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Environment Southland Recreational Shellfish-Gathering Water Monitoring Results: August 2016-2017

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EXECUTIVE SUMMARY

Shellfish are a highly valued natural resource in New Zealand, and are frequently harvested from natural populations by recreational and customary gatherers. Because of their coastal habitat, shellfish may be exposed to faecal and chemical contamination that enters the coastal marine environment from the surrounding landscape. Sources of contamination may include municipal sewage and stormwater, industrial and agricultural wastes and run-off, septic tanks, and direct defecation by animals and birds. There is a risk of illness where people consume shellfish from contaminated environments. The greatest risk is associated with consuming bivalve molluscan shellfish (e.g. mussels, pipi, oysters, cockles) from waters contaminated by human and animal faeces. As most bivalves are filter-feeders, they filter and accumulate environmental contaminants together with their food.

Regional Councils monitor shellfish-gathering waters for faecal contamination using bacteria that are not themselves harmful to humans, but that are present in high concentrations in faeces. The presence of these bacteria in the environment suggest contamination, and that there is a risk that microbial pathogens (i.e. disease-causing bacteria, viruses or protozoa) may also be present. The aim of this report was to review the microbial water quality data collected by Environment Southland during the 2016/2017 season of their State of the Environment recreational shellfish-gathering water monitoring programme. The microbial quality status of the water at the sites, and by extension the safety of shellfish from these sites for consumption, were determined with respect to the Microbiological Guidelines for Shellfish-Gathering Waters.

Eight sites were monitored for levels of faecal indicator bacteria (faecal coliforms, *E. coli* and enterococci) between August 2016 and August 2017. The results suggest that, based on the Guidelines, just one of these sites – Riverton Rocks at Mitchells Bay – should be considered safe for the collection of shellfish intended for human consumption. Faecal source tracking analysis of selected samples established that the predominant sources of faecal contamination across the sites were ruminant animals (including cattle and sheep) and birds (including ducks).

Recommendations for future monitoring are provided. These include consideration of sampling frequency and whether it sufficiently captures temporal variation in water quality that might arise from meteorological and/or hydrological effects (e.g. tides, rainfall). Site-specific sampling and/or trend analysis of historic data could assist in determining an appropriate sampling frequency. There should also be consideration of the microbiological methods used to enumerate faecal coliforms, since current methods (based on colony-forming units, cfu) differ from those specified in the Guidelines (based on Most Probable Number, MPN) estimates. Analysis of shellfish tissues in addition to water samples could be undertaken to provide a clearer understanding of the relationship between microbial concentrations in the water column and in the shellfish tissues themselves. Finally, faecal source tracking analysis is recommended for any sufficiently contaminated samples collected during future monitoring, as understanding the source of contaminants is important in accurately assessing health risk as well as investigating management options.

1. BACKGROUND

Shellfish are a highly valued natural resource in New Zealand, with many different species harvested both commercially and recreationally. Common customary and recreationally-gathered species include cockles (*Austrovenus stutchburyi*), pipi (*Paphies australis*), green-lipped mussels (*Perna canaliculas*), blue mussels (*Mytilus edulis*), oysters (*Crassostrea gigas*, *Saccostrea glomerata*, *Tiostrea chilensis*), tuatua (*Paphies subtriangulata*), scallops (*Pecten novaezelandiae*) pāua (*Haliotis* spp.), kina (*Evechinus chloroticus*) and crayfish (*Jasus edwardsii*).

Because of their coastal habitats, shellfish are exposed to the influences of runoff or discharge from the surrounding land. The nature and extent of this pollution varies depending on land-use in the surrounding catchment, and may include microbiological and chemical contaminants. Contaminant sources include the discharge of municipal sewage, urban stormwater and industrial wastewaters; agricultural and horticultural run-off; seepage from septic tanks; direct defecation to the aquatic environment by animals, especially birds; and the discharge of ballast or sewage from ships (EPA, 2006). In areas where rivers or estuaries discharge to the coastal environment, shellfish may also be exposed to contaminants from within the wider river catchment. Some shellfish harvesting sites may experience little to no contamination, while others may experience intermittent (e.g. post-rainfall) or permanent contamination (e.g. wastewater discharge).

There is a risk of illness when consuming shellfish gathered from contaminated waters. The risk and the severity of illness depends on a number of different factors, including the type of shellfish eaten, the parts of the shellfish eaten, the type and levels of contamination present in the water they were gathered from, the preparatory/cooking methods used, and the age and general health of the consumer (MPI, 2013). Illness typically presents as self-limiting gastrointestinal illness, with vomiting, diarrhoea and fever. Bivalve molluscan shellfish (BMS) such as mussels, cockles, oysters, pipi and tuatua, pose the greatest risk of illness due to their feeding strategy (Iwamoto et al., 2010). Most bivalves are filter feeders, feeding on plankton and other particulates suspended within the water column. However, in addition to filtering food items, bivalve shellfish also filter out environmental contaminants, including microorganisms and chemical contaminants, which accumulate within their digestive tissues (Potasman et al., 2002). Shellfish are capable of filtering large volumes of water (e.g. mussels can filter up to 360 litres per day), and can therefore accumulate high levels of contaminants in a short period of time (Lees, 2000; Greening et al., 2009; MPI, 2015).

The greatest risk to human health associated with the consumption of shellfish is the risk posed by pathogenic (i.e. disease-causing) microorganisms (Hellberg et al., 2012; NSWFA, 2017). Shellfish can filter and retain bacterial, viral and protozoan pathogens if these are present in the overlying water. In this report, we discuss potential sources of microbiological contamination and the associated risks, relevant to shellfish-gathering areas in the Southland region.

1.1 SOURCES OF POTENTIAL HUMAN PATHOGENS

A range of microbial pathogens have been implicated in seafood-related illness, including from both environmental and faecal sources (Wittman and Flick, 1995; Potasman et al., 2002; Lynch et al., 2006). *Vibrio* spp. are bacteria that are naturally present in coastal and estuarine waters, and are a significant cause of shellfish-borne illness internationally (Potasman et al., 2002; Iwamoto et al., 2010). However, *Vibrio* prefer warmer temperatures, and overall infection rates are low in New Zealand (Lake et al., 2003). Pathogens of faecal origin are of far greater concern. These include bacteria such as *Campylobacter*, *Salmonella*, *Shigella*, *E. coli* O157; enteric viruses such as norovirus and adenovirus; and protozoa such as *Cryptosporidium* and *Giardia* (MfE and MoH, 2003; Fong and Lipp, 2005; Greening and Lewis, 2010; Wood et al., 2013). Shellfish-growing waters are therefore monitored routinely for faecal contaminants.

Due to the expense and difficulty in routinely monitoring for the presence of all potential pathogens, bacteria that are known to occur in high concentrations in gut and faeces are used as indicators of faecal contamination and the possible presence of pathogens. These are referred to as faecal indicator bacteria (FIB), and include *Escherichia coli,* faecal coliforms and enterococci. In comparison with pathogen testing, the presence of indicator organisms is quick and inexpensive to test (Field and Samadpour, 2007). The bacteria used depends on the characteristics of the receiving environment (e.g. freshwater or marine) and whether shellfish tissues or water are being tested.

Land use surrounding a waterway or along a coast, as well as across the wider catchment, is known to have a major impact on microbial water quality. Human, livestock and avian faecal materials may enter the coastal environment directly or may reach the coastal zone in discharge from rivers.

1.1.1 Agricultural sources

Cattle are known to have very high concentrations of faecal indicators and pathogens in their faeces, and studies in New Zealand and overseas have demonstrated a link between beef and dairy farming and degraded microbial quality of local surface and groundwaters (Davies-Colley et al., 2004; Close et al., 2008). Zoonotic microorganisms that are excreted by cattle and can cause disease in humans include *Campylobacter*, *E. coli* O157, *Salmonella*, and *Cryptosporidium* (Humphrey et al., 1987; Learmonth et al., 2003; Stanley et al., 1998; Cookson et al., 2006; Sinton et al., 2007; Gilpin et al., 2008; Kunze et al., 2008; Moriarty et al., 2008). Sheep are also recognised as being significant contributors of faecal contaminants to surface waters, as well as excreting zoonotic microorganisms including *Campylobacter*, *E. coli* O157, *Giardia* and *Cryptosporidium* (Kudva et al., 1998; McCluskey et al., 1999; Stanley et al., 2003; Cookson et al., 2006; Oporto et al., 2007; Santin et al., 2007; Mueller-Doblies et al., 2008). A summary report conducted previously by ESR for Environment Southland found that a large number of freshwater sites in Southland are significantly impacted by ovine pollution. New Zealand lambs may excrete a significantly higher quantity of *E. coli*, enterococci and *Campylobacter* than adult sheep (Moriarty et al., 2011b), and therefore microbial loading to the environment may vary between seasons. The impacts of faecal contamination from other livestock on surface waters are less well studied, however deer and horses are known to carry potentially zoonotic strains of *Campylobacter*, *Cryptosporidium*, *Giardia* and *Salmonella* (Grinberg et al., 2009; Pritchard et al., 2009; Jay-Russel et al., 2014; Moriarty et al., 2015).

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. Overland flow occurs following rainfall or irrigation, where the infiltration rate of the soil is exceeded or soils are saturated; microorganisms associated with faecal material on the land are transferred via the flow of water over the land surface to adjacent rivers or coasts. Rainfall-driven overland flow has been identified as one of the largest pathways of faecal microbial losses from agricultural catchments (Muirhead and Monaghan, 2012). Subsurface flow via natural channels (e.g. cracking, subsurface erosion) or artificial channels (e.g. tile drainage) may also carry significant contaminant loads to rivers and streams, where they may ultimately be carried to coastal areas. Agricultural effluents (e.g. dairy shed effluent) may be discharged directly to surface waters, or irrigated to land (and then subject to runoff). The discharge of agricultural effluents to water and direct deposition by livestock into waterways may be significant sources of contamination under base flow conditions.

1.1.2 Avian sources

Concern has arisen over the contribution of bird species to the microbial loading of surface waters, and their subsequent impacts on water quality (Wither et al., 2005). Waterfowl including mallard ducks (*Anas platyrhynchos*), Canada geese (*Branta canadensis*), black swans (*Cygnus atratus*) are abundant throughout New Zealand, frequently occurring in dense populations. These species are predominantly found in estuaries, lakes and wetland habitats, and may also be found around wastewater treatment plant stabilisation ponds. Three species of gull, including the black-billed gull (*Larus bulleri*), are common in New Zealand and are found in a range of habitats from estuaries and harbours, rivers, streams and gravel beds (Heather and Robertson, 2005; Fish and Game, 2019).

Birds may defecate directly into the water, or along beaches, 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 microorganisms have been isolated from the faeces of birds, including *Campylobacter*, *Salmonella*, *Cryptosporidium*, *Listeria* and *Bacillus cereus* (Wahlstrom et al., 2003; Moriarty et al. 2011).

1.1.3 Human sources

Human sewage contains high concentrations of indicator organisms, and a range of pathogens including *Campylobacter*, *Salmonella*, *Cryptosporidium* and *Giardia* may 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). In particular, a range of enteric viral pathogens are excreted in human faeces and are commonly found in human sewage, including noroviruses, adenoviruses, sapoviruses, polyomaviruses, astroviruses and hepatitis A (Lees, 2000; Greening and McCoubrey, 2010; Updyke et al., 2015; Olalemi et al., 2016; Winterbourn et al., 2016). Human faecal materials can make their way into the aquatic environment from a number of point and diffuse sources. These include the discharge of raw and treated sewage, leakage from broken sewerage pipes, urban stormwater and combined sewer overflows, seepage from septic tanks, and the discharge of wastes from boats and ships.

The host-specificity of human viruses means they pose a significant risk to human health. For example, human norovirus is an enteric virus transmitted by the faecal-oral route and is believed to be the most common cause of non-bacterial gastroenteritis worldwide, including in New Zealand, (Koopmans 2008; ESR, 2010). The majority of reported shellfish-borne illness has an unidentified aetiology that is often assumed to be viral (Wittman and Flick, 1995; Lees, 2000; Potasman et al., 2002). Viral contaminants are responsible for the largest outbreaks of shellfish-borne illness, including an outbreak of hepatitis A in China in 1998 that affected more than 290,000 people (Tang et al., 1991). Viruses are less susceptible than bacteria to inactivation by environmental factors such as sunlight, salinity, chloride disinfection and other wastewater treatment processes, thus their presence is not well predicted by bacterial indicators (Updyke et al., 2015; Olalemi et al., 206; Almeida and Soares, 2012; Espinosa et al., 2009; Lin and Ganesh, 2013). In addition, the depuration of viruses from shellfish tissues may take longer than for other pathogens (e.g. weeks compared with days, Scholes et al., 2009). Human norovirus has been detected, and outbreaks have occurred, despite monitoring using faecal indicator bacteria; in these cases, the monitoring incorrectly identified the absence of human faecal contamination in the growing waters (Scholes et al., 2009; Wall et al., 2011; Hay et al., 2014). The impacts of a consumer falling ill from virally-contaminated shellfish may be widespread, as the low infectious dose for many viruses means they are readily spread from person to person, potentially spreading through the whole community (Teunis et al., 2008; Greening and McCoubrey, 2010).

1.2 RECREATIONAL SHELLFISH SAFETY GUIDELINES

Guidelines for coastal waters considered appropriate for the gathering of shellfish intended for human consumption are included in the Ministry for the Environment and Ministry of Health's Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas (MfE and MoH, 2003). These guidelines are followed by regional councils to monitor the safety of shellfish harvesting sites within their region. It should be noted that the indicator organism used for monitoring shellfish-gathering waters (faecal coliforms) differs from that used for the grading and surveillance of marine waters used for recreational activities (enterococci) (MfE and MoH, 2003; Lin and Ganesh, 2013). The guidelines for shellfish-gathering waters specify that:

a) the median faecal coliform content of water samples collected over the shellfish-gathering season shall not exceed an MPN of 14/100 ml; and

b) not more than 10% of the water samples should exceed a faecal coliform MPN of 43/100 ml.

The guidelines are in line with the standards for water deemed appropriate for the commercial harvest of shellfish that will undergo no further treatment (i.e. approved areas from which shellfish may be consumed raw) (MPI, 2018). Commercial standards also allow for the harvesting of shellfish from waters that do not meet these criteria (referred to as restricted harvesting area); however, such shellfish require relaying, depuration or other post-harvest treatment, which are not considered practicable or appropriate for recreational harvesters (MPI, 2018).

There are no specific microbiological guidelines for tissue quality in recreationally or customarily gathered shellfish. The New Zealand Food Safety Authority (NZFSA) advise that the standards for commercial shellfish quality may be used for reference in a non-commercial setting. These limits are outlined in the Ministry for Primary Industry's Animal Products Notice (Specifications for Products Intended for Human Consumption) (MPI, 2018). They specify that in testing a commercial shellfish lot, five samples should be taken, of which

a) not more than one unit should have an *E. coli* MPN greater than 230/100 g tissue
b) no sample should exceed an *E. coli* MPN of 700/100 g

or the lot should be rejected.

