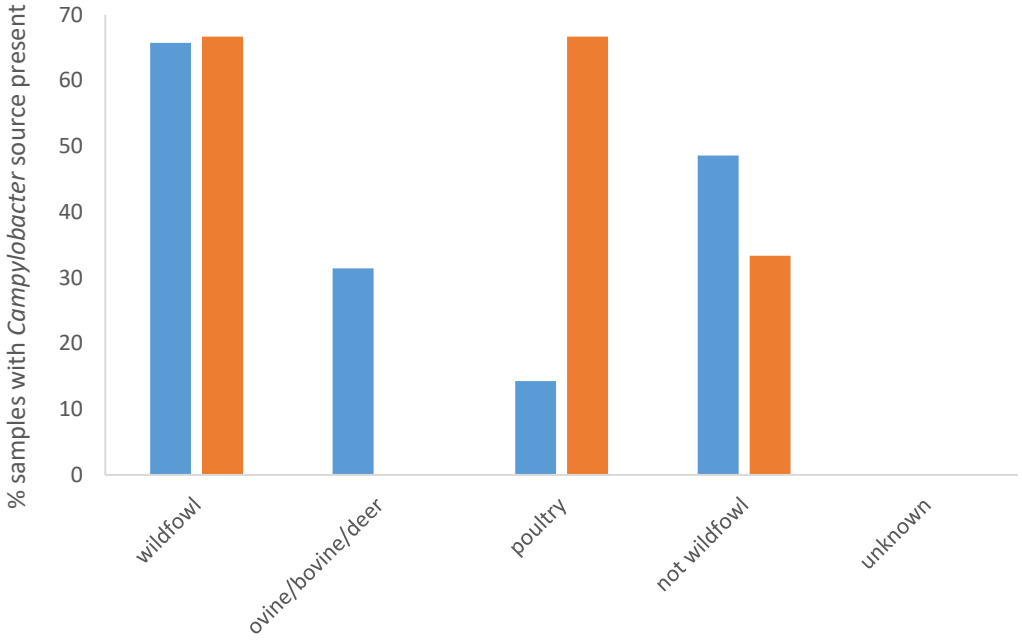

Figure 13. The percentage of *Campylobacter*-positive samples from the Mataura FMU that were identified as having different *Campylobacter* sources present (as determined by MBiT analysis). Samples that were collected following rainfall are shown in blue, and those collected under base flow in orange.

3.3.2 Genotype analysis and comparison with clinical isolates

Comparison of the MBiT genotype data for *Campylobacter* isolates from sites across the Mataura FMU (including isolates that were available from previous studies in the Mataura FMU) revealed a high diversity of genotypes: of 142 isolates analysed, 89 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 Mataura FMU with the isolates from the Waiau, Oreti and Aparima FMUs also shows no clear separation of genotype based on the FMU from which isolates were obtained (Figure 15).

Of the 142 individual isolates recovered from water samples in the Mataura FMU, 53 isolates (37%) representing 26 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 53 isolates, only nine (17%) are likely to have come from wildfowl, compared with 45% 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 nine isolates (5%) were indistinguishable from wildfowl-associated isolates, suggesting wildfowl are a minor source of illness in the community.

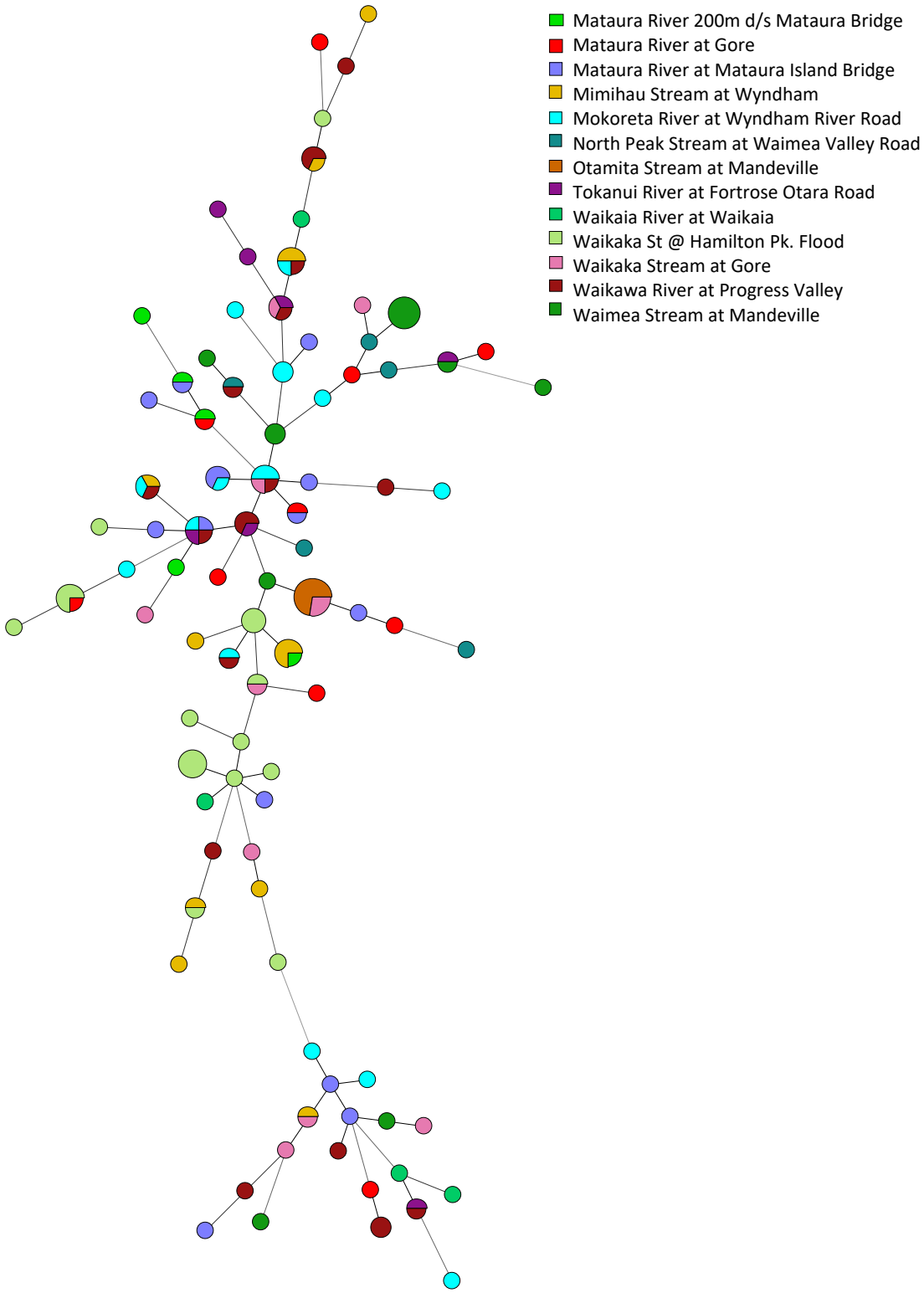

Figure 14. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from sites across the Mataura FMU, based on MBiT analysis. Each circle represents a different genotype, and each colour identifies a site. The number of circles and the spread of colours across the diagram demonstrates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

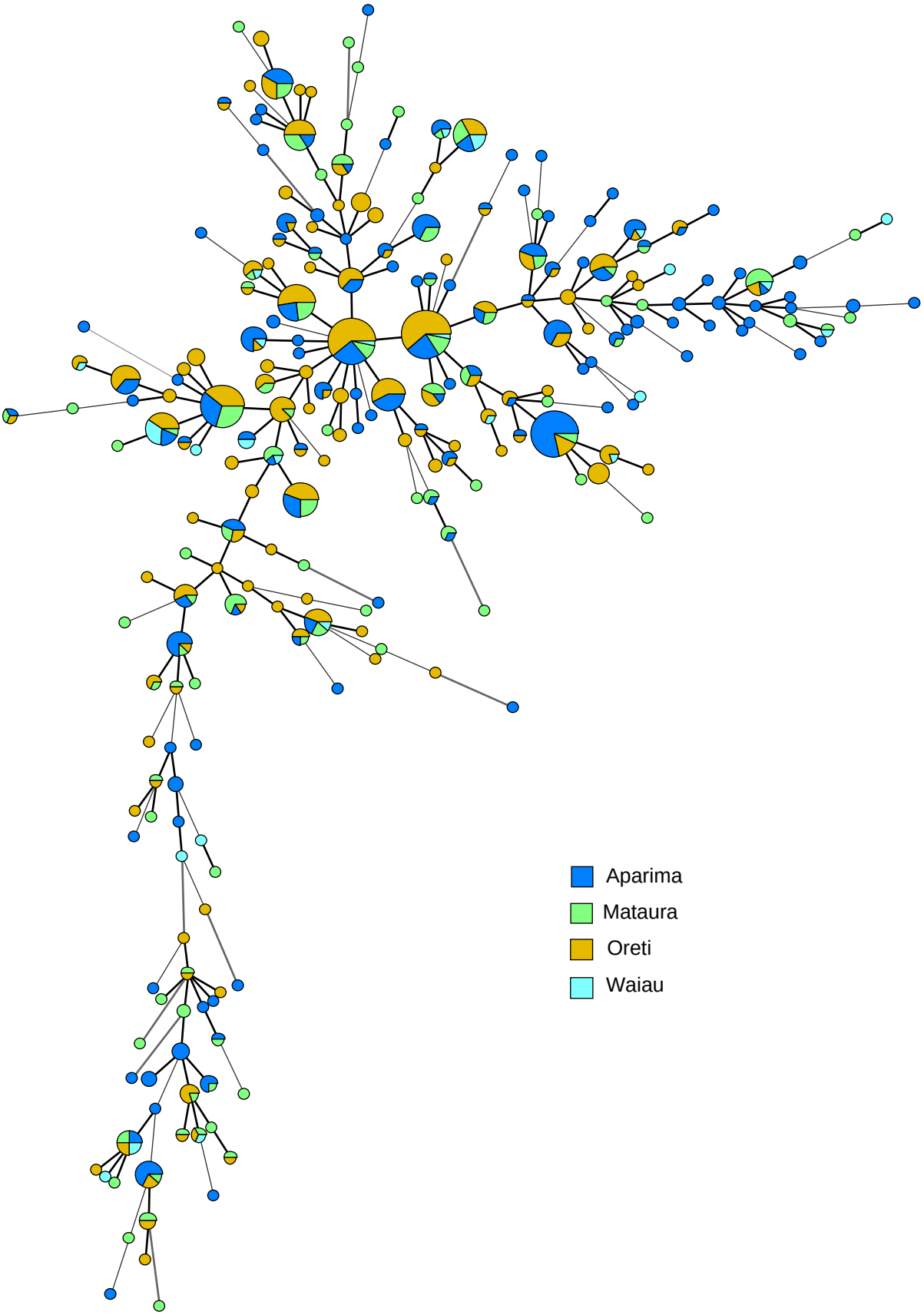

Figure 15. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from across the Southland region, based on MBiT analysis.

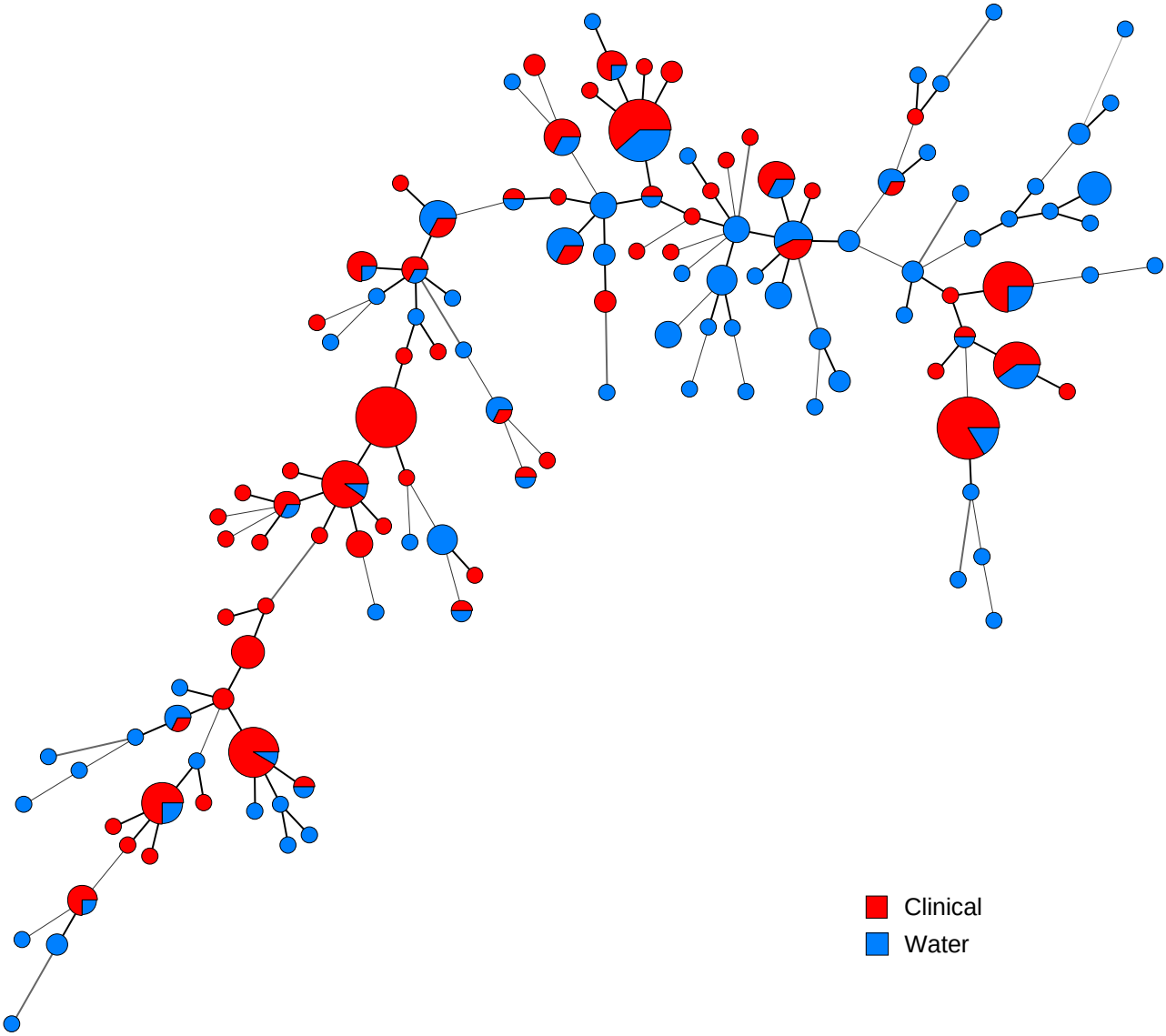

Figure 16. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from water samples from the Mataura FMU (blue) compared with human clinical isolates from the Southland region (red). Circles in which there are both blue and red segments indicate a genotype has been isolated from both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

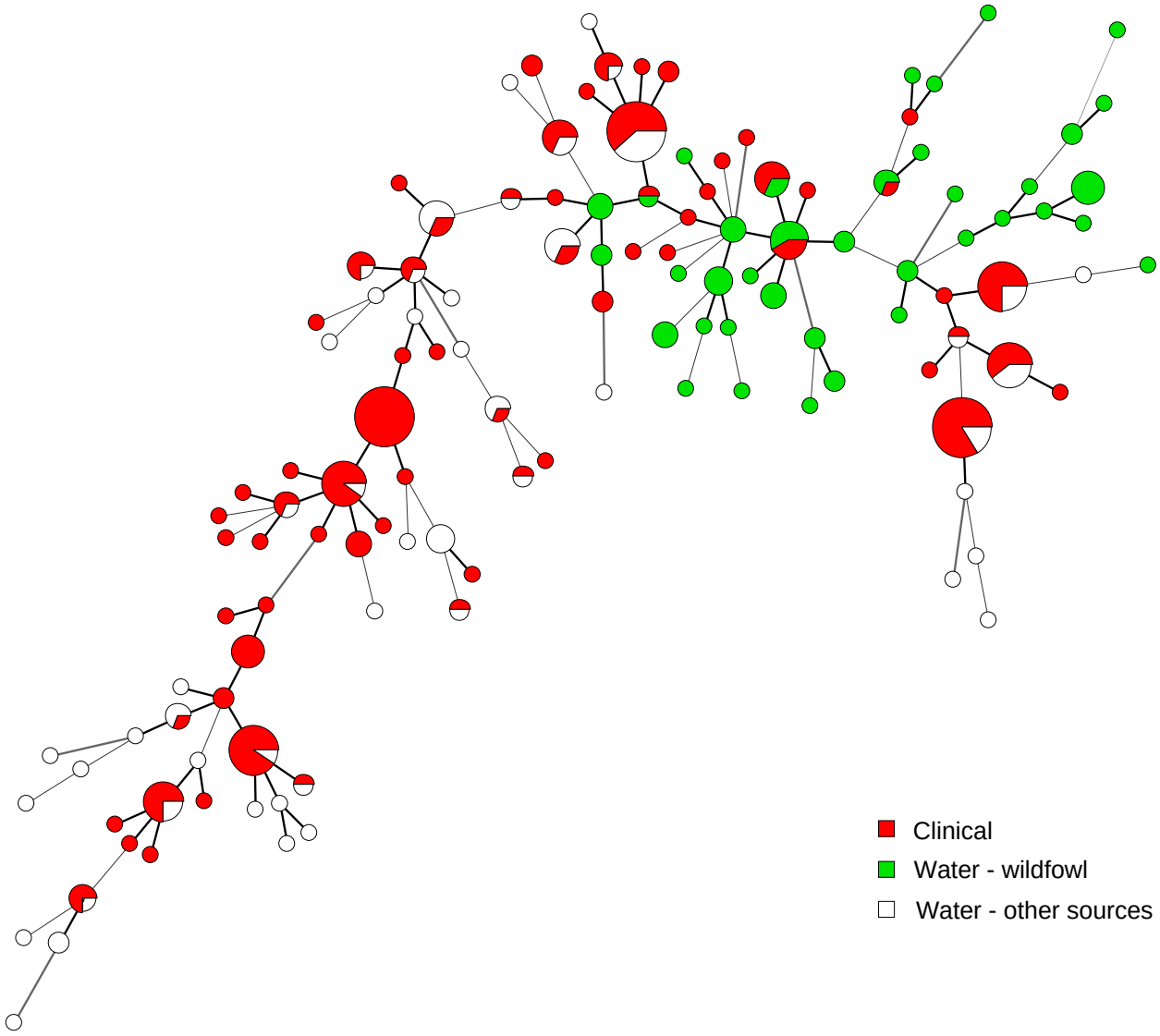

Figure 17. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from water from the Mataura FMU, highlighting those that are wildfowl-associated (green) compared with human clinical isolates (red).

4. DISCUSSION

4.1 MICROBIAL SOURCES AND TRANSMISSION

This study demonstrates that the microbial quality of waterways within the Mataura FMU is highly variable, with the sites sampled being vulnerable to high levels of faecal contamination. Overall microbial concentrations were high, with 12 of the 15 sites having a median *E. coli* level exceeding 550 cfu/100 ml (i.e. the concentration above which the Microbiological Water Quality Guidelines for Freshwater Recreation Areas (MfE and MoH, 2003) recommend avoiding primary recreation such as swimming), and 55% of all samples collected within the FMU exceeding 1,000 cfu/100 ml. 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 (i.e. a site is known to be contaminated to a certain degree, and from certain sources, at least intermittently). Given the limited amount of 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 cattle and sheep). Despite the significant impact of ruminant pollution, microbial concentrations were not correlated with the amount of agricultural activity occurring in the catchment. 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 Mataura 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; these data are 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 Mataura FMU, there are often multiple faecal sources, further compounding these issues (Muirhead et al., 2011).

Although none of the sites in the present study were sampled under both base flow and post-rainfall, overall *E. coli* concentrations were higher under high flow conditions, and ruminant pollution dominated the faecal signature. 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 Mataura FMU. Physiographic data for soils in the Mataura FMU show a prevalence of imperfectly-to-poorly drained gleyed soils, and oxidising soils and bedrock/hill country that are prone to overland flow (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 Mataura 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), or discharge of effluents to rivers. Further resolving the specific source(s) and transmission route(s) will require site visits and examination of consented discharge activity.

The presence of wildfowl pollution was similar irrespective of preceding rainfall, suggesting direct deposition into the waterway under both high and low flow conditions.

Seasonal patterns of agricultural contaminant loss to waterways have been demonstrated in several studies in the Southland and Otago regions, whereby higher 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). 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 spring may reflect greater survival of bacteria in the environment with increasing temperatures, or additional faecal contributions from lambs and calves, which may carry a high microbial burden.

At some sites, the specific faecal source was also observed to vary with season. For example, at the Mimihau Stream and Waikaia River sites, ovine-specific FST markers were present throughout the year, with bovine markers present only in May and/or April. These patterns could result from the different sensitivity of the ovine and bovine markers (the bovine marker is less sensitive and therefore requires a larger amount of pollution be present to attain a positive reaction than does the ovine marker). Alternatively, it might also represent a change in land use or management practice, such as wintering stock. Discussions with the farmers operating within the sub-catchment and/or site visits and visual inspection would assist in better understanding the reason(s) for the changing faecal signature and how this might be managed.

One instance of human contamination was detected, in the Oteramika Stream at Seaward Downs. Review of the land use and discharge information for the Oteramika Stream sub-catchment did not reveal an obvious source of this pollution - much of the land is utilised for dairy (67% including support activity) and beef and sheep farming (24%), with most of the consented discharges to land and water being dairy effluents. There is one consent for the discharge of treated sewage, stormwater and wash water to land, which is possibly the source for this human signal. Alternatively, seepage from a septic tank from one of the nearby farms could be the 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 (78%) in the rural, bird-impacted, Mataura FMU. Interestingly, although *C. jejuni* was the most commonly identified species in the national survey of McBride et al. (2002), it was present in only 48% of *Campylobacter*-positive samples (compared with all positive samples in Mataura). 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 Mataura 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 ingested (i.e. the dose). If it is assumed that all of the *Campylobacter* isolated from these waterways are capable of causing disease, then dose response curves could be used to estimate the health risk to water users. Figure 18 illustrates a dose response curve for *C. jejnui,* which accounts for ~90% of all human cases of campylobacteriosis (Lee and Newell, 2006). It shows that the ingestion of 800 *C. jejuni* is associated with a 50% probability of infection (ID_{50}) (Medema et al., 1996; McBride et al., 2002). Based on this dose response, and the finding of quite high concentrations of *Campylobacter* in a number of samples (200-1100 MPN/100 ml), the ingestion of 70-400 ml of water could carry a fifty-fifty change of infection. 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). However, 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 from *Campylobacter*. Although not conclusive, there is some epidemiological evidence, which is supported by animal models and cell culture, that some strains of *Campylobacter* may be host-specific, and that these different strains have different rates of human infectivity (McBride et al., 2011).

[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

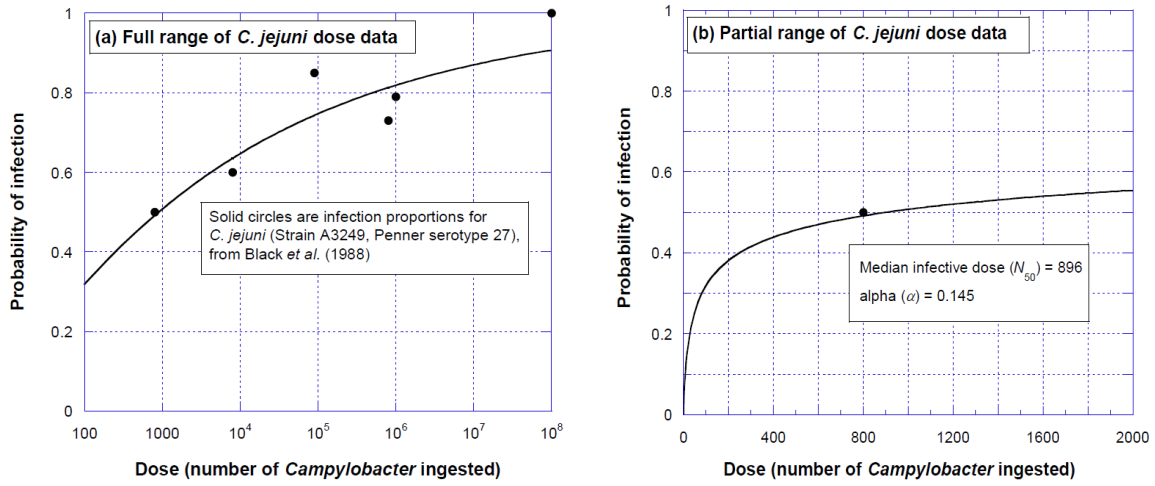

Figure 18. Dose-response curves for *Campylobacter jejuni*, estimating the probability of infection for a given dose. The lowest dose administered during the study was 800 *C. jejuni*, at which point half of the volunteers became infected. Estimating the dose response for lower concentrations requires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

Campylobacter 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 *Campylobacter* genotypes from the Mataura environmental 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 32% of the *Campylobacter*-positive samples were found to contain only isolates of wildfowl origin, the health risk from these samples might be less than that suggested by the data from Black et al. (1988), which is based on clinical isolates.

