ESR
Science for Communities

Sources of Pollution in the Aparima Freshwater Management Unit

May 2019

Dr Elaine Moriarty

Dr Olga Pantos

Dr Sarah Coxon

Prepared with support from Nick Ward, Roger Hodson, Rob Williamson and Matt Couldrey from Environment Southland

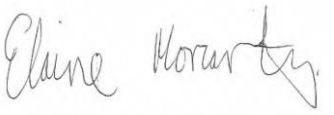
PREPARED FOR: **Environment Southland**

CLIENT REPORT No.: **CSC 19008**

REVIEWED BY: **Dr Brent Gilpin**

ACKNOWLEDGEMENTS

Author



Dr Elaine Moriarty

Senior Scientist,
Health and Environment

Author

Dr Olga Pantos

Senior Scientist,
Health and Environment

Author

Dr Sarah Coxon

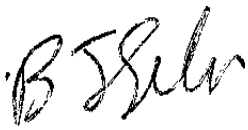
Scientist,
Health and Environment

Manager

Mr Wim Nijhof

Group Leader,
Human and Ecological
Health

Peer reviewer



Dr Brent Gilpin

Manager,
Water and Biowaste
Research and
Development

CONTENTS

EXECUTIVE SUMMARY 1

1. BACKGROUND 4

1.1 MICROBIAL WATER QUALITY 4

1.2 SOURCES OF POLLUTION AND ROUTES OF TRANSMISSION 5

1.2.1 Animal faeces 5

Cattle 5

Sheep 5

Other ruminants 6

Routes of transmission 6

1.2.2 Avian faeces 7

1.2.3 Human sources 8

1.3 FAECAL SOURCE TRACKING 8

1.4 *CAMPYLOBACTER* 9

1.5 REPORT OBJECTIVES 9

2. MATERIALS AND METHODS 11

2.1 SAMPLING SITES 11

2.2 MICROBIOLOGICAL ANALYSIS 14

2.2.1 Coliform and *E. coli* analysis 15

2.2.2 *Campylobacter* isolation 15

2.2.3 *Campylobacter* sub-typing and source attribution 15

2.2.4 Faecal source tracking 15

2.2.5 Faecal sterol analysis 15

2.3 SANITARY SURVEYS 16

3. OVERVIEW OF MICROBIAL WATER QUALITY 17

3.1 OVERVIEW OF MICROBIAL WATER QUALITY IN THE APARIMA FMU 17

3.1.1 General comment on microbial quality of all water samples collected 17

3.1.2 Microbial quality of water samples collected for main Aparima study 17

3.2 SOURCES OF FAECAL POLLUTION 25

3.3 OTAUTAU PLAINS STUDY 27

3.4 CHARACTERISATION OF *CAMPYLOBACTER* IN THE APARIMA FMU 30

3.4.1 MBiT source attribution 30
3.4.2 Genotype analysis and comparison with clinical isolates 31

4. DISCUSSION 36
4.1 MICROBIAL SOURCES AND TRANSMISSION 36
4.2 HEALTH RISK 38
4.3 MANAGEMENT AND MITIGATION 41
4.3.1 Human contamination 41
4.3.2 Direct deposition 42
4.3.3 Indirect sources 42
4.3.4 Wildfowl 43
4.3.5 Prioritising mitigations 43

5. CONCLUSIONS 45

ABBREVIATIONS 46

GLOSSARY 47

APPENDIX A: MICROBIOLOGICAL METHODS AND REPORTING ..49
A.1 COLIFORM AND *E. COLI* ANALYSIS 49
A.2 *CAMPYLOBACTER* SPP. ISOLATION 49
A.3 *CAMPYLOBACTER* SUB-TYPING AND SOURCE ATTRIBUTION BY MBIT ... 49
A.4 PCR MARKERS FOR FAECAL SOURCE TRACKING (FST) 50
A.5 FAECAL STEROL ANALYSIS 55
A.6 PRESENTATION OF RESULTS IN THIS REPORT 55

APPENDIX B: SUBCATCHMENT-SPECIFIC INFORMATION AND MICROBIAL WATER QUALITY 59
B.1 OTAUTAU STREAM AT WAIKOURO 59
B.2 OTAUTAU STREAM AT OTAUTAU-TUATAPERE ROAD 65
B.3 APARIMA RIVER AT DUNROBIN 71
B.4 APARIMA RIVER AT THORNBURY 75
B.5 POURAKINO RIVER AT TRAILL ROAD 80
B.6 CASCADE STREAM AT POURAKINO VALLEY ROAD 85
B.7 OPOURIKI STREAM AT TWEEDIE ROAD 89
B.8 WAIMATUKU STREAM AT LORNEVILLE-RIVERTON HIGHWAY 95
B.9 HAMILTON BURN AT AFFLECK ROAD 101
B.10 OTAUTAU PLAINS DETAILED STUDY 106

B.10.1 Wairio Stream 106
B.10.2 North Head Stream and Upper Otautau River sites..... 109
B.10.3 Confluence of Wairio Stream and Otautau Stream at Waikouro Shortcut 114
B.10.4 Waicolo Stream 116
B.10.5 Opio Stream at Otautau Nightcaps 118
B.10.6 North and South Fern Burn 120

REFERENCES 124

LIST OF TABLES

TABLE 1. SAMPLING SITES SELECTED FOR THE APARIMA FMU, WITH THE CONDITIONS EACH SITE WAS SAMPLED UNDER. 11

TABLE 2. SAMPLING SITES SELECTED FOR THE DETAILED OTAUTAU PLAINS INVESTIGATION, WITH THE CONDITIONS EACH SITE WAS SAMPLED UNDER. 13

TABLE 3. SUMMARY OF MBIT GENE TARGETS AND THEIR METHODOLOGIES. 51

TABLE 4. SUMMARY OF PCR MARKERS USED IN THIS STUDY, INCLUDING MICROBIAL TARGETS, SENSITIVITY AND SPECIFICITY. 54

TABLE 5. FAECAL STEROL RATIOS INDICATIVE OF FAECAL POLLUTION. 56

TABLE 6. GUIDELINE FOR GENERAL DATA, MICROBIAL RESULTS AND MBIT INTERPRETATION 57

TABLE 7. EXPLANATION OF PCR-BASED MARKERS 57

TABLE 8. EXPLANATION OF FAECAL STEROL RESULTS AND INTERPRETATION 58

TABLE 9. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE OTAUTAU STREAM AT WAIKOURO. 61

TABLE 10. NUMBER AND TYPE OF CONSENTED DISCHARGES TO LAND AND WATER IN THE CATCHMENT FOR THE OTAUTAU STREAM AT WAIKOURO. 64

TABLE 11. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE OTAUTAU STREAM AT OTAUTAU-TAUTAPERE ROAD 67

TABLE 12. NUMBER AND TYPE OF CONSENTED DISCHARGES TO LAND AND WATER IN THE CATCHMENT FOR THE OTAUTAU STREAM AT OTAUTAU-TUATAPERE ROAD. 70

TABLE 13. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE APARIMA RIVER AT DUNROBIN. 72

TABLE 14. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE APARIMA RIVER AT THORNBURY 76

TABLE 15. NUMBER AND TYPE OF CONSENTED DISCHARGES TO LAND AND WATER IN THE CATCHMENT FOR THE APARIMA RIVER AT THORNBURY. 79

TABLE 16. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE POURAKINO RIVER AT TRAILL ROAD. 81

TABLE 17. NUMBER AND TYPE OF CONSENTED DISCHARGES TO LAND AND WATER IN THE CATCHMENT FOR THE POURAKINO RIVER AT TRAILL ROAD. 84

TABLE 18. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE CASCADE STREAM AT POURAKINO VALLEY ROAD. 86

TABLE 19. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE OPOURIKI STREAM AT TWEEDIE ROAD. 91

TABLE 20. NUMBER AND TYPE OF CONSENTED DISCHARGES TO LAND AND WATER IN THE CATCHMENT FOR THE OPOURIKI STREAM AT TWEEDIE ROAD. 94

TABLE 21. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE WAIMATUKU STREAM AT THE LORNEVILLE-RIVERTON HIGHWAY 97

TABLE 22. NUMBER AND TYPE OF CONSENTED DISCHARGES TO LAND AND WATER IN THE CATCHMENT FOR THE WAIMATUKU STREAM AT LORNEVILLE-RIVERTON HIGHWAY..... 100

TABLE 23. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM HAMILTON BURN AT AFFLECK ROAD. 102

TABLE 24. NUMBER AND TYPE OF CONSENTED DISCHARGES TO LAND AND WATER IN THE CATCHMENT FOR HAMILTON BURN AT AFFLECK ROAD..... 105

TABLE 25. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE FOUR SITES ALONG THE WAIRIO STREAM, AS PART OF A DETAILED STUDY OF POLLUTION IN THE OTAUTAU PLAINS AREA. 108

TABLE 26. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM TWO SITES ALONG NORTH HEAD STREAM, AS PART OF A DETAILED STUDY OF POLLUTION IN THE OTAUTAU PLAINS AREA. 110

TABLE 27. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM FOUR SITES ALONG THE UPPER OTAUTAU STREAM, AS PART OF A DETAILED STUDY OF POLLUTION IN THE OTAUTAU PLAINS AREA. 113

TABLE 28. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE WAIRIO AND OTAUTAU STREAMS, JUST UPSTREAM OF THEIR CONFLUENCE AT THE WAIKOURO SHORTCUT, AS PART OF A DETAILED STUDY OF POLLUTION IN THE OTAUTAU PLAINS AREA. 115

TABLE 29. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE WAICOLO STREAM, AS PART OF A DETAILED STUDY OF POLLUTION IN THE OTAUTAU PLAINS AREA. 117

TABLE 30. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM THE OPIO STREAM AT OTAUTAU NIGHTCAPS, AS PART OF A DETAILED STUDY OF POLLUTION IN THE OTAUTAU PLAINS AREA. 119

TABLE 31. RESULTS FOR MICROBIAL AND FST ANALYSIS OF WATER SAMPLES COLLECTED FROM NORTH FERN BURN AND SOUTH FERN BURN, AS PART OF A DETAILED STUDY OF POLLUTION IN THE OTAUTAU PLAINS AREA. 122

LIST OF FIGURES

FIGURE 1. AN EXAMPLE OF LAND WITH MULTIPLE SOURCES OF FAECAL POLLUTION WITHIN THE APARIMA FMU, SOUTHLAND. 3

FIGURE 2. THE APARIMA FMU, WITH SUB-CATCHMENTS, SAMPLING SITE LOCATIONS AND RIVERS OF ORDER 4 TO 8 SHOWN. 12

FIGURE 3. MAP OF THE UPSTREAM SITES TESTED AS PART OF THE DETAILED INVESTIGATION OF THE EFFECTS POLLUTION SOURCES ON THE OTAUTAU STREAM. 14

FIGURE 4. OVERVIEW OF MICROBIAL WATER QUALITY IN APARIMA FMU UNDER BASE FLOW CONDITIONS. 19

FIGURE 5. OVERVIEW OF MICROBIAL WATER QUALITY IN APARIMA FMU FOLLOWING RAINFALL. 20

FIGURE 6. *E. COLI* CONCENTRATIONS FOR WATER SAMPLES COLLECTED FROM THE MAIN APARIMA STUDY SITES UNDER BASE FLOW CONDITIONS AND FOLLOWING RAINFALL. 21

FIGURE 7. *E. COLI* CONCENTRATIONS AT SITES FOR WHICH WATER SAMPLES WERE COLLECTED UNDER BOTH BASE FLOWAND HIGH FLOW CONDITIONS. 21

FIGURE 8. CONCENTRATION OF *E. COLI* AT THE MAIN STUDY SITES IN THE APARIMA FMU, ACROSS THE COURSE OF THE YEAR. 22

FIGURE 9. *CAMPYLOBACTER* CONCENTRATIONS FOR WATER SAMPLES COLLECTED FROM THE MAIN APARIMA STUDY SITES UNDER BASE FLOW CONDITIONS AND FOLLOWING RAINFALL. 22

FIGURE 10. *CAMPYLOBACTER* CONCENTRATIONS AT SITES FOR WHICH WATER SAMPLES WERE COLLECTED UNDER BOTH BASE FLOW AND HIGH FLOW CONDITIONS. 23

FIGURE 11. THE PREVALENCE OF DIFFERENT *CAMPYLOBACTER* SPECIES WITHIN *CAMPYLOBACTER*-POSITIVE SAMPLES FROM THE MAIN APARIMA STUDY SITES... 23

FIGURE 12. CONCENTRATION OF *CAMPYLOBACTER* AT THE MAIN STUDY SITES IN THE APARIMA FMU, ACROSS THE COURSE OF THE YEAR. 24

FIGURE 13. RELATIONSHIP BETWEEN *E. COLI* AND *CAMPYLOBACTER* SPP. CONCENTRATIONS IN ALL WATER SAMPLES COLLECTED WITHIN THE APARIMA FMU 24

FIGURE 14. THE PROPORTION OF SAMPLES COLLECTED UNDER BASE FLOW CONDITIONS AND FOLLOWING RAINFALL, THAT WERE AFFECTED BY DIFFERENT LEVELS OF RUMINANT FAECAL POLLUTION. 26

FIGURE 15. THE PERCENTAGE OF SAMPLES COLLECTED FROM WITHIN THE APARIMA FMU THAT WERE POSITIVE FOR FST MARKERS FROM DIFFERENT SOURCES. 26

FIGURE 16. OVERVIEW OF *E. COLI* CONCENTRATIONS AT THE SITES SAMPLED AS PART OF THE DETAILED OTAUTAU PLAINS INVESTIGATION. 28

FIGURE 17. OVERVIEW OF *CAMPYLOBACTER* CONCENTRATIONS AND THE PRESENCE OF FST MARKERS AT THE SITES SAMPLED AS PART OF THE DETAILED OTAUTAU PLAINS INVESTIGATION.. 29

FIGURE 18. THE PERCENTAGE OF *CAMPYLOBACTER*-POSITIVE SAMPLES FROM THE MAIN APARIMA STUDY SITES THAT WERE IDENTIFIED AS HAVING DIFFERENT *CAMPYLOBACTER* SOURCES PRESENT (AS DETERMINED BY MBIT ANALYSIS).. 30

FIGURE 19. BURST DIAGRAM SHOWING PHYLOGENETIC DIVERSITY OF *CAMPYLOBACTER* ISOLATES FROM SITES ACROSS THE APARIMA FMU, BASED ON MBIT ANALYSIS. S..... 32

FIGURE 20. BURST DIAGRAM SHOWING PHYLOGENETIC DIVERSITY OF *CAMPYLOBACTER* ISOLATES FROM ACROSS THE SOUTHLAND REGION, BASED ON MBIT ANALYSIS..... 33

FIGURE 21. BURST DIAGRAM SHOWING PHYLOGENETIC DIVERSITY OF *CAMPYLOBACTER* ISOLATES FROM WATER SAMPLES FROM THE APARIMA FMU COMPARED WITH HUMAN CLINICAL ISOLATES FROM THE SOUTHLAND REGION. .. 34

FIGURE 22. BURST DIAGRAM SHOWING PHYLOGENETIC DIVERSITY OF *CAMPYLOBACTER* ISOLATES FROM WATER FROM THE APARIMA FMU, HIGHLIGHTING THOSE THAT ARE WILDFOWL-ASSOCIATED COMPARED WITH HUMAN CLINICAL ISOLATES 35

FIGURE 23. DOSE-RESPONSE CURVES FOR *CAMPYLOBACTER JEJUNI*, ESTIMATING THE PROBABILITY OF INFECTION FOR A GIVEN DOSE..... 40