IANZ-accredited methods should always be used to determine the levels of faecal coliforms and *E. coli* in the gathering-waters and shellfish tissues, respectively. In assessing water quality, the Guidelines require that the MPN method described in Standard Methods for the Examination of Water and Wastewater (APHA et al., 2012) be followed. To determine *E. coli* levels in shellfish tissues, the MPN method based on the Recommended Procedures for the Examination of Seawater and Shellfish (APHA, 1985) should be followed.

Different shellfish species accumulate and depurate contaminants at different rates, and these rates may vary in response to environmental variables (e.g. temperature, salinity, water movement). Further, shellfish may accumulate and depurate different microorganisms at different rates. As such, there can be a poor correlation between the bacterial counts in the shellfish tissue and the overlying water. This means that there are limitations in using water samples alone as a quality indictor; a programme that combines both water and shellfish monitoring would have greater value and confidence in gauging shellfish safety. In addition, as the source of the contamination can alter the level of associated risk to human health, it can be valuable to determine the source of the FIB using faecal source tracking methods. It should also be noted that compliance with the guidelines does not necessarily guarantee that shellfish are safe to consume, since they relate to the risk posed by faecal pathogens; they do not cover non-faecal pathogens (e.g. *Vibrio* spp.), marine biotoxins, or chemical contaminants (MfE and MoH, 2003).

1.3 FAECAL SOURCE TRACKING

Although identification of FIB provides an indication of potential risk, it does not identify the source of any contamination (Scott et al., 2002; Lin and Ganesh, 2013; Harwood et al., 2014). Discriminating between human and non-human sources of faecal contamination and the subsequent identification of the animal species for non-human sources are essential components of effective water and shellfish quality management (Gourmelon et al., 2010; Cornelisen et al., 2011). By identifying the source, a more thorough risk assessment can take place and appropriate management strategies or engineering implemented. Because of the host-specificity of any pathogens that may be present, human faecal contamination is considered to present the highest human health risk.

In order to identify the source(s) of contamination, a range of microbial and chemical analysis techniques, collectively referred to as Faecal Source Tracking (FST) (Geary and Davies, 2003)

can be used. The process of identifying the source based on microbial indicators is referred to as Microbial Source Tracking (MST), and utilises a group of methodologies that focus mainly on the detection of gut bacteria or enteric viruses that are associated with a human or particular animal host (Gourmelon et al., 2010; Cornelisen et al., 2011; Mieszkin et al., 2013; Wood et al., 2013) DNA is extracted from a water sample and examined for the presence of genetic 'markers' from these source-specific microorganisms; the presence of a marker suggests its host animal is a source of faecal pollution. Chemical methods of FST include the analysis of faecal sterol and stanol fingerprints, which differ between human and animal sources, and the detection of compounds associated with anthropogenic activity, such as laundry detergent whiteners and synthetic drugs (e.g. contraceptives) (Leeming et al., 1994, 1996; Hewitt and Williamson, 2014).

While some source markers, particularly viruses, have a defined host specificity, no single marker will have all the properties required to sufficiently apportion the source(s) of faecal contamination across all situations and environments. For example, MST methods do not work well on BMS tissues (Mieszkin et al., 2013) and therefore additional markers can be used to focus on the environmental microbial communities rather than those within the shellfish. Multiple FST tools are thus almost always required to determine the source. More information can be found at ESR's water quality website: www.waterquality.org.nz.

1.4 REPORT OBJECTIVES

Regional and local councils have responsibilities under the Resource Management Act (1991) and Health Act (1956) to monitor and assess the human health risk associated with the recreational gathering and consumption of shellfish. This involves routine monitoring of bacterial water quality at popular shellfish-gathering sites. In addition, Environment Southland have been working to understand the sources of pollution that may impact these sites, leading to degraded water quality and a health risk to shellfish consumers.

This report reviews the microbial water quality collected by Environment Southland during the 2016/2017 season of their State of the Environment recreational shellfish-gathering water monitoring programme. The quality status of the water at each site, and thus the safety of the shellfish at these sites, is determined with respect to the current Microbiological Guidelines for Shellfish-Gathering Waters (MfE and MoH, 2003). Faecal source tracking analysis was undertaken for samples with sufficiently high levels of contamination to permit analysis.

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

Water samples were collected by Environment Southland staff as a part of their State of the Environment (SoE) monitoring programme for shellfish-gathering waters. Samples were collected from eight shellfish harvesting sites around the Southland region, on a monthly basis between 8th August 2016 and 2nd August 2017. Sampling locations are detailed in Table 1 and shown in Figure 1.

Table 1. Names and coordinates of sites routinely monitored for shellfish-gathering water microbial quality.

Site	Site location (Easting; Northing)
Jacobs River Estuary d/s Fish Co-op	1216487; 4854343
Monkey Island at Frentz Road	1193849; 4859109
New River Estuary at Mokomoko Inlet	1238364; 4837679
New River Estuary at Whalers Bay	1239656; 4841587
Toetoes Harbour at Fortrose	1277716; 4833768
Colac Bay at Bungalow Hill Road	1206185; 4851824
Riverton Rocks at Mitchells Bay	1217389; 4852920
Bluff Harbour at Ocean Beach	1240786; 4829674

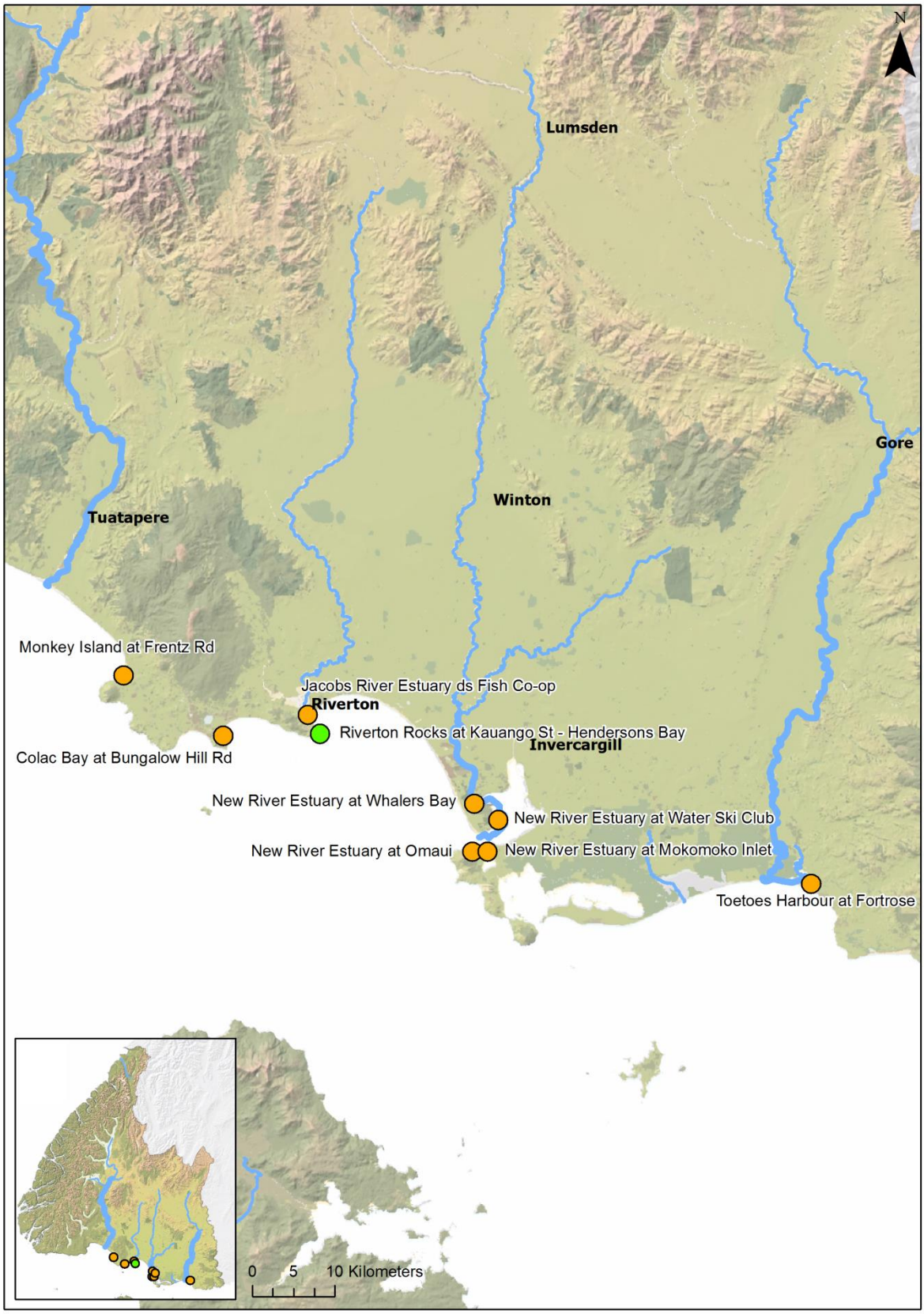


Figure 1. The Southland region, showing sampling locations for monitoring shellfish-gathering water quality and rivers of order 7and 8. Inset: The wider Southland region.

2.2 MICROBIOLOGICAL ANALYSIS

Faecal coliforms, *E. coli* and enterococci were measured as indicators of possible faecal contamination. In addition to identifying the presence of contaminants, selected samples were further analysed using molecular methods to identify the possible source(s) of faecal pollution. A brief summary of the methodologies used for microbiological analysis is described below. Detailed information regarding these methods and the interpretation of results can be found in Appendix A.

2.2.1 Faecal indicator bacteria

Faecal coliforms were analysed using membrane filtration with incubation on mFC agar for 22 hours at 44.5 ^{o}C (Method 9222D, APHA et al., 2012). *E. coli* was analysed by incubating faecal coliform-positive filters with media containing 4-methylumbelliferyl-ß-glucuronidase (MUG) (Method 9222G, APHA et al. 2012). Enterococci were analysed by membrane filtration with incubation on mE agar for 48 hours at 41^{o}C (Method 9230C, APHA et al., 2012).

The results are presented as colony forming units (cfu) per 100 ml of water. At each site, median faecal coliform counts were calculated across the sampling period, for comparison with the MfE/MoH Guidelines for Shellfish-Gathering Waters (MfE and MoH, 2003). Where no more than half of the samples at a site were determined to be below detection limit (indicated by '<' e.g. <10) the actual limit value (e.g. 10) was used in calculating the median. *E. coli* and enterococci counts are also presented for reference. Where faecal coliform concentrations exceeded guideline values, the samples were analysed using molecular faecal source tracking methods.

2.2.2 PCR markers for faecal source tracking

Water samples containing elevated faecal coliform concentrations were assessed for the presence of source-specific genetic markers. Samples (150 ml) 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).

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
- consented effluent application areas
- tile drainage
- consented point source discharge (including municipal or industrial wastewater)
- other relevant activities.

This data is presented in Appendix B.

3. OVERVIEW OF MICROBIAL WATER QUALITY

The following sections describe the overall microbial water quality observed at each site relative to the Guidelines for recreational shellfish-gathering waters, as well as the findings of any faecal source tracking analysis that was undertaken. Detailed microbiological results for each site, together with the results of the sanitary survey, are presented in Appendix B.

Some previous faecal source tracking data is available for several sites, and is presented in Appendix C for reference.

3.1 JACOB'S RIVER ESTUARY DOWNSTREAM OF FISH CO-OP

Twenty samples were collected over the 12-month period beginning in August 2016 (Table 3). This is more than other sites in this report, as the site is utilised for State of the Environment monitoring for both shellfish-gathering waters and coastal water quality. The median faecal coliform concentration was 12 cfu/100 ml and therefore below the guideline level of 14 cfu/100 ml. However, 42% of the samples taken during the sampling period had a faecal coliform concentration exceeding 43 cfu/100 ml, and therefore the overall water quality at the Jacob's River Estuary site is not considered to be safe for the gathering of shellfish intended for human consumption.

One sample was found to have elevated levels of indicator bacteria, and therefore would have been a candidate for faecal source tracking analysis; however, the sample was not received by ESR to undertake this analysis. Previous faecal source tracking analysis of samples from this site (2015, 2016) identified low levels of ruminant faecal contamination (typically 1-10% of the overall faecal load), with an avian signature also detected on one occasion (Appendix C).

3.2 MONKEY ISLAND AT FRENTZ ROAD

Thirteen samples were collected from Monkey Island at Frentz Road throughout the course of the year (Table 5). The median faecal coliform count during this period was 10 cfu/100 ml, and therefore below the guideline level of 14 cfu/100 ml. However, 15% of the samples had a faecal coliform count above 43 cfu/100 ml, and therefore overall water quality at the Monkey Island at Frentz Road site is not considered to be safe for the gathering of shellfish.

Faecal source tracking analysis was undertaken for two samples, both of which had faecal coliform concentrations exceeding 1,000 cfu/100 ml. The results suggest that both samples were dominated by a ruminant animal signature, that accounted for 50-100% of the contamination present. Specifically, contamination from cattle was detected in both samples, with sheep markers also detected in one sample (Table 6).

3.3 NEW RIVER ESTUARY

3.3.1 New River Estuary at Mokomoko Inlet

Thirteen samples were collected from the New River Estuary at Mokomoko Inlet over the 2016/2017 season (Table 7). The median faecal coliform concentration during this period was 9 cfu/100 ml, and therefore below the guideline level of 14 cfu/100 ml. However, 38% of the samples taken during the sampling period had faecal coliform concentrations that exceeded 43 cfu/100 ml. Therefore, overall water quality at Mokomoko Inlet is not considered to be safe for the gathering of shellfish intended for human consumption.

Faecal source tracking analysis was undertaken for two samples, both of which had faecal coliform concentrations exceeding 1,500 cfu/100 ml. The results suggest that both samples were dominated by a ruminant animal signature, that accounted for 50-100% of the contamination present; the ruminant source was further identified as being cattle. An avian signature was also detected in the February sample (Table 9).

3.3.2 New River Estuary at Whaler's Bay

Thirteen samples were also collected from the New River Estuary at Whaler's Bay over the 2016/2017 season (Table 8). The median faecal coliform concentration was 15 cfu/100 ml, and therefore just exceeded the guideline level of 14 cfu/100 ml. In addition, 23% of the samples taken during this period were found to have a faecal coliform concentration exceeding 43 cfu/100 ml. Overall water quality at Whaler's Bay is therefore considered not to be safe for the gathering of shellfish for consumption.

Although one sample was found to have elevated levels faecal coliforms, *E. coli* and enterococci, a sample was not received by ESR for further analysis to determine the source of the contamination.

A previously-analysed (2016) sample from this site identified a faecal source as being present (as indicated by high levels of the GenBac faecal marker), however the source could not be further identified; no specific human, ruminant or wildfowl markers were detected (Appendix C).