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 Mataura 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 Mataura 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 Mataura FMU, with direct deposition, overland flow and subsurface flow via tile drains all important mechanisms for the transfer of faecal microbes to waterways. However, the magnitude of contamination, relative importance of different sources and routes of transmission vary slightly between each of the 15 sites surveyed. Because of the complex interaction of faecal source, land topography, soil type, and climatic factors, one solution will not be suited to or effective for all sites. A site-specific solution that considers these various factors and targets the flow conditions or seasons where contamination is greatest, will yield the greatest benefit for water quality. Visual inspections of the site are highly recommended in providing as much detail as possible on which informed decisions can be made.

4.3.1 Direct deposition

Direct deposition by ruminant animals can be reduced by fencing streams and wetlands to exclude stock, removing the direct source. Fencing also allows for the creation of a riparian buffer strip (RBS), ideally vegetated, that reduces the momentum of surface runoff, aiding in infiltration and promoting the retention of faecal microbes within the soil (Collins et al., 2007). The effectiveness of RBS in attenuating faecal microbes is influenced by the slope of the land, width of the buffer, soil type, amount of runoff and the degree to which microbes are attached to soil particles. Quantitative design guidelines for RBS are described by Collins et al. (2005), based on microbial attenuation modelling. The use of bridges at stream crossing for dairy cattle has also been shown to reduce direct faecal inputs and improve water quality (Collins et al., 2007). Stock exclusion strategies may yield greater benefits where cattle are farmed (i.e. beef or dairy) rather than sheep, since sheep tend to be less attracted to waterways than cattle. A literature review by Muirhead (2011) reported finding no publications on the effectiveness of fencing sheep in reducing *E. coli* concentrations in streams. Deer are also attracted to water, and fencing to exclude deer from wallowing areas that are connected to streams has been shown to reduce contaminant loading to the stream (McDowell, 2008). However, deer have been observed to pace the fenceline and/or create new wallows, undermining the longevity of the water quality benefits. The creation of a new ‘safe’ wallow (not connected to the stream) in combination with the fencing of any connected wallows is recommended as an approach to reducing water contamination associated with deer (McDowell, 2009).

4.3.2 Indirect sources

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

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

4.3.3 Wildfowl

It can be difficult to manage contamination of waterways caused by wildfowl, particularly in large rural catchments. Since wildfowl pollution typically enters waterways via direct deposition, physically separating birds from the water would be expected to be effective in reducing their impact. Unlike livestock, birds cannot be contained by fencing, and so strategies for reducing wildfowl inputs tend to focus on managing population size, or disturbing the birds to discourage settling beside vulnerable waterways.

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

4.3.4 Prioritising mitigations

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

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 Oteramika Stream at Seaward Downs should be undertaken to identify the source of the human signature. 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 unappealing to some within the community. Strategies to reduce ruminant contamination could include fencing for stock exclusion, riparian planting, stock management (intensity, grazing rotation), irrigation management and wastewater treatment.

5. CONCLUSIONS

Waterways in the Mataura FMU are vulnerable to high levels of faecal contamination, particularly following rainfall. 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 significant routes of transmission of faecal materials to waterways. Human sources do not appear to be a significant contributor to faecal contamination in the Mataura FMU, with only a single instance detected. However, human contamination is considered to pose the greatest risk to human health, and further site assessment should be undertaken to try and identify the particular source of this contamination.

Campylobacter was isolated from 78% of samples, at times at quite high concentrations. Wildfowl, ruminants, and poultry were all identified as being sources of *Campylobacter*. *Campylobacter* genotypes that were indistinguishable from human clinical cases in the Southland region were identified. Although there is little data available on the probabilities of infection and/or illness at lower *Campylobacter* concentrations, these finding suggests that there 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.

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

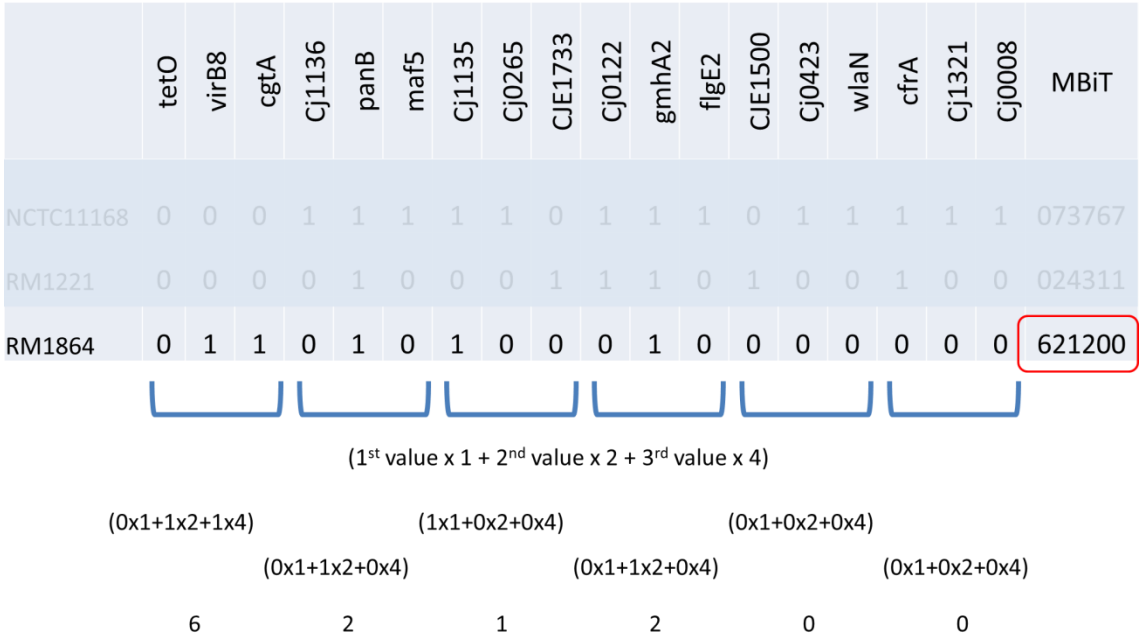

Figure 19. Example of an MBiT pattern naming.

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 MATAURA RIVER AT GORE

Water quality was analysed for the Mataura River at Gore on four occasions through 2015, with two sampling events each in autumn and spring (Table 8). All samples were collected following rainfall. The levels of *E. coli* in the river varied significantly between the seasons, with the highest concentrations observed in April (4,800 cfu/100 ml), reducing at subsequent sampling dates (3,000, 400 and 130 cfu/100 ml in May, October and November, respectively).

Campylobacter spp. was isolated from the three samples that contained the highest concentrations of *E. coli*. Similarly to *E. coli*, *Campylobacter* levels were highest in April (460 MPN/100 ml) and subsequently declined through the year (24, 3 and <0.3 MPN/100 ml). Further analysis revealed the presence of *C. jejuni* in all samples where *Campylobacter* was detected, with *C. coli* also identified in the May sample. MBiT analysis indicated that the *Campylobacter* was mainly of wildfowl origin, although poultry and ruminant sources also contributed to the April and May samples, respectively.

PCR-based faecal source tracking suggested ruminant pollution accounted for up to 50% of the faecal pollution at this site in spring, and 50-100% in April and May. In particular, ovine-specific markers were detected in all four samples, with bovine markers detected also in the May sample. Wildfowl-specific markers were detected in the April, October and November samples.

A review of land use in the Mataura River at Gore sub-catchment shows that sheep and beef farming predominates, with dairy and deer activity also present. Conservation land is also a significant land use (approximately 17%) (Figure 20, Figure 21).

Table 8. Results for microbial and FST analysis of water samples collected from the Mataura River at Gore.

Site		Mataura River at Gore			
Sample #		CMB150387	CMB150498	CMB151772	CMB152082
Client #		20151641	20151846	20153320	20153992
Date Sampled		15/04/2015	13/05/2015	14/10/2015	18/11/2015
Rainfall		Yes	Yes	Yes	Yes
		Microbial Properties			
Faecal coliforms		4,800	3,000	600	150
E. coli		4,800	3,000	400	130
Campylobacter		460	24	3.0	<0.3
***Campylobacter* Species**		*C. jejuni*	*C. jejuni* & *C. coli*	*C. jejuni*	
MBiT *Campylobacter* Source	**Wildfowl**	2		3	
	Ovine/Bovine/ Deer		1		
	Poultry	1			nt
	Not Wildfowl	1		1	
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	++++	+++
Ruminant		50-100%	50-100%	10-50%	10-50%
Human - BacH		-	+	+	-
Human - BiADO		-	-	-	-
Cow		-	+	-	-
Sheep		+	+	+	+
Wildfowl - GFD		+	-	+	-
Wildfowl - E2		+	-	-	+
Canine		nt	nt	nt	-

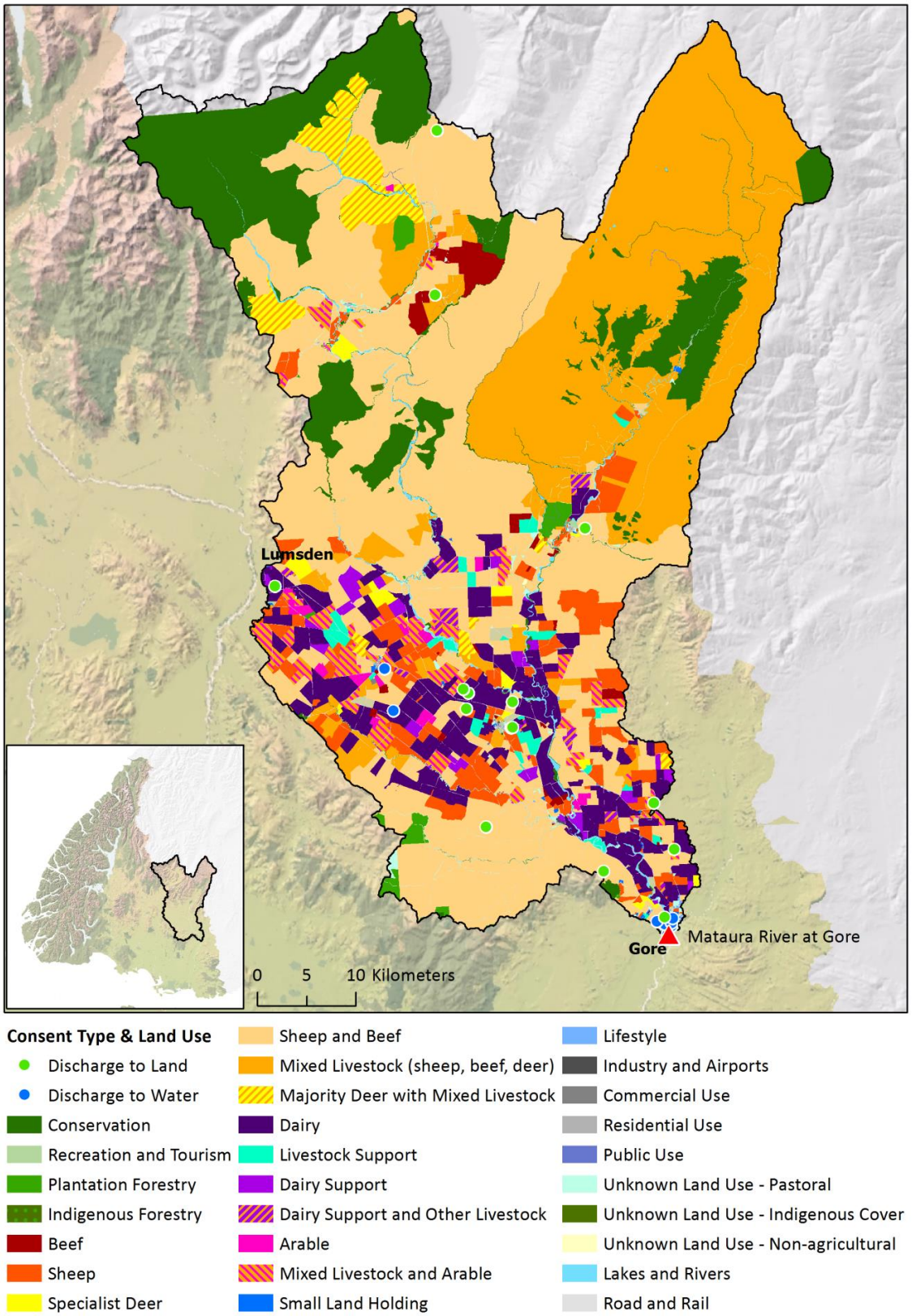

Figure 20. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Mataura River at Gore sampling site.

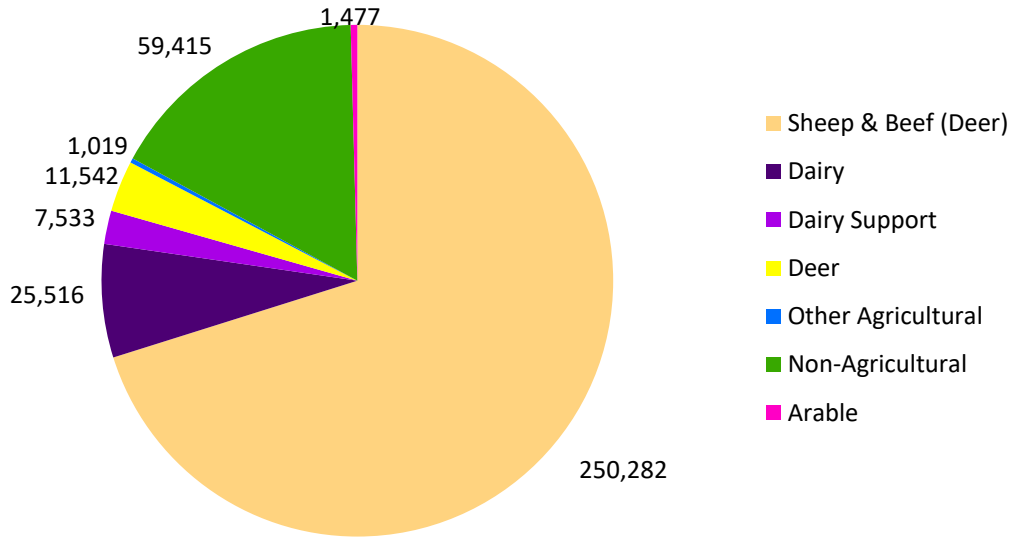

Figure 21. Land use (in hectares) in the catchment for the Mataura River at Gore sampling site.

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), **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

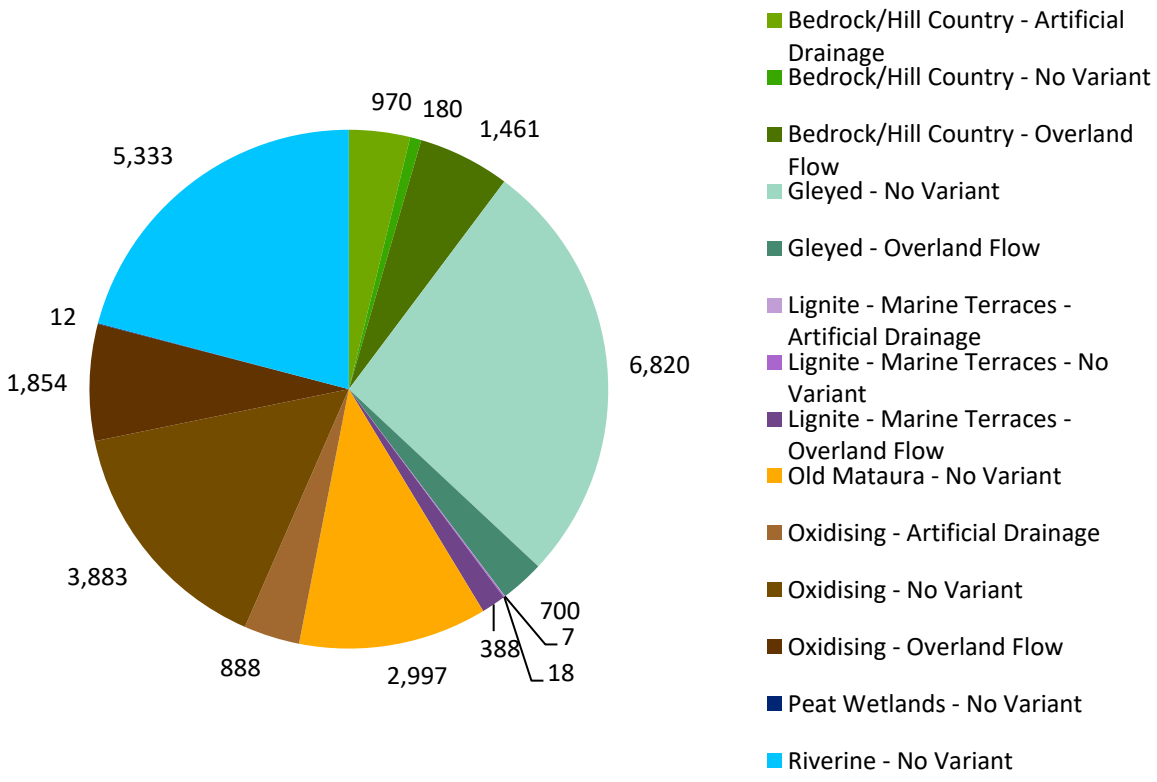

Figure 22. Dairying land (in hectares) in the catchment for the Mataura River at Gore sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016.

Table 9. Number of consented catchment discharges to land and water in the catchment for the Mataura River at Gore sampling site.

Mataura River at Gore		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	16
	1080, Dye	4
	Ash	1
	Dairy Factory Effluent, Wintering Pad/Feedlot Effluent (land)	1
	Dairy Shed Effluent (land)	84
	Dairy Shed Effluent (land), Underpass Effluent	1
	Dairy Shed Effluent (land), Wash Down Effluent, Wash Water, Waste Water	1
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	14
	Green waste	2
	Hazardous Substances	1
	Meat Works Effluent	1
	Meat Works Effluent, Sludge	1
	Meat Works Effluent, Waste Water	7
	Offal	1
	Oil/Grease	8
	Wash Water	5
	Waste Water	3
	Wintering Pad/Feedlot Effluent (land)	6
To Land Total		157
To Water	Ground water, Mine water, Stormwater, Suspended Sediment	1
	Mine water	3
	Mine water, Silt, Waste Water	1
	Mine water, Wash Water	3
	Mine water, Waste Water	2
	Oxidation Pond Effluent, Sewage (Treated), Sewage Package Plant	1
	Silt	1
	Stormwater	7
	Wash Water	2
To Water Total		21
Grand Total		178

Note: Consent information accurate as of April 2017

B.2 MATAURA RIVER, 200m DOWNSTREAM OF MATAURA BRIDGE

Two samples were collected from the Mataura River, 200 m downstream of the Mataura Bridge. The samples were collected in March and August, both under base flow conditions (Table 10).

Faecal coliforms were higher in March (6,000 cfu/100 ml) than August (320 cfu/100 ml). Low levels of *Campylobacter* spp. were present in both samples (2.3-4.3 MPN/100 ml), and was identified as *C. jejuni*. MBiT analysis identified a wildfowl source in both samples, with a poultry source also present in August.

Faecal source tracking analysis suggested that ruminant pollution was not a dominant pollution type at this site, accounting for up to 10% of overall faecal pollution. Considering that much of the land use in the catchment for this site involves beef, sheep, dairy and deer farming (Figure 23, Figure 24), it is also possible that the faecal source tracking results reflect aged ruminant pollution, with both ovine and bovine-specific PCR markers were detected in the August sample. Wildfowl-specific markers were detected in both samples, consistent with MBiT suggesting wildfowl as being the source of the *Campylobacter* recovered.

Table 10. Results for microbial and FST analysis of water samples collected from the Mataura River, 200m downstream of the Mataura Bridge.

Site		Mataura River 200 m downstream of Mataura Bridge	
Sample #		CMB150247	CMB151387
Client #		20151081	20152924
Date Sampled		11/03/2015	12/08/2015
Rainfall		No	No
		Microbial Properties	
Faecal coliforms		6,000	320
E. coli		<1	210
Campylobacter		4.3	2.3
Campylobacter Species		*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl	2	2
	Ovine/Bovine/ Deer		
	Poultry		1
	Not Wildfowl		1
	Unknown		
		Faecal Source Tracking	
General - GenBac3		++++	++++
Ruminant		1-10%	≤1%
Human - BacH		-	+
Human - BiADO		-	-
Cow		-	+
Sheep		-	+
Wildfowl - GFD		+	+
Wildfowl - E2		+	+

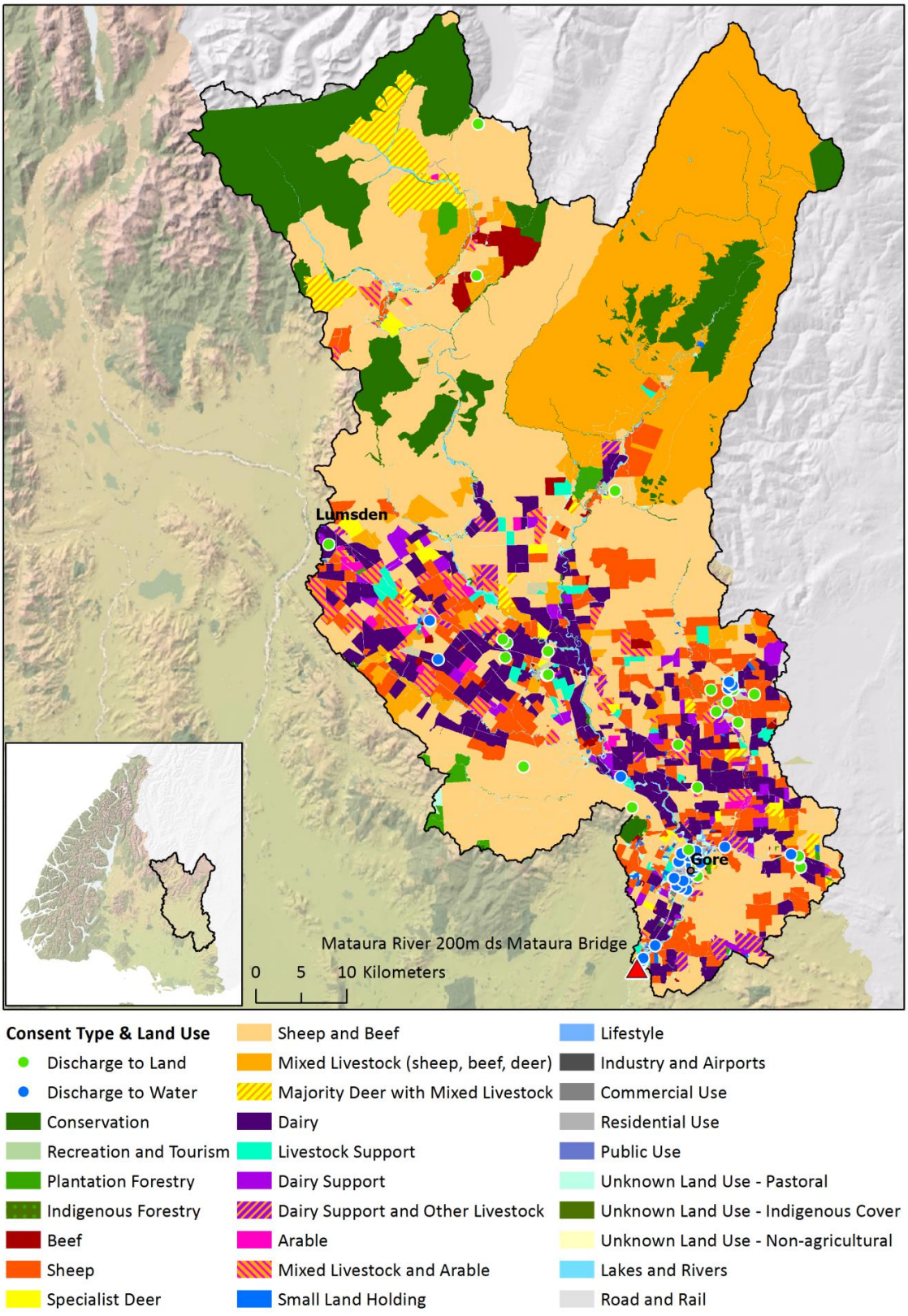

Figure 23. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Mataura River downstream of Mataura Bridge sampling site.