FIGURE 24. EXAMPLE OF AN MBIT PATTERN NAMING..... 52

FIGURE 25. THE OTAUTAU STREAM AT WAIKOURO SHORTCUT. 59

FIGURE 26. LAND USE AND CONSENTS WITH POTENTIAL *E. COLI* CONTAMINATION RISK (NON-DAIRY) IN THE CATCHMENT FOR THE OTAUTAU STREAM AT WAIKOURO SAMPLING SITE. 62

FIGURE 27. LAND USE (IN HECTARES) IN THE CATCHMENT FOR THE OTAUTAU STREAM AT WAIKOURO..... 63

FIGURE 28. DAIRYING LAND (IN HECTARES) IN THE CATCHMENT FOR THE OTAUTAU STREAM WAIKOURO, SEPARATED INTO PHYSIOGRAPHIC UNITS..... 63

FIGURE 29. SAMPLE COLLECTION FROM THE OTAUTAU STREAM AT OTAUTAU-TUATAPERE ROAD. 65

FIGURE 30. LAND USE AND CONSENTS WITH POTENTIAL *E. COLI* CONTAMINATION RISK (NON-DAIRY) IN THE CATCHMENT FOR THE OTAUTAU STREAM AT OTAUTAU-TUATAPERE ROAD SAMPLING SITE. 68

FIGURE 31. LAND USE (IN HECTARES) IN THE CATCHMENT FOR THE OTAUTAU STREAM AT OTAUTAU-TUATAPERE ROAD..... 69

FIGURE 32. DAIRYING LAND (IN HECTARES) IN THE CATCHMENT FOR THE OTAUTAU STREAM AT OTAUTAU-TUATAPERE ROAD, SEPARATED INTO PHYSIOGRAPHIC UNITS..... 69

FIGURE 33. LAND USE AND CONSENTS WITH POTENTIAL *E. COLI* CONTAMINATION RISK (NON-DAIRY) IN THE CATCHMENT FOR THE APARIMA RIVER AT DUNROBIN SAMPLING SITE. 73

FIGURE 34. LAND USE (IN HECTARES) IN THE CATCHMENT FOR THE APARIMA RIVER AT DUNROBIN. 74

FIGURE 35. SAMPLE COLLECTION FROM THE APARIMA RIVER AT THORNBURY..... 75

FIGURE 36. LAND USE AND CONSENTS WITH POTENTIAL *E. COLI* CONTAMINATION RISK (NON-DAIRY) IN THE CATCHMENT FOR THE APARIMA RIVER AT THORNBURY SAMPLING SITE. 77

FIGURE 37. LAND USE (IN HECTARES) IN THE CATCHMENT FOR THE APARIMA RIVER AT THORNBURY. 78
FIGURE 38. DAIRYING LAND (IN HECTARES) IN THE CATCHMENT FOR THE APARIMA RIVER AT THORNBURY, SEPARATED INTO PHYSIOGRAPHIC UNITS. 78
FIGURE 39. THE SAMPLING SITE AT POUROKINO RIVER AT TRAIL ROAD 80
FIGURE 40. LAND USE AND CONSENTS WITH POTENTIAL *E. COLI* CONTAMINATION RISK (NON-DAIRY) IN THE CATCHMENT FOR THE POURAKINO RIVER AT TRAILL ROAD SAMPLING SITE. 82
FIGURE 41. LAND USE (IN HECTARES) IN THE CATCHMENT FOR THE POURAKINO RIVER AT TRAILL ROAD. 83
FIGURE 42. DAIRYING LAND (IN HECTARES) IN THE CATCHMENT FOR THE POURAKINO RIVER AT TRAILL ROAD, SEPARATED INTO PHYSIOGRAPHIC UNITS. . 83
FIGURE 43. LAND USE AND CONSENTS WITH POTENTIAL *E. COLI* CONTAMINATION RISK (NON-DAIRY) IN THE CATCHMENT FOR THE CASCADE STREAM AT POURAKINO VALLEY ROAD SAMPLING SITE. 87
FIGURE 44. LAND USE (IN HECTARES) IN THE CATCHMENT FOR THE CASCADE STREAM AT POURAKINO VALLEY ROAD. 88
FIGURE 45. ENVIRONMENT SOUTHLAND STAFF CONDUCTING WATER SAMPLING IN THE OPOURIKI STREAM AT TWEEDIE RD. 89
FIGURE 46. LAND USE AND CONSENTS WITH POTENTIAL *E. COLI* CONTAMINATION RISK (NON-DAIRY) IN THE CATCHMENT FOR THE OPOURIKI STREAM AT TWEEDIE ROAD SAMPLING SITE. 92
FIGURE 47. LAND USE (IN HECTARES) IN THE CATCHMENT FOR THE OPOURIKI STREAM AT TWEEDIE ROAD. 93
FIGURE 48. DAIRYING LAND (IN HECTARES) IN THE CATCHMENT FOR THE OPOURIKI STREAM AT TWEEDIE ROAD, SEPARATED INTO PHYSIOGRAPHIC UNITS. 93
FIGURE 49. ENVIRONMENT SOUTHLAND STAFF COLLECTING WATER SAMPLES FROM THE WAIMATUKU STREAM AT LORNEVILLE-RIVERTON HIGHWAY. 95
FIGURE 50. LAND USE AND CONSENTS WITH POTENTIAL *E. COLI* CONTAMINATION RISK (NON-DAIRY) IN THE CATCHMENT FOR THE WAIMATUKU STREAM AT LORNEVILLE-RIVERTON HIGHWAY SAMPLING SITE. 98
FIGURE 51. LAND USE (IN HECTARES) IN THE CATCHMENT FOR THE WAIMATUKU STREAM AT LORNEVILLE-RIVERTON HIGHWAY. 99
FIGURE 52. DAIRYING LAND (IN HECTARES) IN THE CATCHMENT FOR THE WAIMATUKU STREAM AT LORNEVILLE-RIVERTON HIGHWAY, SEPARATED INTO PHYSIOGRAPHIC UNITS. 99
FIGURE 53. LAND USE AND CONSENTS WITH POTENTIAL *E. COLI* CONTAMINATION RISK (NON-DAIRY) IN THE CATCHMENT FOR THE HAMILTON BURN AT AFFLECK ROAD SAMPLING SITE. 103
FIGURE 54. LAND USE (IN HECTARES) IN THE CATCHMENT FOR HAMILTON BURN AT AFFLECK ROAD. 104
FIGURE 55. DAIRYING LAND (IN HECTARES) IN THE CATCHMENT FOR HAMILTON BURN AT AFFLECK ROAD, SEPARATED INTO PHYSIOGRAPHIC UNITS. 104
FIGURE 56. WAIRIO STREAM SAMPLING SITES USED IN THE DETAILED OTUATUA STREAM STUDY. A) WAIRIO STREAM AT 20 BIRCHWOOD-WAIRIRIO ROAD; B) WAIRIO

STREAM AT 55 COLLIE ROAD; C) WAIRIO STREAM AT WAICOLO ROAD EAST; D) WAIRIO STREAM AT 100 LINE RD WEST 1050-1076. 106

FIGURE 57. ESR STAFF CARRYING OUT SAMPLING AT NORTH HEAD STREAM SITES: A) 267 WAICOLA ROAD; B) 100 LINE RD WEST 1015-1047. 109

FIGURE 58. SITES ALONG THE OTAUTAU STREAM SAMPLED AS PART OF THE DETAILED STUDY: A) OTAUTAU AT 100 LINE ROAD 1248-1346; B) 99 UPPER SCOTT'S GAP UPPER; C) 122 UPPER SCOTT'S GAP/SYMONS ROAD; D) 232-402 LOWER SCOTT'S GAP/SYMONS ROAD..... 112

FIGURE 59. OTAUTAU STREAM AND WAIRIO STREAM CONFLUENCE AT WAIKOURO SHORTCUT. 114

FIGURE 60. SAMPLING SITES ON WAICOLO STREAM AT: A) 100 LINE RD EAST; B) WAICOLO STREAM AT WAIKOURO. 116

FIGURE 61. SAMPLE COLLECTION FROM THE OPIO STREAM AT OTAUTAU NIGHTCAPS. 118

FIGURE 62. NORTH FERN BURN SAMPLING SITES AT: A) FORESTRY ROAD; AND B) NORTH-FERN BURN AT OTAUTAU-TUATAPERE ROAD. 120

FIGURE 63. SOUTH FERN BURN AT OTAUTAU-TUATAPERE ROAD..... 120

FIGURE 64. INVESTIGATION SAMPLE SITES ON THE OTAUTAU PLAINS, WITH RIVER ORDERS 4-7 SHOWN. 123

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 contamination, and thus of 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 sources of faecal pollution at 9 freshwater sites within the Aparima Freshwater Management Unit (FMU) in Southland, between December 2014 and September 2015. 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. In addition to the main study, an investigation of sites in the Otautau Plains was undertaken to understand the impact of upstream contamination sources. Samples were collected from 18 sites along the tributaries of the Wairio, North Head, Otautau, Waicolo and Opio Streams, and North and South Fern Burn. Samples collected under base flow conditions in March 2015, and were analysed in the same way as samples from the main study (i.e. *E. coli*, *Campylobacter*, FST markers).

The sites sampled within the main Aparima study were vulnerable to high levels of faecal contamination, with 8 of the 9 sites recording *E. coli* concentrations of ≥1,000 colony-forming units (cfu) per 100 ml of water on at least one occasion. Forty-nine percent of individual water samples collected exceeded 1,000 cfu/100 ml, with a maximum concentration 19,000 cfu/100 ml recorded at the Otautau Stream at Waikouro. 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. Ruminant animals (both sheep and cattle) and wildfowl were important sources of pollution in these waterways, and sites were often impacted by multiple sources (e.g. Figure 1). Both wildfowl and ruminant faecal signatures were detected under

both base flow and high flow conditions; however, wildfowl pollution appeared to be 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/or effluent discharge during base flow conditions, with additional inputs via overland flow and artificial tile drains following rainfall. Human faecal pollution was detected at four sites. The specific sources or routes of transmission of this contamination could not be identified, and further investigation is strongly recommended to isolate these.

Campylobacter was detected in 80% of samples, with detections at 7 of the 9 sites. Ninety-four percent of *Campylobacter*-positive samples contained *C. jejuni*, with an unspeciated thermophilic *Campylobacter* and C. coli recovered from 27% and 3% of samples, respectively. *Campylobacter* was present at a similar frequency and at similar concentrations under both base flow and high flow conditions. Wildfowl were determined to be the most common source of *Campylobacter*, followed by those of unknown origin, ruminants (sheep, cattle or deer) and 'not wildfowl.'

The Otautau Plains study also identified variation in microbial burdens between sites, with *E. coli* concentrations ranging from 100 to 7,000 cfu/100 ml, and *Campylobacter* present at 14 of the 18 sites. Ruminant animals (sheep and cattle) and wildfowl were the main sources of faecal pollution, however human faecal signatures were detected at two sites along the Wairio Stream. *Campylobacter* from these sites was also most commonly *C. jejuni* of wildfowl origin, with single isolates of ruminant, poultry and 'not wildfowl' identified. Spatially, the study found that lower *E. coli* and *Campylobacter* concentrations tended to be observed at sites in the upper reaches of tributary waterways, with a progressive increase in concentration as sampling moves downstream. However, some sites (e.g. Wairio Stream at Birchwood Road) may have high levels of local pollution compared with downstream sites, which benefit from the dilution effects of other tributaries. Sites in the upper reaches are also less likely to be impacted by wildfowl pollution,

Molecular MBiT analysis of *Campylobacter* isolates revealed a high diversity of genotypes across the Aparima FMU, and that there was no separation of these to particular sites. Thirty-one percent of the isolates obtained from waters in the Aparima 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. One quarter 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) 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 Aparima FMU, Southland.

2. MATERIALS AND METHODS

2.1 SAMPLING SITES

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

In addition, a detailed study was carried out in the Otautau Plains in an effort to understand the impacts of upstream pollution sources. Samples were collected from a total of 19 sites that ultimately feed into the Otautau Stream, as detailed in Table 2 and Figure 3.

The results described in this report relate to samples collected both as a part of a monthly sampling regime by ES staff, and during targeted sampling events by both ESR and ES staff.

Table 1. Sampling sites selected for the Aparima 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
Otautau Stream at Waikouro	Base-flow and rainfall	Appendix B.1
Otautau Stream at Otautau-Tuatapere Road	Base-flow and rainfall	Appendix B.2
Aparima River at Dunrobin	Rainfall only	Appendix B.3
Aparima River at Thornbury	Base-flow and rainfall	Appendix B.4
Pourakino River at Traill Road	Base-flow and rainfall	Appendix B.5
Cascade Stream at Pourakino Road	Rainfall only	Appendix B.6
Opoutiki Stream at Tweedie Road	Base-flow and rainfall	Appendix B.7
Waimatuku Stream at Lorneville-Riverton Highway	Base-flow and rainfall	Appendix B.8
Hamilton Burn at Affleck Road	Base-flow and rainfall	Appendix B.9

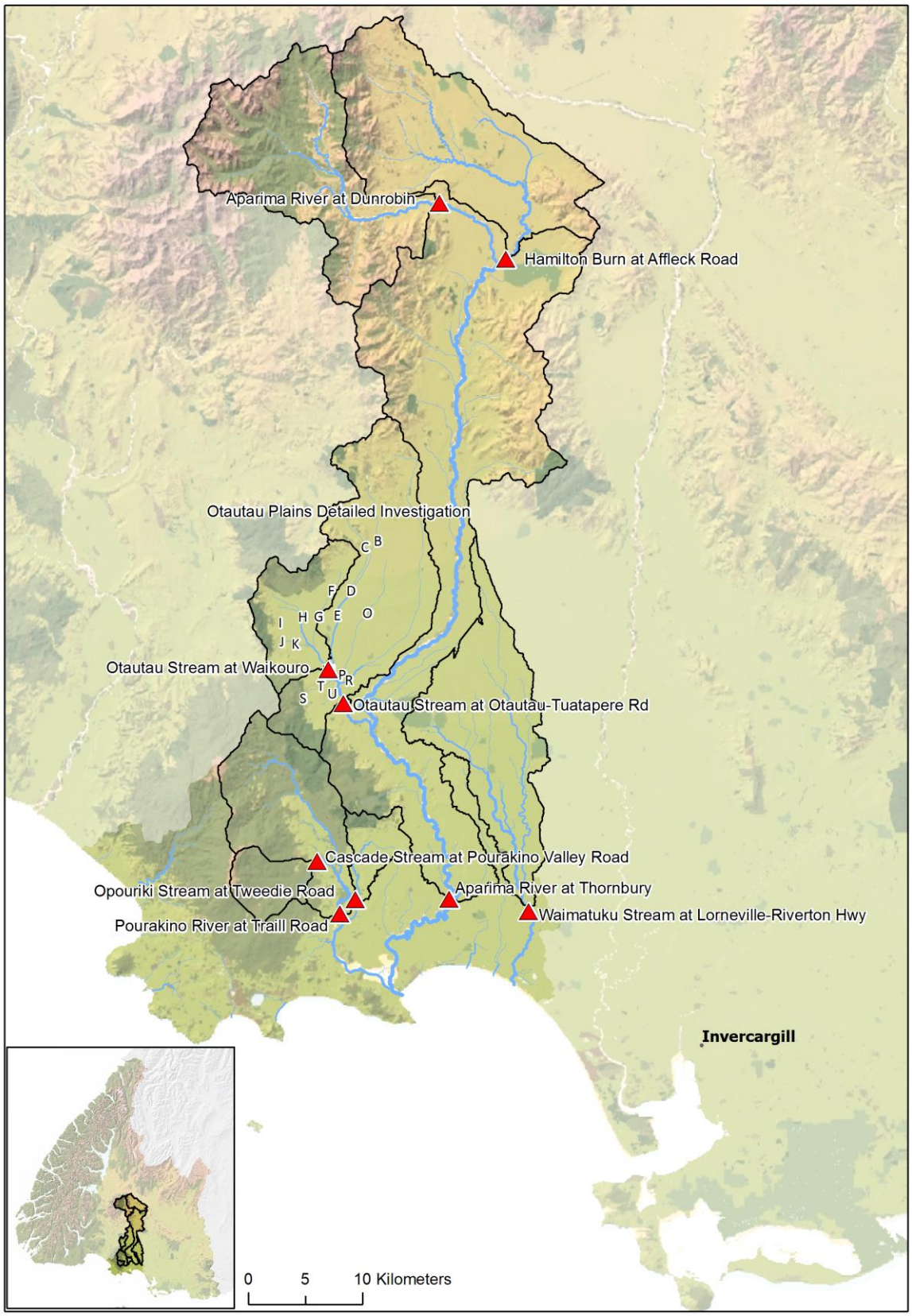


Figure 2. The Aparima FMU, with sub-catchments, sampling site locations and rivers of order 4 to 8 shown. Inset: The Aparima FMU within the wider Southland region.