3.3.3 New River Estuary at Omaui and the Water Ski Club

Two other sites within the New River Estuary – Omaui and the Water Ski Club – are monitored for SoE monitoring and reporting. These sites are designated as marine bathing and recreation sites, with enterococci and *E. coli* concentrations determined as measures of water quality. Guidelines for monitoring water quality at recreation sites differ to those for shellfish-gathering waters, and further details can be found in the MfE/MoH Guidelines (MfE and MoH, 2003).

Faecal source tracking work has been undertaken at these sites, and is described here to add to the picture of faecal sources that may impact the estuary (Table 9). The results show that the Omaui site is impacted intermittently by ruminant faecal pollution. The specific animal source could not be further identified; this could be due to the source being ruminants such as deer for which specific markers were not tested, or the contamination being cattle and/or sheep, but with the presence of specific markers being below detection limits. Avian faecal pollution was also intermittently present at the Omaui site. The site adjacent to the Water Ski

Club was also impacted by varying levels of ruminant pollution, including from cattle and sheep. Avian markers, including the duck-specific E2 marker, were detected in both samples.

3.4 TOETOES HARBOUR AT FORTROSE

Thirteen samples were collected from Toetoes Harbour at Fortrose over the 2016/2017 season (Table 11). The median faecal coliform concentration was 80 cfu/100 ml, and therefore exceeded the guideline level of 14 cfu/100 ml. In addition, 69% of the samples taken during this period were found to have a faecal coliform concentration exceeding 43 cfu/100 ml. Overall water quality at Toetoes Harbour at Fortrose is therefore considered not to be safe for the gathering of shellfish for consumption.

Faecal source tracking analysis was undertaken for three samples (Table 12). The results suggest that samples were dominated by a ruminant animal signature, that consistently accounted for 50-100% of the contamination present. In two of the samples the ruminant source could not be further identified, whilst the third sample identified sheep as being a faecal source. An avian signature was also detected in all three samples.

Previous analysis from this site (2016) similarly identified sheep and birds as being faecal sources (Appendix C).

3.5 COLAC BAY AT BUNGALOW HILL ROAD

Thirteen samples were collected from Colac Bay at Bungalow Hill Road throughout the 2016/2017 season (Table 14). The median faecal coliform concentration was 41 cfu/100 ml, and therefore exceeded the guideline level of 14 cfu/100 ml. In addition, 46% of the samples taken during this period were found to have a faecal coliform concentration exceeding 43 cfu/100 ml. Overall water quality at Colac Bay at Bungalow Hill Road is therefore considered not to be safe for the gathering of shellfish for consumption.

Previous sampling work undertaken at this site and several adjacent sites (Huraki Creek upstream of Foreshore Road, Colay Bay Bridge at Foreshore Road, and Colac Bat at the stream running into Huraki Creek, 2015) also identified high levels of faecal coliforms (34 – 1,000 cfu/100 ml). Analysis of these samples to determine a faecal source(s) found ruminant signatures present at three of the sites, although these could not be further resolved to a particular animal (Appendix C). Wildfowl PCR signatures were detected at the Colac Bay bridge site, with wildfowl faecal sterols detected at three sites. The work also identified the presence of *Campylobacter jejuni* at the Huraki Creek (15 MPN/100 ml) and Colac Bay Bridge (4.3 MPN/100 ml) sites.

3.6 RIVERTON ROCKS AT MITCHELLS BAY

Thirteen samples were collected throughout the course of the year (Table 16). The median faecal coliform count was found to be 2 cfu/100 ml, and therefore below the guideline level of 14 cfu/100 ml. None of the samples collected during this period exceeded 43 cfu/100 ml. Water quality from the Riverton Rocks at Mitchells Bay is therefore considered to be safe for the gathering of shellfish for human consumption at the time of sampling.

Previous faecal source tracking work undertaken in 2015 suggested the presence of a low faecal signature, with canine-specific markers detected (Appendix C). Increased signage to remind people of the importance of collecting and disposing of faecal material from their dog may be warranted.

In 2015, mussels collected from this site were analysed for the presence of faecal indicators and norovirus; neither were detected. Analysis of the tissues for the presence of heavy metals found that all results were within guideline values for human consumption (Appendix D; FSANZ, 2008; New Zealand Gazette, 2015).

3.7 BLUFF HARBOUR AT OCEAN BEACH

Thirteen samples were collected from Bluff Harbour at Ocean Beach over the 2016/2017 season (Table 18). The median faecal coliform concentration during this period was 2 cfu/100 ml, and therefore below the guideline level of 14 cfu/100 ml. However, 23% of the samples taken during the sampling period had faecal coliform concentrations that exceeded 43 cfu/100 ml. Therefore, overall water quality at Bluff Harbour at Ocean Beach is not considered to be safe for the gathering of shellfish intended for human consumption.

4. CONCLUSIONS

Assessment of the microbial water quality of shellfish-gathering waters around the Southland area between August 2016 and August 2017 found that shellfish had a high likelihood of having been exposed to faecal contamination, based on the presence of faecal indicator bacteria. Of the eight sites sampled, only one – Riverton Rocks at Mitchells Bay – was deemed appropriate for the harvesting of shellfish intended for human consumption. The other sites did not meet the MfE/MoH Guidelines (MfE/MoH, 2003) for shellfish-gathering waters, and shellfish from these sites should be considered unsafe to consume, due to the risk of illness from faecal pathogens.

Where water samples contained sufficient levels of contamination to permit further analysis, faecal source tracking identified ruminant animals, including sheep and cattle, and birds to be the major faecal sources. These sources are consistent with the land use within the sub-catchments of each site, which are typically dominated by sheep and beef agriculture, with some catchments also having considerable dairying operations as well. The presence of especially high levels of contamination at multiple sites on 3rd May and 4th of July suggest rainfall-driven overland flow (i.e. run-off) from agricultural land is a significant route of transmission for faecal materials into the coastal environment, either directly or via river discharge. Rainfall data would assist in clarifying the relative impact of run-off on water quality at these sites.

No human faecal contamination was detected during this study.

5. RECOMMENDATIONS

The Microbiological Guidelines for Shellfish-Gathering Waters are meant only as a management tool for monitoring any changes to the microbial quality of shellfish gathering sites (MfE and MoH, 2003). Therefore, where the methods set out in the Guidelines are not strictly followed, it is not a breach of compliance. However, it is advisable to follow the Guidelines as closely as possible to ensure the most accurate conclusions are drawn regarding the microbial safety of shellfish in these areas based on the surrounding water quality.

Membrane filtration methods are currently being used to enumerate the faecal coliforms in the seawater collected from the shellfish gathering sites. This method determines the number of bacteria present in a water sample based on the number of distinguishable colonies that grow on a culture plate (i.e. the number of colony-forming units, cfu). It allows for the analysis of larger volumes of water and for lower concentrations of bacteria to be detected (Gronewold and Wolpert, 2008). However, the Guidelines are based on the use of Most Probable Number (MPN) methods with a 5 tube decimal dilution, or an equivalent validated method that determines faecal coliform levels based on MPN. Most Probable Number is a probabilistic method that estimates the number of faecal coliforms in a water sample, based on the pattern of tubes testing positive for faecal coliforms (McBride et al., 2003). MPN methods tend to yield higher estimates and are more variable than cfu estimates (Gronewold and Wolpert, 2008). The use of MPN methods for future monitoring work would ensure consistency with the Guidelines. However, consideration should be given to the implications of a change in methods, particularly whether the violation frequency or management decisions for a water body (e.g. approval for shellfish gathering) may differ depending on the analysis procedure used (Gronewold and Wolpert, 2008). In addition, if estimates of faecal coliforms do vary depending on the method used, the benefits of merging historic MPN and cfu datasets would be limited (Noble et al., 2003). A study in which samples are analysed by both methods might help to shed light on the differences that might be observed between methods for the sites currently monitored as shellfish-gathering sites.

The Guidelines require that a sufficient number of samples are collected throughout the shellfish-gathering season to provide statistical power in testing for compliance for both the median and the 90% sample limits. All but one of the sites (Jacob's River Estuary downstream of the Fish Co-op) was sampled monthly. However, factors such as tidal state or rain events, which may influence contamination levels, are not reported and therefore cannot be taken into account during analysis. This depth of sampling may not be adequate to capture any temporal variation in water quality, and therefore water quality may not be accurately represented. More in-depth studies should be carried out for each site to determine the optimal number of samples required to capture the level of variation present, as well as the effects of hydrological and meteorological events on contamination levels. This will help to ensure that future sampling is optimised and efficient, whilst allowing the most accurate conclusions to be drawn. Faecal source tracking analysis of appropriate samples (e.g. faecal coliforms >1,000 cfu/100 ml) would help identify pollution sources and may assist in predicting contamination risk. Detailed trend analysis of historic data would also enable the determination of whether

changes in water quality have occurred over time, and if so, determine a suitable sample number for current conditions. This could include whether sampling effort should be altered with season (winter/summer), and the level of impact from meteorological and hydrological events. This work could be carried out by ESR's data scientists.

The microbiological water quality guidelines for freshwater and marine bathing waters have been developed from quantitative microbial risk assessment (QMRA) and epidemiological studies, respectively (MfE and MoH, 2003). Thus, microbial monitoring data can be used directly to estimate the risk of becoming ill. However, while shellfish-gathering guidelines can be used to highlight a risk, they do not quantify the risk to consumers, since only the overlying water is sampled, and not shellfish tissues. As discussed, there may be poor correlation between the concentrations of microorganisms in the water and in the shellfish flesh. In addition, the Guidelines only cover microbiological contamination of faecal origin; they do not incorporate risk from non-faecal pathogens, marine biotoxins or chemical contaminants (MfE and MoH, 2003). The ability of the Guidelines to determine the health risk from contaminated shellfish is limited in some respects. There may be value in including analysis of shellfish tissues during monitoring. This would require ascertaining that shellfish populations could support periodic sampling. A number of site-specific investigations of shellfish tissues, such as the 'Fit for Consumption' study, are currently being undertaken by Environment Southland (Ward N, personal communication with authors).

ABBREVIATIONS AND GLOSSARY

APHA	American Public Health Association
BMS	bivalve molluscan shellfish;
bivalve	shellfish characterised as having a 2-valved shell joined by an elastic ligament; typically with a filter-feeding habit.
cfu	colony-forming unit
ES	Environment Southland
ESR	Institute of Environmental Science and Research
faecal indicator	a microorganisms that is associated with the gut or faeces of an animal and whose presence in environmental waters can be used to indicate faecal contamination
FIB	faecal indicator bacteria
FMU	Freshwater Management Unit
FST	faecal source tracking
MPN	Most Probable Number
pathogen	a bacterium, virus, or other microorganism that can cause disease
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
WWTP	wastewater treatment plant
zoonotic	disease that can be transmitted from animals to people or, more specifically, a disease that normally exists in animals but that can infect 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, *E. COLI* AND ENTEROCOCCI 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±0.2°C 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).

Enterococci were analysed using membrane filtration onto mE agar. Presumptive enterococci colonies were confirmed using appropriate verification tests (following isolation on brain-heart infusion (BHI) agar/broth, colonies are found to be catalase-negative, Gram positive, grow on bile-esculin agar, and grow in BHI broth with 6.5% NaCl) (APHA et al., 2012).

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

A.2 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 of 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 strongly associated with, but not exclusive to, their host animal. Assays for different markers also differ in their sensitivity (Table 2).

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, 2014). The PCR assays applied to water samples are listed in Table 2. 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. (2007). 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.

Markers assays are reported as the gene copies that were detected per 100 ml. If a marker was not detected, it is simply reported as being below the limit of detection (e.g. <110/100 ml). Samples that contained only a low level of faecal pollution (indicated by low levels of GenBac3) may not have sufficient levels of contamination-related microorganisms 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.

In assessing the presence of human faecal contamination, at least two markers must be assayed. Human contamination is supported only when two or more human markers are detected.

Table 2. 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

APPENDIX B: SITE-SPECIFIC WATER QUALITY, SOURCE TRACKING AND CATCHMENT INFORMATION

B.1 JACOBS RIVER ESTUARY DOWNSTREAM OF FISH CO-OP

Table 3. Faecal indicator bacteria results for samples collected at Jacobs River Estuary, downstream of the fish co-op between 8/8/16 and 2/8/17.

Date	Sample ID	ESR sample	Faecal coliforms (cfu/100 ml)	*E. coli* (cfu/100 ml)	Enterococci (cfu/100 ml)
8/08/2016	20162524		40	30	25
11/08/2016	20162088	CMB161047	12	12	5
2/09/2016	20162096	CMB161109	3	2	<1
10/10/2016	20163048		700	700	90
12/10/2016	20162962	CMB161244	9	9	1
3/11/2016	20163413	CMB161316	73	69	4
14/11/2016	20163629		<10	<10	<10
2/12/2016	20163696		170	170	4
12/12/2016	20164126		<10	<10	<10
9/01/2017	20164451		<10	<10	<10
12/01/2017	20163704	CMB170016	170	160	46
1/02/2017	20163712	CMB170155	310	310	27
13/02/2017	20170325		10	<10	<10
2/03/2017	20163720	CMB170303	7	7	1
6/03/2017	20170541		60	50	<10
3/04/2017	20171178	CMB170414	100	90	20
3/05/2017	20171272		63	63	37
6/06/2017	20171350		10	<10	<10
4/07/2017	20171358		8	7	21
2/08/2017	20171366		1	1	5

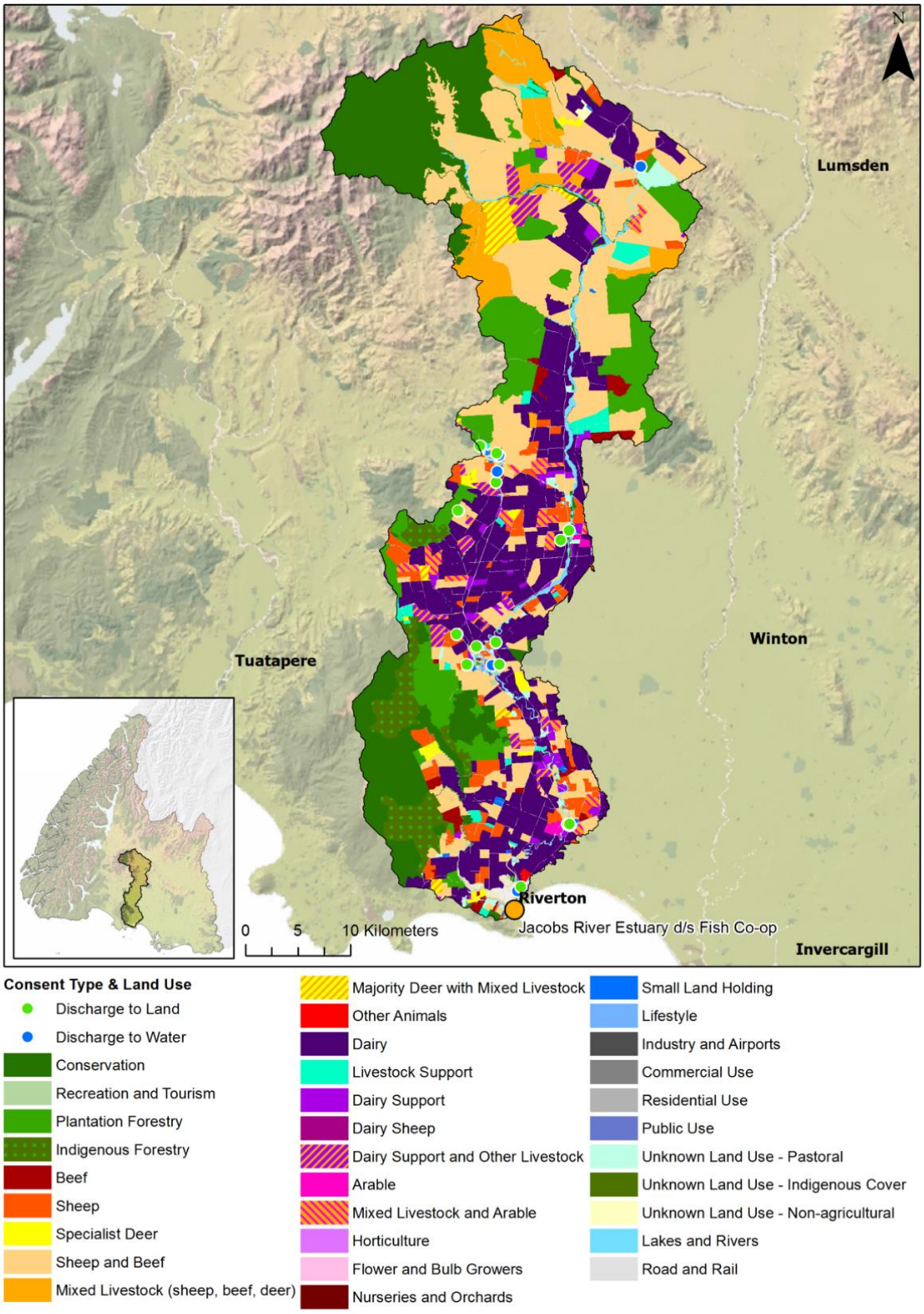


Figure 2. Land use and consented discharges with potential *E. coli* contamination risk (non-dairy) in the catchment for the Jacob's River Estuary sampling site.