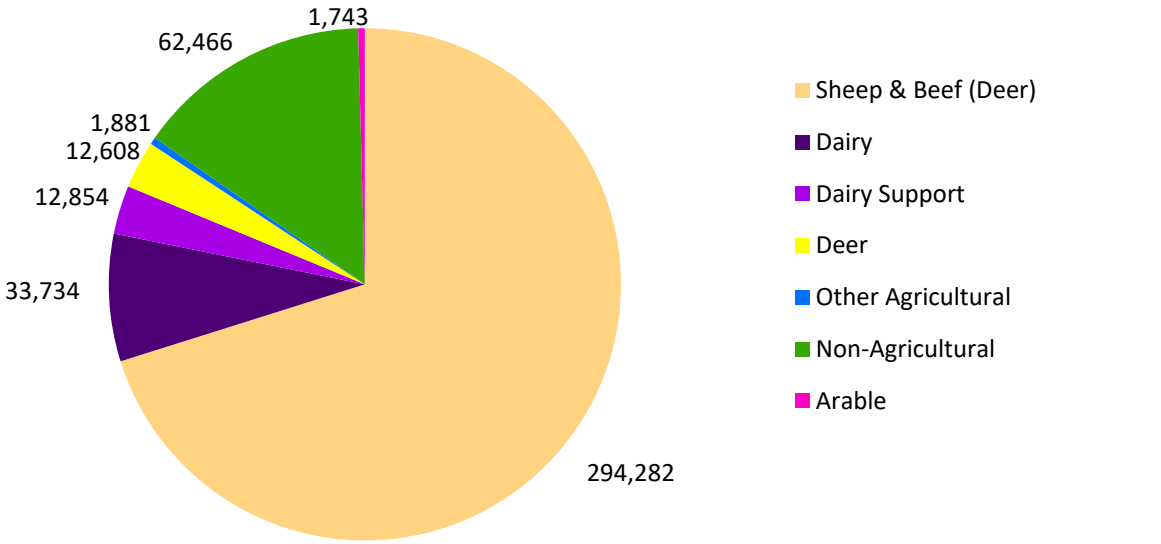

Figure 24. Land use (in hectares) in the catchment for the Mataura River, 200 m downstream of the Mataura Bridge.

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), **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

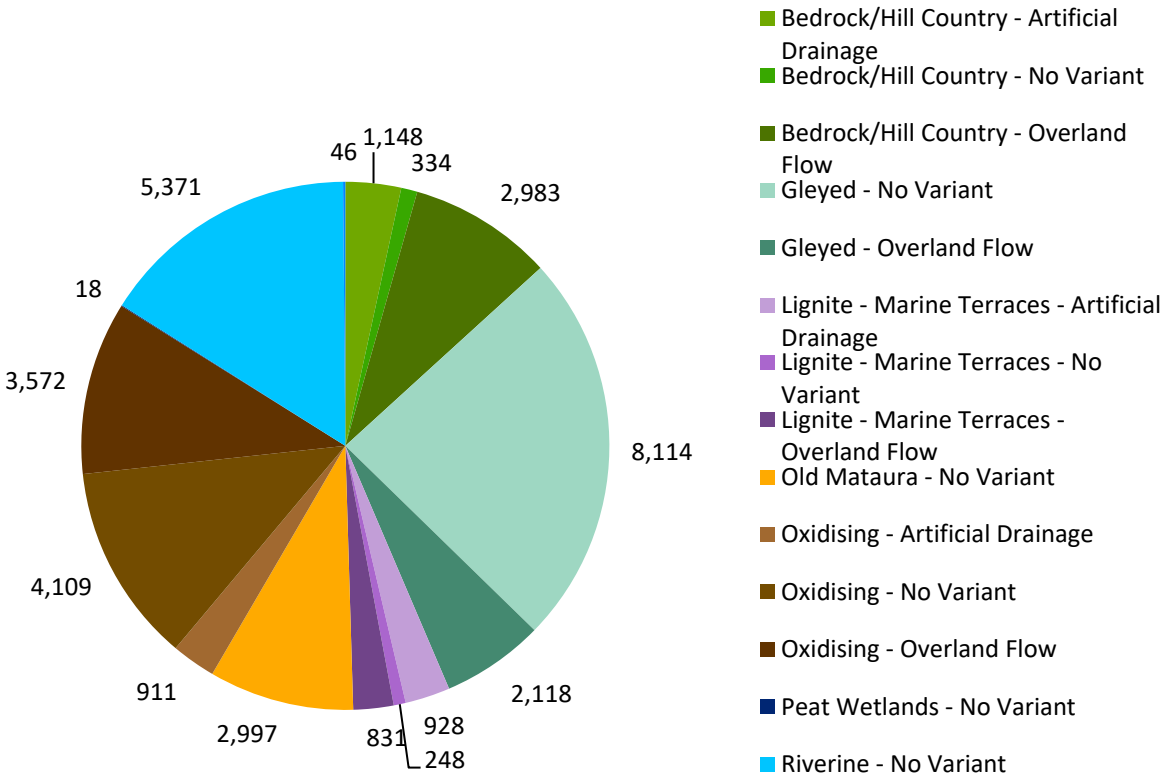

Figure 25. Dairying land (in hectares) in the catchment for the Mataura River, 200 m downstream of the Mataura Bridge, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 11. Number of consented catchment discharges to land and water in the catchment for the Mataura River, 200m downstream of the Mataura Bridge.

Mataura River 200m ds Mataura Bridge		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	17
	1080, Dye	4
	Ash	1
	Blood, Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	2
	Clean Fill	1
	Dairy Factory Effluent, Wintering Pad/Feedlot Effluent (land)	1
	Dairy Shed Effluent (land)	117
	Dairy Shed Effluent (land), Underpass Effluent	1
	Dairy Shed Effluent (land), Wash Down Effluent, Wash Water, Waste Water	1
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	22
	Green waste	2
	Hazardous Substances	1
	Leachate, Refuse - Commercial, Refuse - Domestic	2
	Leachate, Refuse - Commercial, Refuse - Domestic, Refuse - Industrial	1
	Meat Works Effluent	1
	Meat Works Effluent, Sludge	1
	Meat Works Effluent, Wash Down Effluent, Wash Water, Waste Water	1
	Meat Works Effluent, Waste Water	11
	Offal	1
	Oil/Grease	8
	Sewage (Treated)	1
	Tannery Effluent, Wash Water	2
	Wash Down Effluent, Waste Water	1
	Wash Water	5
	Waste Water	3
	Wintering Pad/Feedlot Effluent (land)	7
To Land Total		215
To Water	Other (dewatering construction area)	1
	Cooling Water	2
	Ground water, Mine water, Stormwater, Suspended Sediment	1
	Hydro electric power generation sundry contaminant	1
	Hydro electric power generation sundry contaminant, Water (Hydro)	1
	Meat Works Effluent, Waste Water	1
	Mine water	3
	Mine water, Silt, Waste Water	1
	Mine water, Wash Water	3
	Mine water, Waste Water	2
	Oxidation Pond Effluent, Sewage (Treated)	1
	Oxidation Pond Effluent, Sewage (Treated), Sewage Package Plant	1
	Sewage (Treated), Stormwater, Waste Water	1
	Silt	1
	Silt, Sludge	1
	Stormwater	31
	Wash Water	2
To Water Total		54
Grand Total		269

Note: Consent information accurate as of April 2017

B.3 MATAURA RIVER AT MATAURA ISLAND BRIDGE

The Mataura River was also sampled at the Mataura Island Bridge, with four samples collected between April and July 2015, each following rainfall (Table 12). Similarly to the samples collected near Gore, the microbial load of the autumn samples is an order of magnitude higher than those collected later in the year (autumn: *E. coli* 3,000-6,000 cfu/100 ml*, Campylobacter* 43-93 MPN/100 ml; winter: *E. coli* 400-500 cfu/100 ml. *Campylobacter* 2.3-9.3 MPN/100 ml).

All *Campylobacter* spp. isolates were determined to be *C. jejuni*, with MBiT analysis showing that the majority were of wildfowl origin. Ruminant- and poultry-derived *Campylobacter* strains were also identified in the April sample, with the isolates from the May sample identified only as 'not wildfowl' (i.e. potentially ruminant, poultry or human).

Faecal source tracking suggested that ruminant animals were the dominant source of faecal pollution at this site, accounting for up to 100% of faecal indicators. Ovine and bovine makers were both present in the autumn samples, together with wildfowl markers. However, only wildfowl markers were present in the June sample, and only bovine makers were present in July.

Land use within the sub-catchment is dominated by agricultural activities (approximately 83%), including sheep, sheep and beef, mixed livestock and dairy (Figure 26, Figure 27). There is also a large number of consented discharges to both land and water (Table 13).

Table 12. Results for microbial and FST analysis of water samples collected from the Mataura River at Mataura Island Bridge.

Site		Mataura River at Mataura Island Bridge			
Sample #		CMB150391	CMB150502	CMB150810	CMB150997
Client #		20151647	20151852	20152099	20152685
Date Sampled		15/04/2015	13/05/2015	10/06/2015	8/07/2015
Rainfall		Yes	Yes	Yes	Yes
		Microbial Properties			
Faecal coliforms		3,000	6,000	600	400
E. coli		3,000	6,000	500	400
Campylobacter		43	93	9.3	2.3
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	1		5	3
	Ovine/Bovine/ Deer	1			
	Poultry	1			
	Not Wildfowl		3		
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	+++	++++
Ruminant		50-100%	50-100%	10-50%	50-100%
Human - BacH		+	+	+	-
Human - BiADO		-	-	-	-
Cow		+	+	-	+
Sheep		+	+	-	-
Wildfowl - GFD		+	+	+	-
Wildfowl - E2		+	-	-	-

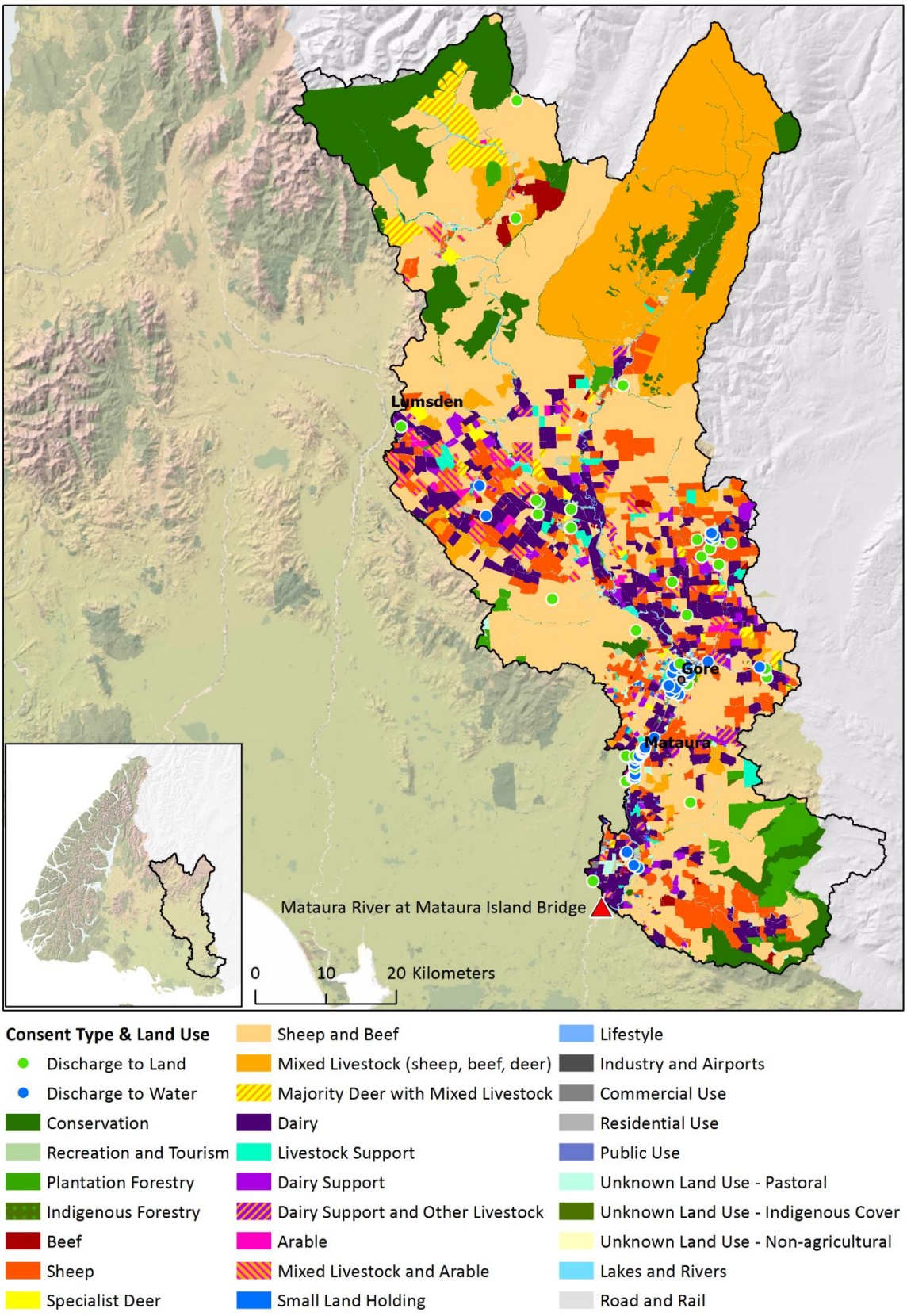

Figure 26. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Mataura River at Mataura Island Bridge sampling site.

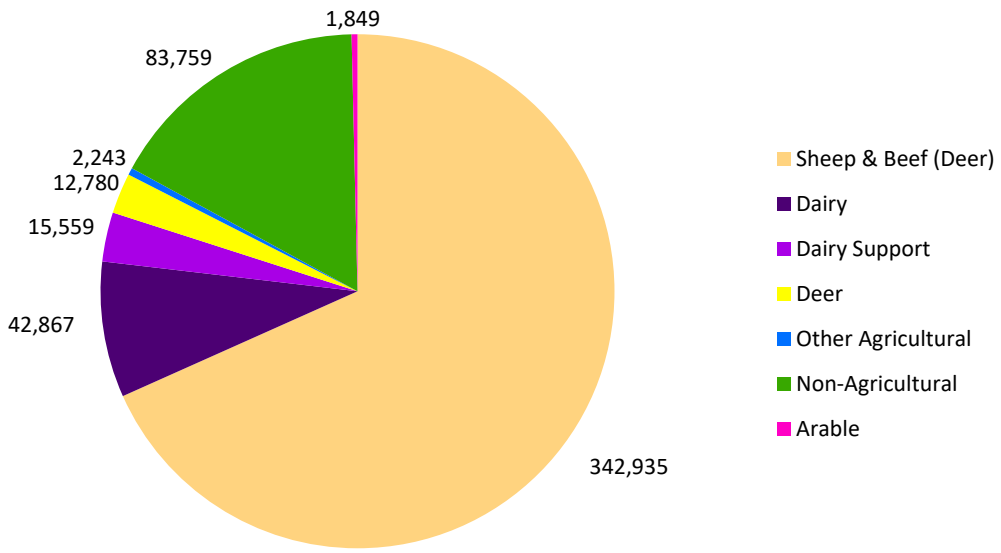

Figure 27. Land use (in hectares) in the catchment for the Mataura River at Mataura Island Bridge.

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), **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

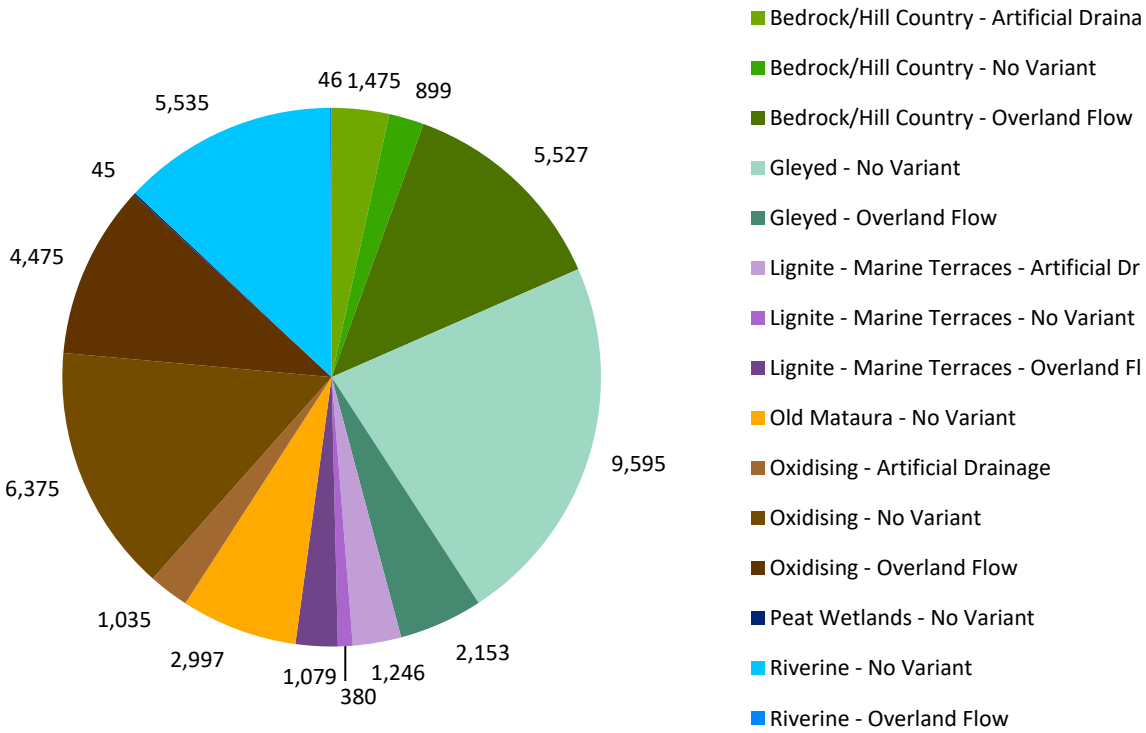

Figure 28. Dairying land (in hectares) in the catchment for the Mataura River at Mataura Island Bridge, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 13. Number of consented catchment discharges to land and water in the catchment for the Mataura River at the Mataura Island Bridge.

Mataura River at Mataura Island Bridge		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	20
	1080, Dye	4
	Ash	1
	Blood, Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	2
	Clean Fill	4
	Dairy Factory Effluent, Wintering Pad/Feedlot Effluent (land)	1
	Dairy Shed Effluent (land)	151
	Dairy Shed Effluent (land), Underpass Effluent	1
	Dairy Shed Effluent (land), Wash Down Effluent, Wash Water, Waste Water	1
	Dairy Shed Effluent (land), Waste Water	1
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	33
	Green waste	2
	Hazardous Substances	1
	Industrial Effluent, Waste Water	2
	Leachate, Refuse - Commercial, Refuse - Domestic	3
	Leachate, Refuse - Commercial, Refuse - Domestic, Refuse - Industrial	1
	Meat Works Effluent	1
	Meat Works Effluent, Sludge	1
	Meat Works Effluent, Wash Down Effluent, Wash Water, Waste Water	1
	Meat Works Effluent, Waste Water	13
	Offal	2
	Oil/Grease	9
	Sewage (Treated)	1
	Silt, Wash Water	1
	Stormwater	1
	Tannery Effluent, Wash Water	2
	Vegetable Wash Water, Wash Water	1
	Wash Down Effluent, Waste Water	1
	Wash Water	6
	Waste Water	4
	Wintering Pad/Feedlot Effluent (land)	8
To Land Total		280
To Water	Other (dewatering construction area)	1
	Boiler Blowdown Water, Waste Water	2
	Cooling Water	2
	Cooling Water, Stormwater, Waste Water	1
	Ground water, Mine water, Stormwater, Suspended Sediment	1
	Hydro electric power generation sundry contaminant	1
	Hydro electric power generation sundry contaminant, Water (Hydro)	1
	Industrial Effluent, Stormwater, Waste Water	1
	Industrial Effluent, Tile drainage	1
	Meat Works Effluent, Waste Water	1
	Mine water	4
	Mine water, Silt, Waste Water	1
	Mine water, Wash Water	3
	Mine water, Waste Water	2

Table 13. Continued

	Oxidation Pond Effluent, Sewage (Treated)	1
	Oxidation Pond Effluent, Sewage (Treated), Sewage Package Plant	1
	Oxidation Pond Effluent, Sewage (Treated), Stormwater, Waste Water	1
	Sewage (Treated), Sewage Package Plant, Waste Water	1
	Sewage (Treated), Stormwater, Waste Water	1
	Silt	1
	Silt, Sludge	1
	Stormwater	36
	Suspended Sediment	1
	Wash Water	3
	Wash Water, Waste Water	1
To Water Total		70
Grand Total		350

Note: Consent information accurate as of April 2017

B.4 WAIMEA STREAM AT MANDEVILLE

Water samples were collected from the Waimea Stream at Mandeville on three occasions during autumn and winter 2015 (Table 14). Each sample was collected following rainfall. The two autumn samples contained high levels of *E. coli*, at 8,000 and 6,000 cfu/100 ml in April and May, respectively. The July sample contained 300 cfu/100 ml *E. coli*.

The water sample collected in April yielded the highest concentration of *Campylobacter* spp. observed in the Mataura FMU – 1,100 MPN/100 ml – while subsequent samples in May and July contained 9.3 and 0.4 MPN/100 ml. A concentration of *Campylobacter* as high as reported for April typically signifies a fresh pollution event. All *Campylobacter* isolates tested were identified as *C. jejuni*, with MBiT analysis suggesting a predominantly wildfowl source.

Faecal source tracking identified ruminant pollution as the dominant pollution type (≤100%) at this site, with both bovine and ovine markers present in all three samples. Wildfowl markers were also present in all three samples.

Land use in the Waimea Stream at Mandeville sub-catchment is almost exclusively agricultural (approximately 98%). This includes a large amount of dairy (24%), as well as sheep and mixed sheep and beef activity (Figure 29, Figure 30).

Table 14. Results for microbial and FST analysis of water samples collected from the Waimea Stream at Mandeville.

Site		Waimea Stream at Mandeville		
Sample #		CMB150394	CMB150563	CMB151035
Client #		20151628	20151861	20152694
Date Sampled		15/04/2015	13/05/2015	9/07/2015
Rainfall		Yes	Yes	Yes
		Microbial Properties		
Faecal coliforms		9,000	7,000	300
E. coli		8,000	6,000	300
Campylobacter		1100	9.3	0.4
Campylobacter Species		*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl	2		1
	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		+	+	+

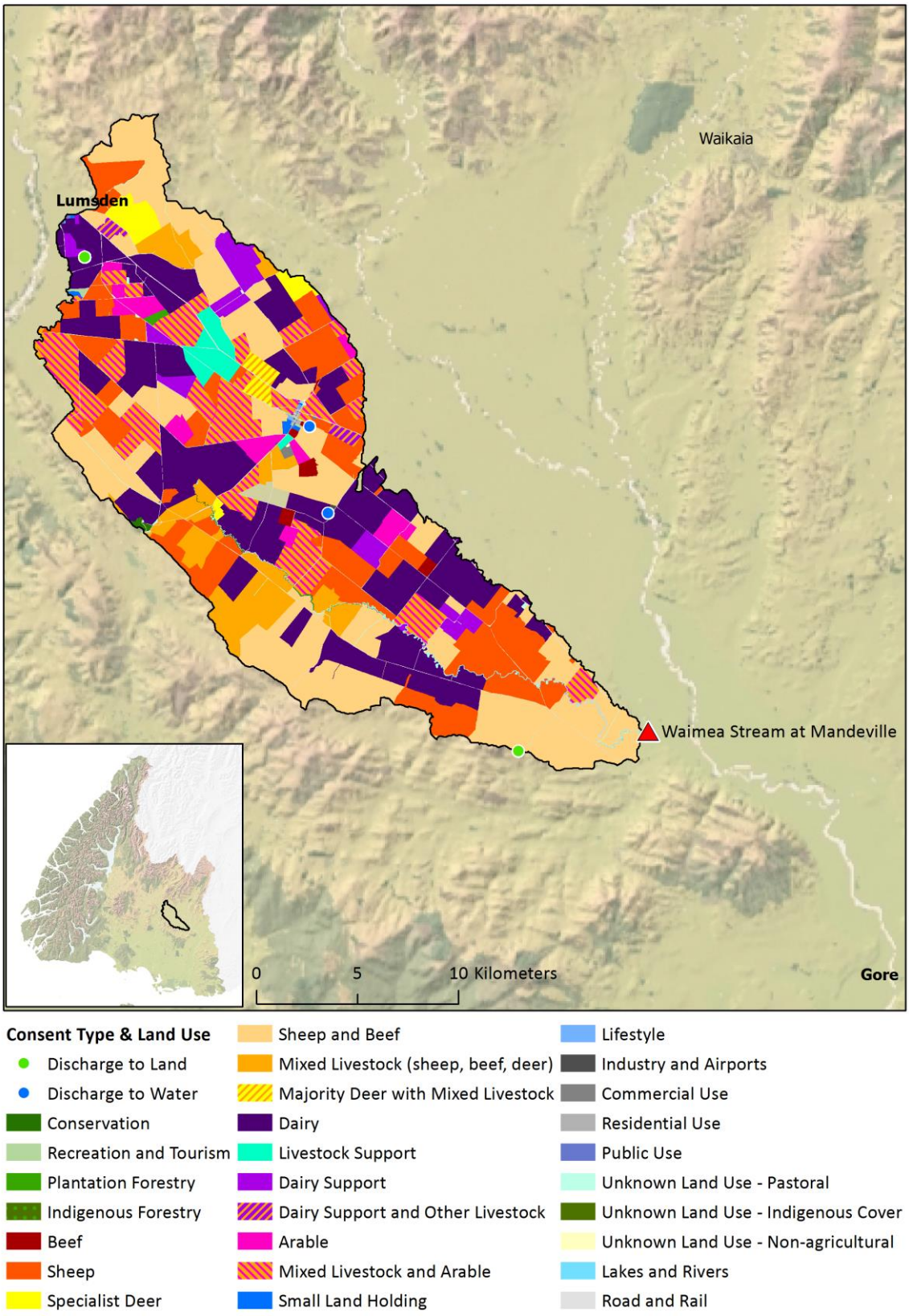

Figure 29. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Waimea Stream at Mandeville sampling site.