Table 2. Sampling sites selected for the detailed Otautau Plains investigation, with the conditions (i.e. base-flow or post-rainfall) each site was sampled under.

Site	Number of sites	Indicative location (Figure 3)	Sampling conditions	Detailed sub-catchment and microbial water quality descriptions
Wairio Stream, upstream of Waikouro Shortcut	4	B, C, D, E.	Base-flow only	Appendix B.10.1
North Head Stream, upstream of Waikouro Shortcut	2	F, G	Base-flow only	Appendix B.10.2
Otautau Stream, upstream of Waikouro Shortcut	4	H, I, J, K	Base-flow only	Appendix B.10.2
Wairio Stream, immediately upstream of Waikouro Shortcut	1	L	Base-flow only	Appendix B.10.3
Otautau Stream, immediately upstream of Waikouro Shortcut	1	M[1]	Base-flow only	Appendix B.10.3
Waicolo Stream	2	O, P	Base-flow only	Appendix B.10.4
Opio Stream	1	R	Base-flow only	Appendix B.10.5
North Fern Burn	2	ST	Base-flow only	Appendix B.10.6
South Fern Burn	1	U	Base-flow only	Appendix B.10.6
Otautau Stream at Otautau-Tuatapere Road	1	W	Base-flow only	Appendix B.2

[1] Represents the combined inputs of both Otautau and North Head Streams

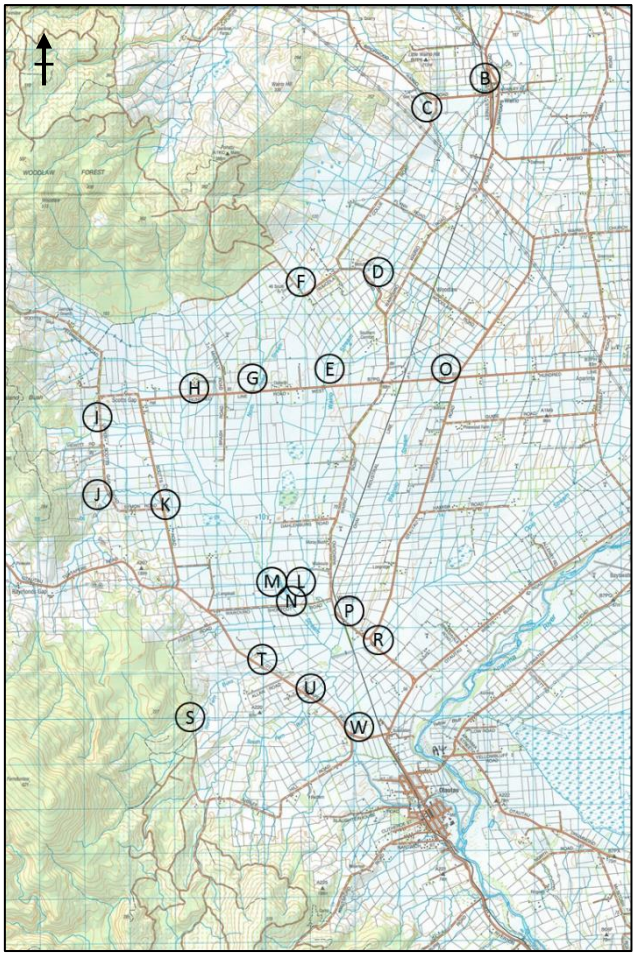


Figure 3. Map of the upstream sites tested as part of the detailed investigation of the effects pollution sources on the Otautau Stream. Scale: 1 square = 1 km.

2.2 MICROBIOLOGICAL ANALYSIS

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

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

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

2.2.1 Coliform and *E. coli* analysis

Faecal coliforms were analysed using membrane filtration with incubation on mFC agar for 22 hours at 44.5°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).

2.2.2 *Campylobacter* isolation

Campylobacter spp. were enumerated using a 3 x 5 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°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

Detailed microbiological results for each site are presented in Appendix B. The overview below includes a general statement that incorporates all data collected from across the Aparima FMU. The results are then discussed for those samples that formed that main study and are regular sampling locations for ES. Results of the detailed Otautau Plains study are then discussed.

3.1 OVERVIEW OF MICROBIAL WATER QUALITY IN THE APARIMA FMU

3.1.1 General comment on microbial quality of all water samples collected

Microbial water quality within the Aparima FMU was highly varied, with *E. coli* concentrations ranging between 100 and 19,000 cfu/100 ml. A large number of sampling locations were vulnerable to high levels of microbial contamination, with 17 of the 27 sites having *E. coli* concentrations exceeding 1,000 cfu/100 ml on at least one occasion. In total, 46% of individual samples collected within the Aparima FMU had an *E. coli* concentration of 1,000 cfu/100 ml or more. The highest *E. coli* concentrations were observed at the Otautau Stream at Waikouro (19,000 and 15,000 cfu/100 ml), Opouriki Stream at Tweedie Road (16,000 cfu/100 m), Otautau Stream at Otautau-Tuatapere Road (8,000 cfu/100 ml) and the Wairio Stream at Birchwood-Wairio Road (7,000 cfu/100 ml) (Figure 4, Figure 5).

Campylobacter was detected at 19 of the 27 sites (70%), and in 79% of water all samples collected within the Aparima FMU. A third of the *Campylobacter*-positive samples contained a concentration of 10 MPN/100 ml of greater, with three samples containing ≥1,100 MPN/100 ml. Ninety-four percent of the *Campylobacter*-positive samples contained *C. jejuni*; *C. coli* and an unspeciated thermophilic *Campylobacter* were also isolated.

3.1.2 Microbial quality of water samples collected for main Aparima study

E. coli concentrations at the nine main study sites ranged from 130 cfu/100 ml to 19,000 cfu/100 ml. Four sites had a median *E. coli* concentration exceeding 550 cfu/100 ml, and two were sampled on only a single occasion, so a median value could not be determined. Samples that were collected following rainfall had higher *E. coli* concentrations than those collected under base follow conditions (Figure 6, Figure 7). There was no discernible seasonal pattern in *E. coli* concentration (Figure 8).

Campylobacter was detected in 80% of samples, and at 7 of the 9 sites. The two exceptions were the Aparima River at Dunrobin and the Cascade Stream at Pourakino Valley Road, each of which were sampled on only a single location. The highest *Campylobacter* concentrations were observed at the Otautau Stream at Otautau-Tuatapere Road (≥1,100 MPN/100 ml,

twice[1]) and at Waikouro (1,100 and 240 MPN/100 ml). *Campylobacter jejuni* was present in 94% of *Campylobacter*-positive samples, with 24% containing both *C. jejuni* and an unspeciated *Campylobacter*. A single sample contained both *C. jejuni* and *C. coli*. An unspeciated thermophilic *Campylobacter* alone was detected in 6% of samples.

The presence of *Campylobacter* did not appear to be influenced by rainfall, being detected in 83% of base flow and 78% of high flow samples (Figure 9). *Campylobacter* concentrations also tended to be similar under both conditions (Figures 4-5, Figures 9-10), although it is notable that the three highest *Campylobacter* concentrations were observed under base flow conditions (Figure 10). The prevalence of the different *Campylobacter* species was similar between base flow and high flow samples (Figure 11). Similarly to *E. coli*, there was no discernible seasonal pattern in *Campylobacter* concentration (Figure 12).

An examination of the relationship between *E. coli* and *Campylobacter* for all samples collected within the Aparima FMU (including the Otautau Plains) reveals a significant positive correlation of data (Spearman rank correlation, r = 0.2932, df = 61, p = 0.0197; Figure 13); thus samples with high levels of *E. coli* were more likely to contain high levels of *Campylobacter*.

[1]Both of these samples were collected on the same day, although at different times. One sample was collected by ES as a part of their routine sampling, and another was collected by ESR as a part of the detailed Otautau Plains study.

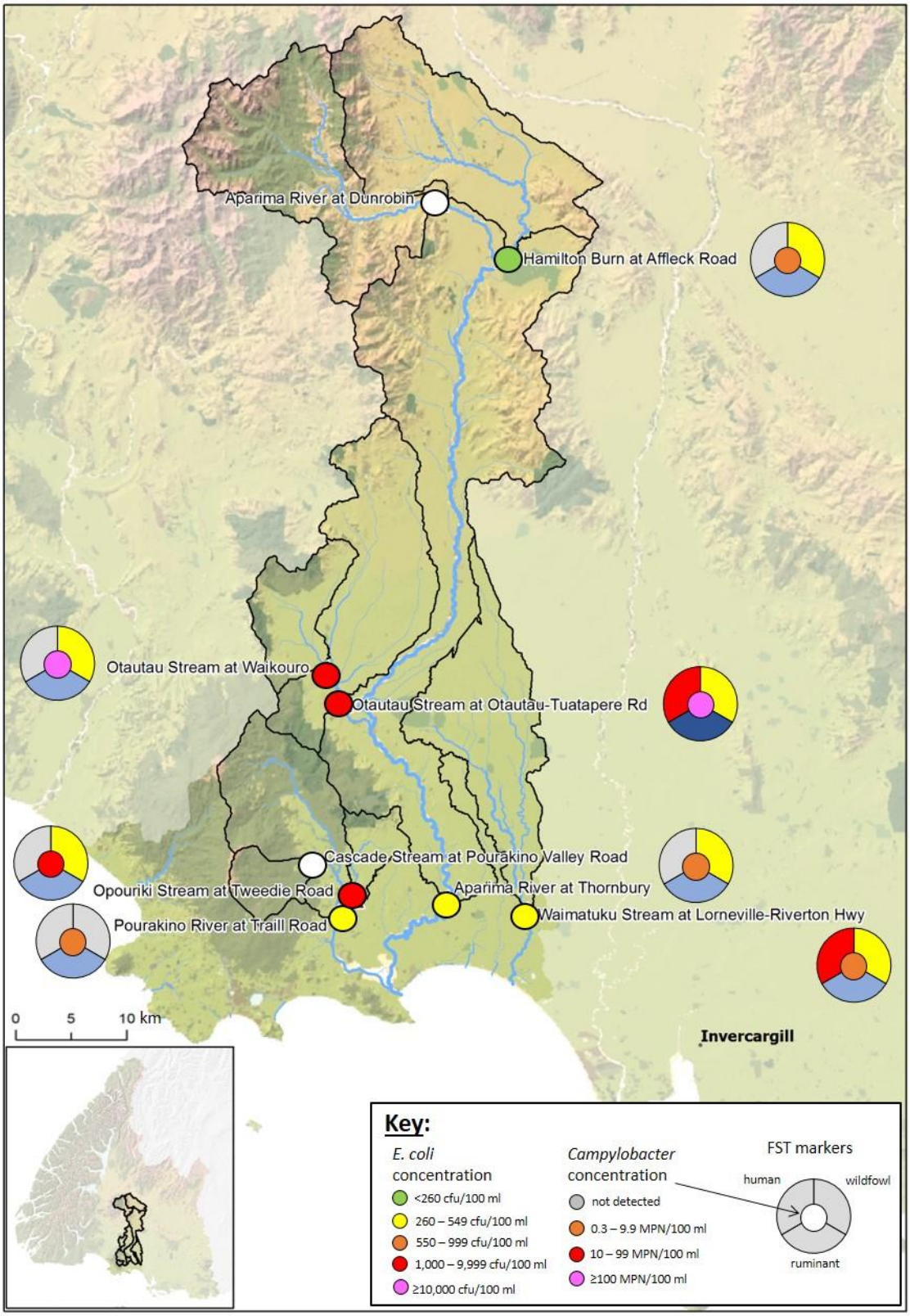


Figure 4. Overview of microbial water quality in Aparima FMU under base flow conditions. Small circles showing sampling locations on the map represent maximum *E. coli* levels for that site; white circles indicate there is no data under these conditions. Larger circles adjacent to site name represent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

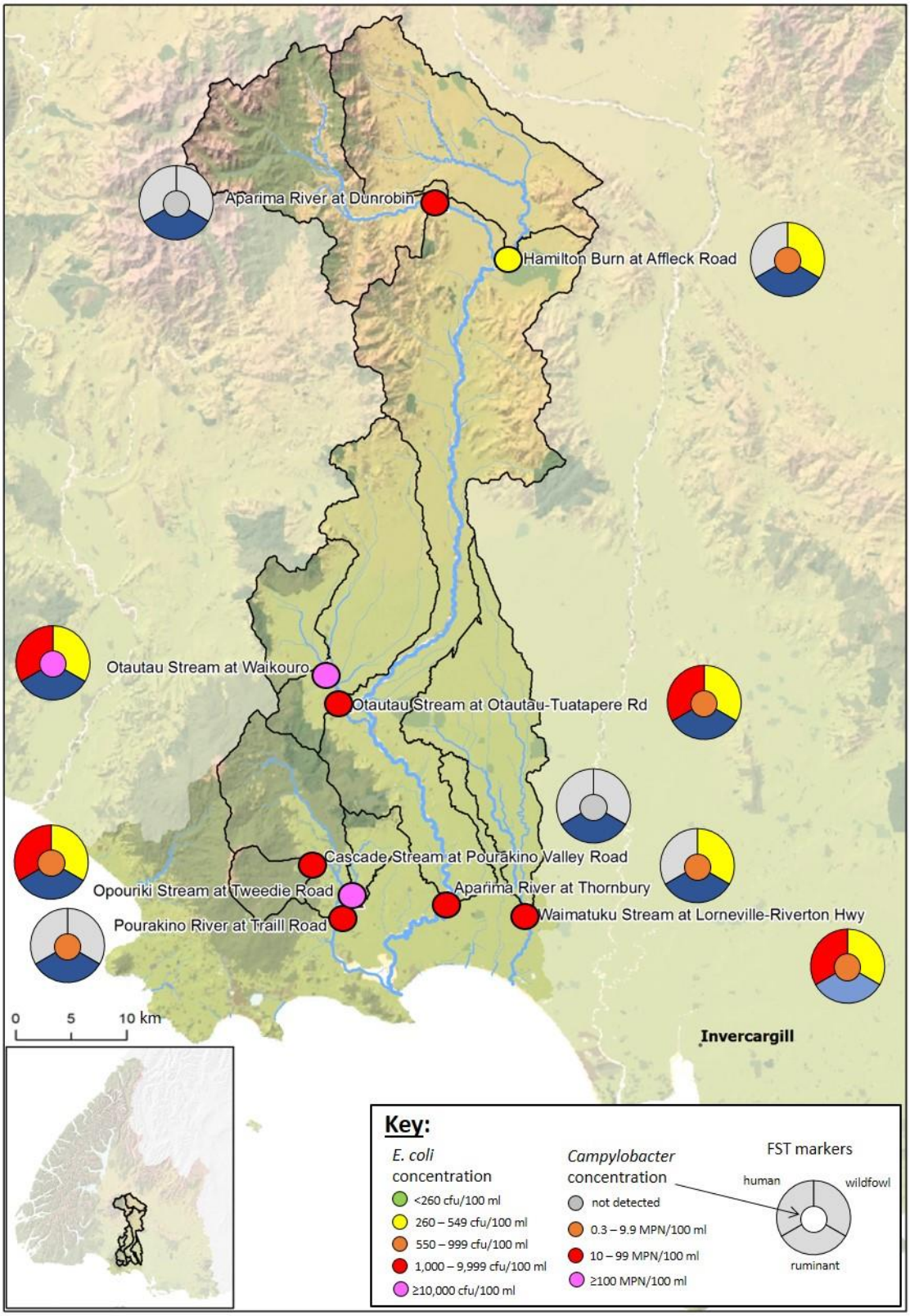


Figure 5. Overview of microbial water quality in Aparima FMU following rainfall. Small circles showing sampling locations on the map represent maximum *E. coli* levels for that site; white circles indicate there is no data under these conditions. Larger circles adjacent to site name represent maximum *Campylobacter* concentration and overall presence/absence of FST markers for that site.