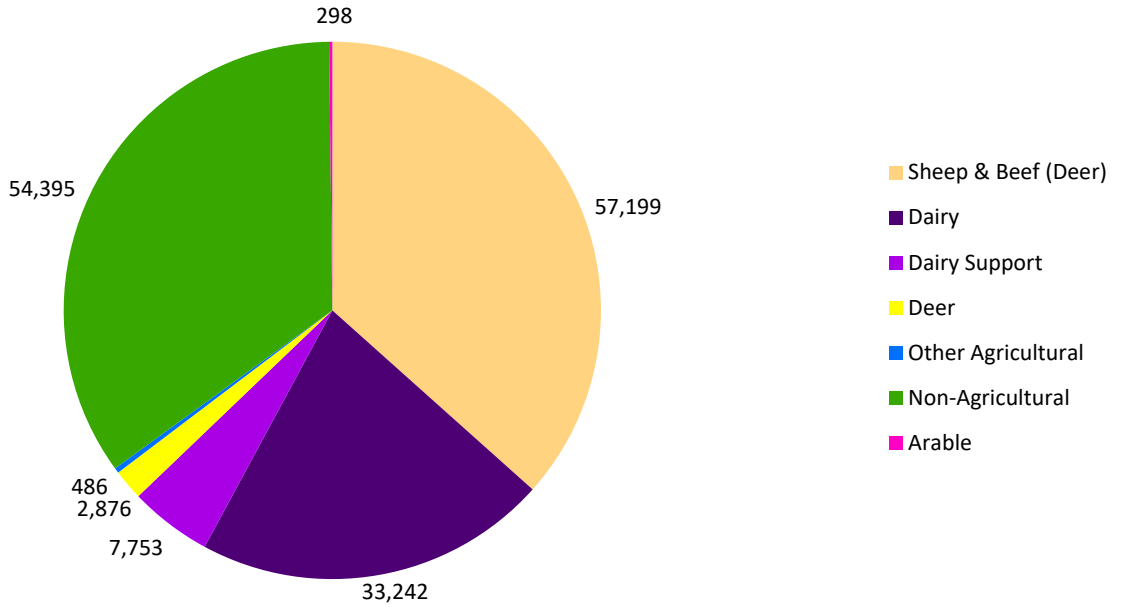


Figure 3. Land use (in hectares) in the catchment for the Jacob's River Estuary, downstream of the Fish Co-op sampling site.

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

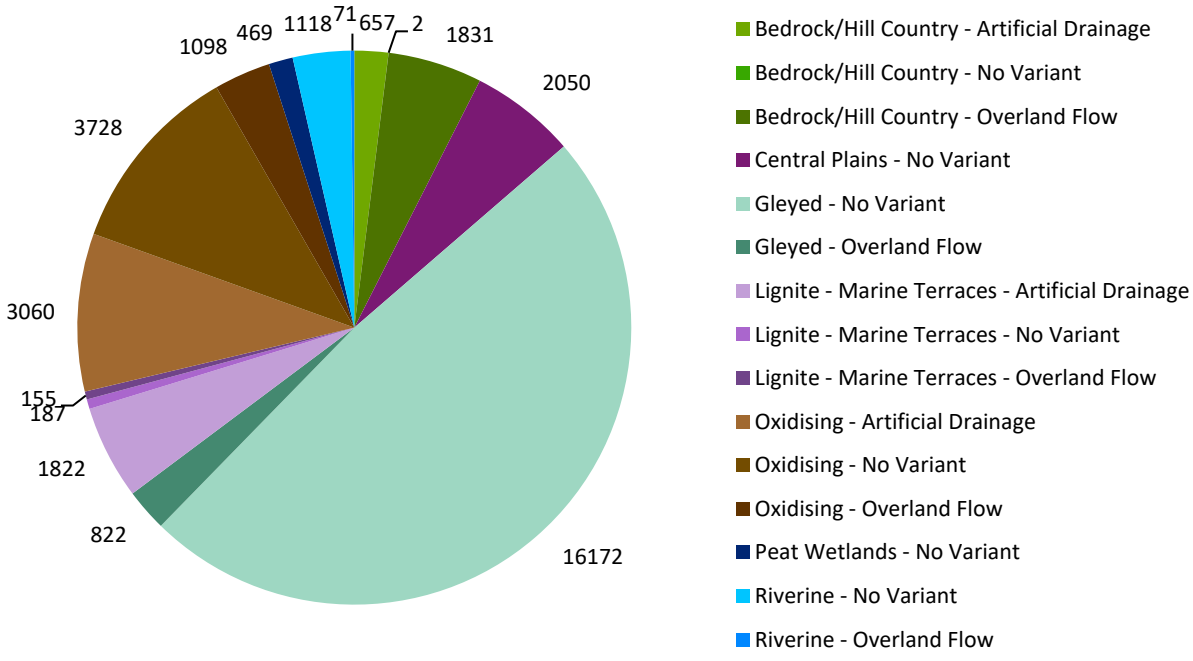


Figure 4. Dairying land (in hectares) in the catchment for Jacob's River Estuary, downstream of the Fish Co-op sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 4. Consented discharges to land and water in the catchment for the Jacob's River Estuary, downstream of the Fish Co-op sampling site.

Jacobs River Estuary d/s Fish Co-op		
Subtype	Contaminant	Total
To Land	Other (whey to pasture 7, dust suppressant 2	9
	1080	2
	Ash	1
	Dairy Shed Effluent (land)	119
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	34
	Filter Backwash, Silt	1
	Fish Processing Waste	1
	Leachate	1
	Meat Works Effluent, Waste Water	1
	Mine water, Stormwater, Wash Water	1
	Sawmilling Waste	1
	Septic Tank Effluent	1
	Septic Tank Effluent, Sewage (Treated)	1
	Sewage (Treated), Sewage Package Plant, Waste Water	1
	Sludge, Wash Water	1
	Stockyard Effluent, Wintering Pad/Feedlot Effluent (land)	1
	Tannery Effluent	1
	Wash Down Effluent	1
	Wash Down Effluent, Wash Water	1
	Wash Water	1
	Wash Water, Waste Water	1
	Wintering Pad/Feedlot Effluent (land)	1
To Land Total		182
To Water	Clean Fill	1
	Filter Backwash, Silt	1
	Mine water	2
	Silt, Suspended Sediment	1
	Stormwater	3
	Waste Water	1
To Water Total		9
Grand Total		191

Note: Consent information accurate as of April 2017

B.2 MONKEY ISLAND AT FRENTZ ROAD

Table 5. Faecal indicator bacteria results for samples collected at Monkey Island at Frentz Road between 11/8/16 and 2/8/17. Samples that were subsequently analysed by faecal source tracking are indicated in bold text.

Date	Sample ID	ESR sample	Faecal coliforms (cfu/100ml)	*E. coli* (cfu/100 ml)	Enterococci (cfu/100 ml)
11/08/2016	20162087	CMB161046	2	2	2
2/09/2016	20162095	CMB161108	1	1	1
12/10/2016	20162961	CMB161243	27	23	1
3/11/2016	20163412	CMB161315	33	25	1
2/12/2016	20163695		9	9	1
12/01/2017	20163703	CMB170015	12	12	12
1/02/2017	20163711	CMB170154	28	21	7
2/03/2017	20163719	CMB170302	3	3	1
3/04/2017	20171175	CMB170413	9	9	11
3/05/2017	**20171269**	**CMB170716**	**1,800**	**1,800**	**1,200**
6/06/2017	20171347		10	10	10
4/07/2017	**20171355**	**CMB171168**	**1,400**	**1,000**	**2,600**
2/08/2017	20171363		4	4	9

Table 6. Faecal source tracking results for samples collected from Monkey Island at Frentz Road during the 2016/2017 monitoring period.

Date	ES Sample #	ESR Sample #	General GenBac / 100 ml	Human BacH / 100 ml	Human BiADO / 100 ml	Ruminant BacR / 100 ml	Proportion Ruminant	Ruminant Sheep / 100 ml	Ruminant Cow / 100 ml	Avian GFD / 100 ml	Avian E2 / 100 ml	Conclusion
4/5/17	20171269	CMB170716	2,400,000	<83	<110	440,000	50-100%	<100	1400	<72	<99	Faecal source: - ruminant (cow) 50-100%
4/7/17	20171355	CMB171168	180,000	<33	<43	32,000	50-100%	110	120	<29	<40	Faecal source: - ruminant (sheep + cow) 50-100%

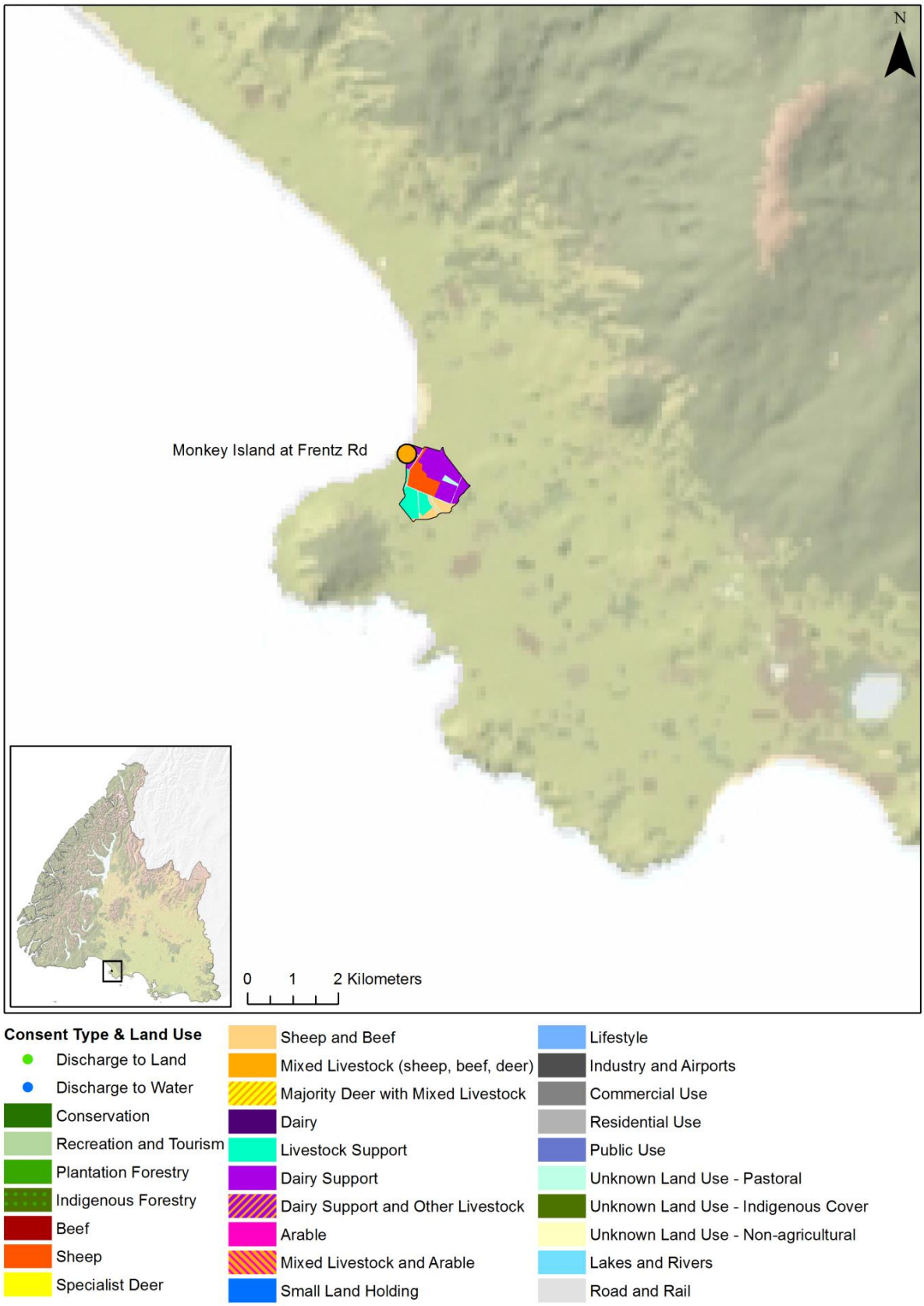


Figure 5. Land use and consented discharges with potential *E. coli* contamination risk (non-dairy) in the catchment for the Monkey Island at Frentz Road sampling site.