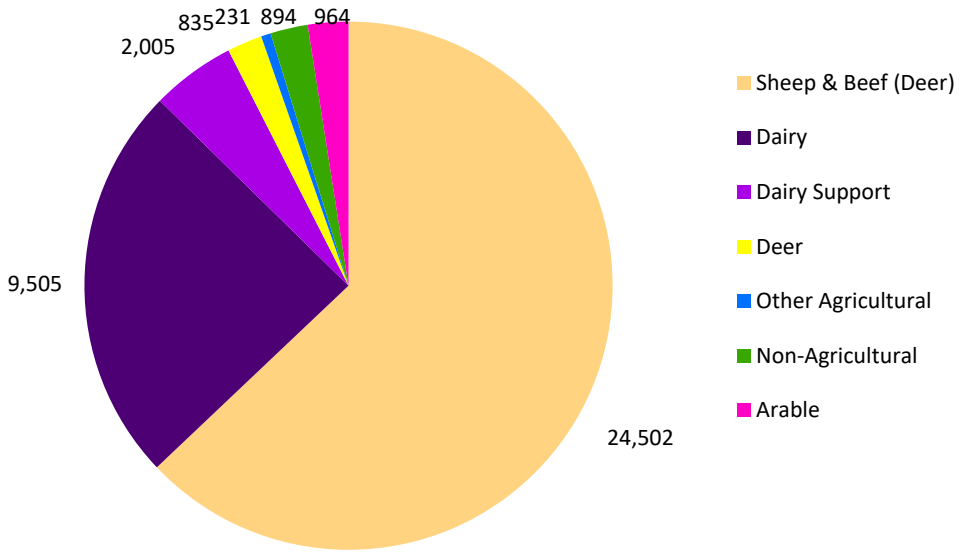

Figure 30. Land use (in hectares) in the catchment for Waimea Stream at Mandeville.

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), **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

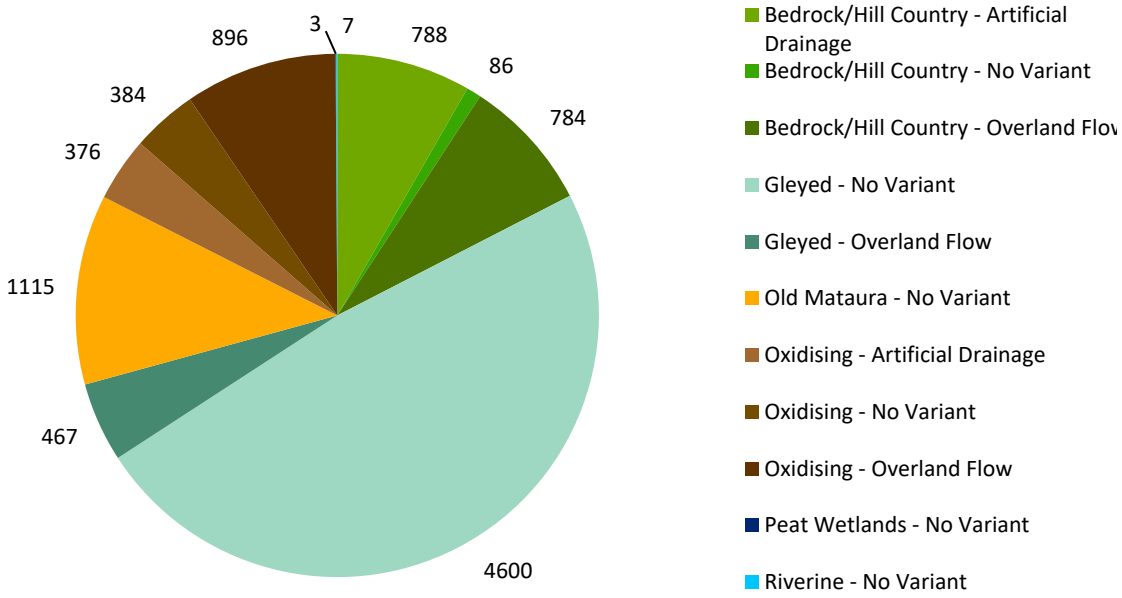

Figure 31. Dairying land (in hectares) in the catchment for Waimea Stream at Mandeville, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 15. Number of consented catchment discharges to land and water in the catchment for Waimea Stream at Mandeville.

Waimea Stream at Mandeville		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	3
	Dairy Factory Effluent, Wintering Pad/Feedlot Effluent (land)	1
	Dairy Shed Effluent (land)	28
	Dairy Shed Effluent (land), Wash Down Effluent, Wash Water, Waste Water	1
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	9
	Meat Works Effluent, Waste Water	1
	Offal	1
	Wintering Pad/Feedlot Effluent (land)	2
To Land Total		46
To Water	Oxidation Pond Effluent, Sewage (Treated), Sewage Package Plant	1
	Stormwater	1
To Water Total		2
Grand Total		48

Note: Consent information accurate as of April 2017

B.5 WAIKAIA RIVER AT WAIKAIA

Water samples were collected from the Waikaia River at Waikaia in April, May and June 2015 (Table 16). Each sample collection was preceded by a rainfall event. Microbial loading of the water samples demonstrated a similar seasonal variation to that seen at other sites, with the highest levels of *E. coli* detected in April (3,100 cfu/100 ml), and subsequent samples containing progressively lower counts (600 and 180 cfu/100ml respectively).

A similar pattern was observed for *Campylobacter*, with the highest levels observed in April (15 MPN/100 ml), declining in May (0.9 MPN/100 ml) and falling below detection limits in June (<0.3 MPN/100 ml). *C. jejuni* was identified in both April and May samples, with *C. coli* also present in the April sample. MBiT analysis identified the *Campylobacter* as being from a ruminant or 'not wildfowl' source.

Faecal source tracking suggested that ruminant animals were the dominant source of faecal pollution at this site, accounting for up to 100% of faecal indicator markers. Analysis of the PCR markers identified only ovine-specific markers. Eighty-five percent of the land in the catchment is used for either mixed sheep/beef/deer or sheep-only agriculture (Figure 32, Figure 33).

Table 16. Results for microbial and FST analysis of water samples collected from the Waikaia River at Waikaia.

Site		Waikaia River at Waikaia		
Sample #		CMB150392	CMB150560	CMB150811
Client #		20151623	20151856	20152103
Date Sampled		15/04/2015	13/05/2015	10/06/2015
Rainfall		Yes	Yes	Yes
		Microbial Properties		
Faecal coliforms		3,100	600	210
E. coli		3,100	600	180
Campylobacter		15	0.9	<0.3
Campylobacter Species		*C. jejuni* & *C. coli*	*C. jejuni*	nt
MBiT *Campylobacter* Source	Wildfowl			
	Ovine/Bovine/ Deer	2		
	Poultry			
	Not Wildfowl	1	1	
	Unknown			
		Faecal Source Tracking		
General - GenBac3		++++	++++	++++
Ruminant		≤100%	≤100%	≤100%
Human - BacH		+	+	+
Human - BiADO		-	-	-
Cow		-	-	-
Sheep		+	+	+
Wildfowl - GFD		-	-	-
Wildfowl - E2		-	-	-

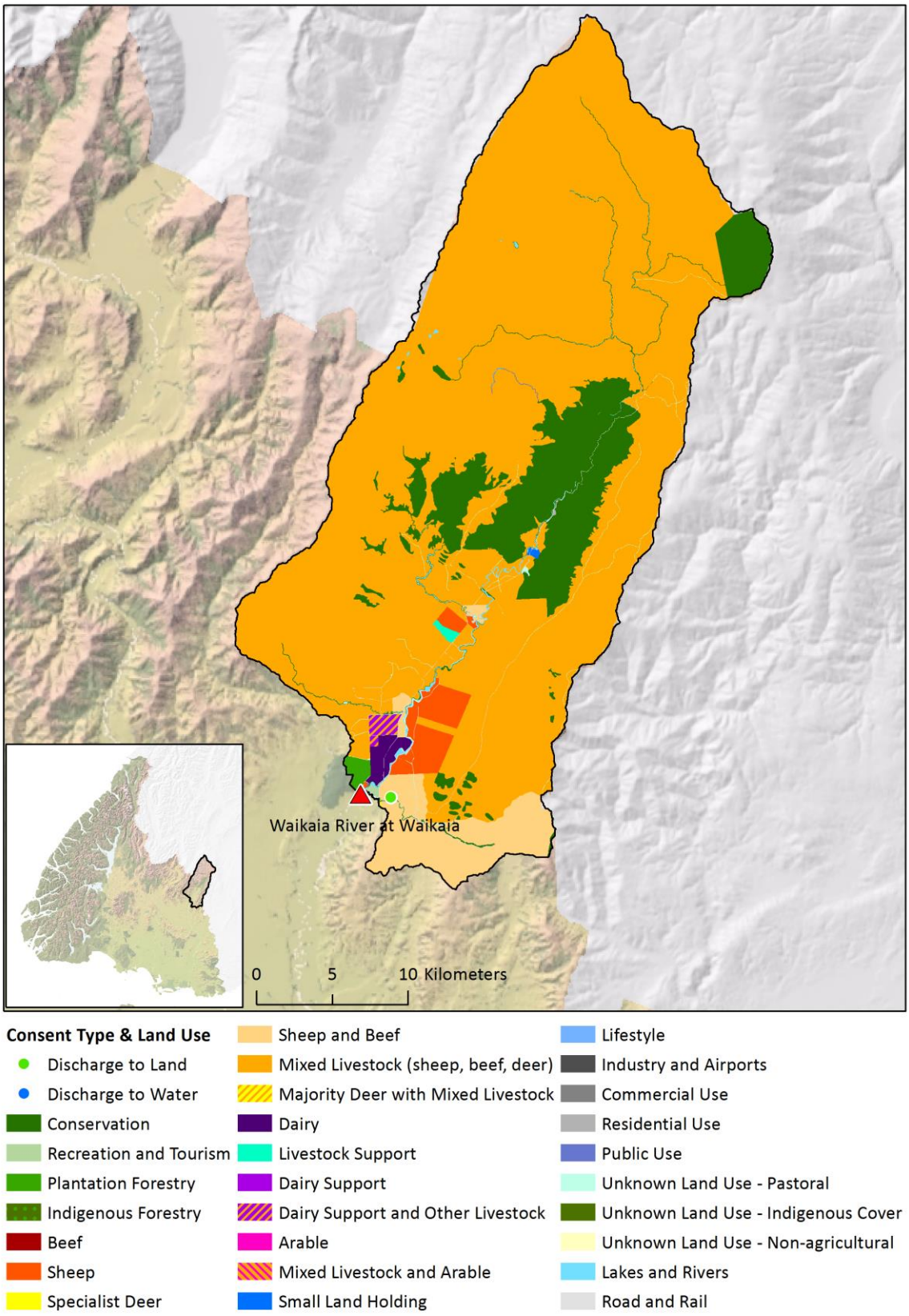

Figure 32. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Waikaia River at Waikaia sampling site.

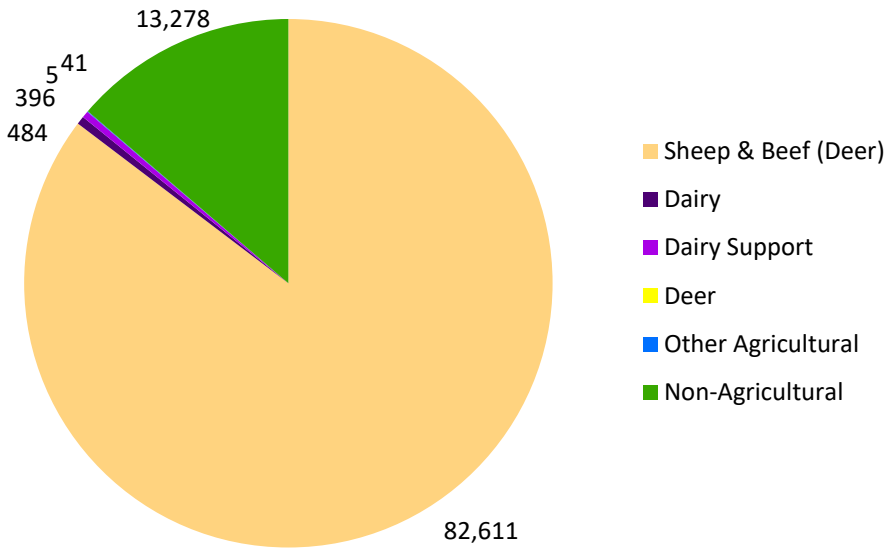

Figure 33. Land use (in hectares) in the catchment for the Waikaia River at Waikaia.

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), **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

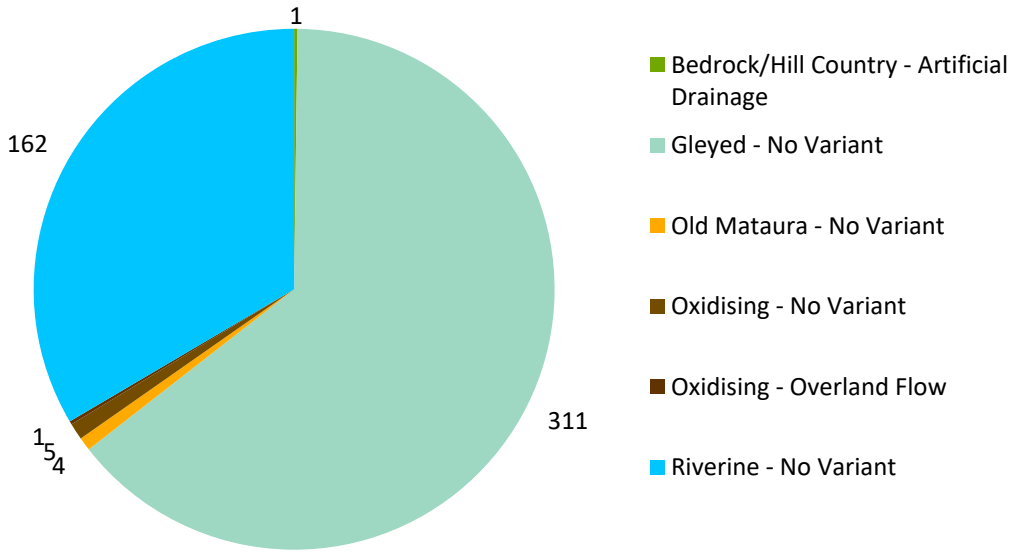

Figure 34. Dairying land (in hectares) in the catchment for the Waikaia River at Waikaia, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 17. Number of consented catchment discharges to land and water in the catchment for the Waikaia River at Waikaia.

Waikaia River at Waikaia		
Subtype	Contaminant	Total
To Land	1080, Dye	4
	Dairy Shed Effluent (land)	1
	Green waste	1
	Wintering Pad/Feedlot Effluent (land)	1
To Land Total		7
To Water	Mine water	3
	Mine water, Silt, Waste Water	1
	Mine water, Wash Water	2
To Water Total		6
Grand Total		13

Note: Consent information accurate as of April 2017

B.6 OTAMITA STREAM AT MANDEVILLE

Water samples were collected from the Otamita Stream at Mandeville during the autumn, winter and summer of 2015 (Table 18). Water samples were all collected under base flow conditions. *E. coli* levels varied between samples, with concentrations of 330, 20 and 1,000 cfu/100 ml observed in the March, August and December samples respectively.

The observation of high levels of *E. coli* in December was coincident with the only instance of *Campylobacter* being detected at this site (9.3 MPN/100 ml). Isolates were identified as *C. jejuni* from a poultry source.

Faecal source tracking revealed that ruminant pollution accounted for less than half of the overall faecal pollution present at this site. No source-specific PCR markers were identified other than a wildfowl marker in August. This may result from the relatively low levels and/or possibility of aged faecal pollution at this site, making it more difficult to recover source-specific markers.

More than 90% of the sub-catchment is used for beef and sheep farming, with much of the remainder being plantation forestry (Figure 35, Figure 36).

Table 18. Results for microbial and FST analysis of water samples collected from the Otamita Stream at Mandeville.

Site		Otamita Stream at Mandeville		
Sample #		CMB150246	CMB151390	CMB152238
Client #		20151078	20152938	20154466
Date Sampled		11/03/2015	12/08/2015	9/12/2015
Rainfall		No	No	No
		Microbial Properties		
Faecal coliforms		330	20	1,100
E. coli		330	20	1,000
Campylobacter		<0.3	<0.3	9.3
Campylobacter Species		nt	nt	*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl			
	Ovine/Bovine/ Deer			
	Poultry			5
	Not Wildfowl			
	Unknown			
		Faecal Source Tracking		
General - GenBac3		+++	+++	++++
Ruminant		1-10%	10-50%	10-50%
Human - BacH		-	-	-
Human - BiADO		-	-	-
Cow		-	-	-
Sheep		-	-	-
Wildfowl - GFD		-	-	-
Wildfowl - E2		-	+	-

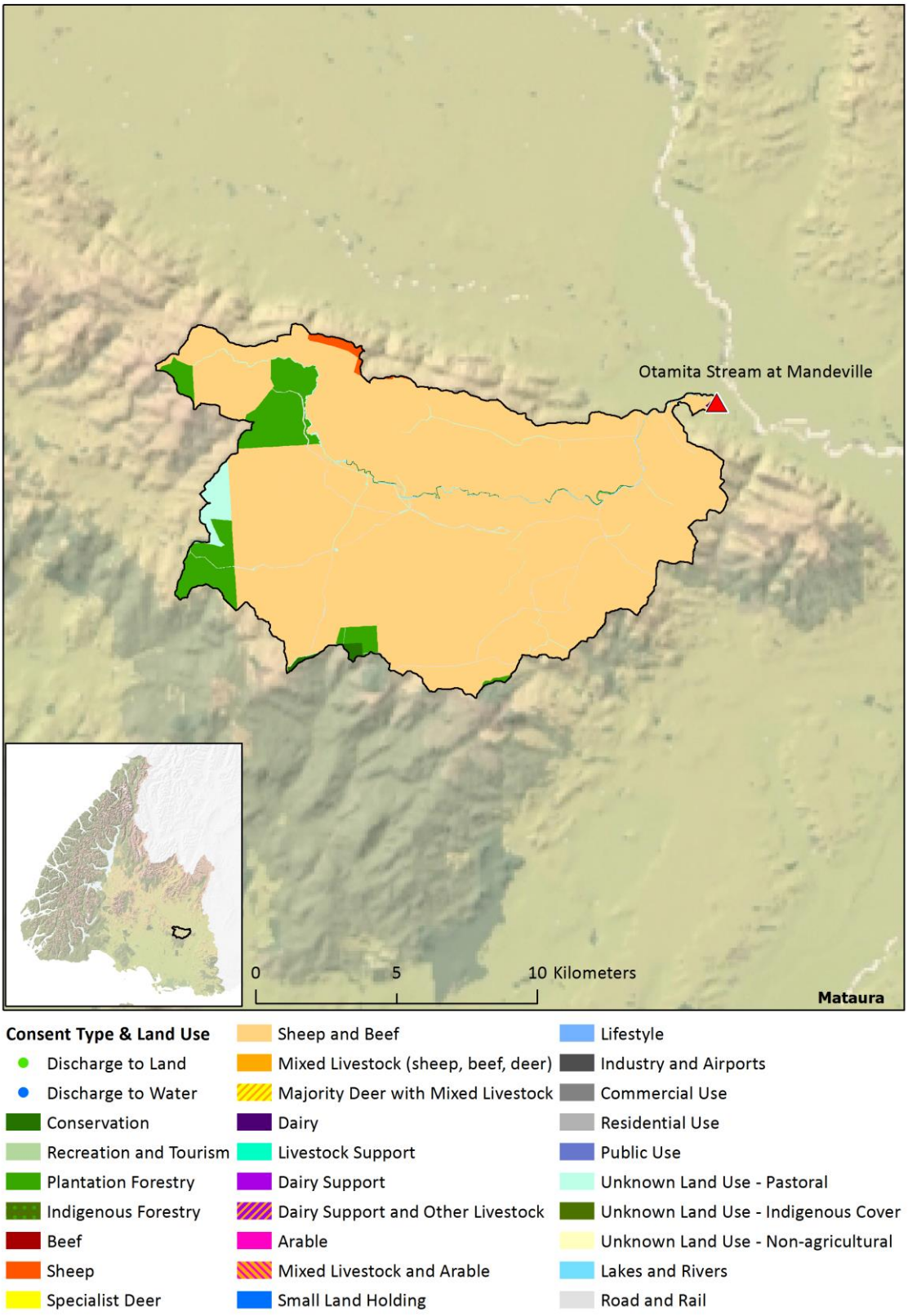

Figure 35. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Otamita Stream at Mandeville sampling site.

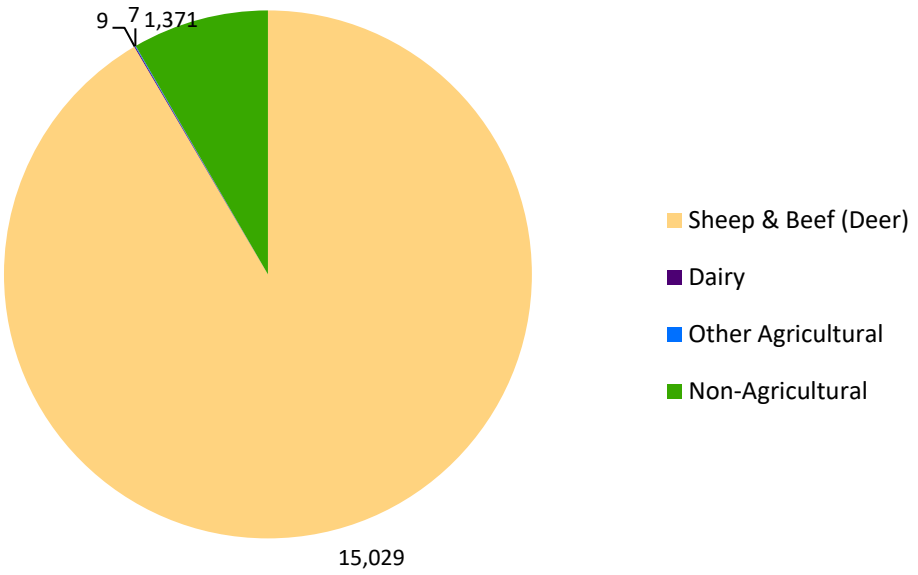

Figure 36. Land use (in hectares) in the catchment for Otamita Stream at Mandeville.