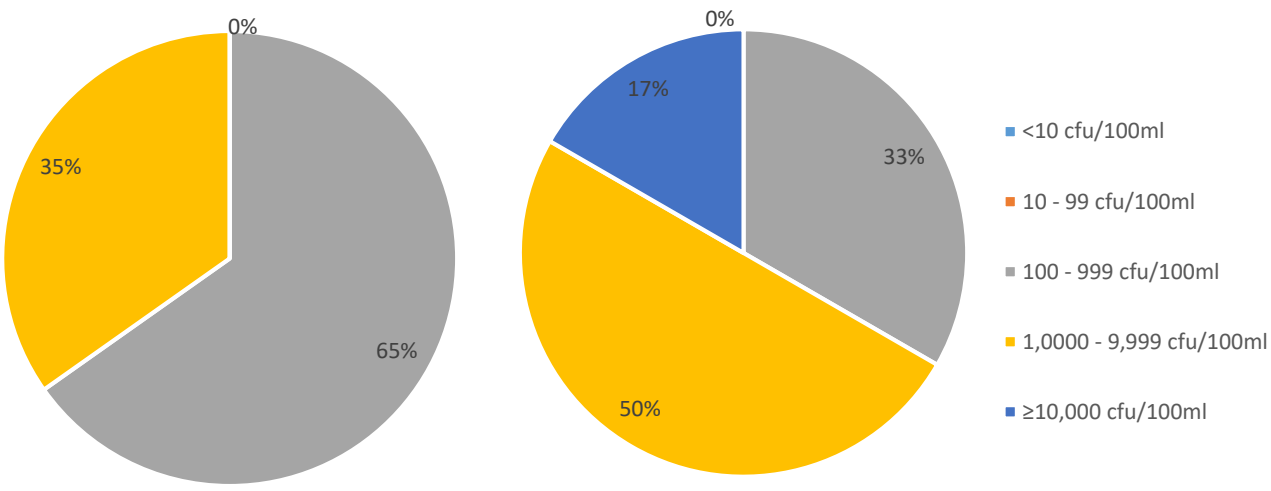


Figure 6. *E. coli* concentrations for water samples collected from the main Aparima study sites under base flow conditions (left, n=23) and following rainfall (right, n=18).

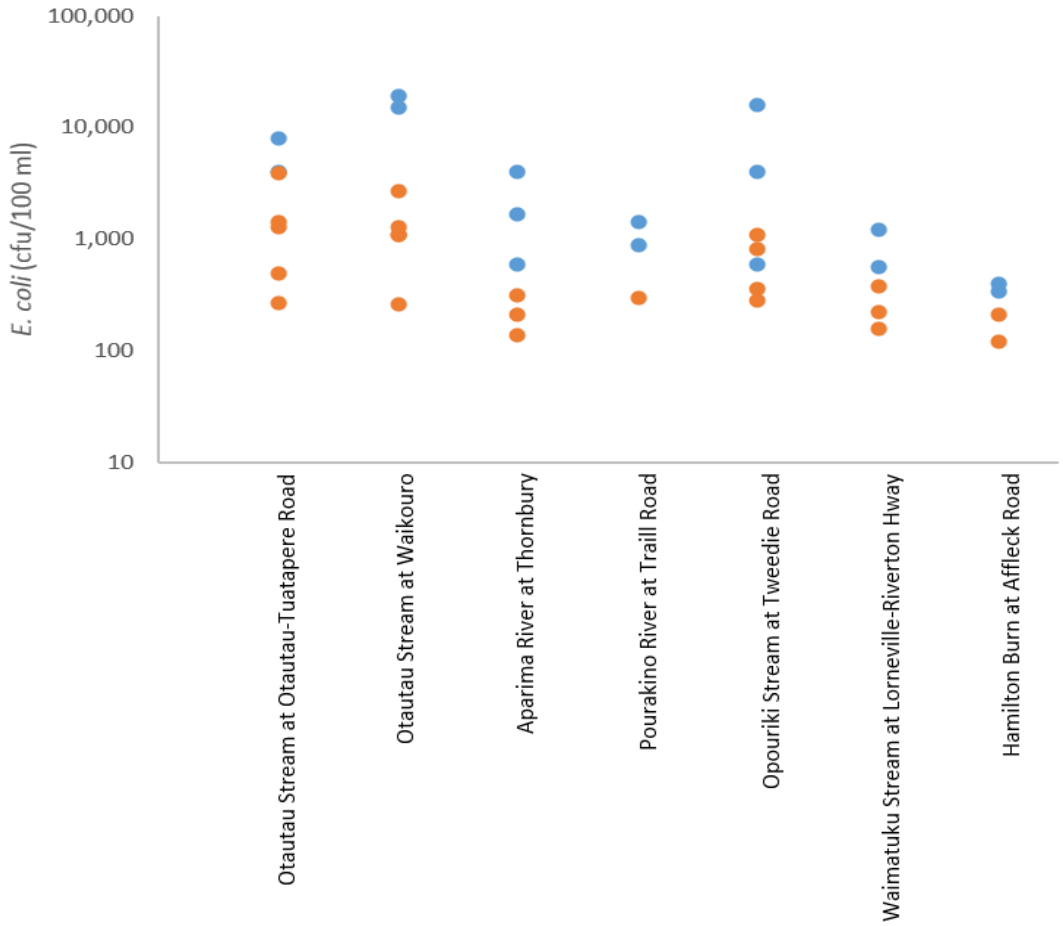


Figure 7. *E. coli* concentrations at sites for which water samples were collected under both base flow (orange) and high flow (blue) conditions.

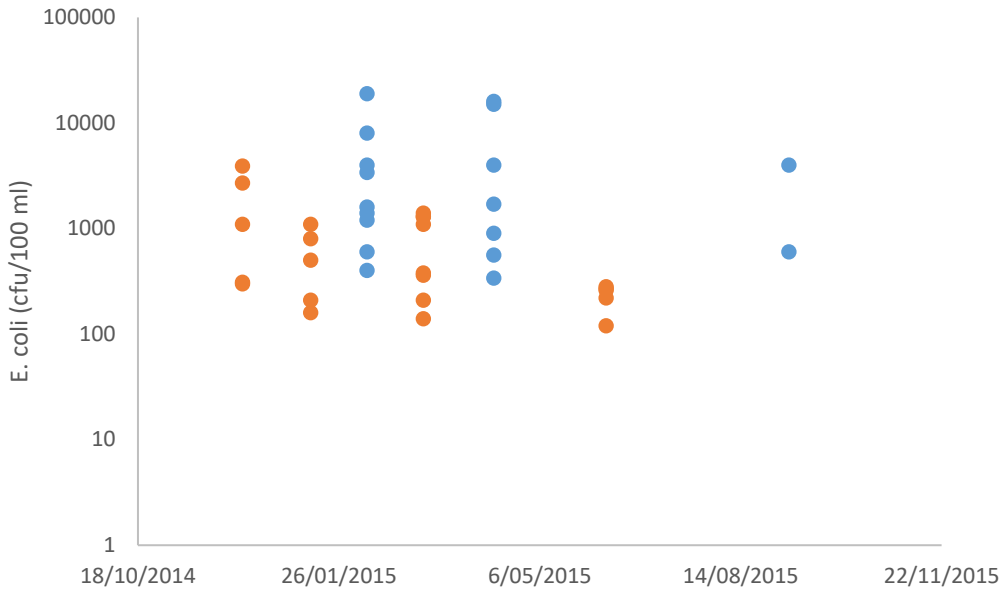


Figure 8. Concentration of *E. coli* at the main study sites in the Aparima FMU, across the course of the year. Samples collected following rainfall are shown in blue, and those collected under base flow conditions are in orange.

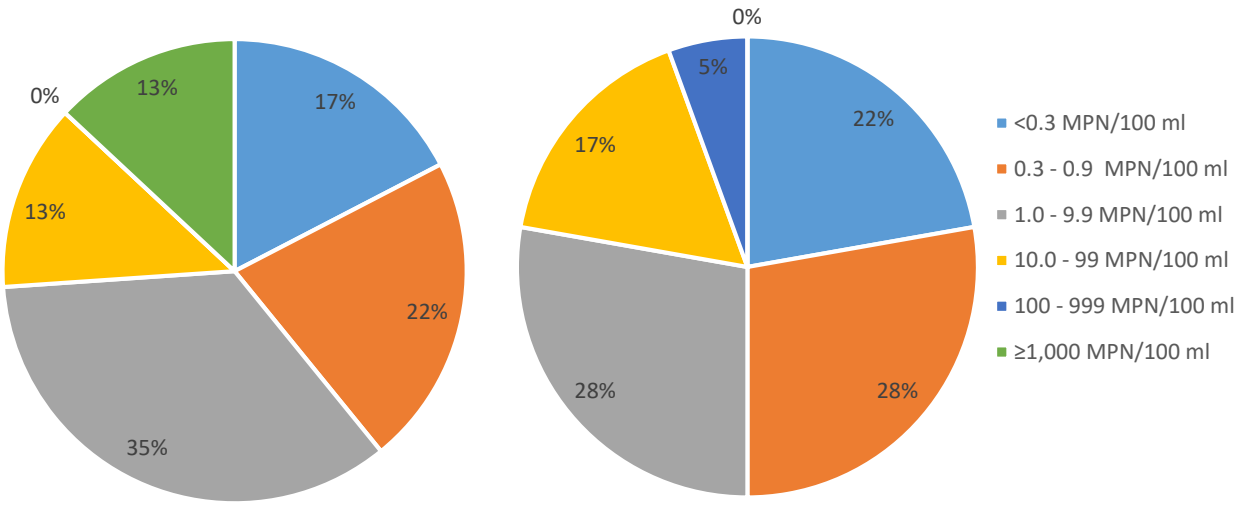


Figure 9. *Campylobacter* concentrations for water samples collected from the main Aparima study sites under base flow conditions (left, n=23) and following rainfall (right, n=18).

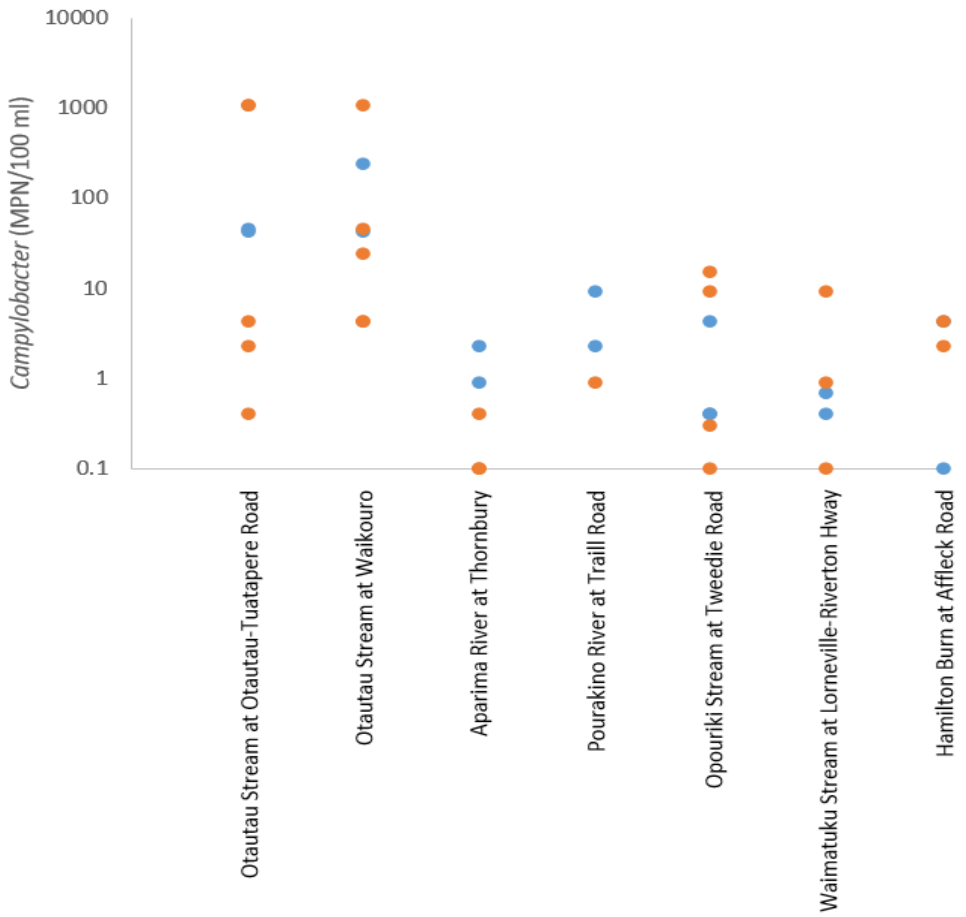


Figure 10. *Campylobacter* concentrations at sites for which water samples were collected under both base flow (orange) and high flow (blue) conditions.

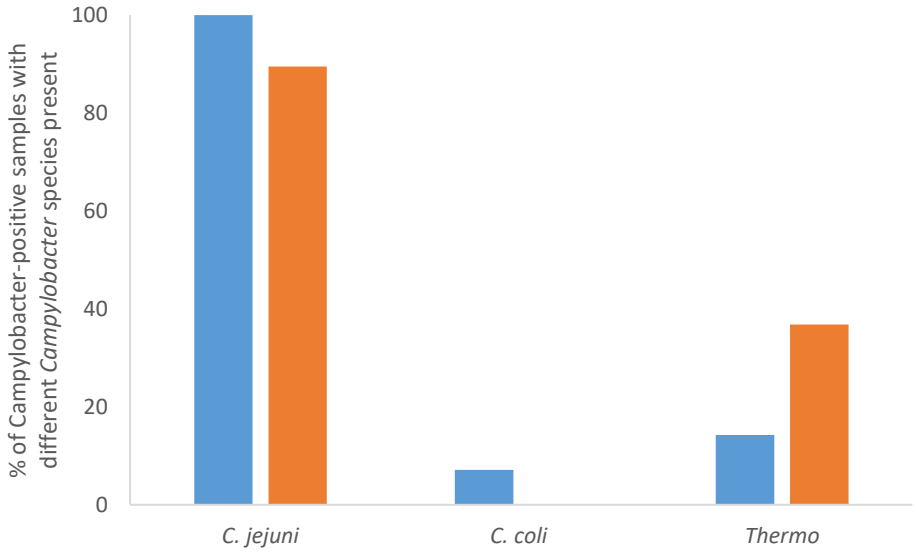


Figure 11. The prevalence of different *Campylobacter* species within *Campylobacter*-positive samples from the main Aparima study sites (n=33). Blue bars represent samples collected following rainfall, and orange bars represent samples collected under base flow.