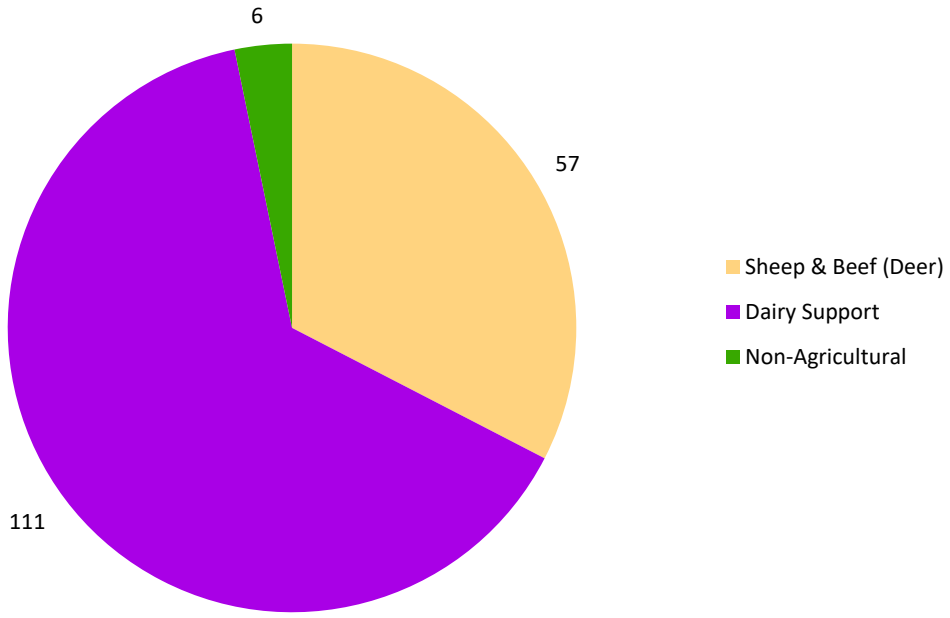


Figure 6. Land use (in hectares) in the catchment for the Monkey Island at Frentz Road sampling site.

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

There is no dairy farming in this sub-catchment.

There are no consented discharges in this sub-catchment.

B.3 NEW RIVER ESTUARY AT MOKOMOKO INLET AND WHALER'S BAY

Table 7. Faecal indicator bacteria results for samples collected at New River Estuary at Mokomoko Inlet between 10/8/16 and 2/8/17. Samples that were subsequently analysed by faecal source tracking are indicated in bold text.

Date	Sample ID	ESR sample	Faecal coliforms (cfu/100ml)	*E. coli* (cfu/100 ml)	Enterococci (cfu/100 ml)
10/08/16	20162084	CMB161044	7	7	5
1/09/16	20162092	CMB161106	4	4	10
12/10/16	20162958	CMB161241	2	2	1
3/11/16	20163409	CMB161313	35	27	9
1/12/16	20163692		440	350	22
12/01/17	20163700	CMB170013	70	70	10
1/02/17	**20163708**	**CMB170157**	**5,600**	**5,600**	**30**
28/02/17	20163716	CMB170296	5	5	1
3/04/17	20171172	CMB170411	9	9	11
3/05/17	**20171266**	**CMB170714**	**1,700**	**1,600**	**220**
6/06/2017	20171344		<1	<1	<1
4/07/2017	20171352		70	50	35
2/08/2017	20171360		6	6	6

Table 8. Faecal indicator bacteria results for samples collected at New River Estuary at Whaler's Bay between 10/8/16 and 2/8/17.

Date	Sample ID	ESR sample	Faecal coliforms (cfu/100ml)	*E. coli* (cfu/100 ml)	Enterococci (cfu/100 ml)
10/08/2016	20162083	CMB161043	40	40	17
1/09/2016	20162091	CMB161105	10	10	8
12/10/2016	20162957	CMB161240	3	3	1
3/11/2016	20163408	CMB161312	150	110	8
1/12/2016	20163691		1,600	1,300	26
12/01/2017	20163699	CMB170012	23	20	8
1/02/2017	20163707	CMB170156	25	25	12
28/02/2017	20163715	CMB170295	15	13	1
3/04/2017	20171171	CMB170410	6	6	<1
3/05/2017	20171265		3	3	8
6/06/2017	20171343		<1	<1	2
4/07/2017	20171351		87	86	33
2/08/2017	20171359		9	6	9

Table 9. Faecal source tracking results for samples collected from the New River Estuary at Mokomoko Inlet, Omaui and the Water Ski Club during the 2016/2017 monitoring period.

Site	Date	ES Sample #	ESR Sample #	General GenBac / 100 ml	Human BacH / 100 ml	Human BiADO / 100 ml	Ruminant BacR / 100 ml	Proportion Ruminant	Ruminant Sheep / 100 ml	Ruminant Cow / 100 ml	Avian GFD / 100 ml	Avian E2 / 100 ml	Conclusion
New River Estuary at Mokomoko Inlet	01/02/17	20163708	CMB170157	580,000	290	<110	130,000	50-100%	<100	110	810	250	Faecal source: - ruminant (cow) 50-100% - avian, duck
New River Estuary at Mokomoko Inlet	04/05/17	20171266	CMB170714	250,000	<83	<110	53,000	50-100%	<100	12	<72	<99	Faecal source: - ruminant (cow) 50-100%
New River Estuary at Omaui	17/01/17	20163817	CMB170055	140,000	<83	<110	5,100	10-50%	<100	<11	<72	<99	Faecal source: - ruminant (not further identified) 10-50%
New River Estuary at Omaui	14/02/17	20163857	CMB170213	100,000	<83	<110	14,000	50-100%	<100	<11	100	<99	Faecal source: - ruminant (not further identified) 50-100% - avian
New River Estuary at Omaui	21/03/17	20163907	CMB170387	150,000	<83	<110	<91	ND	<100	<11	390	320	Faecal source: - avian, duck
New River Estuary at Water Ski Club	24/01/17	20163831	CMB170076	600,000	420	<110	110,000	50-100%	820	320	510	210	Faecal source: - ruminant (cow, sheep) 50-100% - avian, duck
New River Estuary at Water Ski Club	31/01/17	20163841	CMB170153	73,000	170	<110	2,900	10-50%	<100	<11	380	420	Faecal source: - ruminant (not further identified) 10-50% - avian, duck

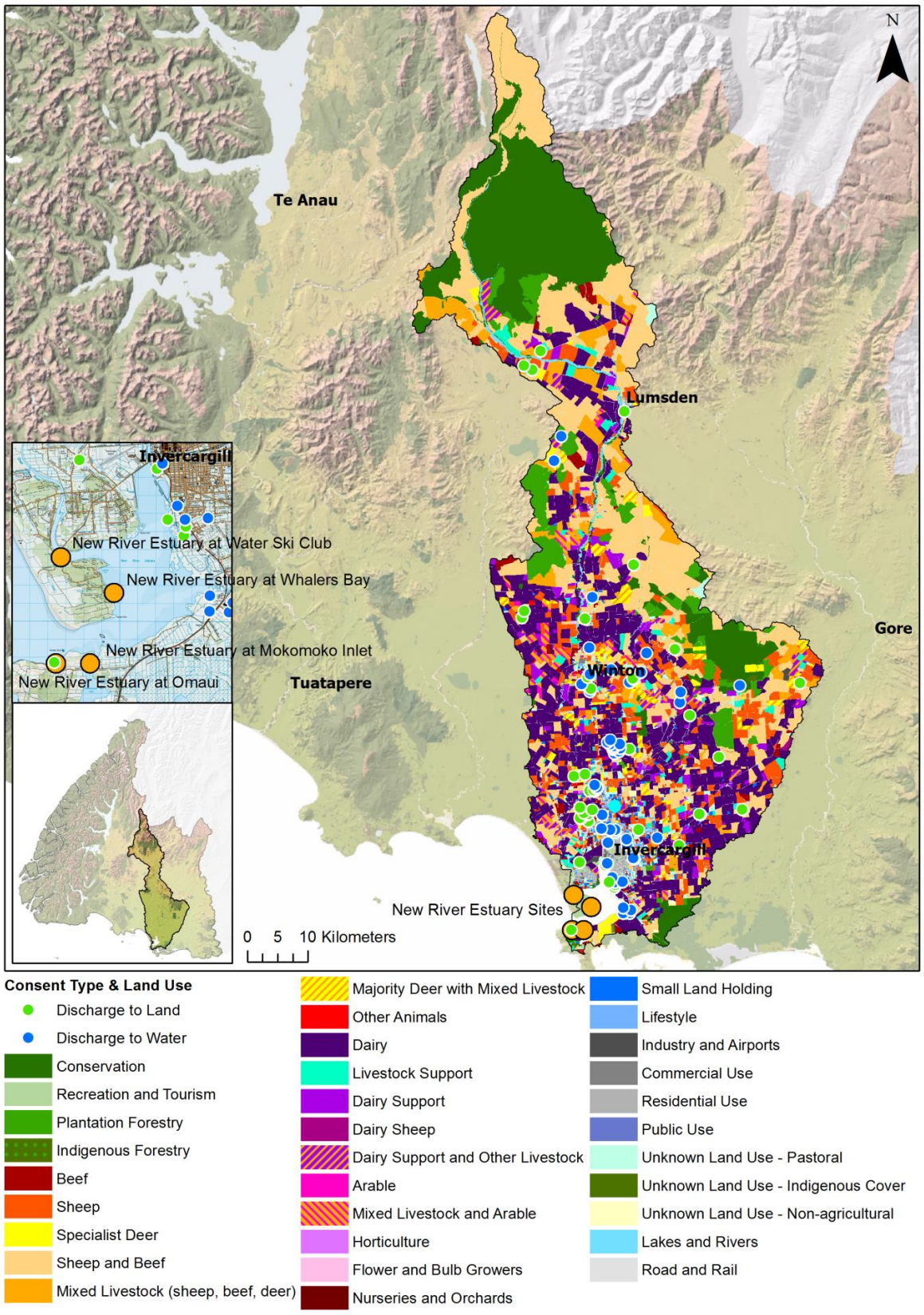


Figure 7. Land use and consented discharges with potential *E. coli* contamination risk (non-dairy) in the catchment for the New River Estuary, including Mokomoko Inlet and Whaler's bay sampling sites.

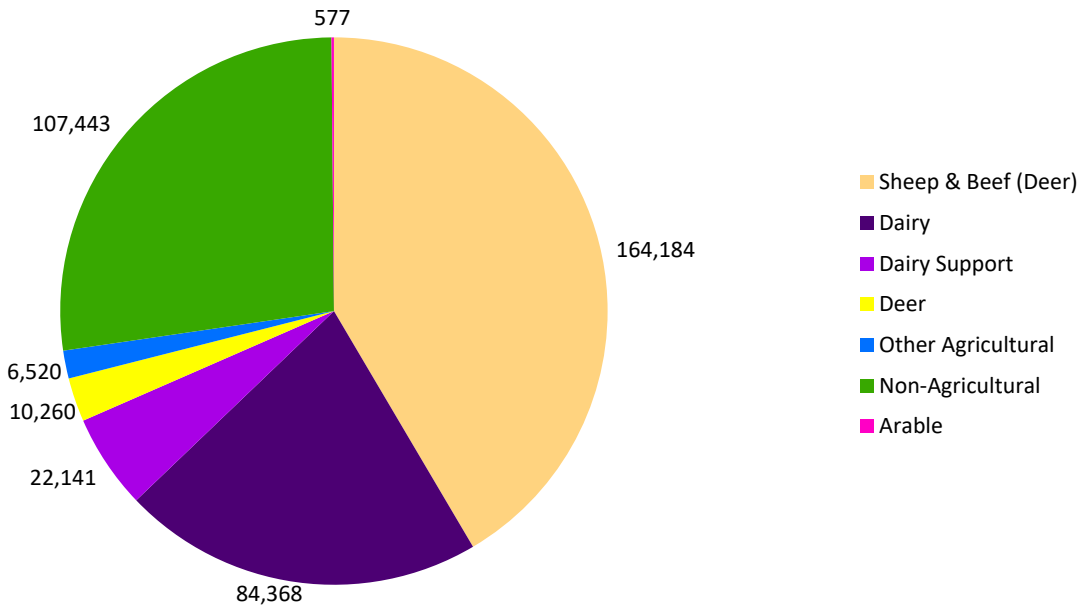


Figure 8. Land use (in hectares) in the catchment for the New River Estuary at Mokomoko Inlet and Whaler's Bay sampling sites.

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

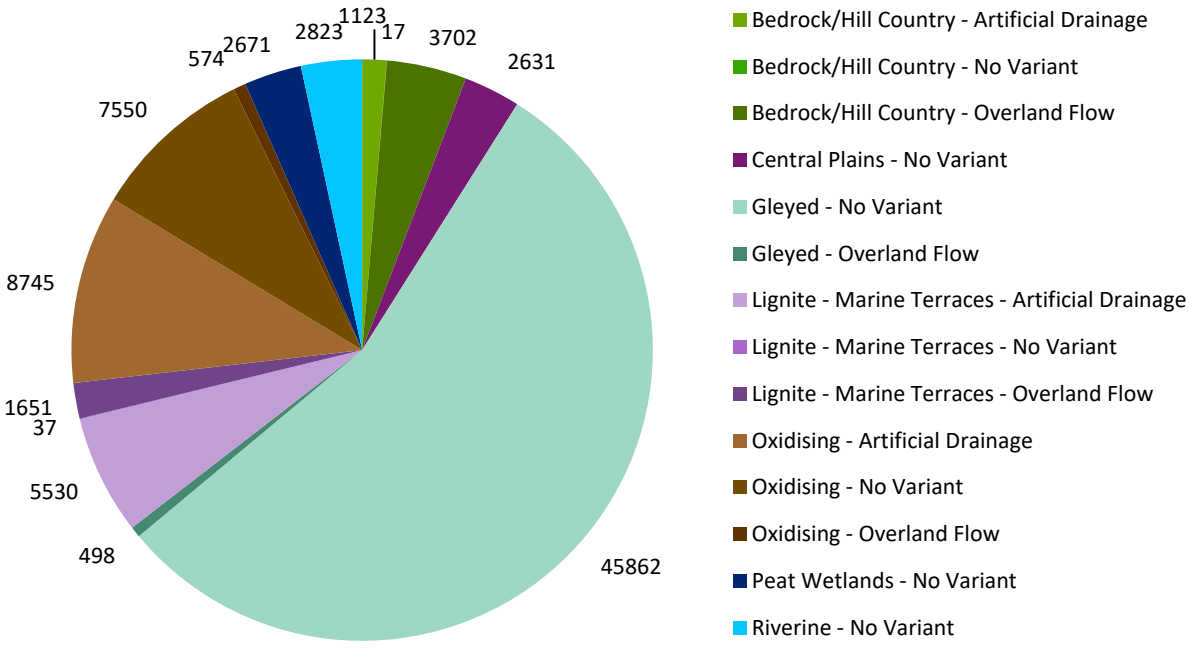


Figure 9. Dairying land (in hectares) in the catchment for the New River Estuary, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 10. Consented discharges to land and water in the catchment for the New River Estuary.