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), **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

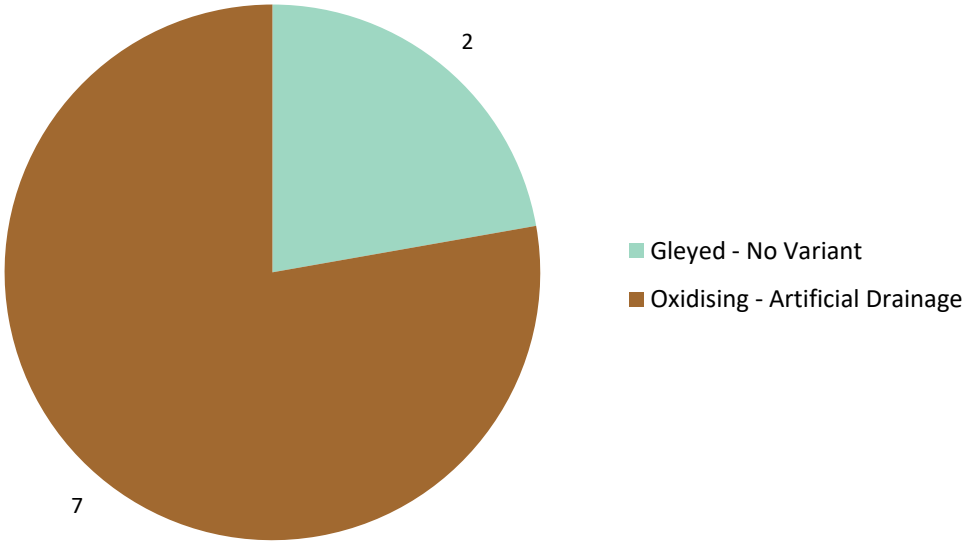

Figure 37. Dairying land (in hectares) in the catchment for the Otamita Stream at Mandeville, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 19. Number of consented catchment discharges to land and water in the catchment for the Otamita Stream at Mandeville.

Otamita Stream at Mandeville	
There are no consented discharges in the Otamita Stream at Mandeville sub-catchment.	
Grand Total	0

Note: Consent information accurate as of April 2017

B.7 WAIKAKA STREAM AT GORE

The Waikaka Stream was sampled at Gore on four occasions during the autumn and spring of 2015. All sampling was preceded by a rainfall event (Table 20). *E. coli* levels in the April and May samples contained the second- and third-highest concentrations of *E. coli* observed across all samples collected within the Mataura FMU (17,000 and 14,000 cfu/100 ml respectively). *E. coli* levels were greatly reduced later in the year (1,000 and 280 cfu/100 ml).

Campylobacter was detected in all four samples. Similarly to *E. coli*, the highest concentrations of *Campylobacter* were observed during April and May (240 and 43 MPN/100 ml), with lower levels in the spring (1.5 and 0.4 MPN/100 ml). *C. jejuni* was isolated from all four samples, with *C. coli* also isolated from the April sample, and an unspeciated thermophillic *Campylobacter* from the November sample. MBiT analysis suggested a range of possible sources for *Campylobacter*, including wildfowl, poultry and ruminant sources.

Faecal source tracking determined that during autumn, 50-100% of the faecal pollution was from a ruminant source, and that this was reduced to 10-50% in spring. Autumn samples contained both bovine and ovine markers, while the October sample was also positive for ovine markers. Neither bovine- nor ovine-specific markers were detected in the November sample, possibly because of the lower pollution level. Wildfowl PCR markers were identified in each of the four samples.

Land use within the Waikaka Stream at Gore sub-catchment is almost exclusively agricultural (97%), comprising a mix of dairy, sheep, and sheep and beef ventures. There is also a small amount of mixed livestock farming in which deer are present (Figure 38, Figure 39).

Table 20. Results for microbial and FST analysis of water samples collected from the Waikaka Stream at Gore.

Site		Waikaka Stream at Gore			
Sample #		CMB150388	CMB150499	CMB151773	CMB152083
Client #		20151642	20151847	20153322	20153994
Date Sampled		15/04/2015	13/05/2015	14/10/2015	18/11/2015
Rainfall		Yes	Yes	Yes	Yes
		Microbial Properties			
Faecal coliforms		18,000	14,000	1,000	390
E. coli		17,000	14,000	1,000	280
Campylobacter		240	43	1.5	0.4
Campylobacter Species		*C. jejuni & C. coli*	*C. jejuni*	*C. jejuni*	*C. jejuni & Thermo*
MBiT *Campylobacter* Source	Wildfowl	2		1	1
	Ovine/Bovine/ Deer	2			
	Poultry		1	1	1
	Not Wildfowl		2	1	
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	++++	++++
Ruminant		≤100%	≤100%	≤50%	≤50%
Human - BacH		+	+	+	-
Human - BiADO		-	-	-	-
Cow		+	+	-	-
Sheep		+	+	+	-
Wildfowl - GFD		+	+	+	-
Wildfowl - E2		+	-	+	+

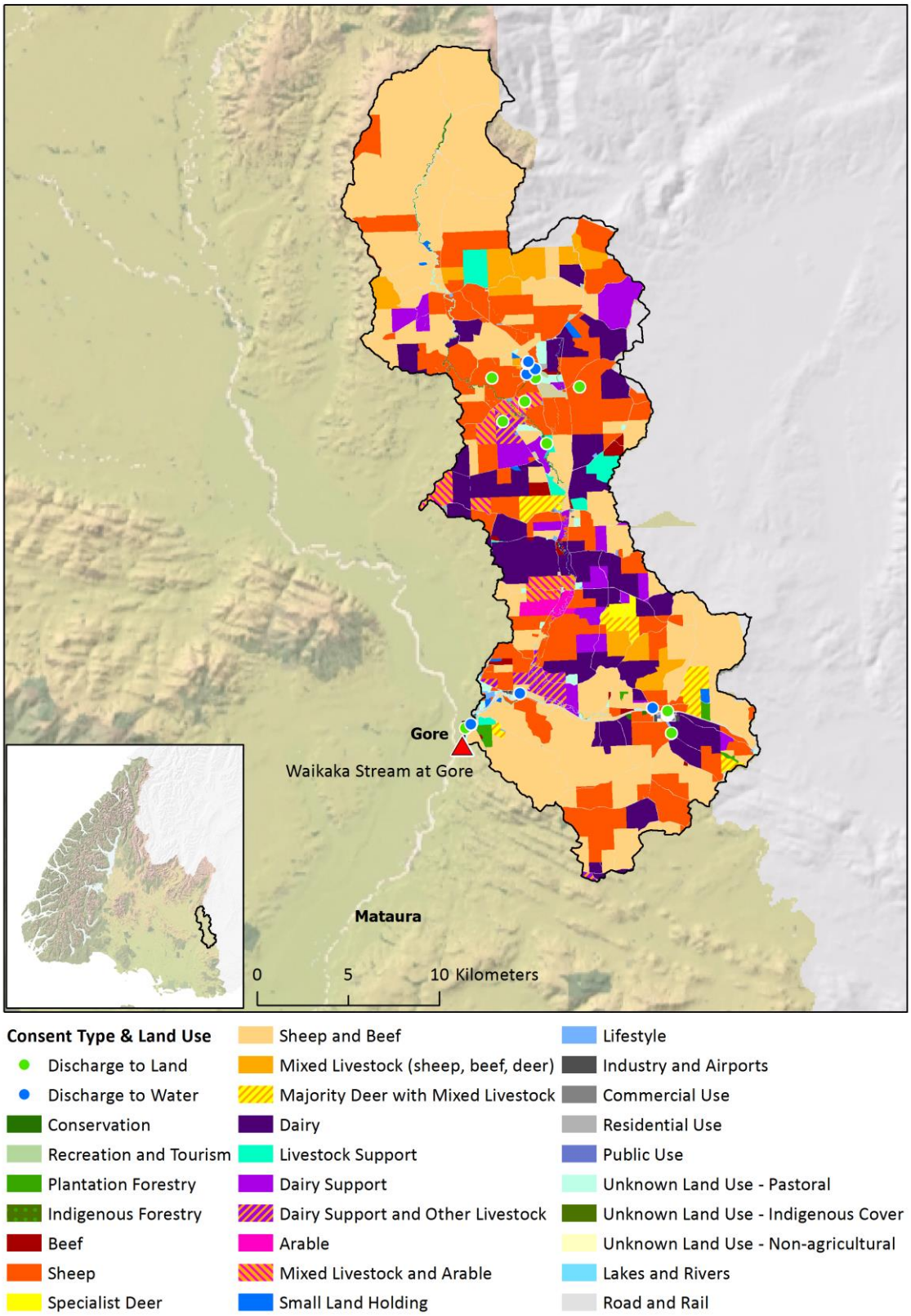

Figure 38. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Waikaka Stream at Gore sampling site.

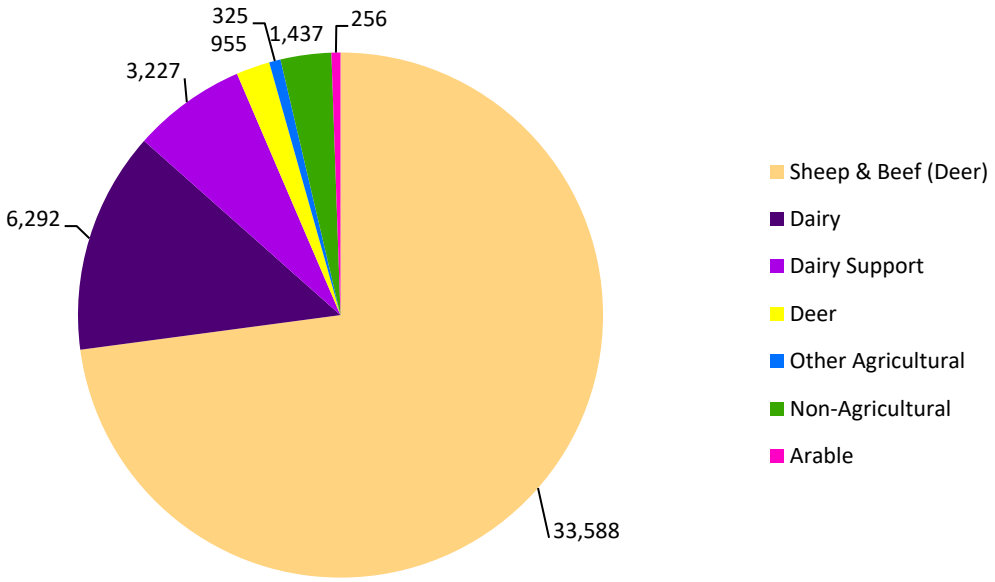

Figure 39. Land use (in hectares) in the catchment for Waikaka Stream at Gore.

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), **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

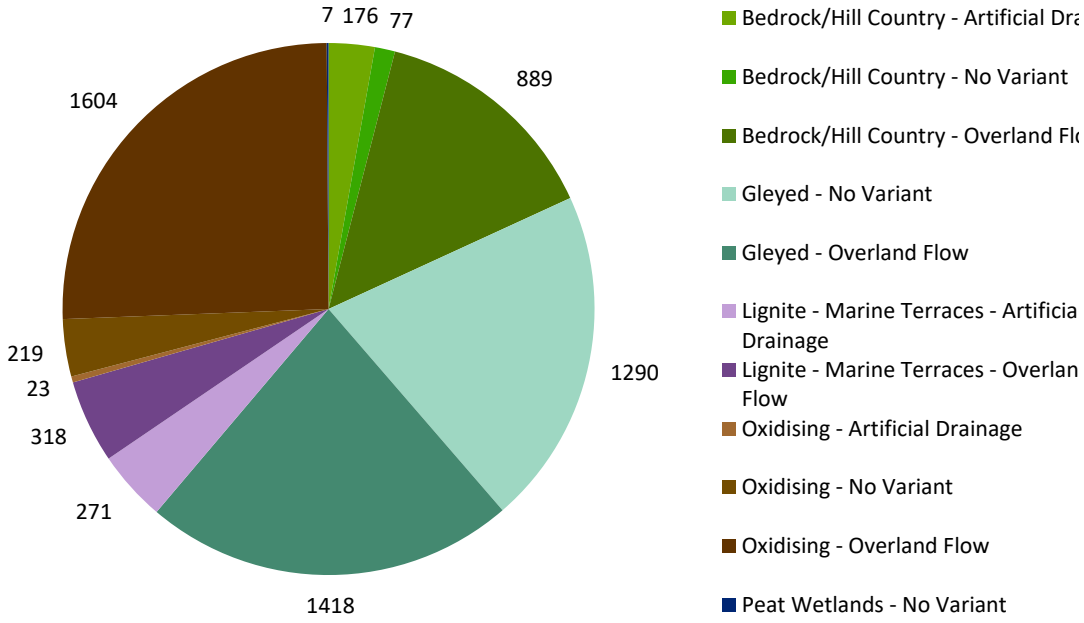

Figure 40. Dairying land (in hectares) in the catchment for Waikaka Stream at Gore, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 21. Number of consented catchment discharges to land and water in the catchment for Waikaka Stream at Gore.

Waikaka Stream at Gore		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	25
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	5
	Leachate, Refuse - Commercial, Refuse - Domestic	2
	Leachate, Refuse - Commercial, Refuse - Domestic, Refuse - Industrial	1
	Meat Works Effluent, Waste Water	4
	Sewage (Treated)	1
	Tannery Effluent, Wash Water	2
	Wash Down Effluent, Waste Water	1
	Wintering Pad/Feedlot Effluent (land)	1
To Land Total		42
To Water	Oxidation Pond Effluent, Sewage (Treated)	1
	Stormwater	9
To Water Total		10
Grand Total		52

Note: Consent information accurate as of April 2017

B.8 NORTH PEAK STREAM AT WAIMEA VALLEY ROAD

Water samples were collected from North Peak Stream at Waimea Valley Road in April, May and June 2015 (Table 22). Each sample collection was preceded by a rainfall event. *E. coli* levels were highest in April (2,800 cfu/100 ml) and declined in subsequent samples (1,800 and 60 cfu/100 ml in May and June, respectively).

A similar pattern was observed for *Campylobacter* concentration (240, 2.3 and <0.3 MPN/100 ml), with levels falling below the limits of detection in June. Where *Campylobacter* was detected, isolates were identified as being *C. jejuni* of wildfowl origin.

Faecal source tracking suggested that 50-100% of contamination in the two autumn samples was from a ruminant source, falling to 10-50% in winter. Bovine and wildfowl markers were identified in the April sample, and bovine and ovine markers detected in the May sample. No specific PCR markers were detected in the June sample, likely as a result of the low levels of contamination present in this sample.

The land in the North Peak Stream catchment is split between sheep and beef, and dairy farming (Figure 41, Figure 42).

Table 22. Results for microbial and FST analysis of water samples collected from North Peak Stream at Waimea Valley Road.

Site		North Peak Stream at Waimea Valley Road		
Sample #		CMB150393	CMB150562	CMB150812
Client #		20151626	20151859	20152106
Date Sampled		15/04/2015	13/05/2015	10/06/2015
Rainfall		Yes	Yes	Yes
		Microbial Properties		
Faecal coliforms		3,300	2,400	70
E. coli		2,800	1,800	60
Campylobacter		240	2.3	<0.3
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	nt
MBiT *Campylobacter* Source	**Wildfowl**	3	1	
	Ovine/Bovine/ Deer			
	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		+	-	-

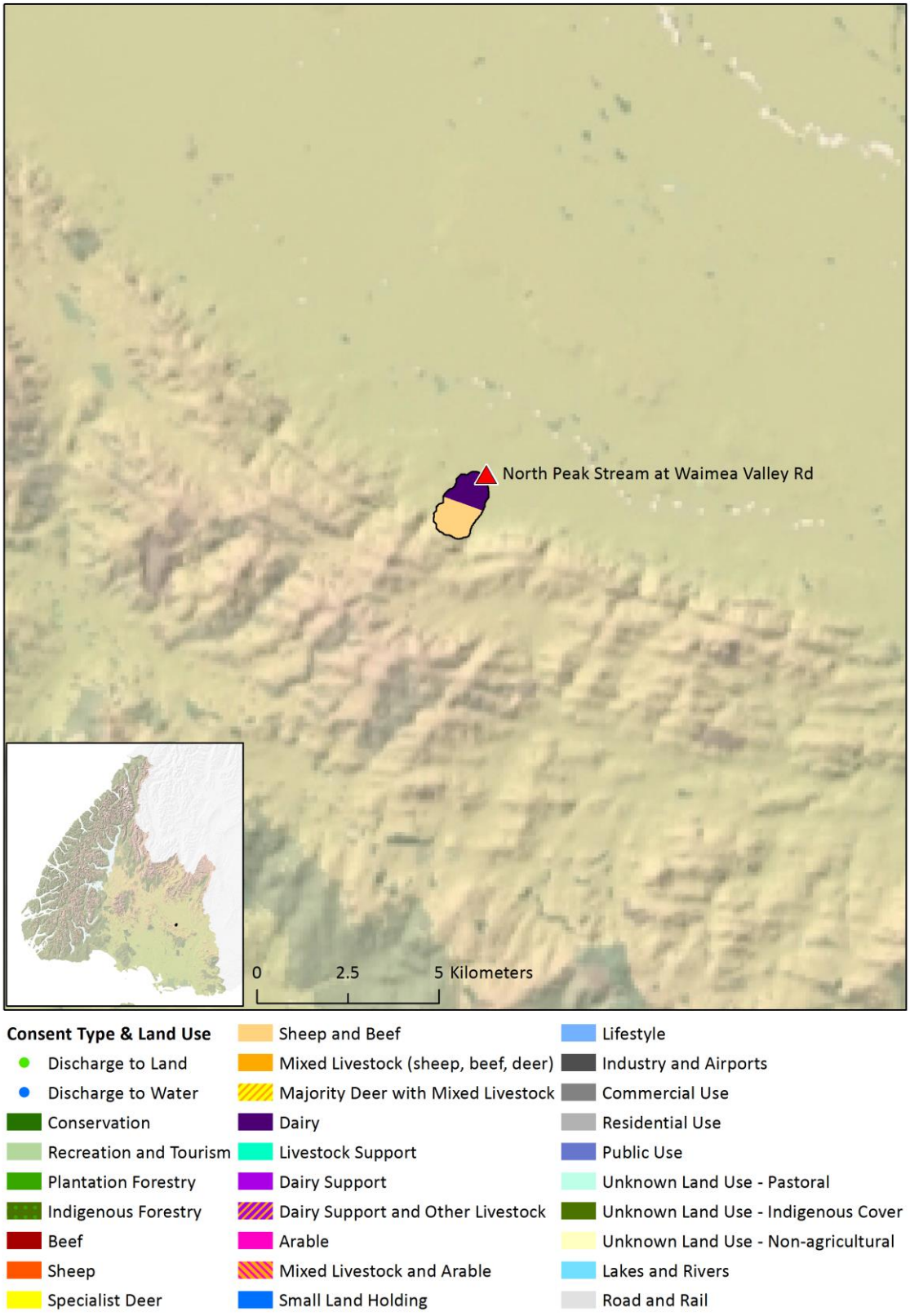

Figure 41. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the North Peak Stream at Waimea Valley Road sampling site.

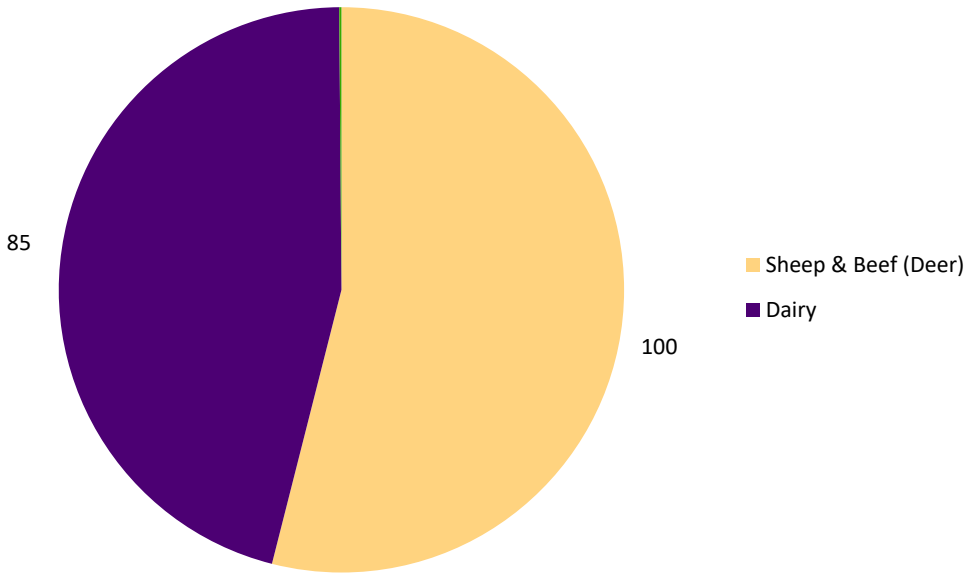

Figure 42. Land use (in hectares) in the catchment for North Peak Stream at Waimea Valley Road.

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), **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

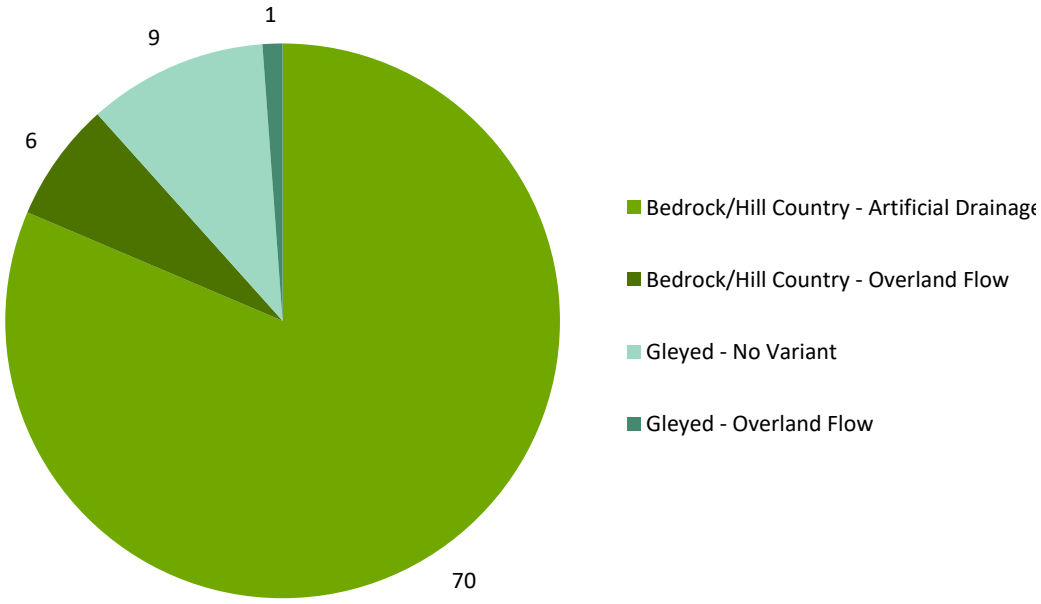

Figure 43. Dairying land (in hectares) in the catchment for North Peak Stream at Waimea Valley Road, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 23. Number of consented catchment discharges to land and water in the catchment for the North Peak Stream at Waimea Valley Road.

North Peak Stream at Waimea Valley Rd		
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.9 SANDSTONE STREAM AT KINGSTON CROSSING ROAD

Water samples were collected from Sandstone Creek at Kingston Crossing in August and December 2015 (Table 24). Both samples were taken under base flow conditions. There was a significant difference in the microbial loading of the water between the two samples: *E. coli* levels were 80 cfu/100 ml in August and 5,000 cfu/100 ml in December. No *Campylobacter* was detected in either sample.