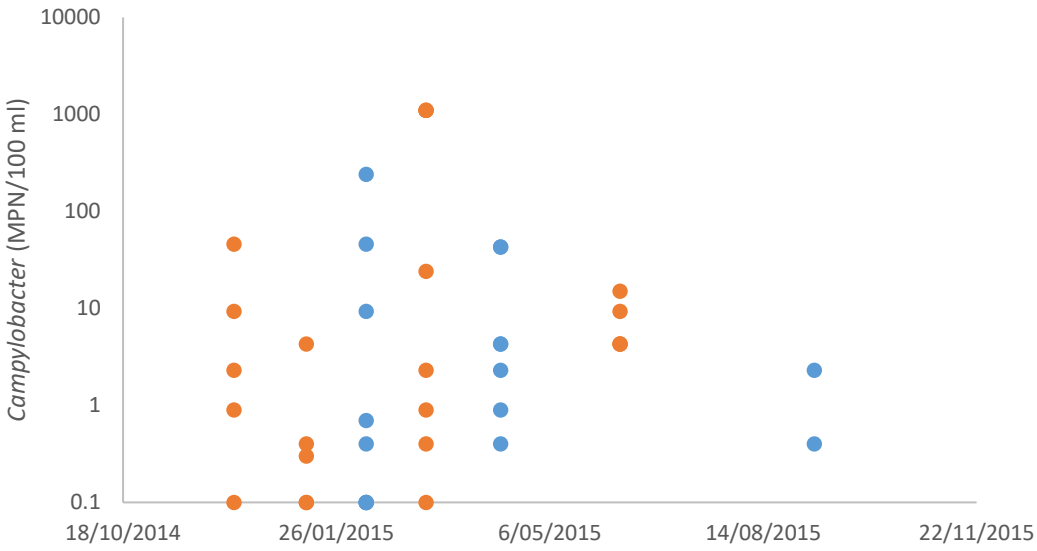


Figure 12. Concentration of *Campylobacter* at the main study sites in the Aparima FMU, across the course of the year. Samples collected following rainfall are shown in blue, and those collected under base flow conditions are in orange.

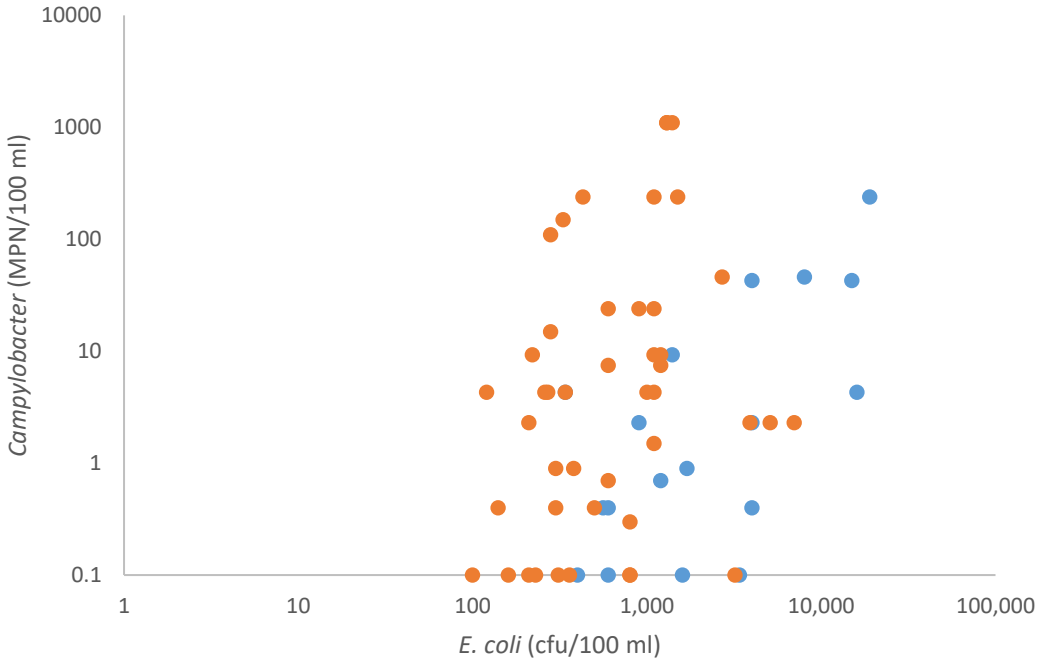


Figure 13. Relationship between *E. coli* and *Campylobacter* spp. concentrations in all water samples collected within the Aparima FMU (n=63). For display purposes, samples collected following rainfall are shown in blue, and those collected under base flow conditions in orange.

3.2 SOURCES OF FAECAL POLLUTION

Faecal source tracking analysis detected ruminant animal pollution at all nine of the sites comprising the main Aparima study (Figure 4, Figure 5). Ovine-specific FST markers were detected at 8 sites, with bovine markers detected at 4 sites. The relative impact of ruminant sources was found to increase following rainfall (Figures 4-5, Figure 14). For example, in 56% of the samples collected under base flow, ruminant pollution accounted for ≤10% of the total faecal pollution present. In contrast, 72% of the samples collected following rainfall were dominated by ruminant pollution (i.e. ruminant pollution accounted for 50-100% of all pollution). Fifty percent of the samples collected following rainfall had a bovine-specific faecal signature identified, compared with 13% of base flow samples. The presence of ovine contamination was similar for both high and base flow conditions, at 44% and 48%, respectively (Figure 15).

Wildfowl faecal pollution was detected at six sites. At sites where a wildfowl signature was identified, it was ubiquitous – five of these sites had a wildfowl signature present in every sample collected there, whilst the sixth site had a wildfowl signature present in four of six samples.

Human faecal pollution was detected in six samples across four sites – the Otautau Stream at Otautau-Tuatapere Road and at Waikouro, the Opouriki Stream at Tweedie Road, and the Waimatuku Stream at the Lorneville-Riverton Highway. Two samples were collected under base flow and four following rainfall (Figure 15).

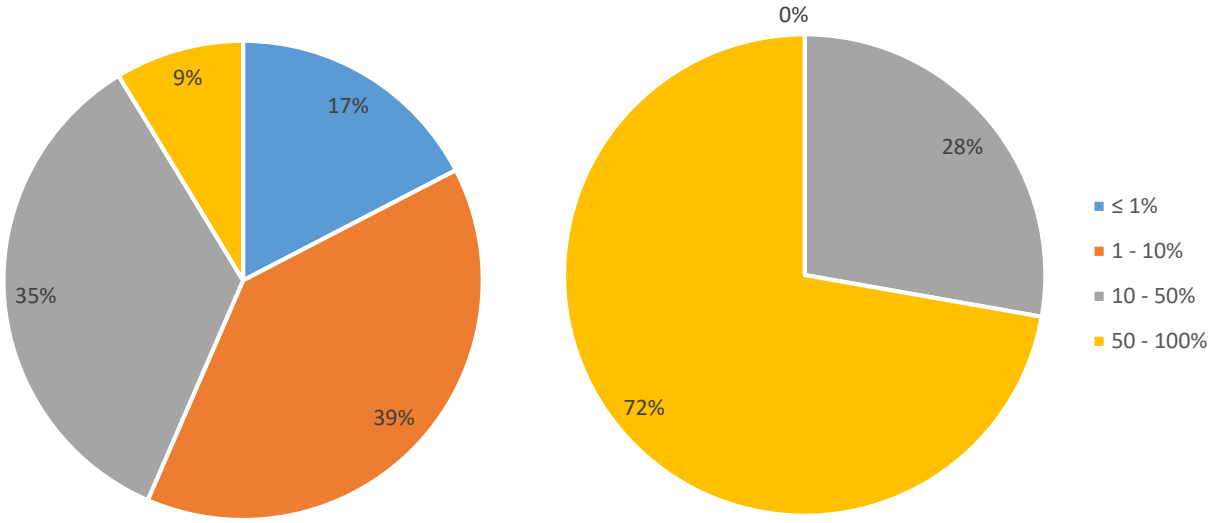


Figure 14. The proportion of samples collected under base flow conditions (left) and following rainfall (right), that were affected by different levels of ruminant faecal pollution.

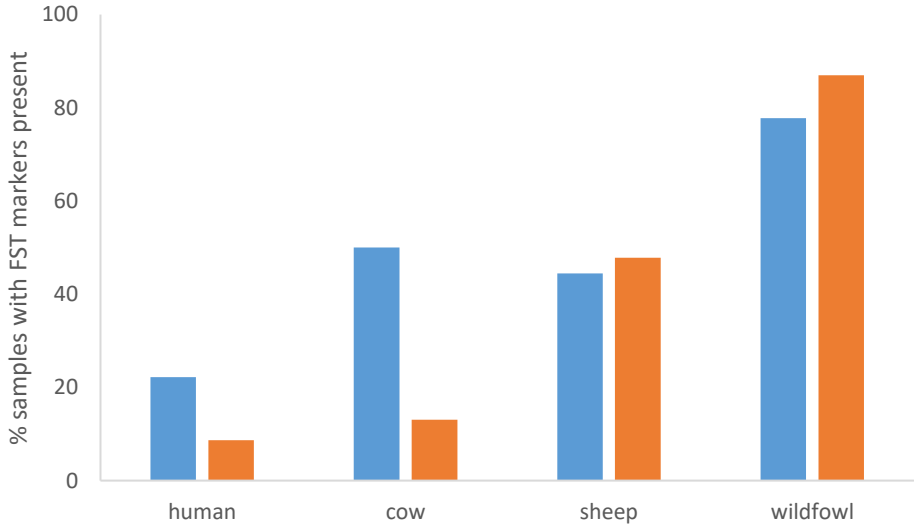


Figure 15. The percentage of samples collected from within the Aparima FMU that were positive for FST markers from different sources. Samples that were collected following rainfall are shown in blue, and those collected under base flow in orange.

3.3 OTAUTAU PLAINS STUDY

Eighteen sites were sampled as part of the Otautau Plains study. Each of these sites had a water sample collected on 9 March 2015. Four sites also had water samples collected on 9 December 2014. All samples were collected under base flow conditions.

Microbial water quality across the Otautau Plains was variable, with *E. coli* concentrations ranging between 100 and 7,000 cfu/100 ml. *Campylobacter* was detected in 77% of samples, representing 14 sites. The highest concentration of *Campylobacter* was 240 MPN/100 ml, observed at the Opio Stream at Otautau Nightcaps, and both the Wairio and Otautau Streams upstream of their confluence with each other. All but one of these isolates was identified as *C. jejuni*; the other was an unspeciated thermophilic *Campylobacter*.

Ruminant faecal pollution was detected at all sites, although the relative impacts were highly variable between sites. For example, ruminant pollution was estimated to account for less than 1% of the overall faecal load at five sites, and up to 100% of the faecal load at four others. Ovine-specific markers were identified at 5 sites and bovine markers at 4 sites. Wildfowl pollution was widespread, being detected at 14 sites. Human faecal contamination was detected in samples collected from two sites in the Wairio Stream (20 Birchwood-Wairirio Road and upstream confluence with Otautau Stream).

When microbial concentrations and faecal source data are overlain with a map of the Otautau Plains study area, several high-level trends become apparent (Figure 16, Figure 17). Firstly, *E. coli* concentrations tend to be lowest in the upper reaches of tributary streams, such as the Wairio, Waicolo, and North Head streams, and North Fern Burn. However, this is not universally true - some upstream or tributary sites (e.g. one tributary of the Otautau Stream and one of the Wairio Stream) do have high microbial loads. As the sampling moves downstream of the respective waterways, the *E. coli* concentrations tend to increase, until the highest concentrations are observed in the Otautau Stream or tributaries (especially the Waicolo and Opio streams) shortly prior to the point at which they drain into the Otautau.

A similar pattern is observed with *Campylobacter*. Concentrations at the upstream sites are either very low or below detection limits. As sampling progresses downstream, *Campylobacter* concentrations increase, with the highest concentrations present in the Otautau Stream and several tributaries just prior to their confluence with it.

Analysis of FST markers again demonstrates the presence of ruminant pollution at each of the sites. There is no specific pattern as to the locations of sites that are more or less heavily dominated by ruminant pollution. For example, four sites are dominated by ruminant pollution, and while they are all located in tributary streams, they represent both upper and lower reaches, and do not necessarily relate to those with the highest *E. coli* and/or *Campylobacter*. The three sites that are not impacted by wildfowl pollution are located upstream. Interestingly, they also represent three or the four sites at which *Campylobacter* was not detected. This suggest that where wildfowl contamination is present, there is also a high likelihood of *Campylobacter* being present.

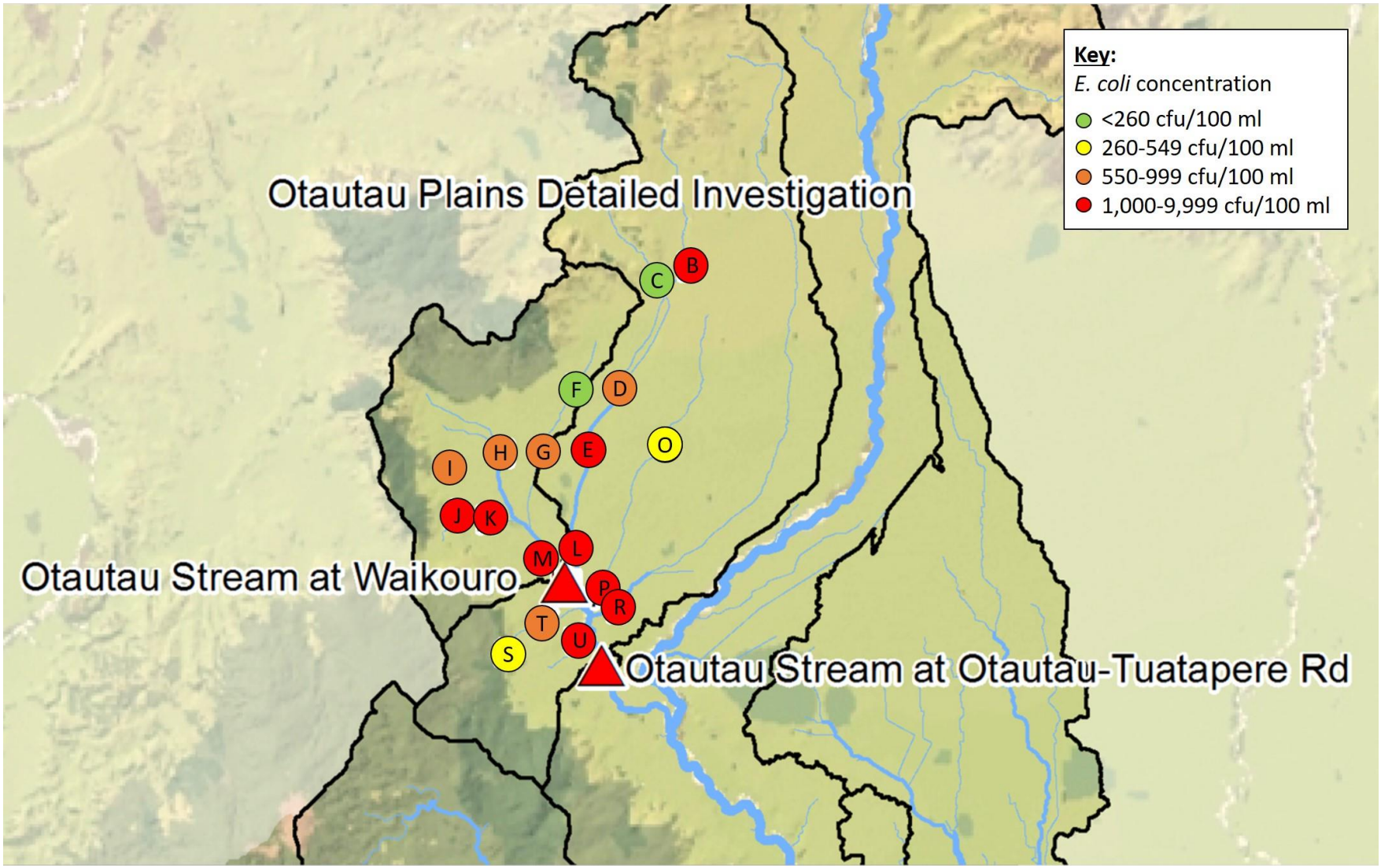


Figure 16. Overview of *E. coli* concentrations at the sites sampled as part of the detailed Otautau Plains investigation. For each site, the *E. coli* concentration is indicated by the coloured circle. The letters within each circle identify the site, as per Table 2 and Figure 3.