New River Estuary		
Subtype	Contaminant	Total
To Land	Other (whey to pasture 9, dust suppressant 3)	12
	1080	2
	Ash	2
	Ash, Dairy Factory Effluent, Wash Water, Waste Water	1
	Blood	2
	Blood, Meat Works Effluent, Wash Water, Waste Water	1
	Blood, Wash Down Effluent	1
	Calcium Magnesium Acetate	1
	Cereal bait	1
	Clean Fill	15
	Dairy Shed Effluent (land)	312
	Dairy Shed Effluent (land), Leachate	2
	Dairy Shed Effluent (land), Underpass Effluent	3
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	75
	Filter Backwash	1
	Green waste	1
	Hazardous Substances, Wash Water	1
	Industrial Effluent	1
	Industrial Effluent, Meat Works Effluent, Wash Water	1
	Leachate, Sewage Sludge, Sludge, Stormwater	1
	Meat Works Effluent	1
	Meat Works Effluent, Offal, Tannery Effluent, Wool Scour Effluent	1
	Meat Works Effluent, Sludge	1
	Meat Works Effluent, Wash Water	1
	Meat Works Effluent, Waste Water	2
	Mine water, Stormwater	1
	Offal	1
	Oil/Grease	4
	Oxidation Pond Effluent, Sewage (Treated), Waste Water	2
	Particulate, Wash Water	1
	Refuse - Commercial	3
	Refuse - Commercial, Refuse - Domestic, Refuse - Industrial	2
	Refuse - Industrial	1
	Septic Tank Effluent	1
	Septic Tank Effluent, Sewage (Treated), Waste Water	1
	Septic Tank Effluent, Waste Water	1
	Sewage (Treated), Sewage Package Plant	1
	Sewage (Treated), Waste Water	3
	Stockyard Effluent	1
	Stormwater	2
	Vegetable Wash Water, Wash Water	1
	Wash Down Effluent	2
	Wash Down Effluent, Wash Water	2
	Wash Down Effluent, Wash Water, Waste Water	1
	Wash Water	8
	Wash Water, Waste Water	1
	Waste Water	3
	Wintering Pad/Feedlot Effluent (land)	4
To Land Total		489
To Water	Other (sediment laden water to settling pond)	1

	Filter Backwash	2
	Floodwaters	2
	Ground water	1
	Ground water, Mine water, Stormwater	1
	Ground water, Stormwater	3
	Hazardous Substances	2
	Hot Water, Stormwater	1
	Industrial Effluent, Wash Water	1
	Meat Works Effluent, Waste Water	2
	Mine water	1
	Oxidation Pond Effluent, Sewage (Treated), Sewage Package Plant, Waste Water	1
	Pumped Drainage	1
	Sewage (Treated)	1
	Sewage (Treated), Waste Water	1
	Silt, Stormwater	1
	Sludge, Wash Water	1
	Stormwater	32
	Stormwater, Wash Down Effluent	1
	Wash Water	1
	Wash Water, Waste Water	1
	Waste Water	2
To Water Total		60
Grand Total		549

Note: Consent information accurate as of April 2017

B.4 TOETOES HARBOUR AT FORTROSE

Table 11. Faecal indicator bacteria results for samples collected at Toetoes Harbour at Fortrose between 10/8/16 and 2/8/17. Samples that were subsequently analysed by faecal source tracking are indicated in bold text.

Date	Sample ID	ESR sample	Faecal coliforms (cfu/100ml)	*E. coli* (cfu/100 ml)	Enterococci (cfu/100 ml)
10/08/16	20162085	CMB161045	80	80	9
1/09/16	20162093	CMB161107	50	50	3
12/10/16	20162959	CMB161242	<5	<5	<5
3/11/16	20163410	CMB161314	260	150	33
1/12/16	20163693		270	270	12
12/01/17	20163701	CMB170014	340	220	20
1/02/17	**20163709**	**CMB170158**	**800**	**800**	**15**
28/02/17	20163717	CMB170297	41	32	<1
3/04/17	20171174	CMB170412	2	1	<1
3/05/17	**20171268**	**CMB170715**	**300**	**300**	**200**
6/06/17	20171346		66	44	4
4/07/17	**20171354**	**CMB171167**	**2,600**	**2,200**	**330**
2/08/17	20171362		<1	<1	13

Table 12. Faecal source tracking results for samples collected from Toetoes Harbour at Fortrose during the 2016/2017 monitoring period.

Date	ES Sample #	ESR Sample #	General GenBac / 100 ml	Human BacH / 100 ml	Human BiADO / 100 ml	Ruminant BacR / 100 ml	Proportion Ruminant	Ruminant Sheep / 100 ml	Ruminant Cow / 100 ml	Avian GFD / 100 ml	Avian E2 / 100 ml	Conclusion
1/2/17	20163709	CMB170158	64,000	<33	<43	4,900	50 - 100%	<41	<5	200	60	Faecal source: - ruminant (not further identified) 50 - 100% - avian, duck
3/5/17	20171268	CMB170715	46,000	75	<110	4,000	50-100%	<100	<11	71	<99	Faecal source: - ruminant (not further identified) 50 -100% - avian
4/7/17	20171354	CMB171167	63,000	89	<43	6,000	50-100%	220	<5	46	<40	Faecal source: - ruminant (sheep) 50 -100% - avian

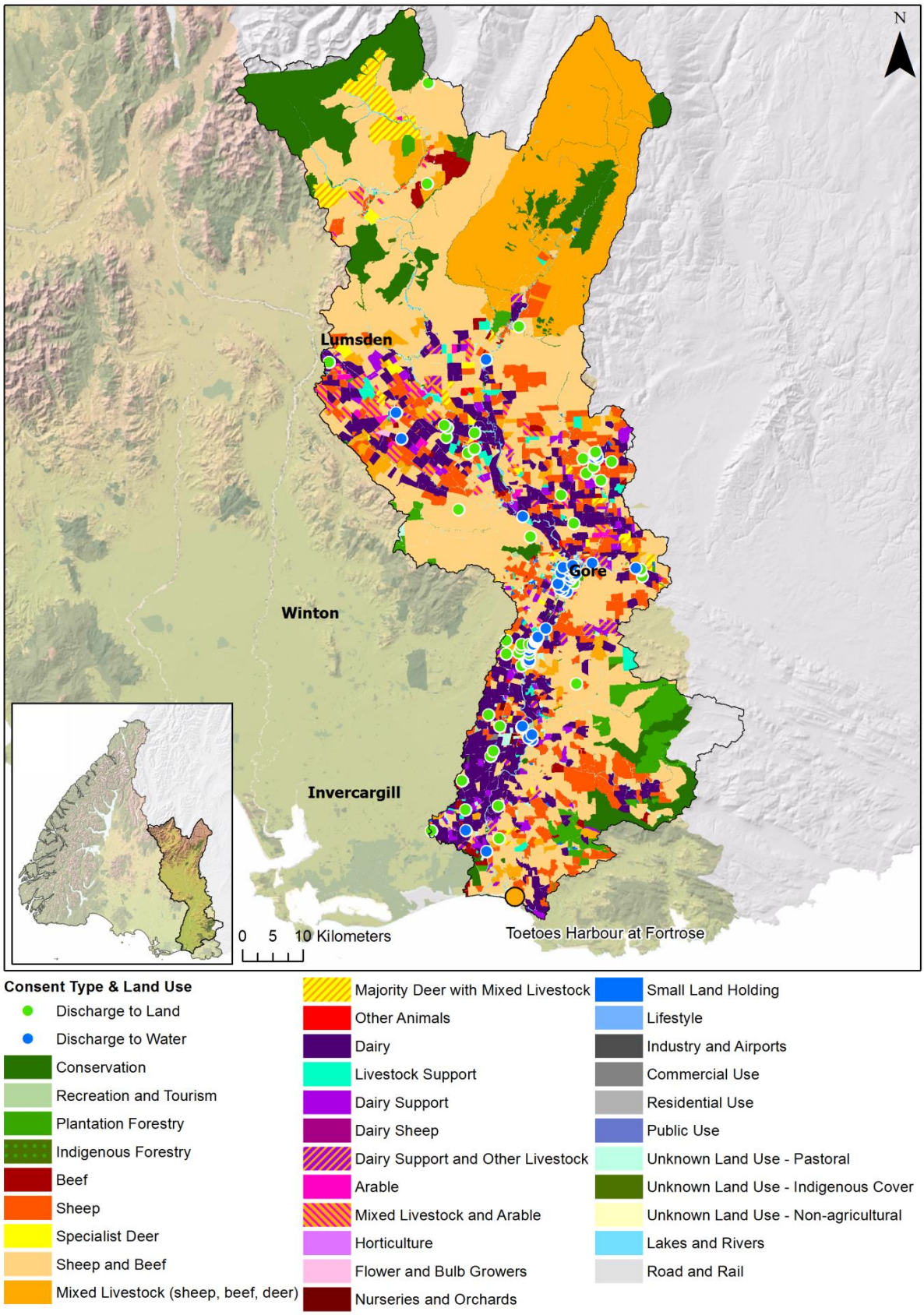


Figure 10. Land use and consented discharges with potential *E. coli* contamination risk (non-dairy) in the catchment for the Toetoes Harbour at Fortrose sampling site.

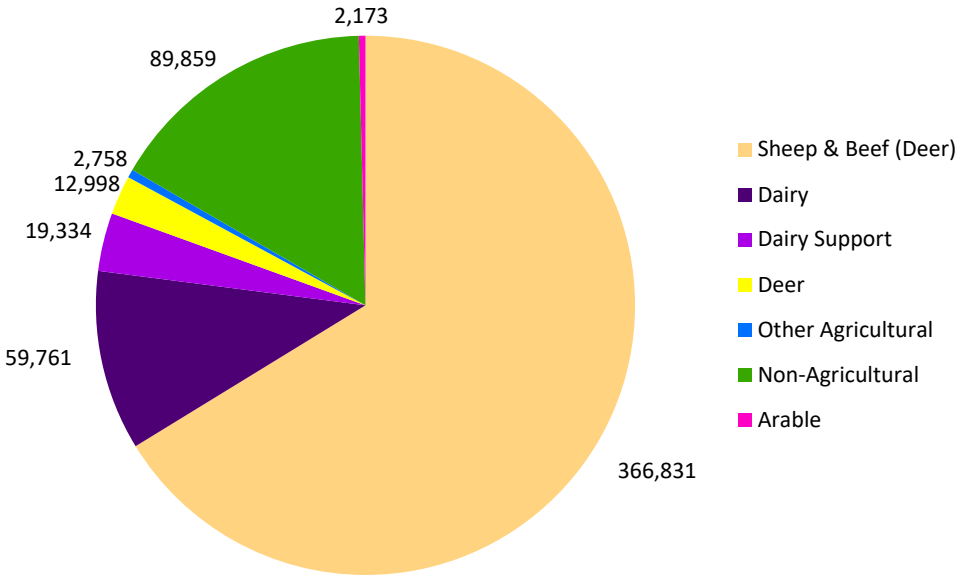


Figure 11. Land use (in hectares) in the catchment for the Toetoes Harbour at Fortrose sampling site.

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

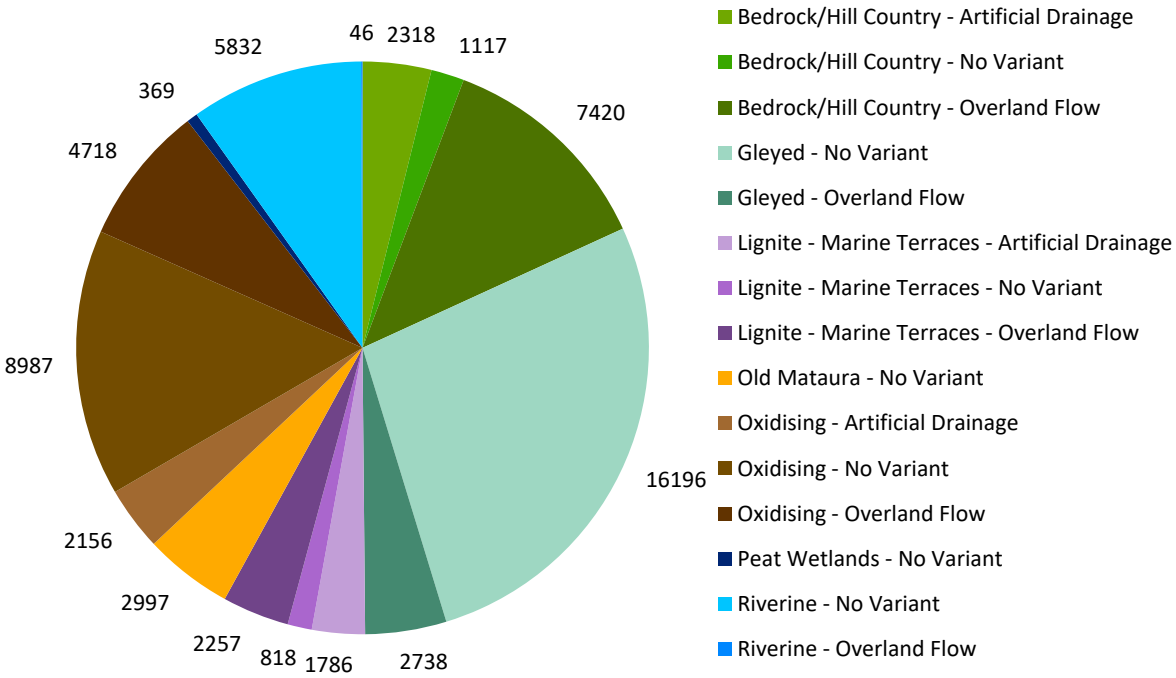


Figure 12. Dairying land (in hectares) in the catchment for the Toetoes Harbour at Fortrose sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 13. Consented discharges to land and water in the catchment for Toetoes Harbour at Fortrose.

Toetoes Harbour at Fortrose		
Subtype	Contaminant	Total
To Land	Other (whey to pasture)	29
	1080, Dye	4
	Ash	1
	Blood, Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	2
	Clean Fill	9
	Dairy Factory Effluent	3
	Dairy Factory Effluent, Wintering Pad/Feedlot Effluent (land)	1
	Dairy Shed Effluent (land)	220
	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)	50
	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	17
	Offal	2
	Oil/Grease	9
	Sewage (Treated)	1
	Sewage (Treated), Stormwater, Wash Water, Waste Water	1
	Silt, Wash Water	1
	Stormwater	2
	Tannery Effluent, Wash Water	2
	Vegetable Wash Water, Wash Water	1
	Wash Down Effluent, Wash Water	1
	Wash Down Effluent, Waste Water	1
	Wash Water	10
	Waste Water	4
	Wintering Pad/Feedlot Effluent (land)	12
To Land Total		398
To Water	Other (dewatering construction area)	1
	Boiler Blowdown Water, Waste Water	2
	Cooling Water	2
	Cooling Water, Stormwater, Waste Water	1
	Floodwaters	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
	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
	Oxidation Pond Effluent, Sewage (Treated)	1
	Oxidation Pond Effluent, Sewage (Treated), Sewage Package Plant	1
	Oxidation Pond Effluent, Sewage (Treated), Stormwater, Waste Water	1
	Oxidation Pond Effluent, Sewage (Treated), 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	4
	Wash Water, Waste Water	1
	Waste Water	1
To Water Total		75
Grand Total		473

Note: Consent information accurate as of April 2017

B.5 COLAC BAY AT BUNGALOW HILL ROAD

Table 14. Faecal indicator bacteria results for samples collected at Colac Bay at Bungalow Hill Road between 11/8/16 and 2/8/17.