Faecal source tracking analysis identified ruminant animals as being the primary source of contamination at this site (50-100%). Bovine-specific PCR markers were identified in the summer sample, with wildfowl markers present in both samples. The primary activity within the sub-catchment is dairy (56%; Figure 44, Figure 45)

Table 24. Results for microbial and FST analysis of water samples collected from Sandstone Stream at Kingston Crossing Road

Site		Sandstone Stream at Kingston Crossing Road	
Sample #		CMB151389	CMB152237
Client #		20152936	20154464
Date Sampled		12/08/2015	09/12/2015
Rainfall		No	No
		Microbial Properties	
Faecal coliforms		90	5,200
E. coli		80	5,000
Campylobacter		<0.3	<0.3
***Campylobacter* Species**		nt	nt
MBiT *Campylobacter* Source	**Wildfowl**		
	Ovine/Bovine/ Deer		
	Poultry		
	Not Wildfowl		
	Unknown		
		Faecal Source Tracking	
General - GenBac3		+++	++++
Ruminant		50-100%	50-100%
Human - BacH		-	-
Human - BiADO		-	-
Cow		-	+
Sheep		-	-
Wildfowl - GFD		-	+
Wildfowl - E2		+	-

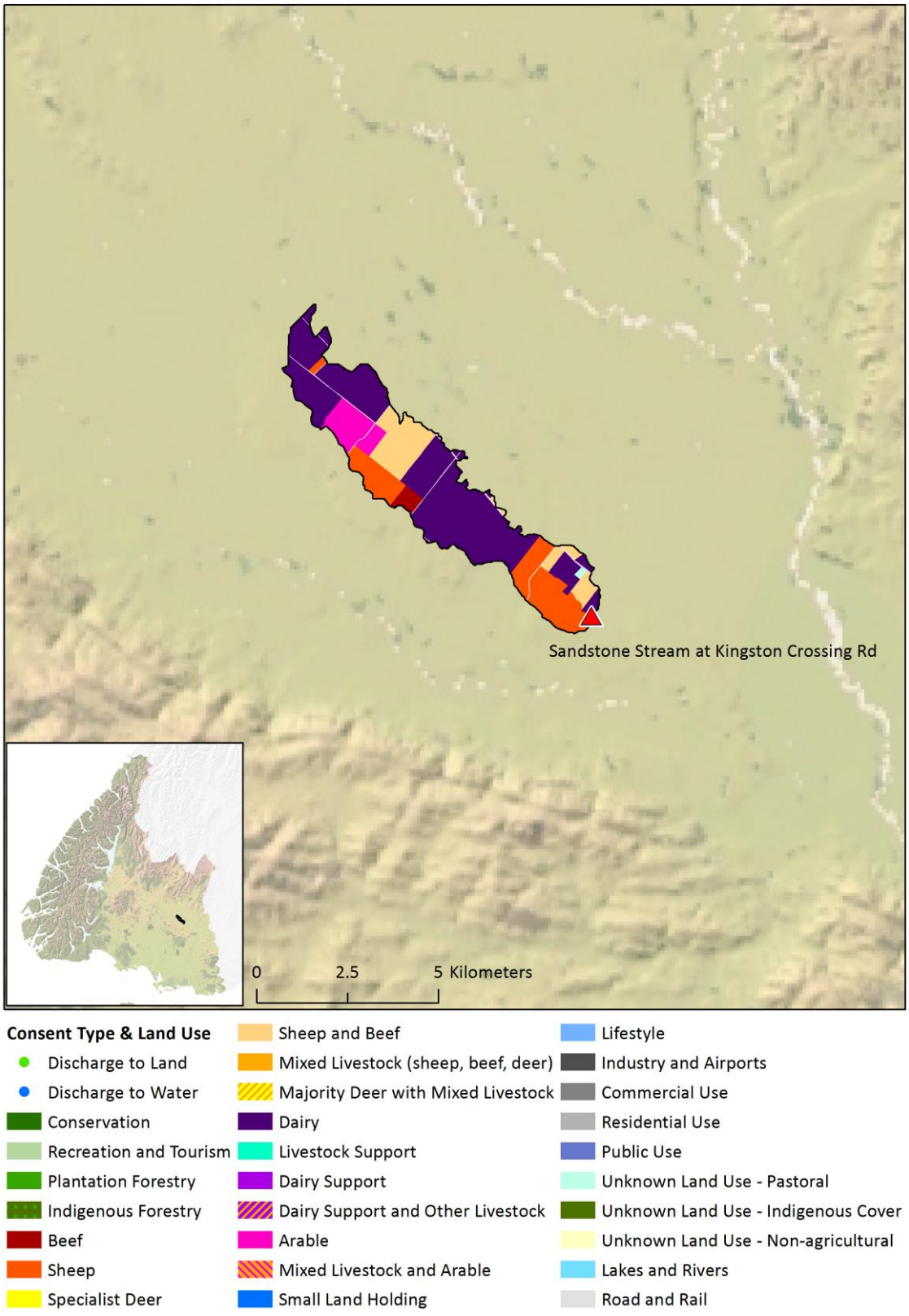

Figure 44. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Sandstone Stream at Kingston Crossing Road sampling site.

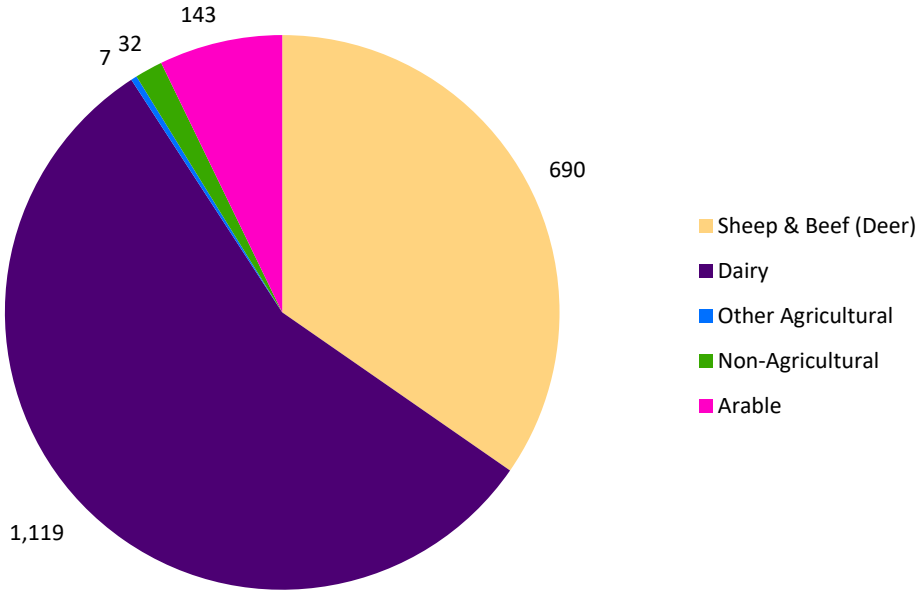

Figure 45. Land use (in hectares) in the catchment for the Sandstone Stream at Kingston Crossing Road.

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), **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

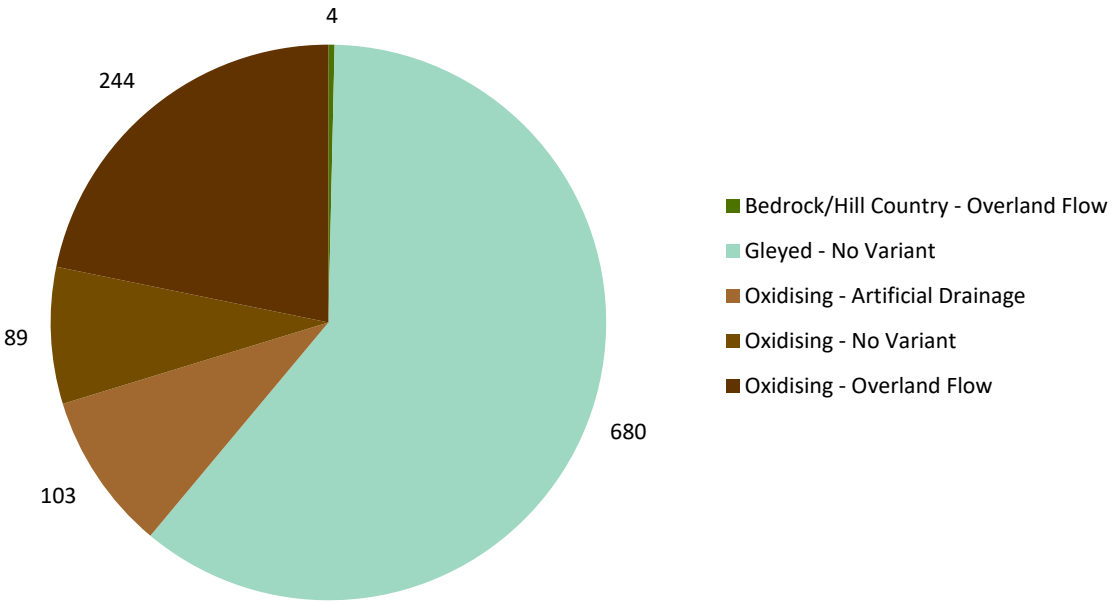

Figure 46. Dairying land (in hectares) in the catchment for Sandstone Stream at Kingston Crossing Road, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 25. Number of consented catchment discharges to land and water in the catchment for Sandstone Stream at Kingston Crossing Road.

Sandstone Stream at Kingston Crossing Rd		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	1
	Dairy Shed Effluent (land)	6
	Wintering Pad/Feedlot Effluent (land)	1
To Land Total		8
Grand Total		8

Note: Consent information accurate as of April 2017

B.10 LONGRIDGE STREAM AT SANDSTONE

Water samples were collected from Longridge Stream at Sandstone in May, July and December 2015. Each sampling event was preceded by rainfall (Table 26). Water samples collected in May yielded especially high levels of *E. coli* at 19,000 cfu/100 ml, with lower levels in subsequent samples (70 and 700 cfu/100 ml in July and December, respectively).

Campylobacter was detected in the May sample only (9.3 MPN/100 ml), and was determined to be *C. jejuni* of ruminant origin.

FST analysis determined that ruminant pollution was the dominant pollution type present in the May sample, with both ovine- and bovine-specific PCR markers detected. The prevalence of ruminant pollution declined in subsequent samples (10-50% in July and 1-10% in December), with ovine-specific markers detected in July. Wildfowl PCR markers were detected in all three water samples.

Land use in the Longridge Stream sub-catchment is dominated by sheep and beef farming (including large sheep-only blocks), with smaller amounts of dairy and deer farming (Figure 47, Figure 48).

Table 26. Results for microbial and FST analysis of water samples collected from Longridge Stream at Sandstone.

Site		Longridge Stream at Sandstone		
Sample #		CMB150561	CMB151034	CMB152264
Client #		20151858	20152691	20154554
Date Sampled		13/05/2015	09/07/2015	16/12/2015
Rainfall		Yes	Yes	Yes
		Microbial Properties		
Faecal coliforms		19,000	70	700
E. coli		19,000	70	700
Campylobacter		9.3	<0.3	<0.3
***Campylobacter* Species**		*C. jejuni*	nt	nt
MBiT *Campylobacter* Source	**Wildfowl**			
	Ovine/Bovine/ Deer	1		
	Poultry			
	Not Wildfowl	1		
	Unknown			
		Faecal Source Tracking		
General - GenBac3		++++	+++	++++
Ruminant		50-100%	10-50%	1-10%
Human - BacH		-	-	-
Human - BiADO		-	-	-
Cow		+	-	-
Sheep		+	+	-
Wildfowl - GFD		+	+	+
Wildfowl - E2		+	+	+

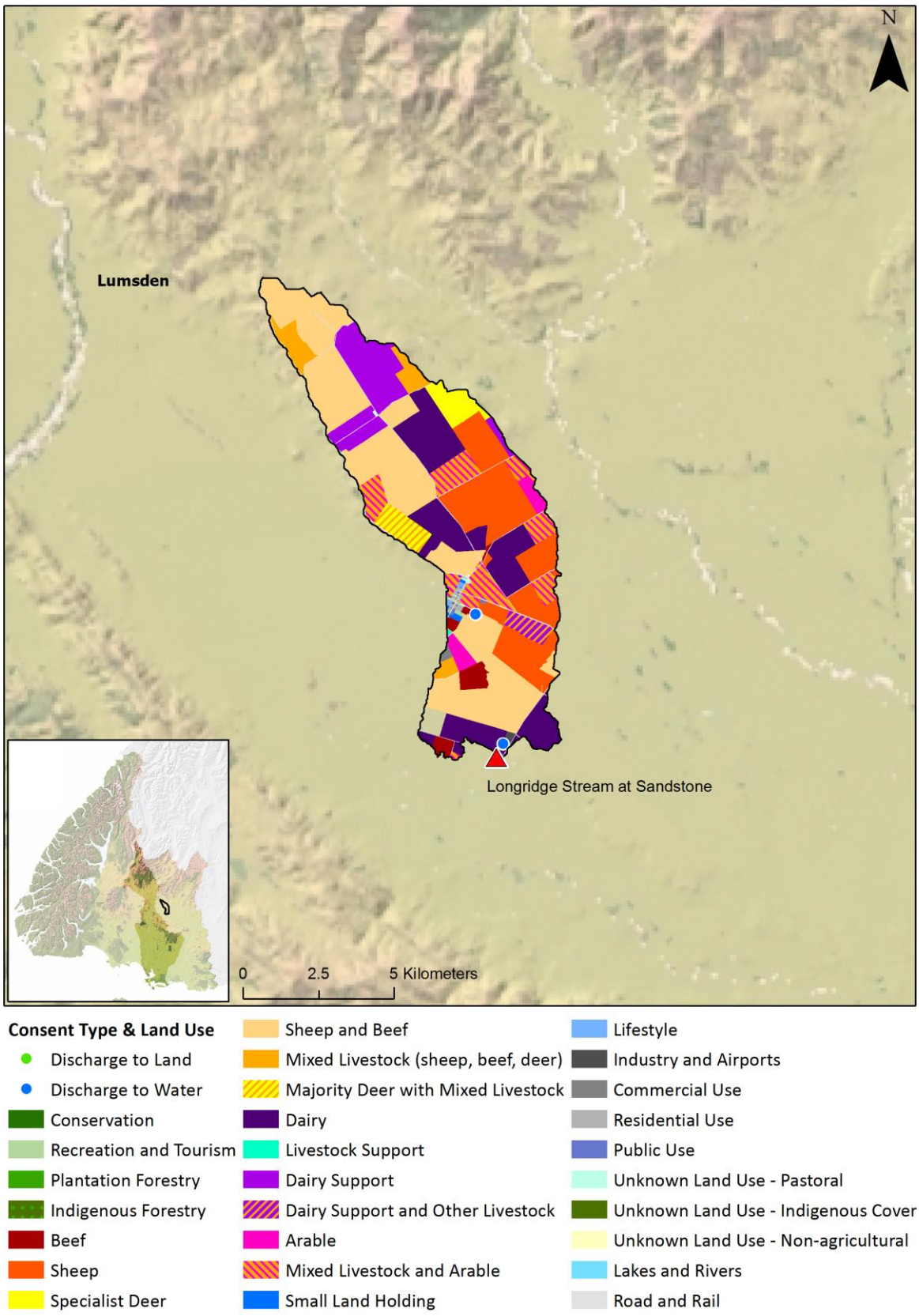

Figure 47. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Longridge Stream at Sandstone sampling site.

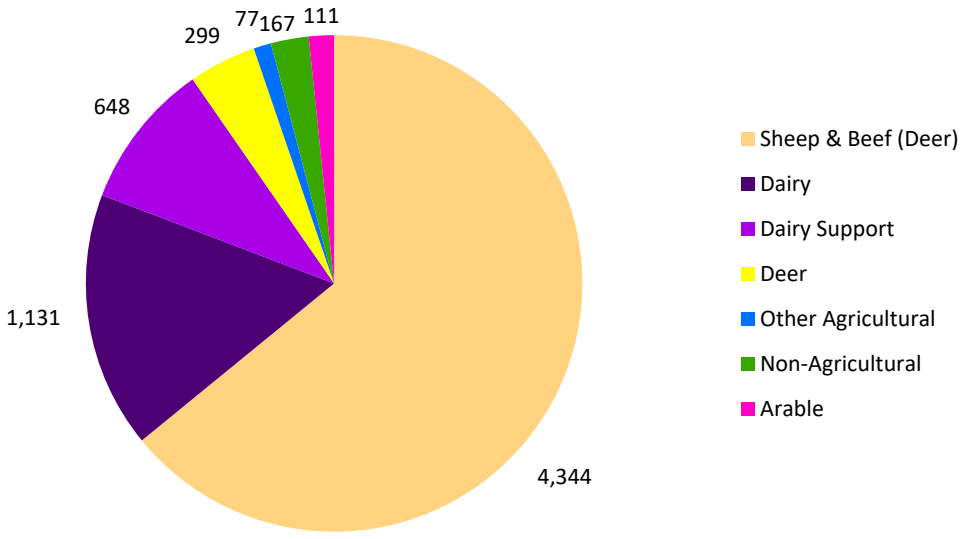

Figure 48. Land use (in hectares) in the catchment for the Longridge Stream at Sandstone sampling site.

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

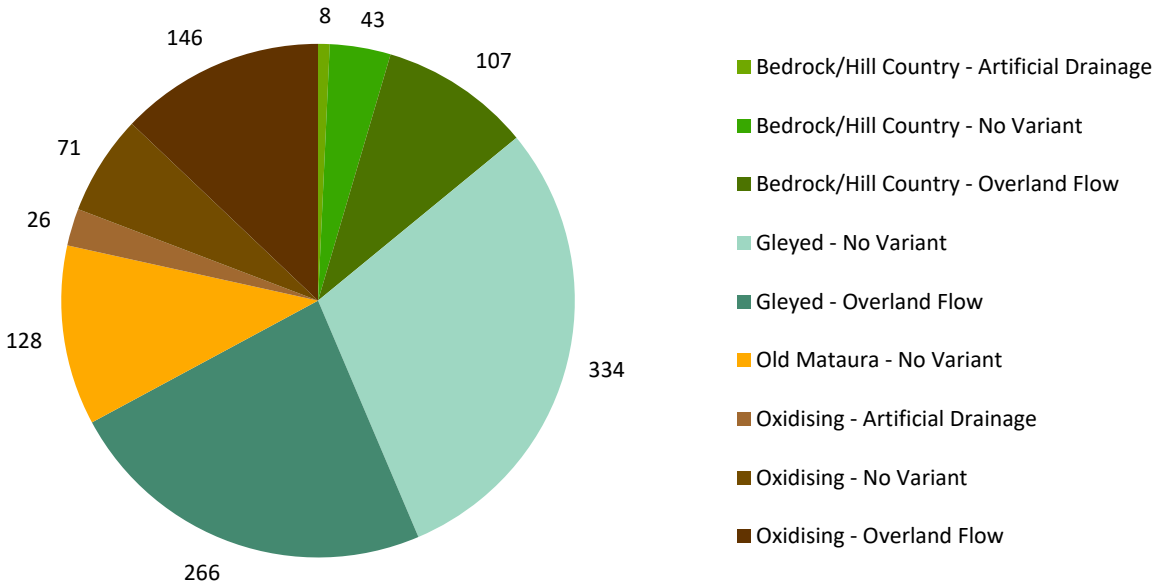

Figure 49. Dairying land (in hectares) in the catchment for the Longridge Stream at Sandstone sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016.

Table 27. Number of consented catchment discharges to land and water in the catchment for the Longridge Stream at Sandstone sampling site.

Longridge Stream at Sandstone		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	1
	Dairy Shed Effluent (land)	3
	Dairy Shed Effluent (land), Wash Down Effluent, Wash Water, Waste Water	1
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
To Land Total		6
To Water	Oxidation Pond Effluent, Sewage (Treated), Sewage Package Plant	1
	Stormwater	1
To Water Total		2
Grand Total		8

Note: Consent information accurate as of April 2017

B.11 MIMIHAU STREAM AT WYNDHAM

Mimihau Stream was sampled at Wyndham on four occasions, between autumn and summer 2015 (Table 28). All samples were collected following a rainfall event. Very high levels of *E. coli* were observed in the December sample – the highest for any site in the Mataura FMU at 22,000 cfu/100 ml. *E. coli* levels present in the other samples varied between 700 and 3,400 cfu/100 ml.

Campylobacter followed the seasonal pattern that was observed at other sites, with the highest concentrations being in April (93 MPN/100 ml), and lower levels in samples collected later in the year (43, 0.9 and 2.3 MPN/100 ml during May, October and December, respectively). All *Campylobacter* isolates were identified as *C. jejuni.* MBiT analysis identified both wildfowl and ruminant sources for the *Campylobacter* present in the April sample; however, the source of isolates from the other samples could be identified only as 'not wildfowl' (i.e. ruminant, poultry or human).

Faecal source tracking revealed ruminant animals were a dominant source of pollution, accounting for 50-100% of contamination in all samples collected at this site. Ovine-specific markers were present in all samples, with bovine markers also evident in the two autumn samples. Wildfowl FST marker were identified in the May and December samples. This is consistent with land-use in the sub-catchment, which is dominated by sheep and beef (including sheep only and mixed sheep-beef-deer) farming (63%; Figure 50, Figure 51).

Table 28. Results for microbial and FST analysis of water samples collected from Mimihau Stream at Wyndham.

Site		Mimihau Stream at Wyndham			
Sample #		CMB150389	CMB150500	CMB151774	CMB152265
Client #		20151644	20151849	20153324	20154555
Date Sampled		15/04/2015	13/05/2015	14/10/2015	16/12/2015
Rainfall		Yes	Yes	Yes	Yes
		Microbial Properties			
Faecal coliforms		3,000	3,500	700	22,000
E. coli		3,000	3,400	700	22,000
Campylobacter		93	43	0.9	2.3
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	1			
	Ovine/Bovine/ Deer	4			
	Poultry				
	Not Wildfowl		3	1	3
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	++++	++++
Ruminant		50-100%	50-100%	50-100%	50-100%
Human - BacH		+	+	+	+
Human - BiADO		-	-	-	-
Cow		+	+	-	-
Sheep		+	+	+	+
Wildfowl - GFD		-	+	-	-
Wildfowl - E2		-	+	-	+

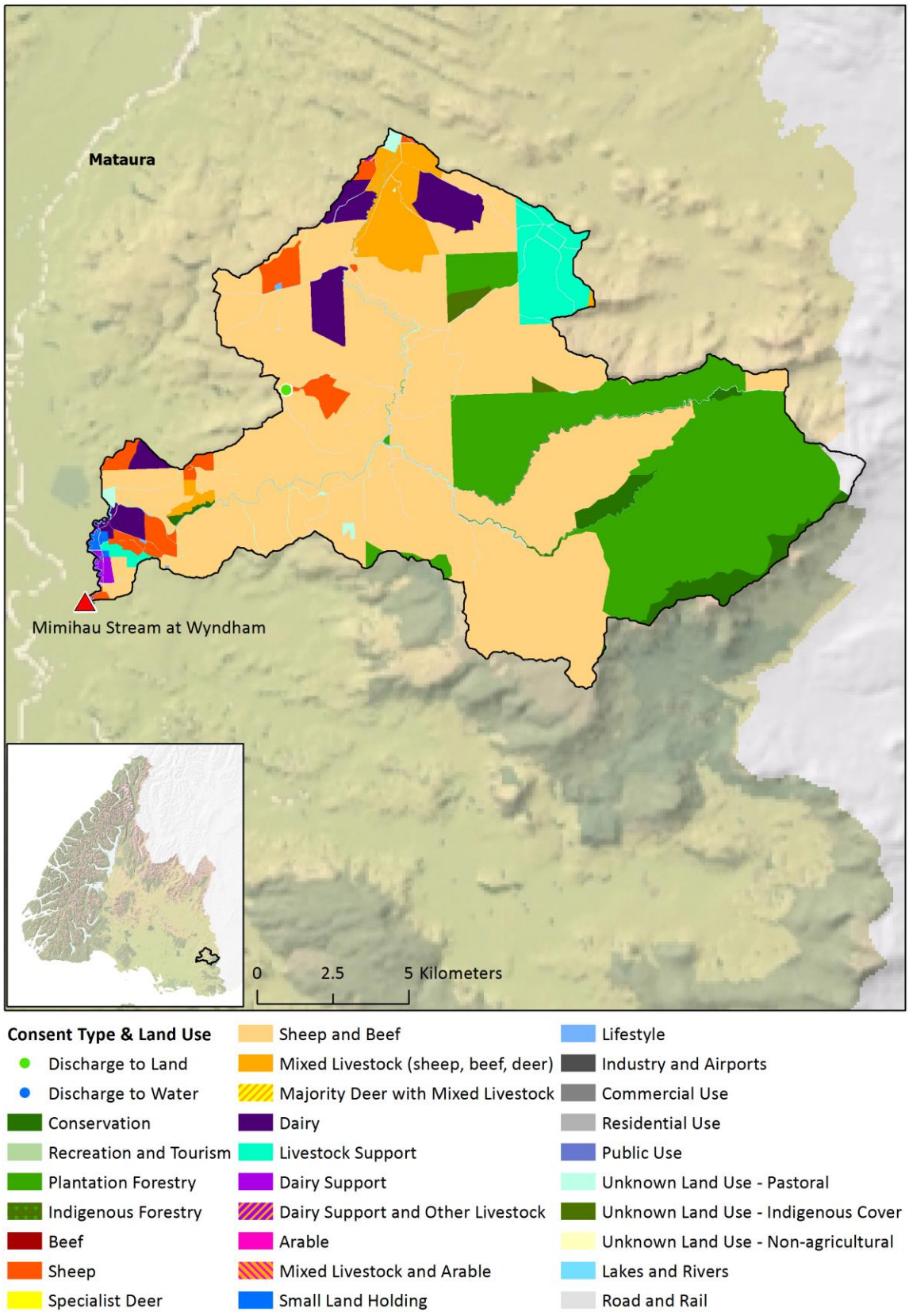

Figure 50. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Mimihau Stream at Wyndham sampling site.