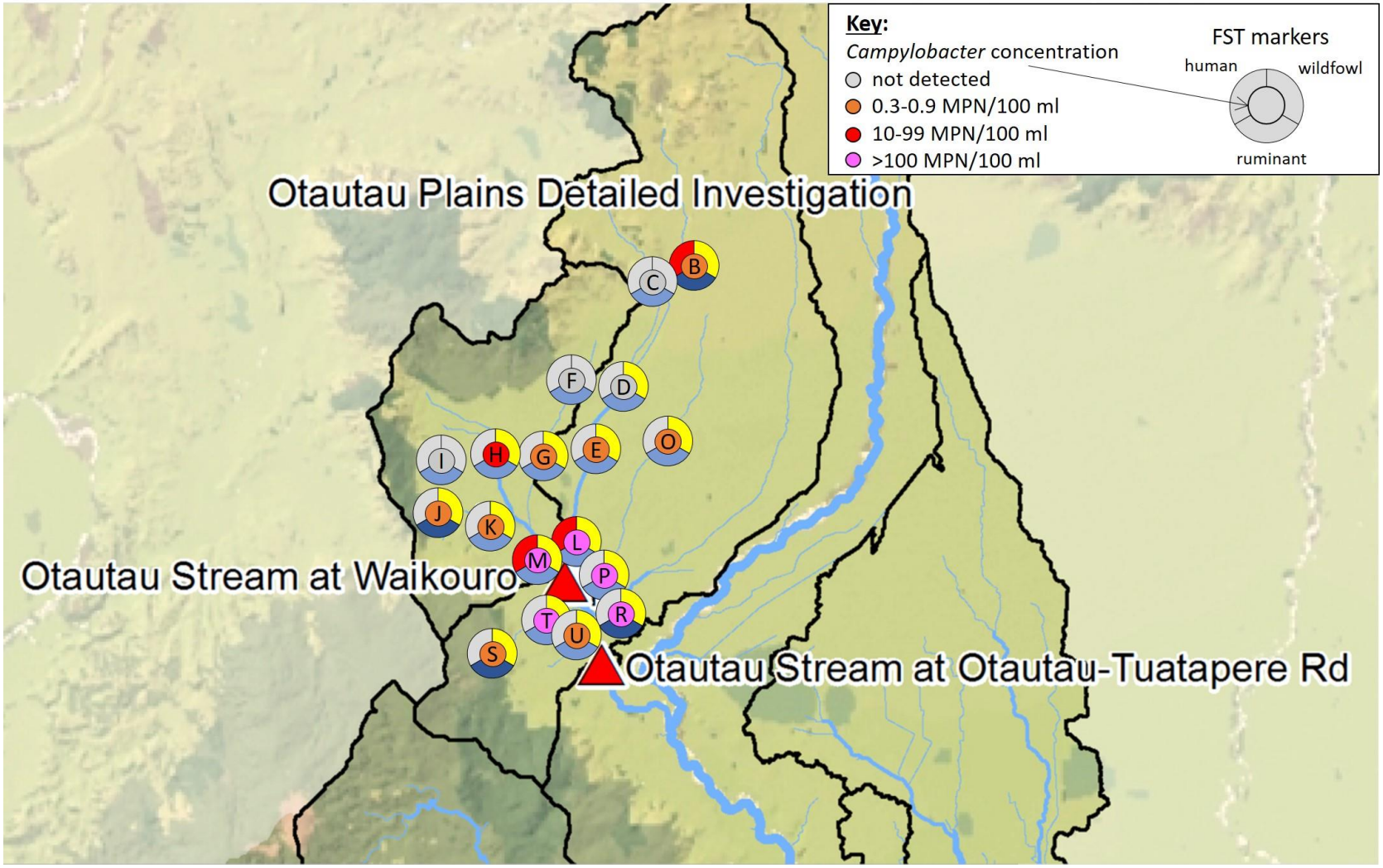


Figure 17. Overview of *Campylobacter* concentrations and the presence of FST markers at the sites sampled as part of the detailed Otautau Plains investigation. For each site, the *Campylobacter* concentration recorded is indicated by the coloured central circle. The presence or absence of specific FST markers at a site is indicated in the peripheral thirds of each circle, as per Figures 4 and 5. The letters within each circle identify the site, as per Table 2 and Figure 3.

3.4 CHARACTERISATION OF *CAMPYLOBACTER* IN THE APARIMA FMU

3.4.1 MBiT source attribution

MBiT source attribution analysis found that the *Campylobacter* isolated from the various sites across the Aparima FMU were of wildfowl, ruminant (ovine, bovine and/or deer), poultry and unknown sources. Several sites were found to have *Campylobacter* from more than one source.

At the main study sites, wildfowl were the most common source of *Campylobacter*, with 64% of *Campylobacter*-positive samples being positive for a wildfowl strain, followed by unknown sources (33%), ovine/bovine/deer (15%) and 'not wildfowl' (9%). No poultry-associated *Campylobacter* was isolated from these sites. 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. *Campylobacter* isolates of unknown origin were twice as common under base flow conditions than they were following rainfall (Figure 18). Rainfall did not appear to greatly influence the relative importance of *Campylobacter* from other sources (i.e. their occurrence of isolates from each source type was similar under both base and high flow conditions).

For samples collected as part of the Otautau Plains study, wildfowl were also the most common source of *Campylobacter* - 77% of *Campylobacter*-positive samples had a wildfowl-associated strain identified. Eighteen percent of positive samples had a *Campylobacter* strain of unknown origin, and ovine/bovine/deer, poultry and not-wildfowl strains were each identified on one occasion.

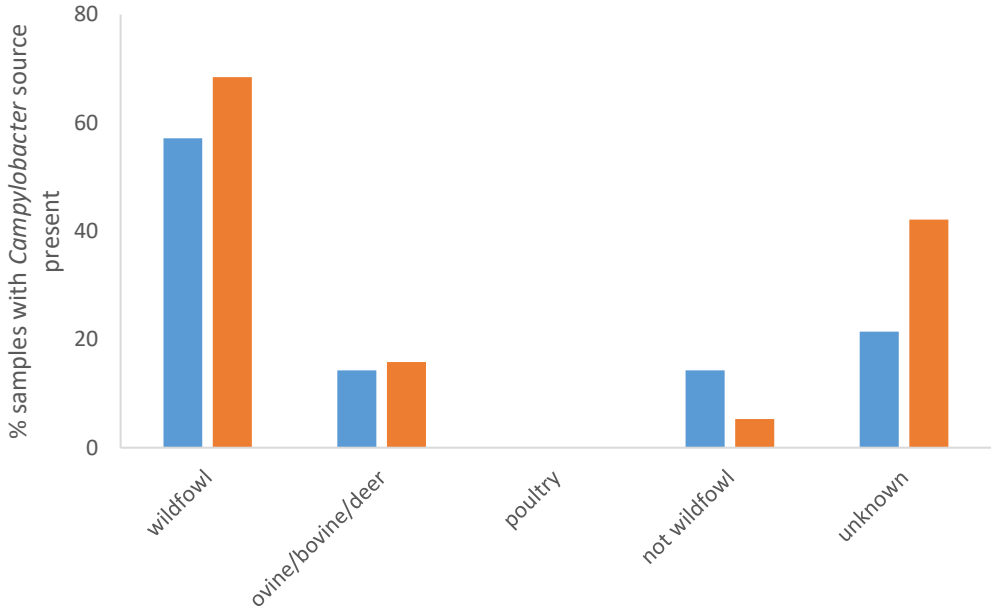


Figure 18. The percentage of *Campylobacter*-positive samples from the main Aparima study sites that were identified as having different *Campylobacter* sources present (as determined by MBiT analysis). Samples that were collected following rainfall are shown in blue, and those collected under base flow in orange.

3.4.2 Genotype analysis and comparison with clinical isolates

Comparison of the MBiT genotype data for *Campylobacter* isolates from sites across the Aparima FMU (including isolates that were available from previous studies in the Aparima FMU) revealed a high diversity of genotypes: of 262 isolates analysed, 129 different genotypes were identified. No clear pattern or separation of genotypes was observed based on the site from which isolates were collected (Figure 19). Comparison of the genotypes of isolates from the Aparima FMU with the isolates from the Waiau, Oreti and Mataura FMUs also shows no clear separation of genotype based on the FMU from which isolates were obtained (Figure 20).

Of the 262 individual isolates recovered from water samples in the Aparima FMU, 80 isolates (31%) representing 24 genotypes were found to 'overlap' with (i.e. were indistinguishable from) human clinical isolates from the Southland region (Figure 21). 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 80 isolates, 20 (25%) are likely to have come from wildfowl, compared with 63% of the isolates from water samples being wildfowl-associated (Figure 22). This suggests that *Campylobacter* from a wildfowl origin may present a lesser risk to human health than *Campylobacter* from other sources, e.g. humans or ruminants. This is also suggested by general analysis of the clinical isolates, which shows only ten isolates (6%) were indistinguishable from wildfowl-associated isolates, suggesting wildfowl are a minor source of illness in the community.

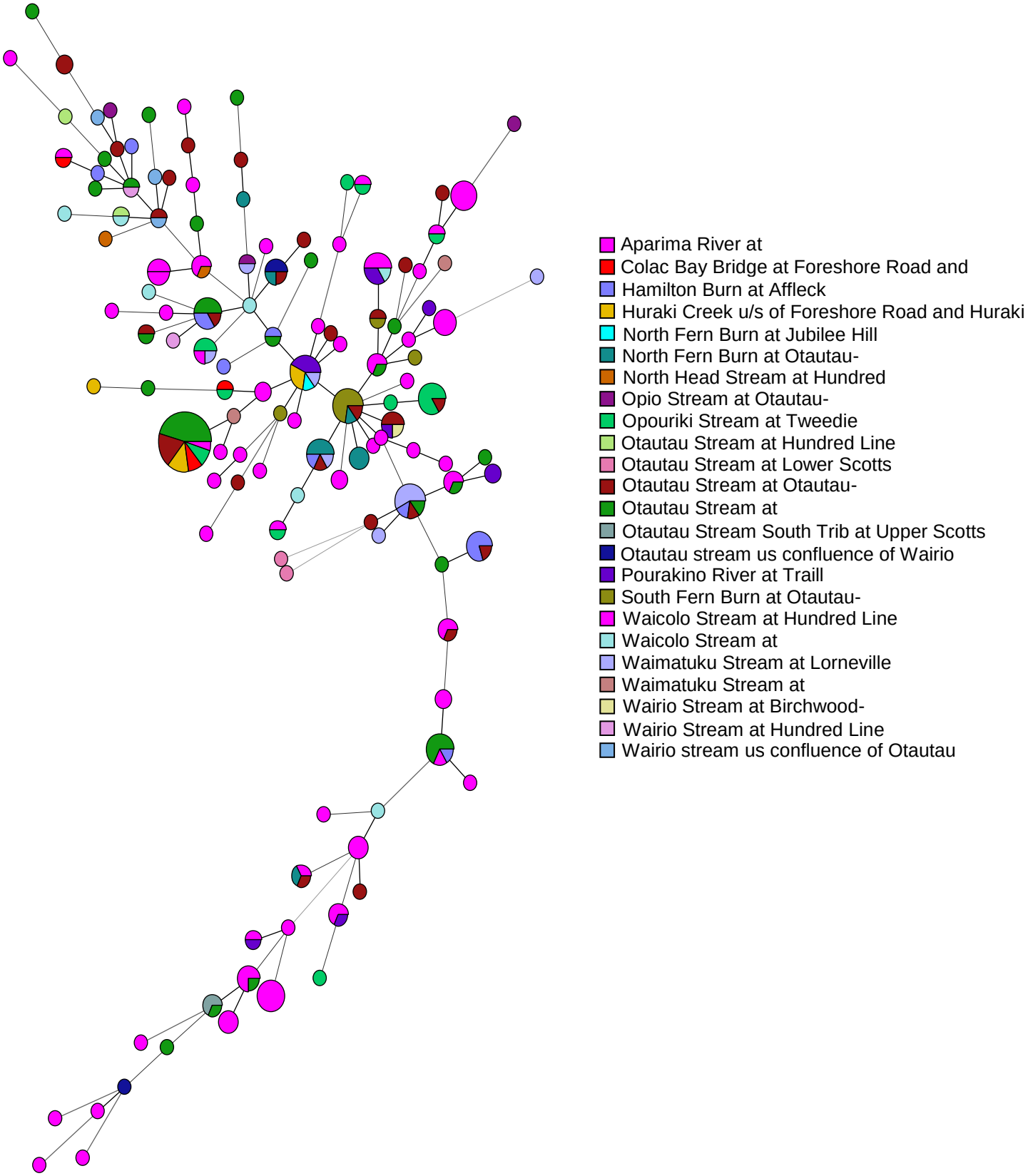


Figure 19. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from sites across the Aparima FMU, based on MBiT analysis. Each circle represents a different genotype, and each colour identifies a site. The number of circles and the spread of colours across the diagram demonstrates the diversity of genotypes within the Aparima FMU, and at individual sites.

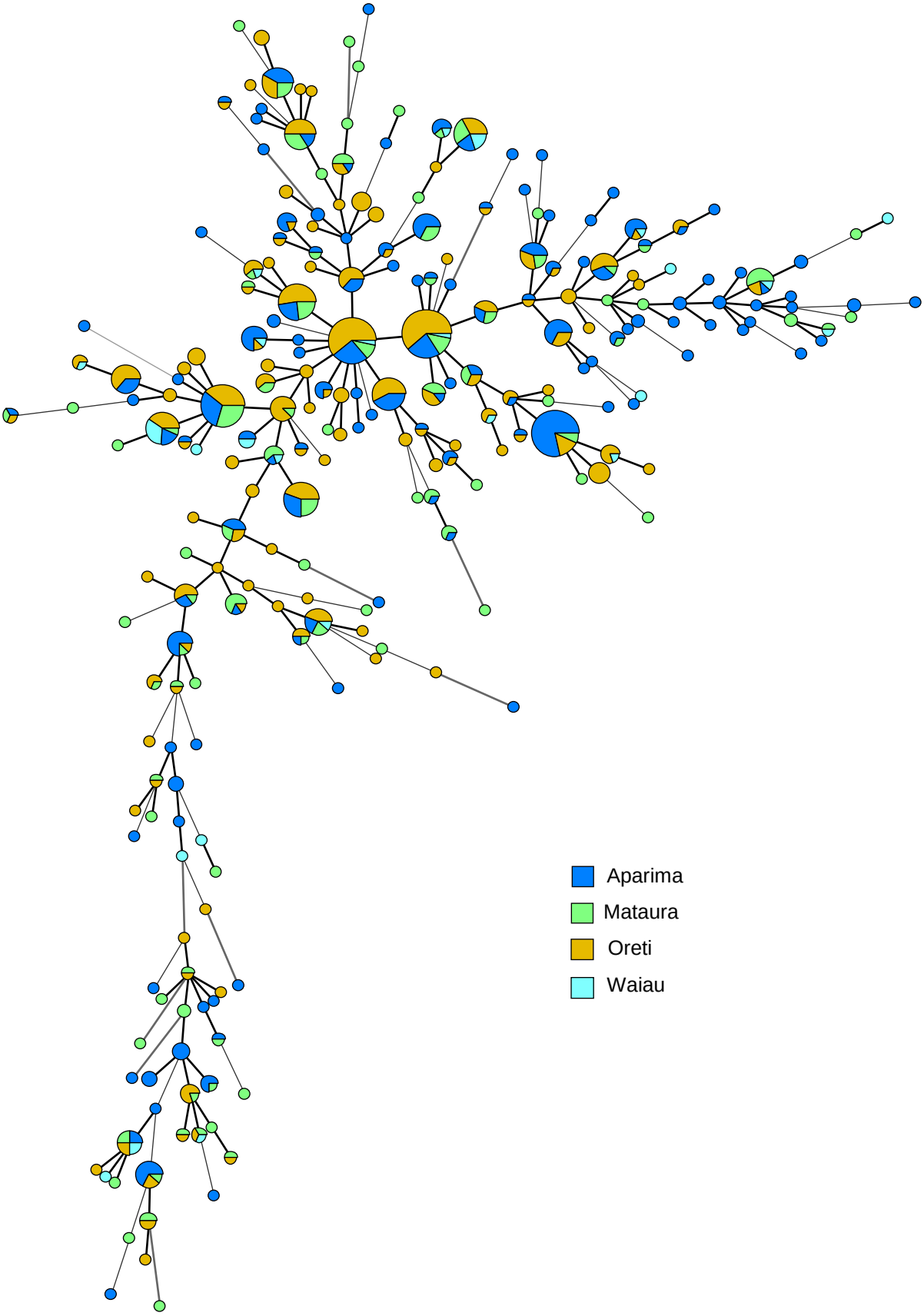


Figure 20. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from across the Southland region, based on MBiT analysis.