Date	Sample ID	Faecal coliforms (cfu/100ml)	*E. coli* (cfu/100 ml)	Enterococci (cfu/100 ml)
11/08/16	20162089	2	2	4
2/09/16	20162097	9	8	1
12/10/16	20162963	170	150	18
3/11/16	20163414	<1	<1	1
2/12/16	20163697	58	53	11
12/01/17	20163705	30	25	36
1/02/17	20163713	250	250	29
2/03/17	20163721	3	1	<1
3/04/17	20171176	41	36	18
3/05/17	20171270	2000	2000	450
6/06/17	20171348	10	10	<10
4/07/17	20171356	2500	1900	530
2/08/17	20171364	240	230	320

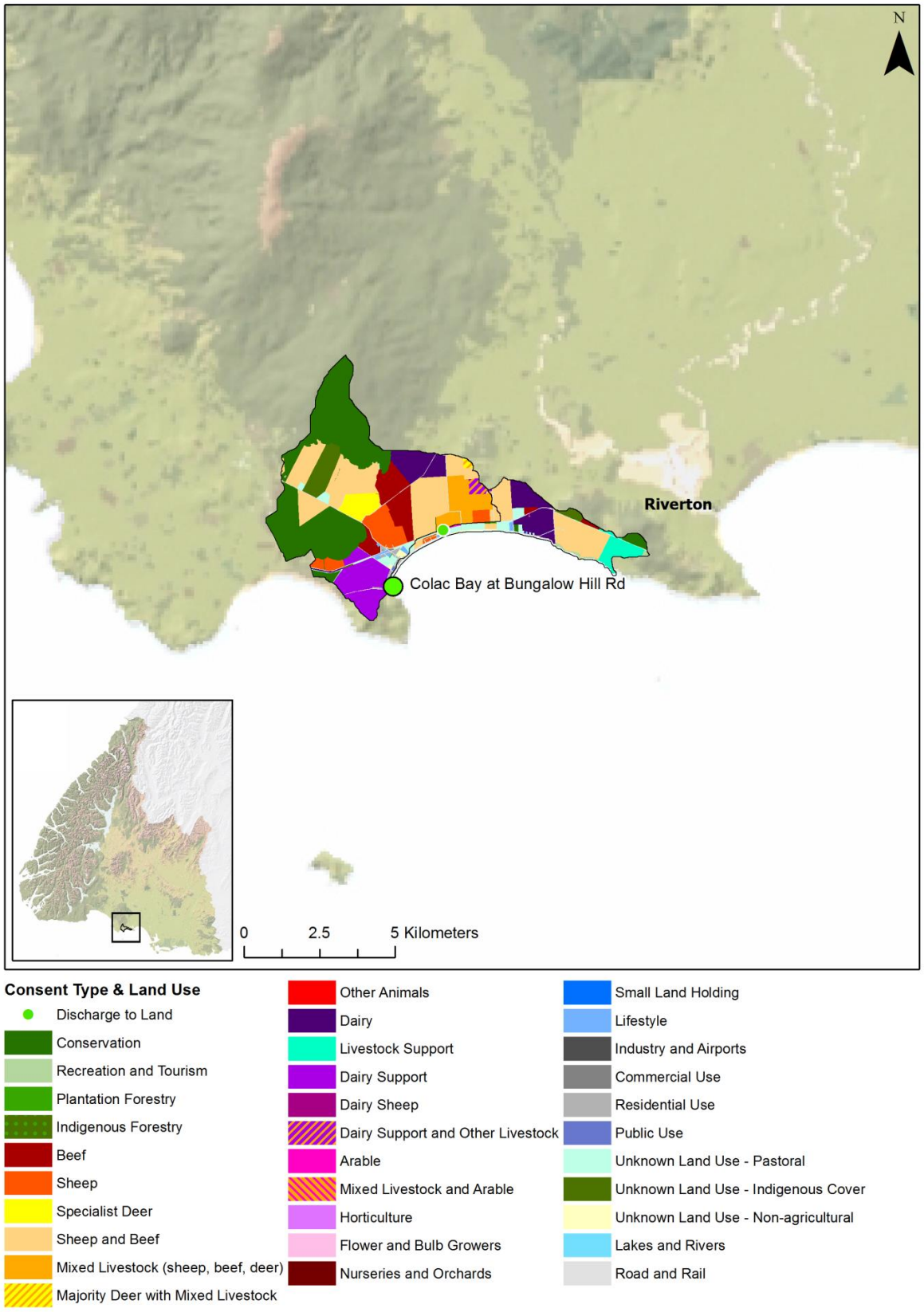


Figure 13. Land use and consented discharges with potential *E. coli* contamination risk (non-dairy) in the catchment for the Colac Bay at Bungalow Hill sampling site.

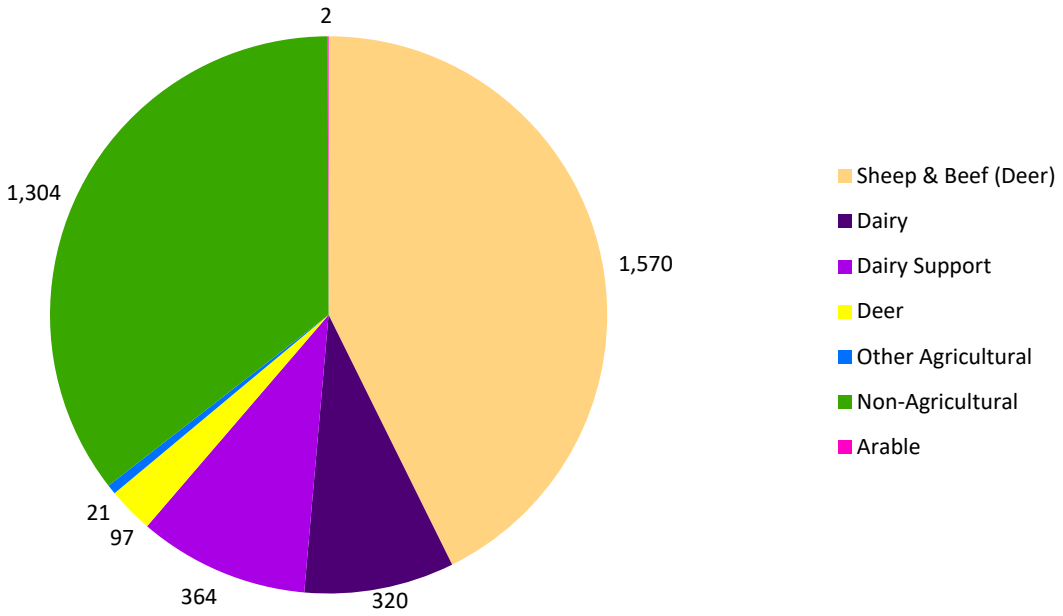


Figure 14. Land use (in hectares) in the catchment for the Colac Bay at Bungalow Hill sampling site.

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

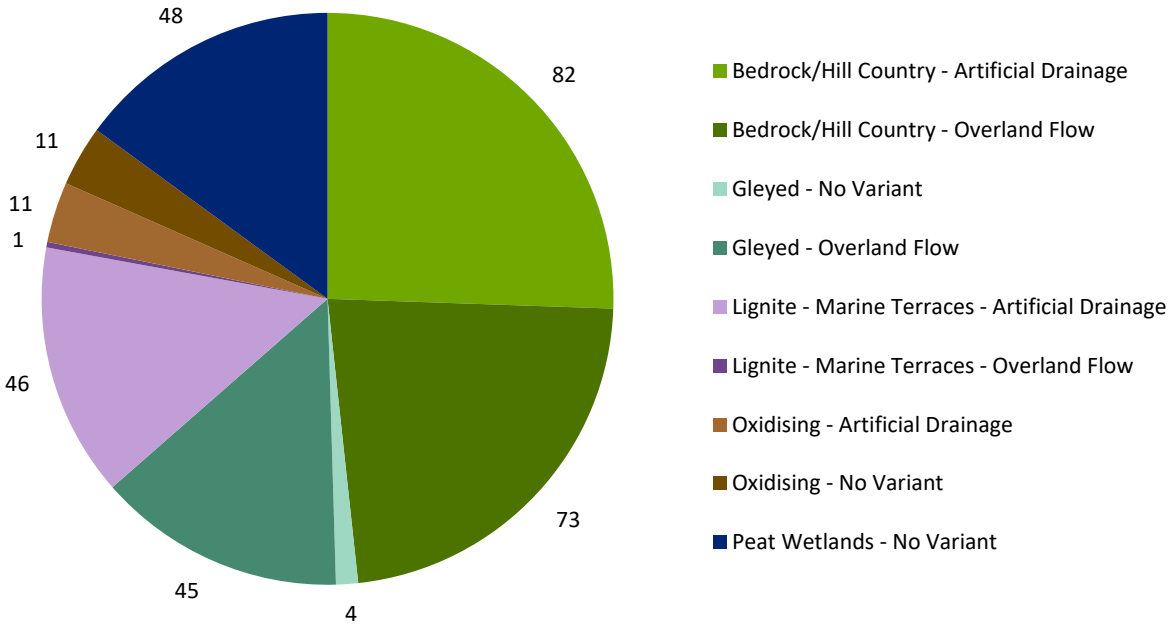


Figure 15. Dairying land (in hectares) in the catchment for the Colac Bay at Bungalow Hill sampling site, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 15. Consented discharges to land and water in the catchment for the Colac Bay at Bungalow Hill Road.

Colac Bay at Bungalow Hill Road		
Subtype	Contaminant	Total
To Land	Dairy Shed Effluent (land)	1
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
	Mine water, Wash Water	1
	Oil/Grease	1
	Sewage (Treated)	1
To Land Total		5
Grand Total		5

B.6 RIVERTON ROCKS AT MITCHELLS BAY

Table 16. Faecal indicator bacteria results for samples collected at Riverton Rocks at Mitchells Bay between 11/8/16 and 2/8/17.

Date	Sample ID	Faecal coliforms (cfu/100ml)	*E. coli* (cfu/100 ml)	Enterococci (cfu/100 ml)
11/08/16	20162086	1	<1	3
2/09/16	20162094	<1	<1	<1
12/10/16	20162960	2	<1	<1
3/11/16	20163411	5	5	1
2/12/16	20163694	<1	<1	<1
12/01/17	20163702	3	2	<1
1/02/17	20163710	3	1	3
2/03/17	20163718	3	3	<1
3/04/17	20171177	2	2	5
3/05/17	20171271	1	1	4
6/06/17	20171349	20	<10	<10
4/07/17	20171357	15	13	9
2/08/17	20171365	2	2	2

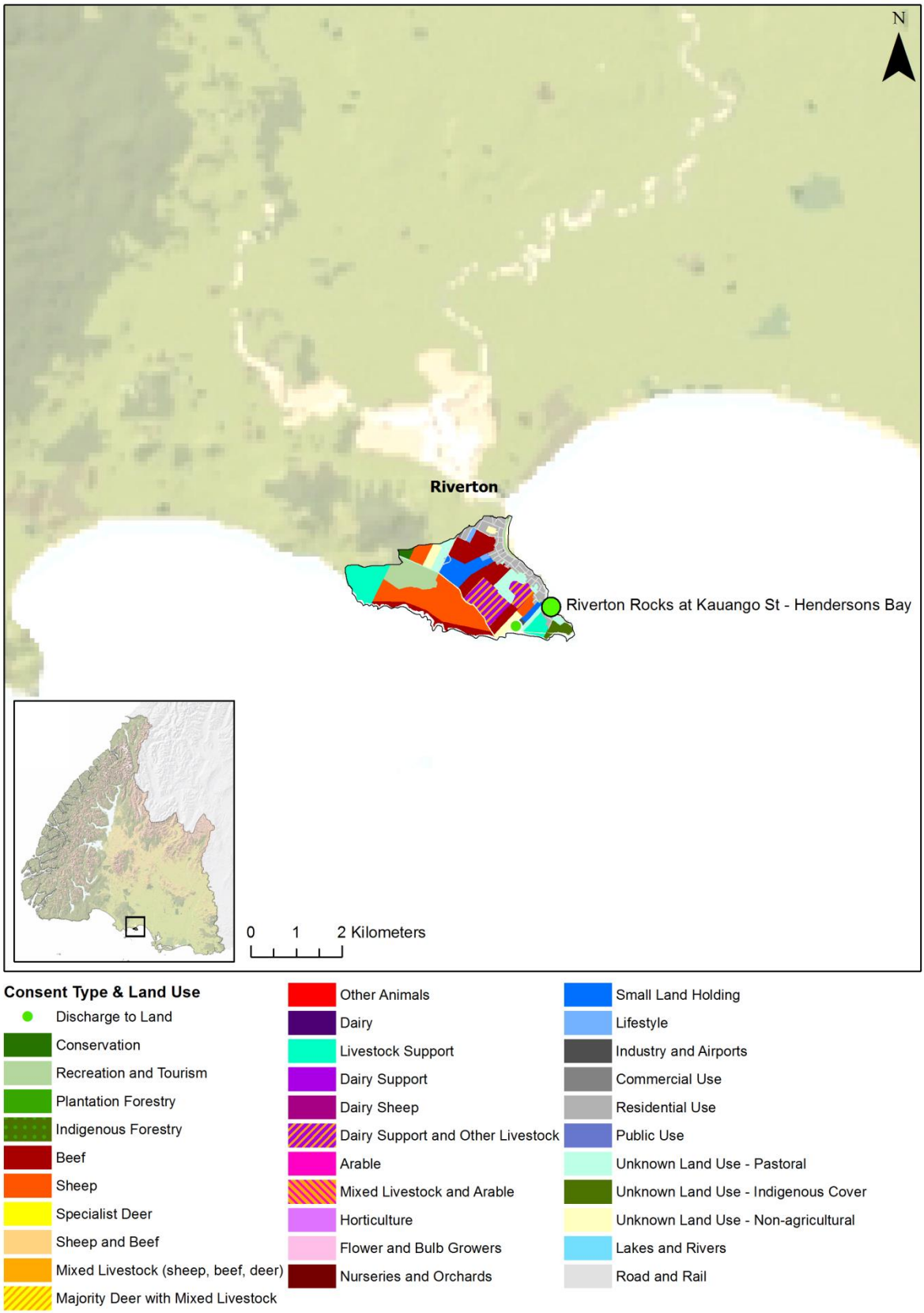


Figure 16. Land use and consented discharges with potential *E. coli* contamination risk (non-dairy) in the catchment for Riverton Rocks at Kauango Street, Henderson's Bay, which is adjacent to the Mitchell's Bay sampling site.