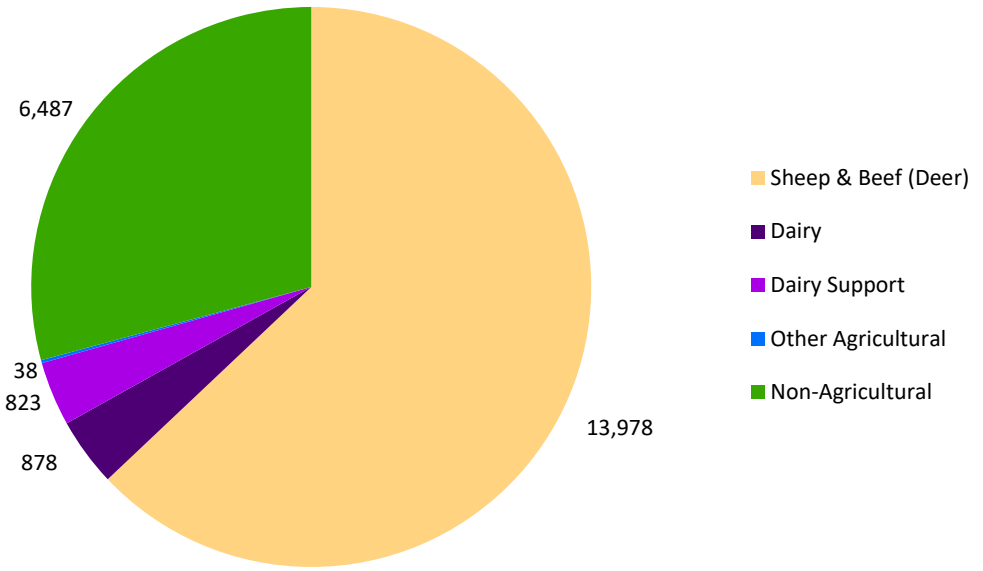

Figure 51. Land use (in hectares) in the catchment for Mimihau Stream at Wyndham.

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), **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

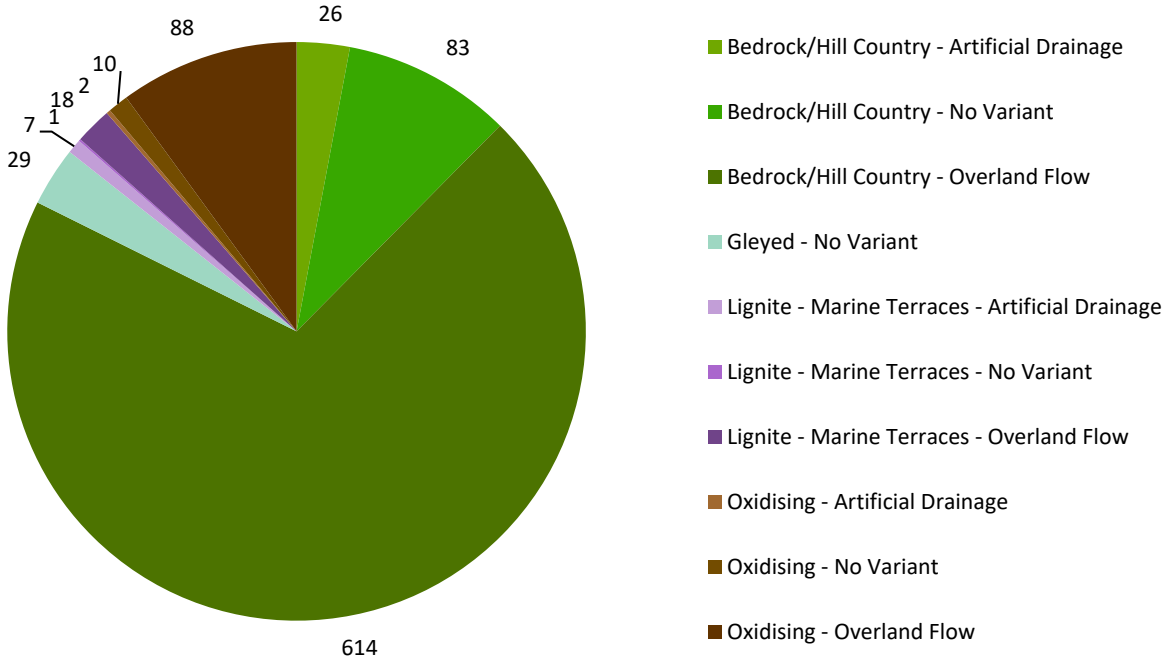

Figure 52. Dairying land (in hectares) in the catchment for Mimihau Stream at Wyndham, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 29. Number of consented catchment discharges to land and water in the catchment for Mimihau Stream at Wyndham.

Mimihau Stream at Wyndham		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	1
	Clean Fill	1
	Dairy Shed Effluent (land)	3
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
	Offal	1
	Waste Water	1
To Land Total		8
To Water	Suspended Sediment	1
To Water Total		1
Grand Total		9

Note: Consent information accurate as of April 2017

B.12 MOKORETA RIVER AT WYNDHAM RIVER ROAD

Water samples were collected from the Mokoreta River at Wyndham River Road on four occasions, two each during autumn (April and May) and winter (June and July) 2015 (Table 30). All four sampling events were preceded by rainfall. *E. coli* concentrations between 2,800 and 3,100 cfu/100 ml were reported for three of the samples, with the fourth containing 410 cfu/100 ml.

Campylobacter was detected in all four samples, with concentrations between 4.3 and 150 MPN/100 ml. The highest concentration was observed in May. *C. jejuni* was isolated in each of the samples, with an unspeciated thermophilic *Campylobacter* also identified in June. MBiT analysis indicated a range of potential sources for *Campylobacter*: a wildfowl source was evident in all four samples, a ruminant source identified in May, and a 'not wildfowl' source in April, May and June.

Faecal source tracking suggested ruminant animals were the dominant source of faecal pollution at this site (50-100%). Ovine-specific pollution markers were detected in all four samples, with bovine markers additionally detected in April and July. Wildfowl PCR markers were detected in April and June samples.

Land use in the Mokoreta River at Wyndham River Road sub-catchment is dominated by sheep and beef (55%, including significant blocks of sheep-only farming), followed by non-agricultural use (36%, conservation and plantation forestry) and dairy (7%) (Figure 53, Figure 54).

Table 30. Results for microbial and FST analysis of water samples collected from the Mokoreta River at Wyndham River Road.

Site		Mokoreta River at Wyndham River Road			
Sample #		CMB150390	CMB150501	CMB150809	CMB150996
Client #		20151646	20151851	20152098	20152684
Date Sampled		15/04/2015	13/05/2015	10/06/2015	08/07/2015
Rainfall		Yes	Yes	Yes	Yes
		Microbial Properties			
Faecal coliforms		2,800	3,100	410	3,000
E. coli		2,800	3,100	410	3,000
Campylobacter		24	150	24	4.3
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni & Thermo*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	1	1	5	4
	Ovine/Bovine/ Deer		1		
	Poultry				
	Not Wildfowl	1	2	1	
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	++++	++++
Ruminant		50-100%	50-100%	50-100%	50-100%
Human - BacH		+	+	+	+
Human - BiADO		-	-	-	-
Cow		+	-	-	+
Sheep		+	+	+	+
Wildfowl - GFD		+	-	+	-
Wildfowl - E2		+	-	+	-

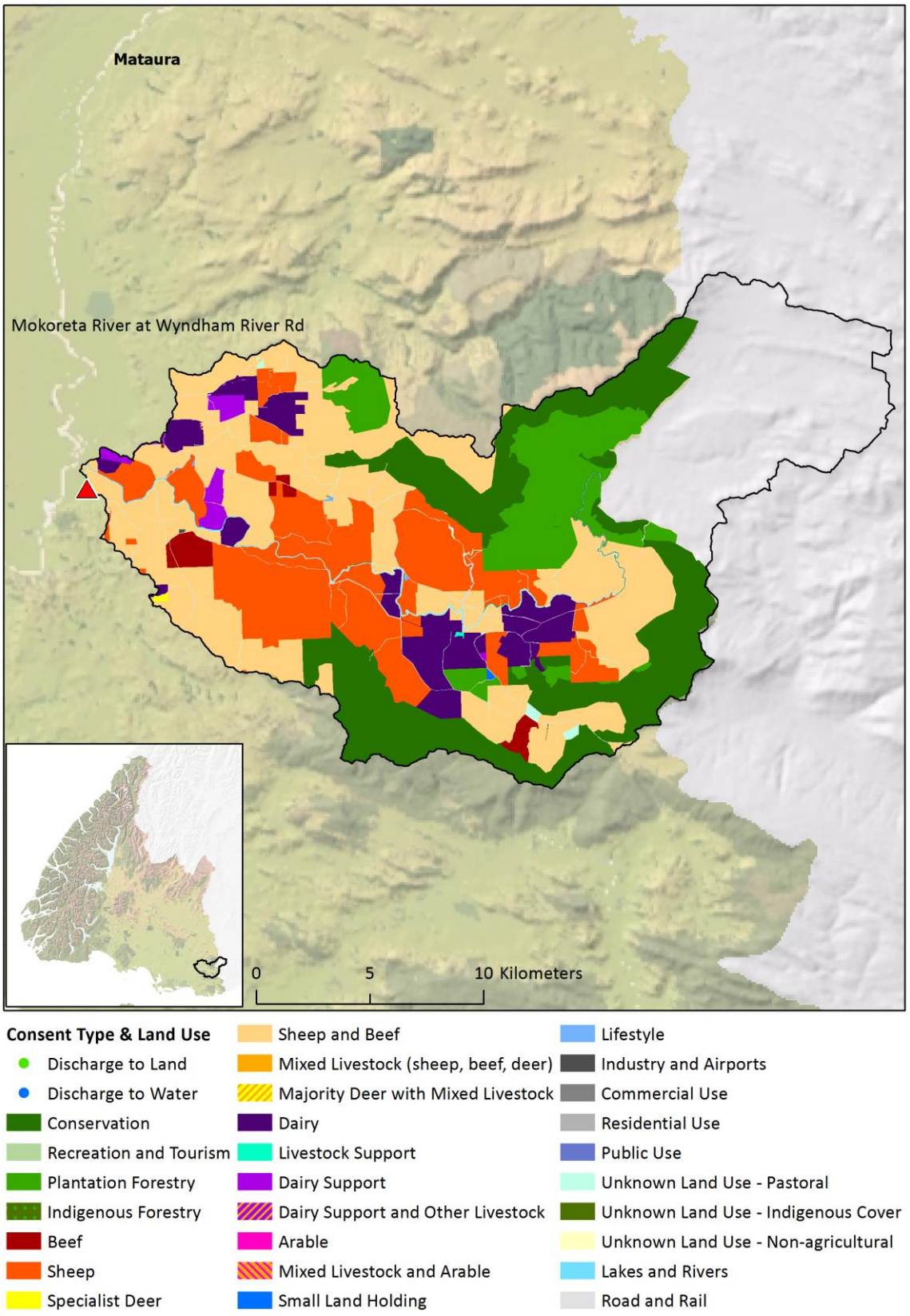

Figure 53. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Mokoreta River at Wyndham River Road sampling site.

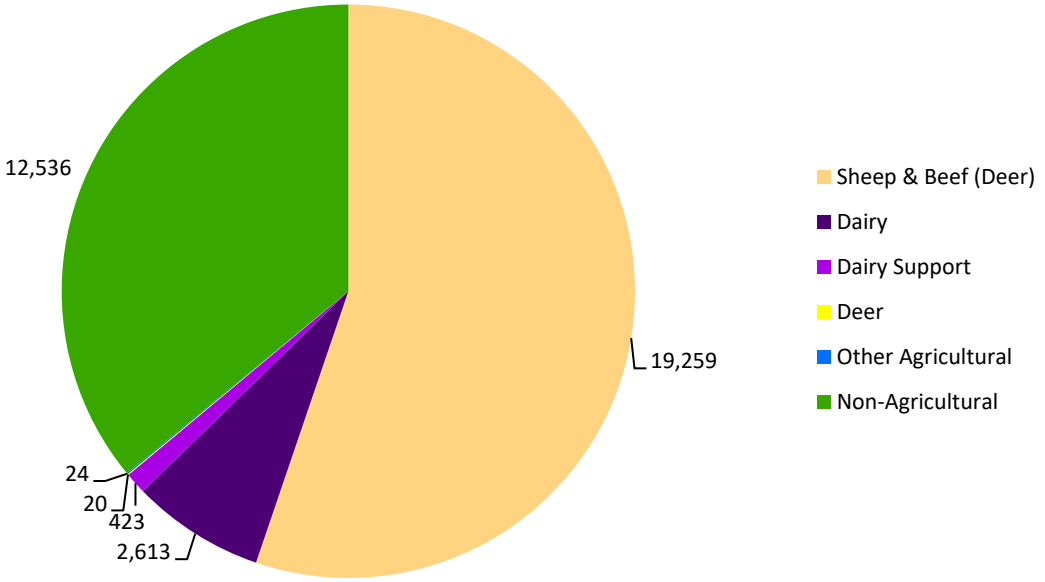

Figure 54. Land use (in hectares) in the catchment for the Mokoreta River at Wyndham River Road.

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), **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

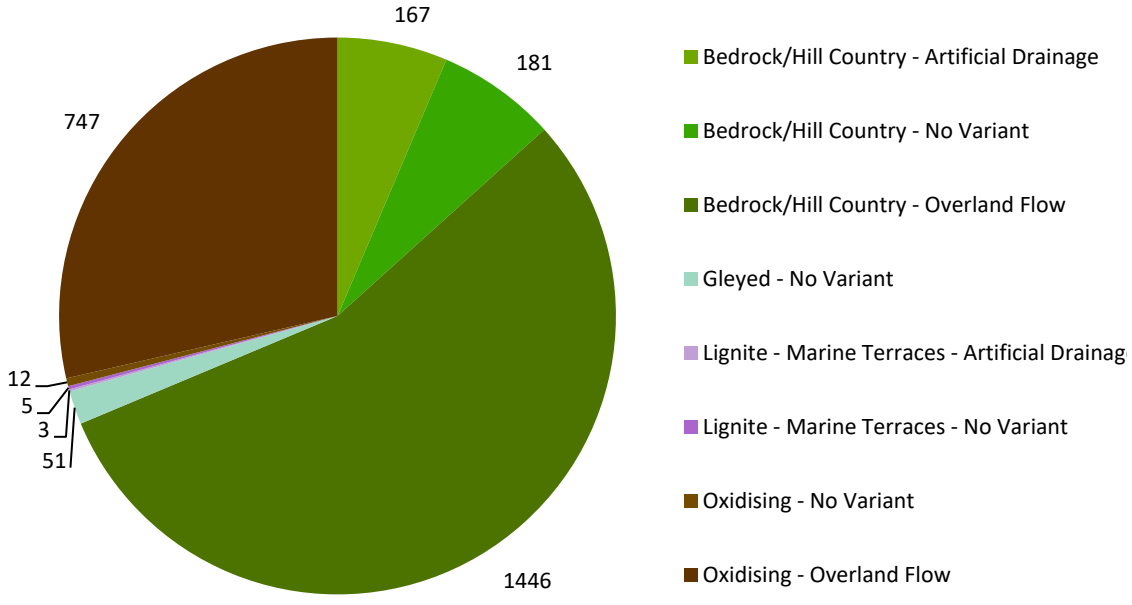

Figure 55. Dairying land (in hectares) in the catchment for the Mokoreta River at Wyndham River Road, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 31. Number of consented catchment discharges to land and water in the catchment for the Mokoreta River at Wyndham River Road

Mokoreta River at Wyndham River Rd		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	8
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	3
To Land Total		11
Grand Total		11

Note: Consent information accurate as of April 2017

B.13 OTERAMIKA STREAM AT SEAWARD DOWNS

Water samples were collected from the Oteramika Stream at Seaward Downs in March and August 2015, under base flow conditions (Table 32). Microbial loadings were greater in autumn than they were in winter, with *E. coli* levels of 1,300 and 140 cfu/100 ml, respectively. *Campylobacter* was not detected in either sample.

Faecal source tracking determined that ruminant pollution was negligible (≤1%) in autumn, and accounted for ≤10% of faecal contamination in the winter sample. No specific markers of ovine or bovine pollution were detected. Wildfowl markers were detected in both samples. Specific markers of human faecal pollution were detected in the March sample.

Faecal sterol properties were analysed for the March sample only. The results suggested that human and ruminant pollution was present in the sample, but did not identify a wildfowl signature. The reason for the differences in contamination signals suggested by FST and sterol analysis remains unclear, but is likely a result of methodological differences and the use of chemical versus molecular markers to identify contamination sources, as well as the complexity of interpreting sterol signatures from environmental samples with mixed faecal inputs.

Land use in this sub-catchment is predominantly dairy and associated activities (approximately 67%), followed by sheep and sheep and beef farming (Figure 56, Figure 57). There is one consent to discharge treated sewage and stormwater to land, however it is unclear whether this would be the source of the human contamination detected, given the samples were collected under base flow. The collection of additional samples from this location, including under different flow conditions, might provide a clearer understanding of contamination sources at this site.

Table 32. Results for microbial and FST analysis of water samples collected from Oteramika Stream at Seaward Downs.

Site		Oteramika Stream at Seaward Downs	
Sample #		CMB150248	CMB151388
Client #		20151086	20152929
Date Sampled		11/03/2015	12/08/2015
Rainfall		No	No
		Microbial Properties	
Faecal coliforms		1,300	190
E. coli		1,300	140
Campylobacter		<0.3	<0.3
***Campylobacter* Species**		nt	nt
MBiT *Campylobacter* Source	**Wildfowl**		
	Ovine/Bovine/ Deer		
	Poultry		
	Not Wildfowl		
	Unknown		
		Faecal Source Tracking	
General - GenBac3		++++	++++
Ruminant		≤1%	1-10%
Human - BacH		+	-
Human - BiADO		+	-
Cow		-	-
Sheep		-	-
Wildfowl - GFD		+	+
Wildfowl - E2		+	-
		Sterol Properties	
Total Sterols		7070	nt
Coprostanol		580	
Faecal		F1+F2	
Human		Yes (2)	
Ruminant		Yes (1)	
Wildfowl		No	

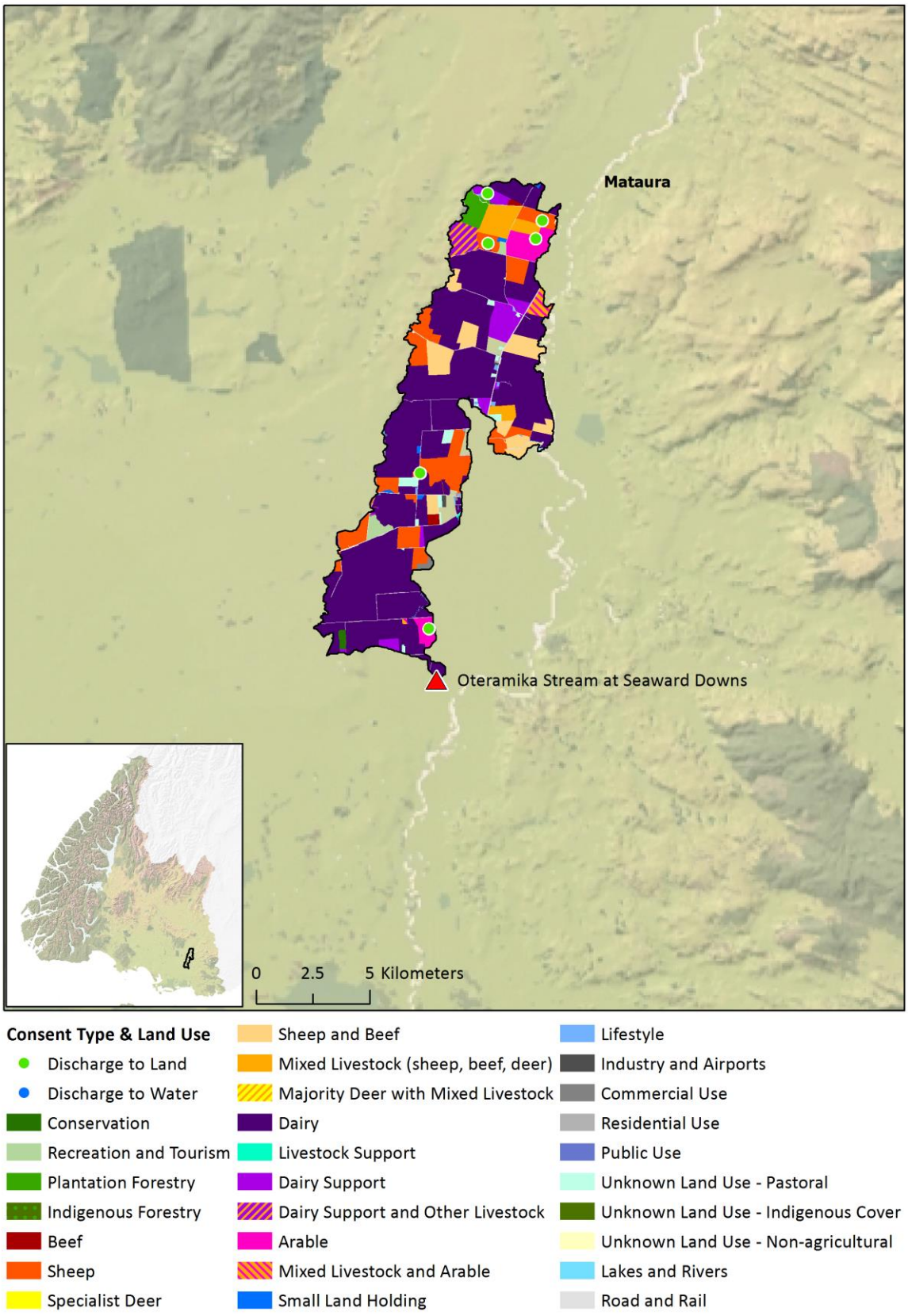

Figure 56. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Oteramika Stream at Seaward Downs.

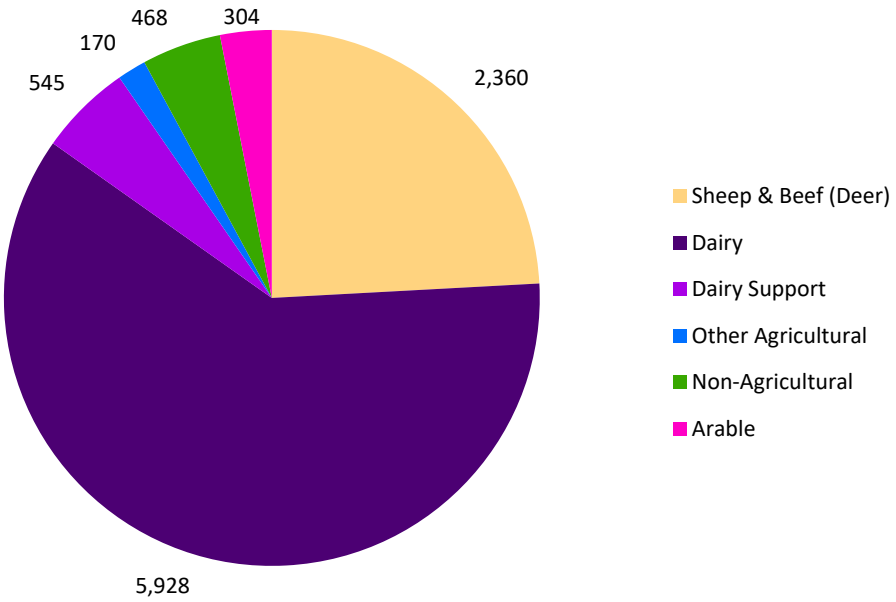

Figure 57. Land use (in hectares) in the catchment for the Oteramika Stream at Seaward Downs.