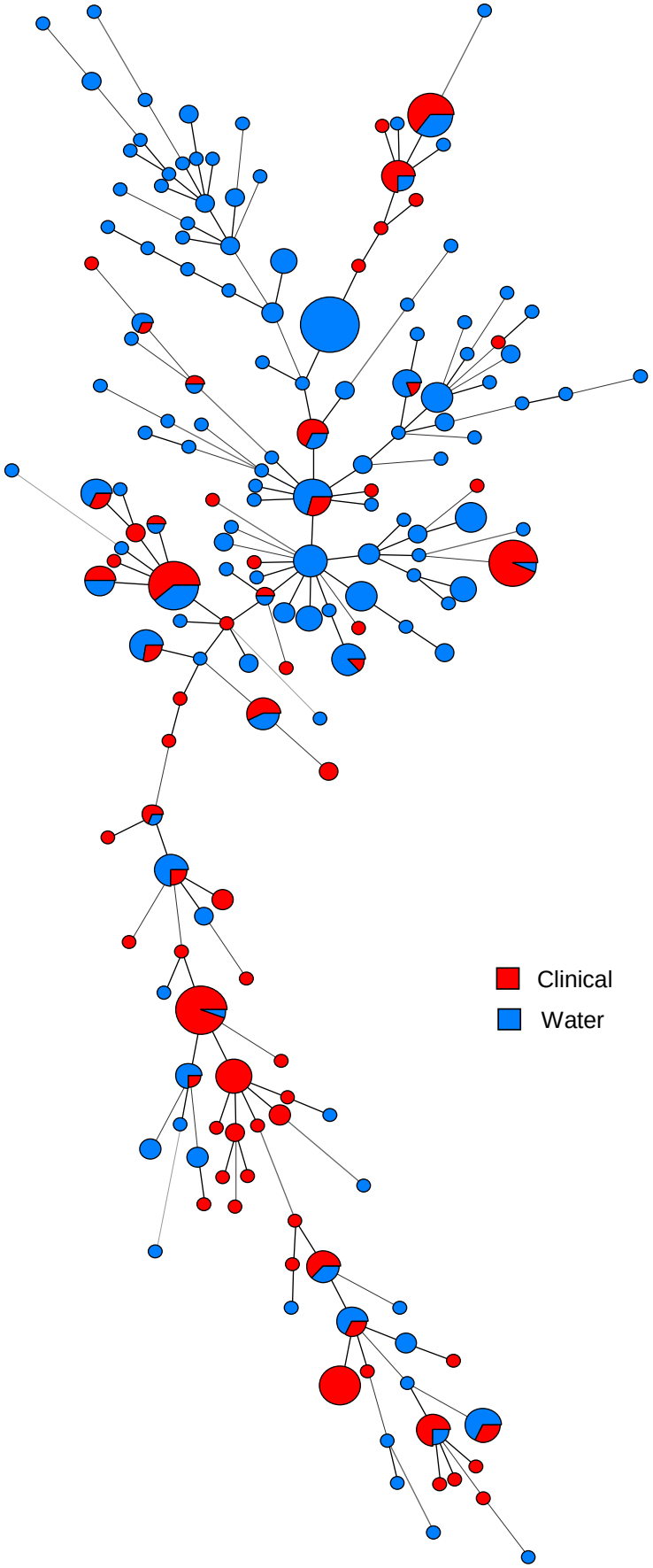


Figure 21. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from water samples from the Aparima FMU (blue) compared with human clinical isolates from the Southland region (red). Circles in which there are both blue and red segments indicate a genotype has been isolated from both the environment and clinical samples, representing the potential for human infection from waterborne sources.

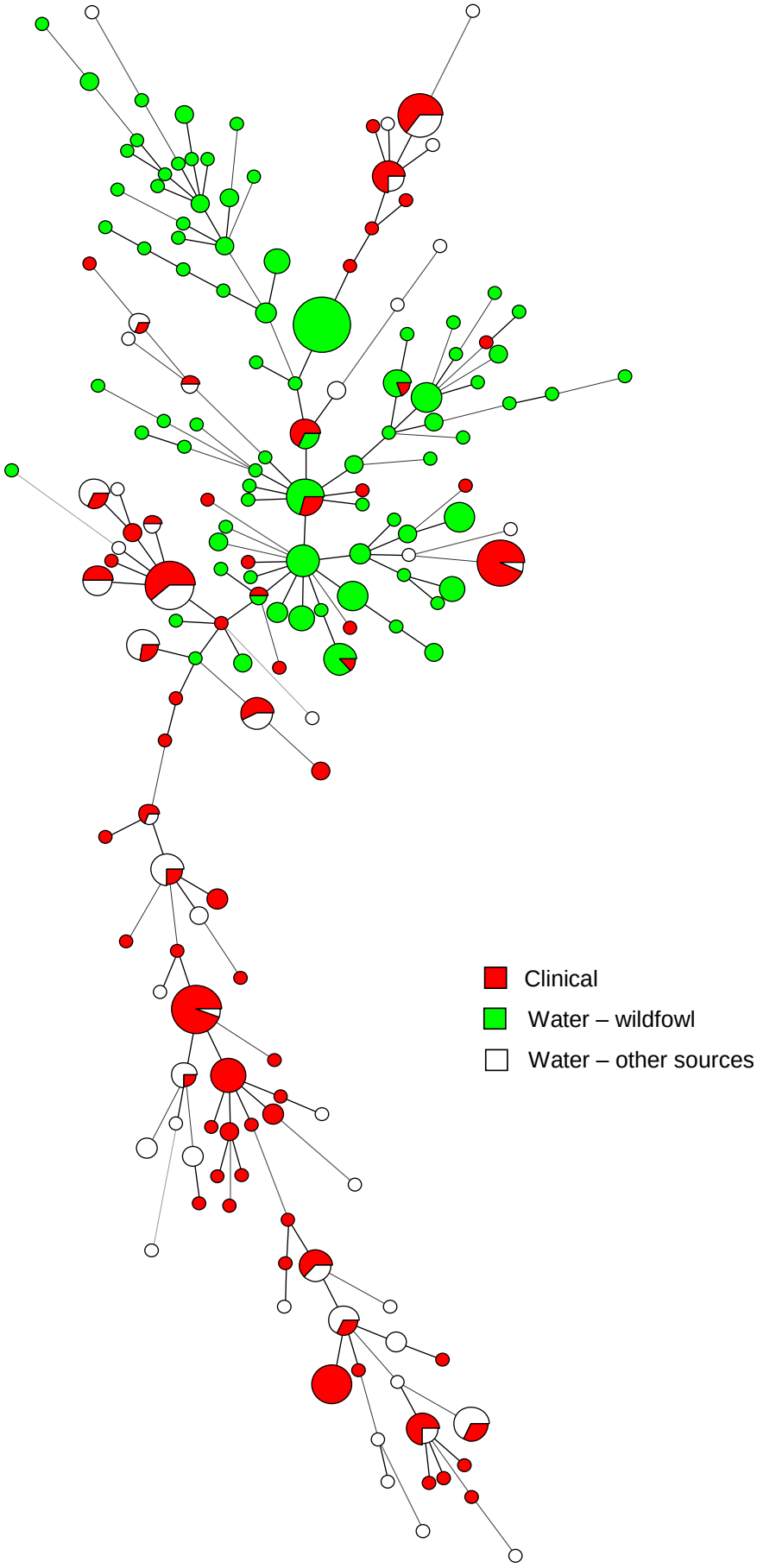


Figure 22. Burst diagram showing phylogenetic diversity of *Campylobacter* isolates from water from the Aparima FMU, highlighting those that are wildfowl-associated (green) compared with human clinical isolates (red).

4. DISCUSSION

4.1 MICROBIAL SOURCES AND TRANSMISSION

This study demonstrates that the microbial quality of waterways in the Aparima FMU is highly variable, with most of the sites being vulnerable to high levels of faecal contamination. *E. coli* concentrations varied between 100 and 19,000 cfu/100 ml, and 46% of all samples collected exceeded 1,000 cfu/100 ml. Within the main Aparima study, four of the seven sites that were sampled on more than one occasion had a median *E. coli* concentration exceeding 550 cfu/100 ml (i.e. the concentration above which the 2003 MfE/MoH Water Quality Guidelines recommend avoiding primary recreation such as swimming). High levels of variability in microbial concentration have previously been reported for waterways draining large, sparsely-populated rural catchments (e.g. Crowther et al., 2002, 2003), such as the Aparima FMU. Variables such as land use, topography and rainfall are known to influence the microbial burden of waterways (Collins et al., 2007), with additional factors such as stocking densities, application of effluent to land, and livestock access to waterways also impacting microbial water quality. This latter data is more difficult to obtain, particularly for large catchments, making it difficult to link water quality at individual sampling site to a single source, land use or management practice (Crowther et al., 2003; Monaghan et al., 2010). Further, as was the case for many of the sites sampled within the Aparima FMU, there are often multiple faecal sources, further compounding these issues (Muirhead et al., 2011). The map-based display of microbial data (Figure 3, Figure 4) shows the peak *E. coli* and *Campylobacter* concentrations recorded for each site, with the overall presence or absence of FST markers also shown. In essence, these figures demonstrate a 'worst-case scenario' for each site, based on the data available. Given the limited amount of data and variable nature of the data collected for each site, this was considered to be the most informative way to represent the public health risk that could be associated with contact with these waterways, and the possible sources of that risk.

The main sources of faecal pollution were wildfowl and ruminant animals. Across all of the sites sampled within the Aparima FMU (i.e. both the main Aparima study and the Otautau Plains study), ruminant pollution was detected at all sites, although at some sites this was only trace amounts (e.g. ≤1% total pollution). The presence of both ovine and bovine PCR markers is consistent with the large amount of agricultural activity in the catchment, and the greater prevalence of ovine markers likely reflects sheep and beef agriculture being the predominant activity (Appendix C). Wildfowl pollution was detected at 74% of all sites. Human contamination was also identified at six sites.

Within the main Aparima study, seven sites were sampled under both base flow conditions and following rainfall. Comparison of the microbial burden at each site under the different conditions shows *E. coli* concentrations (and at some sites, *Campylobacter* concentrations) are higher following rainfall, and that there was a shift in the dominant faecal signature. Under base flow, ruminant pollution typically accounted for 1-10 or 10-50% of the total faecal load, depending on the site. However, following rainfall, there was an almost universal shift to a dominant (i.e. 50-100%) ruminant signature. This suggests that rainfall-driven overland flow and/or preferential subsurface flow (e.g. via tile drains) from agricultural land are significant routes for the transmission of faecal microbes to waterways in the Aparima FMU.

Physiographic data for soils in the Aparima show a prevalence of imperfectly-to-poorly drained gleyed soils, oxidising soils with artificial drainage, and bedrock/hill country that is prone to overland flow or with artificial drainage (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 Aparima FMU; an estimated 76% of agricultural land within the Southland region likely has some form of artificial drainage (Monaghan, 2014; Pearson, 2015). The relative loss of faecal contaminants via runoff relative to drainage will differ between sites according to local characteristics such as soil type, land contour and density of drainage structures. The presence of ruminant pollution in waterways under base flow conditions likely results from direct deposition (e.g. stock access to unfenced waterways in pasture, passage through streams during stock movement between paddocks or to milking sheds), and/or discharge of effluents to rivers.

The similarity in the prevalence of wildfowl pollution under both base flow and high flow conditions suggests that direct deposition occurs irrespective of rainfall.

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

- Otautau Stream at Waikouro. A single base flow sample from this site was positive for human contamination, suggesting direct discharge or seepage to the environment. There are no obvious sources for the contamination - the catchment is dominated by agricultural activity (dairy, sheep and beef) with a large amount of conservation land. Available information shows the consented discharge of dairy shed and meat works effluents to the environment. There is a small number of residential dwellings in the area upstream of this site, so contamination from septic tanks might be an issue; however, although other sites near these dwellings (e.g. around Scott's Gap) did not have human contamination detected.
- Otautau Stream at Otautau-Tuatapere Road. Two samples from this site – one base flow and one high flow – were positive for human contamination. Although there are no obvious sources in the immediate vicinity of the site, the wider catchment for this site includes the settlements of Wairio and Nightcaps. Consented discharges include dairy and stockyard effluents, various industrial effluents (mine, meatworks, sawmilling), and stormwater and washdown/wastewater. Stormwater or seepage from septic tanks or the Nightcaps sewerage scheme could therefore be sources of pollution.
- Waimatuku Stream at Lorneville-Riverton Highway. Two of the five samples collected here were positive for human contamination. One sample was collected under base flow and the other following rainfall. The sub-catchment is dominated by dairy and sheep and beef agriculture, with consented discharges for dairy shed effluents, wash down effluents and oil and grease. The stream passes approximately 500 m to the east of the small Waimatuku township. Seepage from septic tanks at local properties and/or runoff might be potential sources of contamination. Septic tanks from the small number of farm houses upstream of the sampling site (<5 over several kilometres) might also be a potential source of contamination.

- Opouriki Stream at Tweedie Road. A single post-rainfall sample from this site tested positive for human contamination. Based on the available data, the catchment is dominated by sheep and beef and dairy agriculture, with some conservation land. Consented discharges to land or water are few and relate to dairy shed effluents. There is therefore no obvious source of the human contamination detected. Satellite imagery shows a small number of farm houses upstream of the sampling site (<10 within 500 m from stream over several kilometres) – seepage from septic tanks at these dwellings should be investigated as a potential source of contamination.