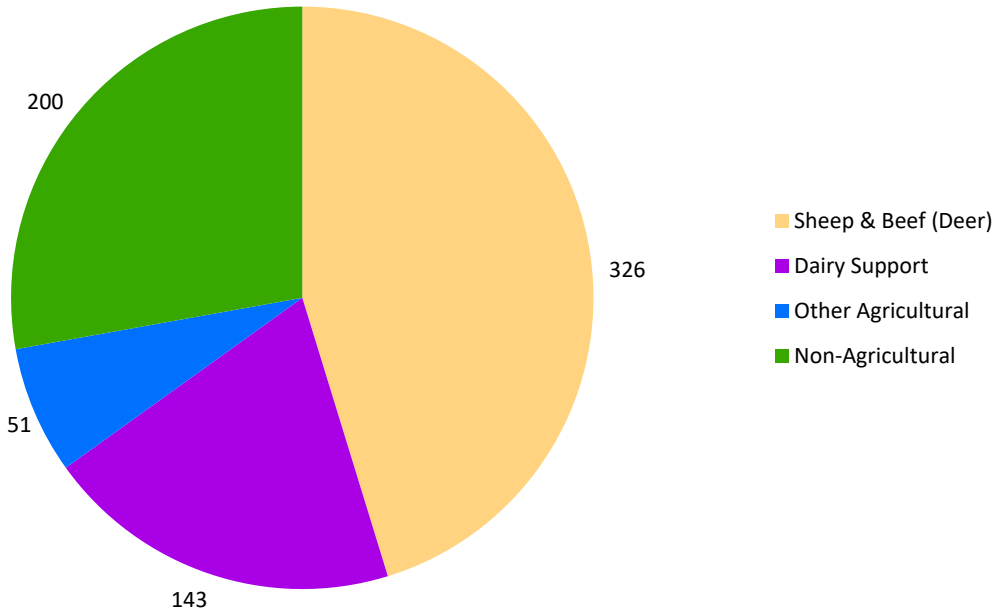


Figure 17. Land use (in hectares) in the catchment for Riverton Rocks at Kauango Street.

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

There is no dairying in the Riverton Rocks at Kauango Street sub-catchment.

Table 17. Consented discharges to land and water in the catchment for Riverton Rocks at Kauango Street.

Riverton Rocks at Kauango St – Hendersons Bay		
Subtype	Contaminant	Total
To Land	Waste Water	1
To Land Total		1
Grand Total		1

B.7 BLUFF HARBOUR AT OCEAN BEACH

Table 18. Faecal indicator bacteria results for samples collected at Bluff Harbour at Ocean Beach between 10/8/16 and 2/8/17.

Date	Sample ID	Faecal coliforms (cfu/100ml)	*E. coli* (cfu/100 ml)	Enterococci (cfu/100 ml)
10/08/16	20162082	<1	<1	1
1/09/16	20162090	<1	<1	<1
12/10/16	20162956	800	200	2300
3/11/16	20163407	<1	<1	<1
1/12/16	20163690	<1	<1	3
12/01/17	20163698	38	25	23
1/02/17	20163706	10	10	2
28/02/17	20163714	<1	<1	<1
3/04/17	20171173	2	2	5
3/05/17	20171267	90	70	3000
6/06/17	20171345	2	1	9
4/07/17	20171353	4	3	5
2/08/17	20171361	50	50	3

APPENDIX C: PREVIOUS FAECAL SOURCE TRACKING DATA

The following table (Table 19) documents the results of the faecal source tracking analysis that was undertaken for selected samples collected in 2015 and 2016.

Table 19. Faecal source tracking results for samples collected in 2015 and 2016.

Site	Date	ES Sample #	ESR Sample #	General GenBac / 100 ml	Human BacH / 100 ml	Human BiADO / 100 ml	Ruminant BacR / 100 ml	Proportion Ruminant	Ruminant Sheep / 100 ml	Ruminant Cow / 100 ml	Avian GFD / 100 ml	Avian E2 / 100 ml	Conclusion
Jacobs River Estuary d/s Fish Co-op	12/1/15	20150842	CMB150149	53,000	<83	<110	170	1 - 10%	<100	<11	140	<99	Faecal source: - low level ruminant (not further identified) 1-10% - avian
Jacobs River Estuary d/s Fish Co-op	18/2/16	20154270	CMB160398	13,000	<33	<43	160	1 - 10%	<41	<5	<29	<40	Faecal source: - low level ruminant (not further identified) 1-10%
Jacobs River Estuary d/s Fish Co-op	12/4/16	20160976	CMB160555	19,000	<83	<110	370	10 - 50%	<100	<11	<72	<99	Faecal source: - ruminant 10 - 50%
New River Estuary at Whalers Bay	11/4/16	20160971	CMB160546	42,000	<83	<110	<91	ND	<100	<11	<72	<99	Faecal source: - source present but not identified
Toetoes Harbour at Fortrose	21/6/16	20160991	CMB160934	120,000	69	<110	3,300	10 - 50%	130	<11	88	<99	Faecal source: - ruminant (sheep) 10 - 50% - avian
Riverton Rocks at Kauango Street	18/2/15	20150846	CMB150152	2,600	<83	<110	<91	ND	<100	<11	<72	<99	Faecal source: = canine markers detected

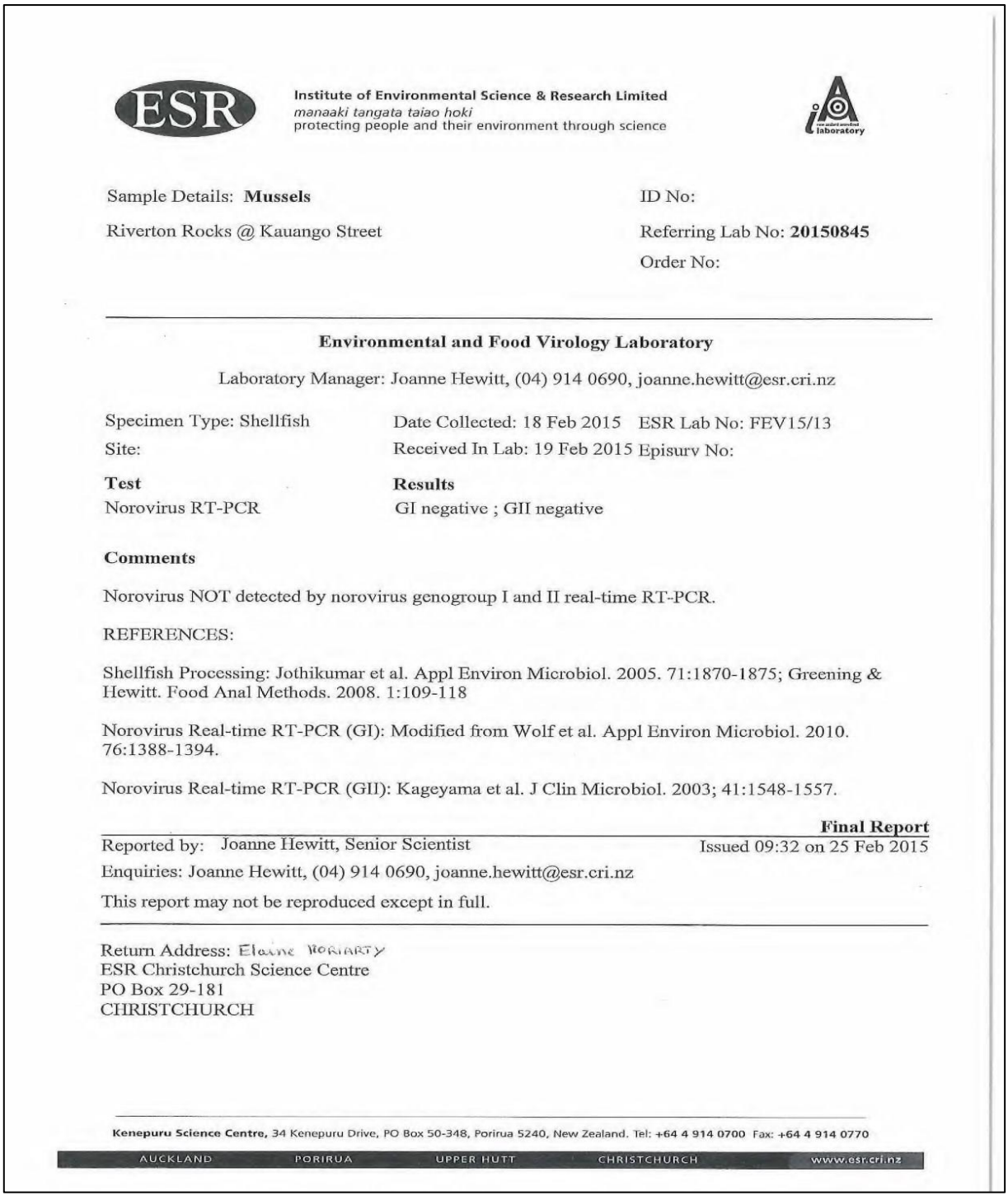
Table 19 continued. Faecal source tracking results for samples collected in 2015 and 2016.

Site	Date	ES Sample #	ESR Sample #	General GenBac / 100 ml	Human BacH / 100 ml	Human BiADO / 100 ml	Ruminant BacR / 100 ml	Proportion Ruminant	Ruminant Sheep / 100 ml	Ruminant Cow / 100 ml	Avian GFD / 100 ml	Avian E2 / 100 ml	Conclusion
Colac Bay at Bungalow Hill Road	12/1/15	20150843	CMB150150	35,000	<83	<110	3,400	50 - 100%	<100	<11	<72	<99	Faecal source: - ruminant (not further identified) 50-100%
Colac Bay – Huraki Creek u/s Foreshore Road	18/2/15	20150847	CMB150153	66,000	120	<110	730	1-10%	<100	<11	<72	<99	Faecal source: - ruminant (not further identified) 1-10% - ruminant and wildfowl faecal sterols detected
Colac Bay Bridge at Foreshore Road	18/2/15	20150844	CMB150151	63,000	<83	<110	570	1-10%	<100	<11	<72	<99	Faecal source: - ruminant (not further identified) 1-10% - ruminant and wildfowl faecal sterols detected
Colac Bay at Stream running into Huraki Creek	18/2/15	20150848	CMB150154	5,000	150	<110	<91	ND	<100	<11	<72	<99	Faecal source: - source present but not Identified - wildfowl faecal sterols detected

ND – not detected

APPENDIX D: ANALYSIS OF MUSSELS FROM RIVERTON ROCKS

D.1 TESTING OF MUSSEL TISSUE FOR NOROVIRUS



ESR

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protecting people and their environment through science

Sample Details: **Mussels**
Riverton Rocks @ Kauango Street

ID No:
Referring Lab No: **20150845**
Order No:

Environmental and Food Virology Laboratory

Laboratory Manager: Joanne Hewitt, (04) 914 0690, joanne.hewitt@esr.cri.nz

Specimen Type: Shellfish
Site:

Date Collected: 18 Feb 2015 ESR Lab No: FEV15/13
Received In Lab: 19 Feb 2015 Episurv No:

Test	**Results**
Norovirus RT-PCR	GI negative ; GII negative

Comments

Norovirus NOT detected by norovirus genogroup I and II real-time RT-PCR.

REFERENCES:

Shellfish Processing: Jothikumar et al. Appl Environ Microbiol. 2005. 71:1870-1875; Greening & Hewitt. Food Anal Methods. 2008. 1:109-118

Norovirus Real-time RT-PCR (GI): Modified from Wolf et al. Appl Environ Microbiol. 2010. 76:1388-1394.

Norovirus Real-time RT-PCR (GII): Kageyama et al. J Clin Microbiol. 2003; 41:1548-1557.

Final Report

Reported by: Joanne Hewitt, Senior Scientist
Issued 09:32 on 25 Feb 2015

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D.2 TESTING OF MUSSEL TISSUE FOR HEAVY METAL CONTAMINANTS

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ANALYSIS REPORT

Client:	Environment Southland	**Lab No:**	1386854 SPv1
Contact:	E Moriarty C/- ESR - Christchurch C/- Science Centre PO Box 29181 CHRISTCHURCH 8540	**Date Registered:** **Date Reported:** **Quote No:** **Order No:** **Client Reference:** **Submitted By:**	20-Feb-2015 04-Mar-2015 66448 4060.1375.412 Sediment & Shellfish testing Nick Ward

Sample Type: Raw whole shell mussel

	Sample Name:	20150845 18-Feb-2015 7:00 am				
	Lab Number:	1386854.1				
Antimony	mg/kg as rcvd	< 0.10	-	-	-	-
Arsenic	mg/kg as rcvd	2.0	-	-	-	-
Bismuth	mg/kg as rcvd	< 0.010	-	-	-	-
Cadmium	mg/kg as rcvd	0.079	-	-	-	-
Copper	mg/kg as rcvd	0.77	-	-	-	-
Lead	mg/kg as rcvd	0.058	-	-	-	-
Mercury	mg/kg as rcvd	< 0.010	-	-	-	-
Silver	mg/kg as rcvd	< 0.010	-	-	-	-
Tin	mg/kg as rcvd	< 0.05	-	-	-	-
Total Heavy Metals*	mg/kg as rcvd	3.0	-	-	-	-

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Raw whole shell mussel

Test	Method Description	Default Detection Limit	Sample No
Shucking of Shellfish*	Removal of tissue from shell. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	-	1
Homogenise*	Mincing, chopping, or blending of sample to form homogenous sample fraction. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	-	1
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, 85°C for 1 hour. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	-	1
Antimony	Biological materials digestion, ICP-MS.	0.02 mg/kg as rcvd	1

Arsenic	Biological materials digestion, ICP-MS.	0.02 mg/kg as rcvd	1
Bismuth	Biological materials digestion, ICP-MS.	0.002 mg/kg as rcvd	1
Cadmium	Biological materials digestion, ICP-MS.	0.0004 mg/kg as rcvd	1
Copper	Biological materials digestion, ICP-MS.	0.010 mg/kg as rcvd	1
Lead	Biological materials digestion, ICP-MS.	0.002 mg/kg as rcvd	1
Mercury	Biological materials digestion, ICP-MS.	0.002 mg/kg as rcvd	1
Silver	Biological materials digestion, ICP-MS.	0.002 mg/kg as rcvd	1
Tin	Biological materials digestion, ICP-MS.	0.010 mg/kg as rcvd	1
Total Heavy Metals*	Calculation: sum of individual metals (antimony, arsenic, bismuth, cadmium, copper, lead, mercury, silver, tin). Heavy Metals Test (as lead sulfide), Food Chemicals Codex 4th Edition, 1996 (modified - ICP-MS analysis).	1.0 mg/kg as rcvd	1

This Laboratory is accredited by International Accreditation New Zealand (IANZ), which represents New Zealand in the International Laboratory Accreditation Cooperation (ILAC). Through the ILAC Mutual Recognition Arrangement (ILAC-MRA) this accreditation is internationally recognised.
The tests reported herein have been performed in accordance with the terms of accreditation, with the exception of tests marked *, which are not accredited.

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

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