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), **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

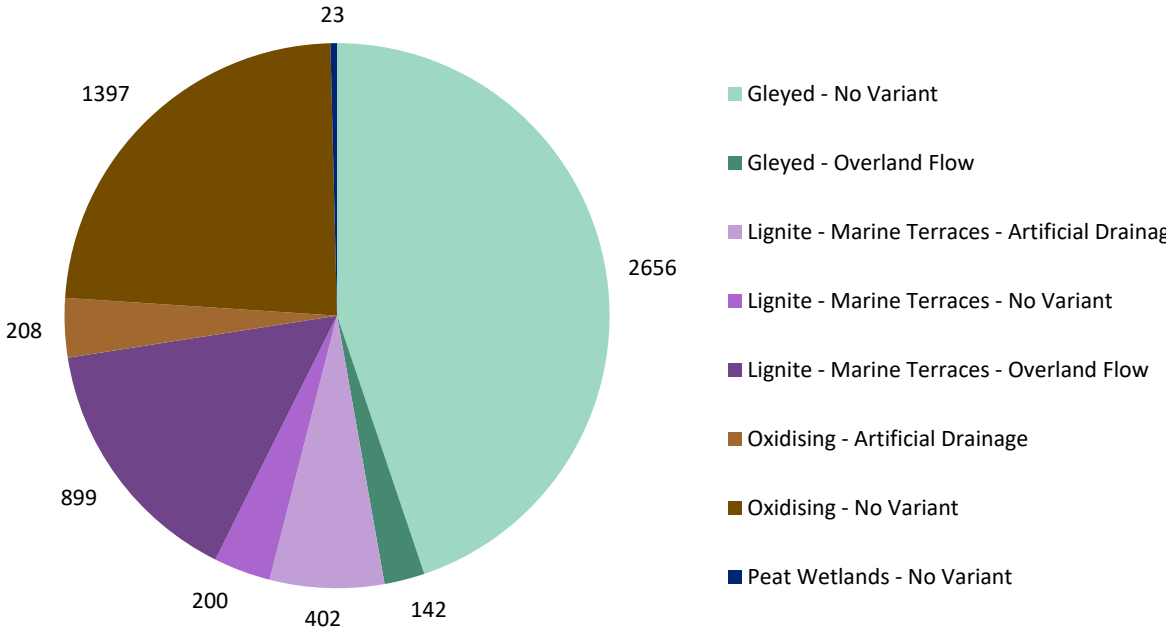

Figure 58. Dairying land (in hectares) in the catchment for the Oteramika Stream at Seaward Downs, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 33. Number of consented catchment discharges to land and water in the catchment for the Oteramika River at Seaward Downs.

Oteramika Stream at Seaward Downs		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	8
	Clean Fill	5
	Dairy Factory Effluent	1
	Dairy Shed Effluent (land)	25
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	8
	Meat Works Effluent, Waste Water	4
	Sewage (Treated), Stormwater, Wash Water, Waste Water	1
	Wintering Pad/Feedlot Effluent (land)	1
To Land Total		53
To Water	Wash Water	1
	Waste Water	1
To Water Total		2
Grand Total		55

Note: Consent information accurate as of April 2017

B.14 WAIKAWA RIVER AT PROGRESS VALLEY

Water samples were collected from the Waikawa River at Progress Valley on four occasions between March and July 2015 (Table 34). Each collection event was preceded by rainfall. *E. coli* concentrations were elevated in all samples, with 10,000 cfu/100 ml recorded in April, and 1,000-2,800 cfu/100 ml in the other samples.

Campylobacter was isolated from all four samples, in a pattern that mirrored *E. coli* concentrations: the highest levels of *Campylobacter* were observed in April (210 MPN/100 ml), with March, May and July samples containing 24, 15 and 21 MPN/100 ml. *C. jejuni* was isolated from all four samples, with *C. coli* additionally isolated from the April sample, and an unspeciated thermophilic *Campylobacter* from the May sample. MBiT analysis found the *Campylobacter* to be from a combination of ruminant and wildfowl sources.

Faecal source tracking results indicated that ruminant animals were a dominant source of pollution, accounting for up to 50% of contamination in March, and up to 100% at the three later sampling dates. Ovine pollution markers were identified in all four samples, and bovine pollution markers identified in all but the May sample; however, the lower sensitivity of the bovine marker relative to the ovine marker means that cattle cannot be excluded as a pollution source in the May sample as well. Wildfowl pollution markers were also identified in the March, April and July samples.

The FST results are consistent with the land use in the Waikawa River at Progress Valley sub-catchment, which is largely sheep and beef farming (53%, including some sheep-only blocks), and non-agricultural use (45%, mostly conservation land) (Figure 59, Figure 60).

Table 34. Results for microbial and FST analysis of water samples collected from the Waikawa River at Progress Valley.

Site		Waikawa River at Progress Valley			
Sample #		CMB150242	CMB150359	CMB150467	CMB150951
Client #		20150995	20151539	20151808	20152638
Date Sampled		09/03/2015	13/04/2015	11/05/2015	06/07/2015
Rainfall		Yes	Yes	Yes	Yes
		Microbial Properties			
Faecal coliforms		3,300	12,000	1,000	2,500
E. coli		2,800	10,000	1,000	2,400
Campylobacter		24	210	15	21
Campylobacter Species		*C. jejuni*	*C. jejuni & C. coli*	*C. jejuni & Thermo*	*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl	1		4	2
	Ovine/Bovine/ Deer	1	3		3
	Poultry				
	Not Wildfowl		2		1
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	+++	++++
Ruminant		10-50%	50-100%	50-100%	50-100%
Human - BacH		+	+	-	+
Human - BiADO		-	-	-	-
Cow		+	+	-	+
Sheep		+	+	+	+
Wildfowl - GFD		+	+	-	+
Wildfowl - E2		-	-	-	+

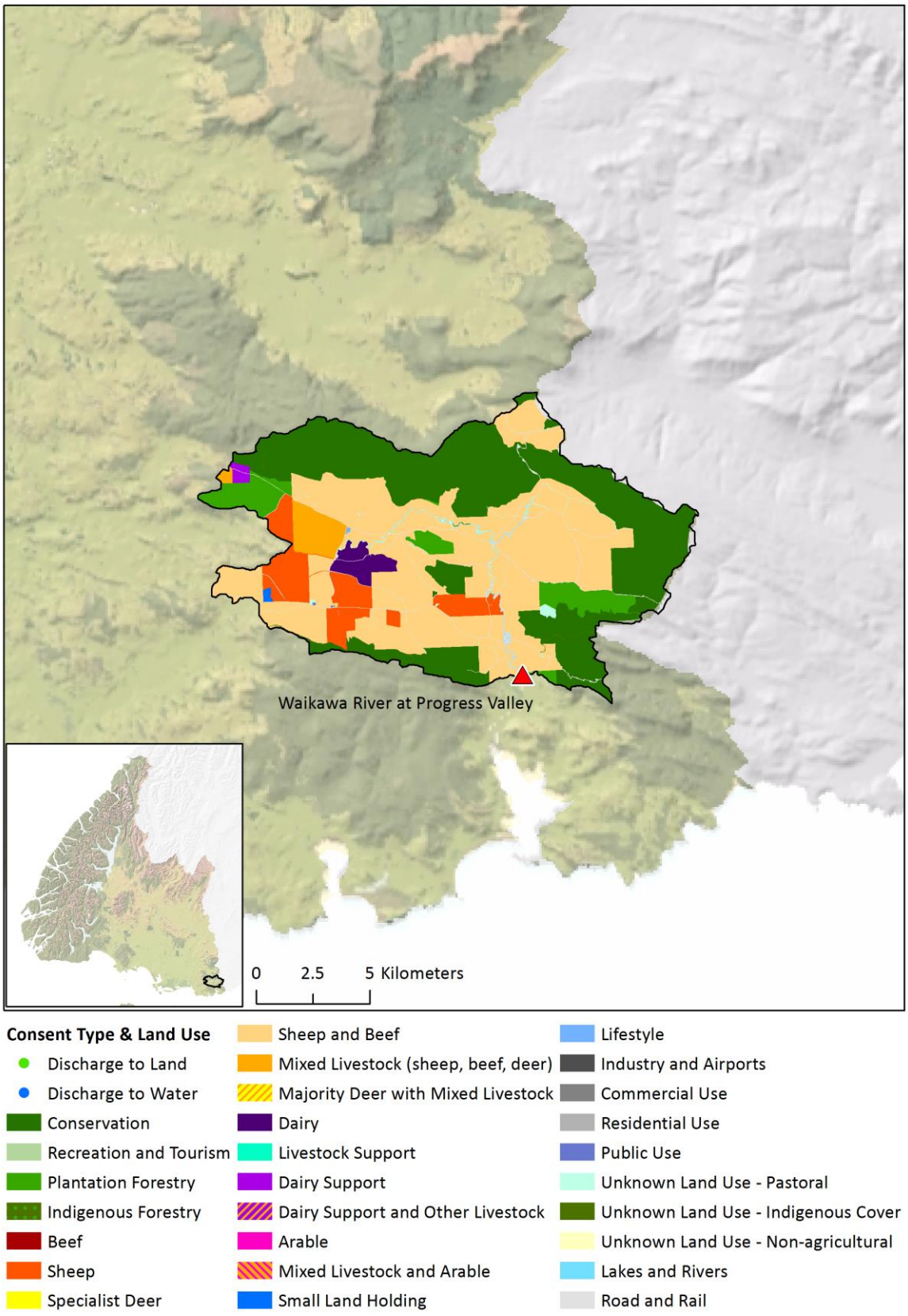

Figure 59. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Waikawa River at Progress Valley sampling site.

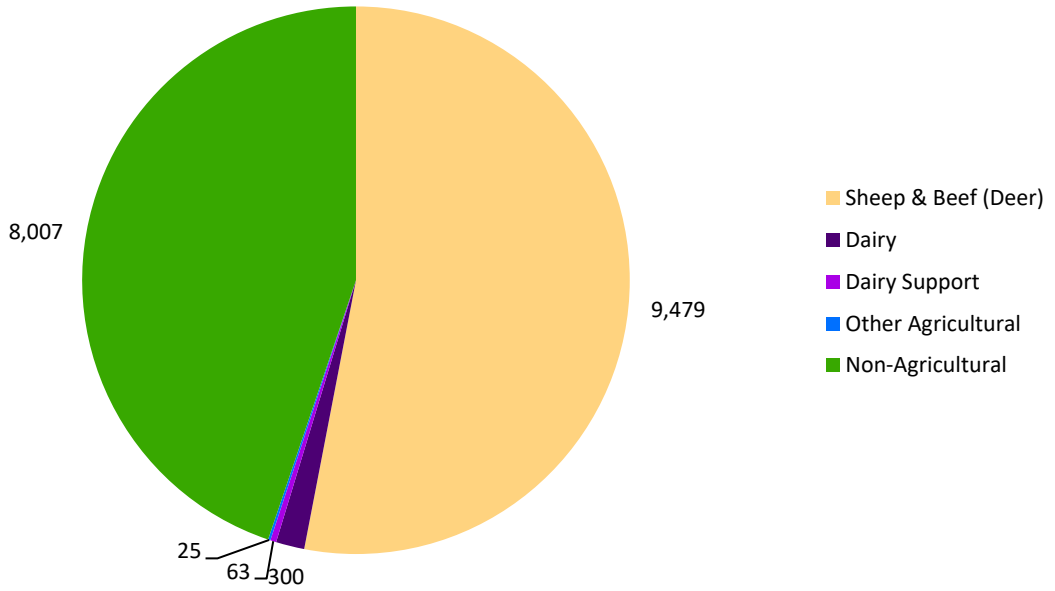

Figure 60. Land use (in hectares) in the catchment for the Waikawa River at Progress Valley.

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), **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

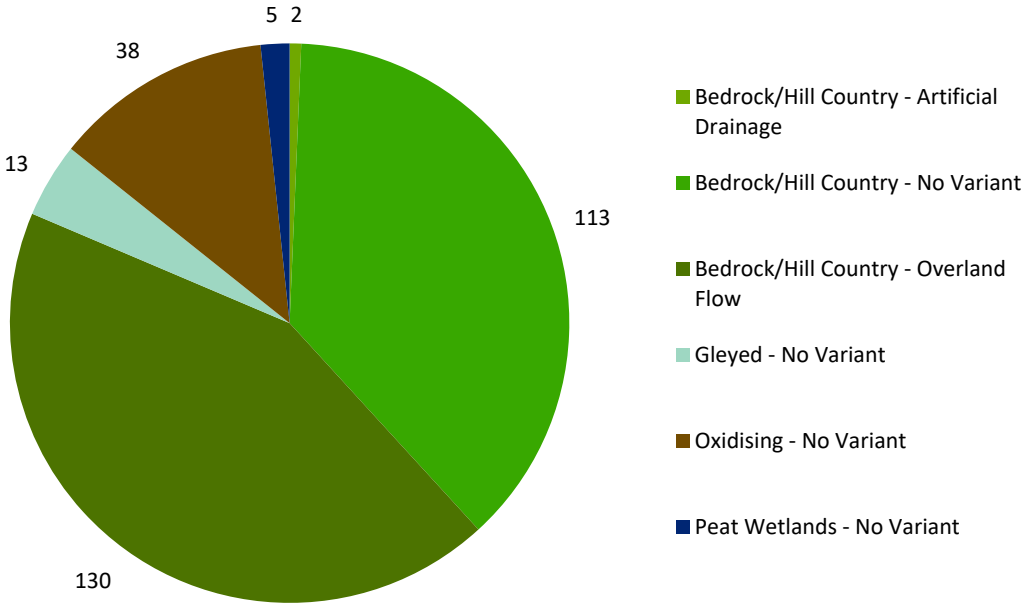

Figure 61. Dairying land (in hectares) in the catchment for the Waikawa River at Progress Valley, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 35. Number of consented catchment discharges to land and water in the catchment for the Waikawa River at Progress Valley.

Waikawa River at Progress Valley		
Subtype	Contaminant	Total
To Land	Cereal bait	1
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
	Wash Down Effluent, Wash Water	1
To Land Total		3
To Water		1
To Water Total		1
Grand Total		4

Note: Consent information accurate as of April 2017

B.15 TOKANUI RIVER AT FORTROSE OTARA ROAD

The Tokanui River was sampled at Fortose Otara Road on four occasions between during autumn and winter 2015 (Table 36). Each sampling event was preceded by rainfall. *E. coli* levels were variable across the sampling events, with peak *E. coli* observed in April (3,700 cfu/100 ml), and lower levels present in the other samples (700, 400 and 200 MPN/100 ml in March, May and August, respectively).

Low levels of *Campylobacter* were detected in all four water samples, with the highest levels also detected in April (9.3 MPN/100 ml). *C. jejuni* was identified in all four samples, with an unspeciated thermophilic *Campylobacter* also present in August. MBiT analysis determined that the *Campylobacter* was of wildfowl origin in three of the four samples, the exception being the April sample, which was found to be from a ruminant source.

Faecal source tracking found that ruminant pollution accounted for 1-10% of the pollution present in the March sample, increasing to 50-100% in April when microbial loading was highest, and falling to 10-50% in May and August. Specifically, ovine markers were detected in all four samples, with bovine markers also present in April and August. Wildfowl pollution was detected in the samples collected in March and April.

Land use in the sub-catchment is predominantly sheep and beef farming (68%, including sheep-only blocks), with some dairy (10% plus support), and non-agricultural use (12%, conservation and plantation forestry) (Figure 62, Figure 63).

Table 36. Results for microbial and FST analysis of water samples collected from the Tokanui River at Fortrose Otara Road.

Site		Tokanui River at Fortose Otara Road			
Sample #		CMB150243	CMB150355	CMB150468	CMB151376
Client #		20150998	20151542	20151811	20152895
Date Sampled		09/03/2015	13/04/2015	11/05/2015	10/08/2015
Rainfall		Yes	Yes	Yes	Yes
		Microbial Properties			
Faecal coliforms		700	3,700	410	200
E. coli		700	3,700	400	200
Campylobacter		2.3	9.3	4.3	4.3
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni & Thermo*
MBiT *Campylobacter* Source	**Wildfowl**	1		4	4
	Ovine/Bovine/ Deer		2		
	Poultry				
	Not Wildfowl				
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	+++	++++
Ruminant		1-10%	50-100%	10-50%	10-50%
Human - BacH		+	-	-	+
Human - BiADO		-	-	-	-
Cow		-	+	-	+
Sheep		+	+	+	+
Wildfowl - GFD		+	+	-	-
Wildfowl - E2		+	+	-	-

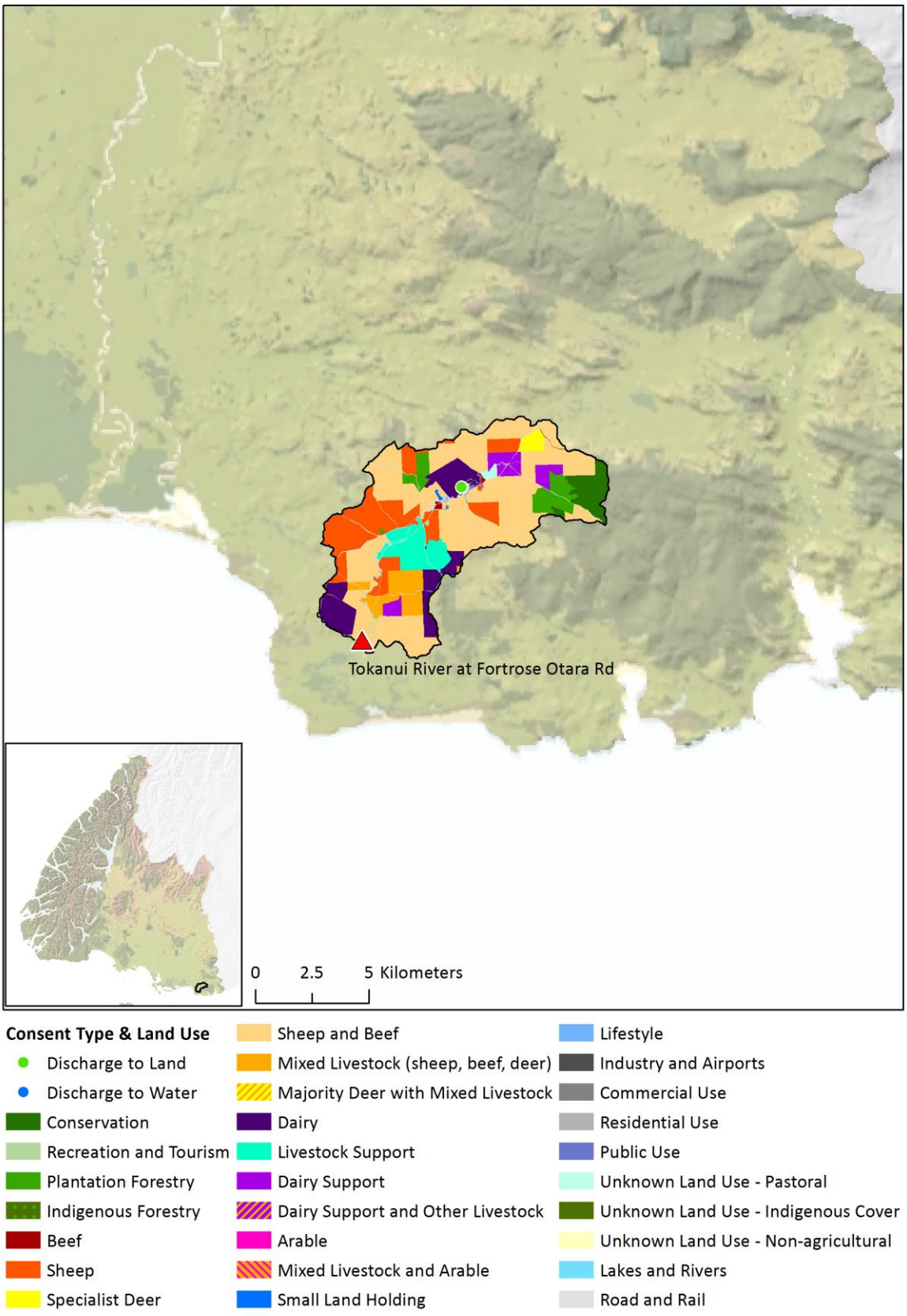

Figure 62. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Tokanui River at Fortrose Otara Road sampling site.

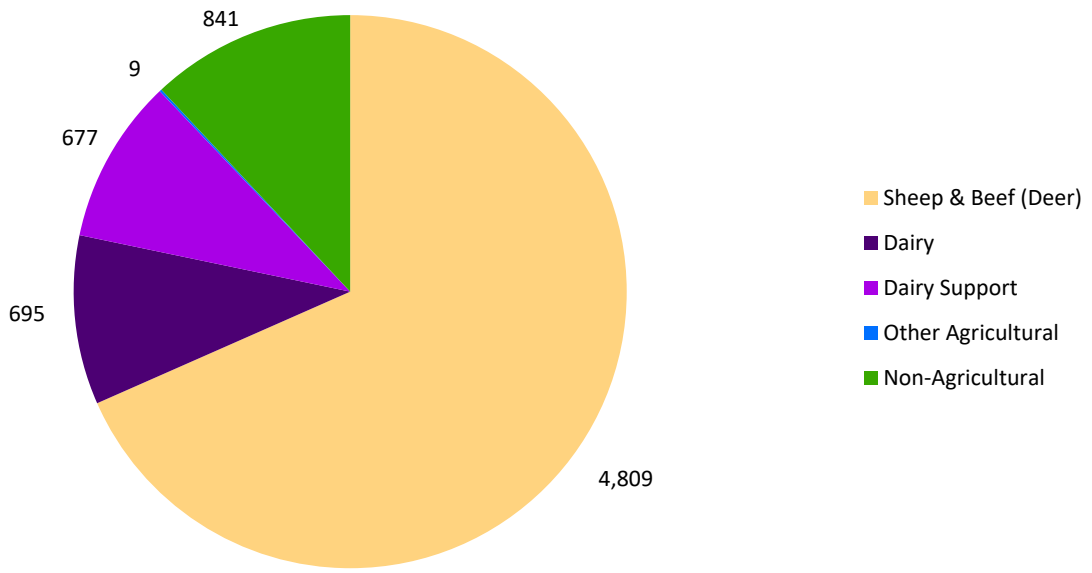

Figure 63. Land use (in hectares) in the catchment for the Tokanui River at Fortrose Otara Road.

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), **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

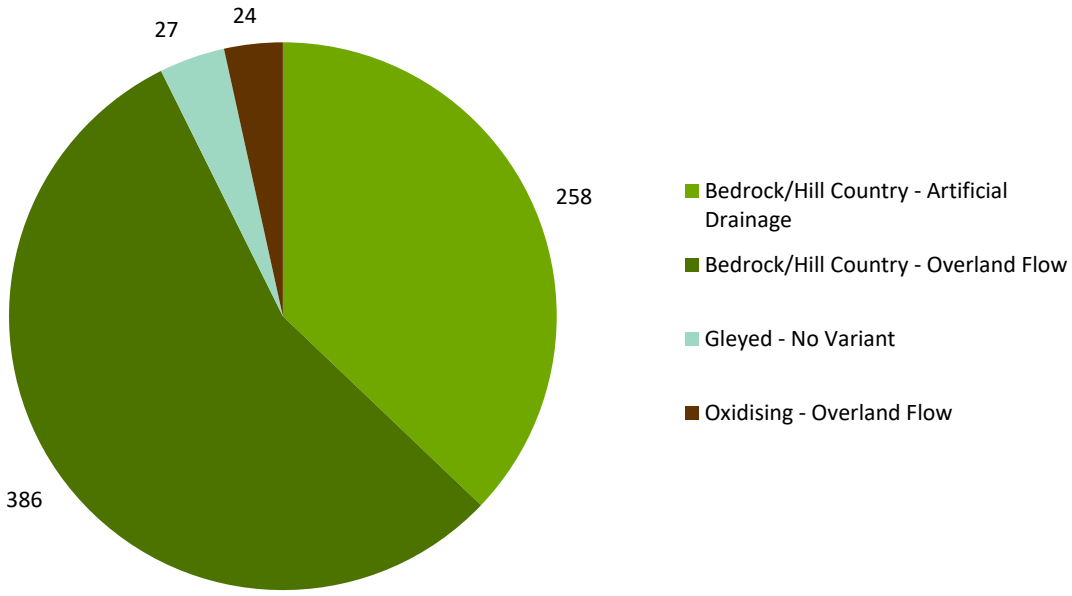

Figure 64. Dairying land (in hectares) in the catchment for the Tokanui River at Fortrose Otara Road, separated into physiographic units.

Southland Physiographic information accurate as of June 2016.

Table 37. Number of consented catchment discharges to land and water in the catchment for the Tokanui River at Fortrose Otara Road.

Tokanui River at Fortrose Otara Rd		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	3
	Oxidation Pond Effluent, Sewage (Treated), Waste Water	1
To Land Total		4
To Water	Oxidation Pond Effluent, Sewage (Treated), Waste Water	1
To Water Total		1
Grand Total		5

Note: Consent information accurate as of April 2017

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