In the Otautau Plains study, the general increase in *E. coli* and *Campylobacter* concentrations with progressive downstream sampling shows that the impacts of faecal pollution are widespread across the area, with increasing microbial concentrations reflecting the larger catchment and cumulative inputs at the downstream sites. There was no obvious spatial pattern regarding sites that were more or less heavily impacted by ruminant pollution, however the generally low proportion of ruminant pollution observed likely reflects the base flow conditions. The high *E. coli* concentrations and/or percentage of ruminant pollution at a small number of upstream sites (e.g. Wairio Stream at Birchwood Road, Otautau at 122 Upper Scott's Gap/Symons Road) suggests point sources such as dairy shed effluents may have a disproportionate effect on water quality at these sites. It is also possible that local diffuse sources such as irrigation-driven overland flow may contribute. The dilution of these localised inputs can then be seen at sites downstream. As only a single sample was collected from each site, the relationship between the contamination observed at each site is therefore only reflective of base flow conditions. Further, it is difficult to gauge how representative a single sample is of those conditions. Gaining a full understanding of the impacts of upstream contamination sources on downstream sites will require repeated sampling events under both base and high flow conditions.

Human contamination was detected at two sites along the Wairio Stream (20 Birchwood Road and upstream of confluence with the Otautau Stream). There is no catchment data available for these two sites, however given the proximity of the Birchwood Road site to the settlement of Wairio, septic tanks from the area are a strong candidate for a source. From the current data we cannot know if the human contamination present in the sample collected upstream of the confluence is the same as that present at Birchwood Road. However, there are two sites along the the Wairio between these two contaminated sites, which did not have a human signal detected. This suggests that the input at Birchwood Road is subsequently diluted below the detection limit, with a further human input upstream of the confluence with the Otautau Stream.

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 (79%) in the rural, bird-impacted, Aparima catchment. Interestingly, although *C. jejuni* was the most commonly identified species in the national survey McBride et al. (2002), it was present in only 48% of *Campylobacter*-positive samples (compared with 94% of positive samples in the Aparima). Further, McBride et al. (2002) detected *Campylobacter lari* in 33% of positive samples from predominantly sheep-impacted sites.[2] These differences might reflect geographic differences, or differences in land use in the Aparima 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 23 illustrates a dose response curve for *C. jejnui.,* which is accounts for ~90% of all human cases of campylobacteriosis (Lee and Newell, 2006). It shows that the ingestion of 800 *C. jejuni* is associated with a 50% probability of infection (ID_{50}) (Medema et al., 1996; McBride et al., 2002). Ingestion rates for primary recreation have been estimated at between 10 and 100 ml per hour, with average exposure between 0.25 and 2 hours (McBride, 2012); estimates of water ingested therefore range between 2.5 ml and 200 ml. Approximately two thirds of the *Campylobacter*-positive samples collected across the Aparima FMU had a concentration of less than 10 MPN/100ml, meaning very large volumes of water (e.g. >8 litres) would be required to attain ID_{50}. However, a small number of samples contained high concentrations, (240-1,100 MPN/100 ml), such that the ingestion of 70-330 ml of water could carry a fifty-fifty chance of infection. The Guidelines define a risk of infection of 5% as being the upper limit for tolerable or acceptable risk; clearly a much smaller volume again will be required to meet this risk. Further, the dose response for *Campylobacter* was derived from a feeding study involving adult volunteers (Black et al., 1988), and more recent studies suggest that the infective dose may be much lower, particularly for susceptible population subgroups, such as children or people who are immunocompromised (Teunis et al., 2005). If this is so, the exposure required for infection (e.g. volume of water ingested) will be lower than suggested above. Despite the significance of campylobacteriosis to public health, dose response information on *Campylobacter* infection is scarce, and confounded by limited exposure doses. In particular, the risk associated with exposure to low doses of *Campylobacter* is not well known, although its success as a parasite (i.e. one of the most common in the western world), suggests high infectivity (Teunis et al., 2005). The probability of illness resulting from *Campylobacter* infection is also not well known (Teunis et al., 2005); one estimate suggests 28% of infections result in illness (Soller et al., 2010).

[2] 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 20% of *Campylobacter*-positive samples

There are further uncertainties around the risks of infection and illness by *Campylobacter*. Although not conclusive, there is some epidemiological evidence, which is supported by animal models and cell culture, that some strains of *Campylobacter* may be host-specific, and that these different strains have different rates of human infectivity (McBride et al., 2011). *Campylobacter* from avian sources are suggested to pose a limited threat to human health (McBride et al., 2011), although they remain implicated in cases of human disease (French et al., 2009; Mohan et al. 2013). Indeed, a number of wildfowl-associated isolates from this study 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 48% of the *Campylobacter*-positive samples were found to contain only isolates of a wildfowl origin, the health risk in some instances might be less than that suggested by the data from Black et al. (1988), which is based on clinical isolates.

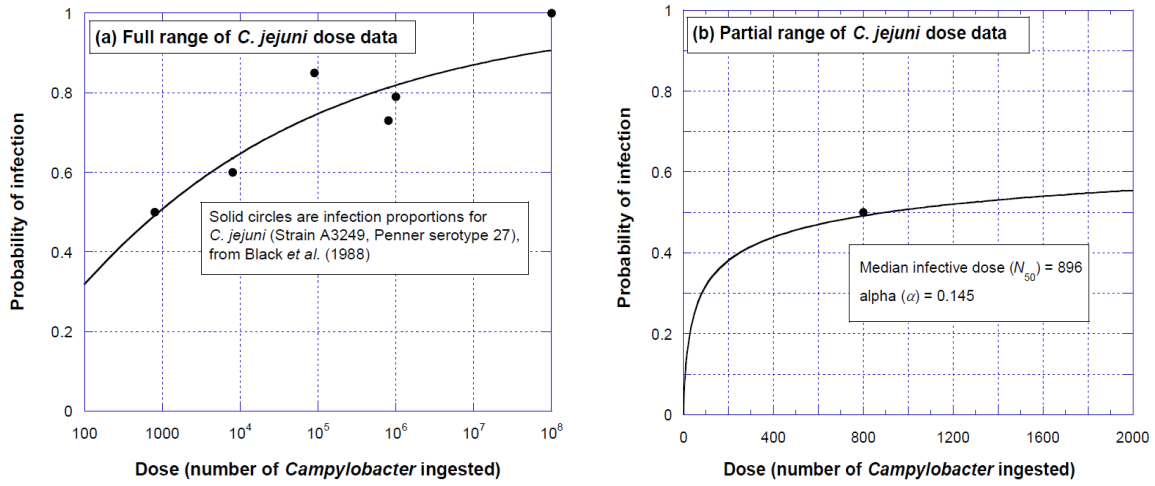


Figure 23. Dose-response curves for *Campylobacter jejuni*, estimating the probability of infection for a given dose. The lowest dose administered during the study was 800 *C. jejuni*, at which point half of the volunteers became infected. Estimating the dose response for lower concentrations requires extrapolation. From McBride et al. (2002).

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

4.3.1 Human contamination

The routes by which human contamination enters waterways in the Aparima FMU are not particularly clear. The catchments for the sites at which human contamination was detected are typically sparsely populated, with agricultural activity and conversation lands being dominant land use. However, the settlements of Nightcaps and Wairio are in the catchments of some of these sites, suggesting stormwater (or combined sewer overflows), leaking or cross-connected sewerage pipes, and/or seepage from septic tanks may be a source of contamination. Septic tanks are more likely to be the source in the more remote sites. Further investigation is highly recommended to identify the specific contaminant site(s) and transmission pathway(s) that impact each site. This could include assessment of the condition of the Nightcaps stormwater network and oxidation ponds for potential integrity issues, and the use of chemical, biological or DNA tracers to identify a point of origin for contamination (Harvey and Harms, 2002; Richards et al., 2016, 2017; Pang et al., 2017). Tracers are commonly used to model microbial transport through the subsurface – they are added at a

potential point of contamination (e.g. a septic tank), and their recovery in an aquifer or waterway monitored. Chemical tracers and dyes tend to require large inputs, which may be toxic to aquatic life. However, synthetic DNA tracers have a particular advantage in that they are environmentally safe, since they are not derived from any organisms and hence have no genetic functionality. Analysis is rapid and extremely sensitive, so that only trace amounts of DNA are required. In addition, multiple DNA tracers, each with a unique sequence, can be used to allow concurrent tracking of multiple potential sources and pathways (Pang et al., 2017). Once the specific source(s) has been identified, informed mitigation options can be explored. These may include the repair or replacement of failing septic and sewerage infrastructure, remediation of any identified cross-connections, separation of sewage and stormwater networks to avoid partially or untreated wastes being discharged to the environment, or incorporation of wetlands or other treatment options to stormwater or washwater discharge systems.

4.3.2 Direct deposition

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

4.3.3 Indirect sources

Strategies that can be used to reduce ruminant contamination associated with overland and/or subsurface flow will depend on characteristics of the land and farm management practices. Identifying locations that are associated with a high risk of microbial transfer to waterways is a key step in adjusting agricultural practices to improve water quality. For example, the ability of soils to attenuate faecal microbes depends on soil type and slope. Poorly drained soils, soils with low infiltration rates, soils with high preferential flow (macropores or cracking), land with artificial drainage, or hilly terrain, have a high risk of transferring microbes to waterways.

High intensity grazing should be avoided on such land. During periods of wet weather, grazing rotation and exclusion of stock from paddocks adjacent to waterways, or that are prone to saturation and/or pugging, can help reduce runoff and wash-in of faeces following rainfall.

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

4.3.4 Wildfowl

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

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

4.3.5 Prioritising mitigations

The benefits of these various mitigation strategies need to be balanced against the cost that will inevitably be associated with their implementation, such as material and labour costs for fencing and planting riparian zones, upgrades to effluent treatment systems or reduced productivity associated with reduced stock densities. Mitigations should be prioritised based on risk assessments that identify priority areas for improvement, whilst also considering which particular strategies provide the ‘greatest return for investment’ (i.e. greatest reduction in microbial contamination). Catchment water quality models such as CLUES (Catchment Land Use for Environmental Sustainability model, ftp://ftp.niwa.co.nz/clues) allow users to assess

the effects of changes in land use and farm practice (e.g. stocking rates, fencing), and can help in ranking various mitigation scenarios. The protection of public health must be at the forefront of this decision-making. Discussions around mitigation options should also be held in consultation with landowners and the public.

Since faecal pollution of waters by humans is considered the greatest risk to human health, these sources should be addressed first. Additional monitoring and site assessment at the six sites at which human contamination was present should be undertaken to identify the particular source(s) of the human signature (e.g. leaking sewerage network, septic tanks etc). However, faecal pollution of waters by livestock or wildfowl represent a real human health risk that should not be diminished or dismissed. Population control through hunting is likely the most cost-effective means to reduce wildfowl contamination of waterways, but may be unacceptable to some members of the community. Strategies to reduce ruminant contamination could include fencing for stock exclusion, riparian planting, stock management (intensity, grazing rotation), irrigation management and wastewater treatment.

5. CONCLUSIONS

Waterways in the Aparima 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 were detected at all sites for which base flow samples were collected, although at times only at trace levels. This suggests that direct deposition by livestock, either as a result of free access to the stream or wash in at dairy crossings, and/or the discharge of farm effluents to the water are routes of transmission for faecal material to waterways. Following rainfall, ruminant animals are the dominant faecal source, with both overland flow/surface runoff and subsurface flow through tile drains being significant routes of transmission of faecal materials to waterways. Human faecal contamination was identified at six sites, with repeated detection at two of these. Potential sources are difficult to identify, but may include failing septic tanks, stormwater and urban run-off, or leaking or cross-connected sewerage infrastructure. Human faecal contamination is considered to pose the greatest risk to human health, and further investigations at these sites should be undertaken to identify the specific source(s) and transmission routes. This could include the inspection of sewerage infrastructure and/or the use of tracers such as synthetic DNA.

Campylobacter was isolated from 79% of samples, occasionally at quite high concentrations. Wildfowl, ruminants, poultry and humans were all identified as being sources of *Campylobacter*. *Campylobacter* genotypes that were indistinguishable from human clinical cases in Southland region were identified. Although there is little information available on the probabilities of infection and/or illness at lower *Campylobacter* concentrations, these findings suggest 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 that this is also a possibility.

Because of the interaction between faecal source, soil type, land contour, artificial drainage and climate factors in determining contaminant transfer to waterways, and the variation in these between sites, a single mitigation strategy will not be effective for all sites. Risk assessments should be used in conjunction with water quality models to prioritise approaches to mitigate the greatest health risks and that afford the greatest improvements to water quality for a given investment. Population control through hunting is likely the most cost-effective means to reduce wildfowl contamination of waterways, but may be unacceptable to some within the community. Strategies to reduce ruminant contamination could include fencing for stock exclusion, riparian planting, stock management (intensity, grazing rotation), irrigation management and wastewater treatment. Management of human contamination may involve the repair or replacement of septic tanks or sewerage infrastructure, or installation of stormwater and washwater treatment systems.

ABBREVIATIONS

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

GLOSSARY

attenuation	the reduction of contaminant concentrations in the environment
base flow	the portion of stream flow that is sustained between rainfall events; stream flow during fair weather
bovine	relating to cattle
colony-forming units	method of estimating the concentration of bacteria in a water sample, based on the number of distinguishable colonies that grown in a culture plate
enteric pathogen	microorganisms that live in the intestine and can cause illness
faecal indicator organism	a microorganism that is associated with the gut or faeces of an animal and whose presence in environmental waters can be used to indicate faecal contamination
faecal source tracking	a 'toolbox' of methods that can be used to determine the source of faecal contamination (e.g. whether it is of human, ruminant, wildfowl etc origin)
genotypes	the genetic makeup or DNA sequence of an organism
illness	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±0.2°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).

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 3) 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 24). 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 3. 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

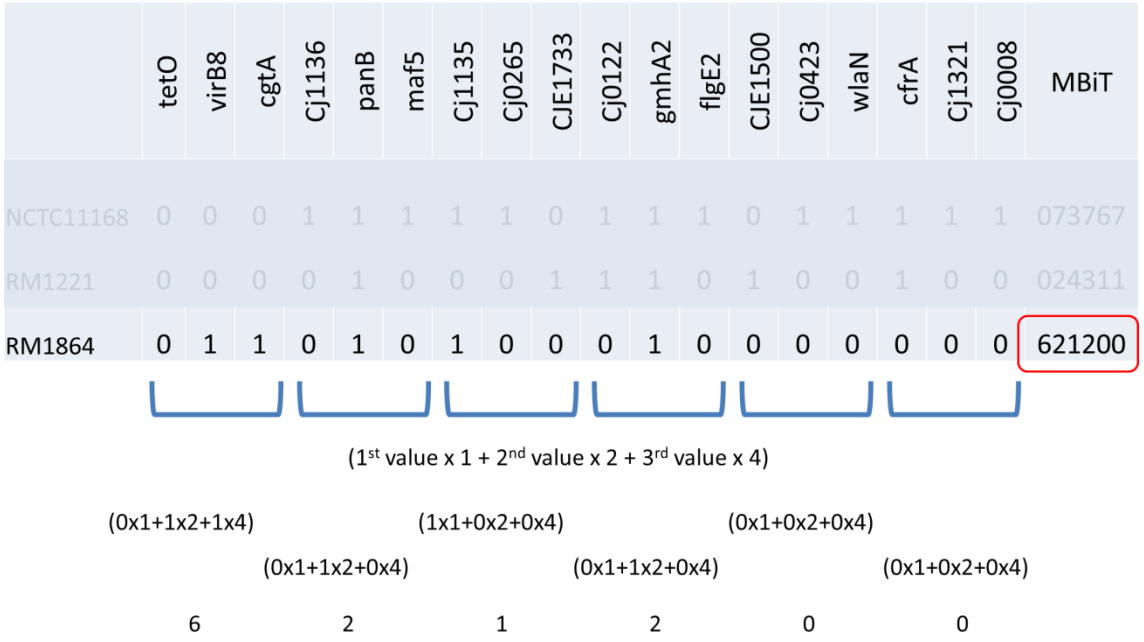


Figure 24. Example of an MBiT pattern naming.

strongly associated with, but not exclusive to, their host animal. Assays for different markers also differ in their sensitivity (Table 4).

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 4. 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 4. 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 5 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 5.

A.6 PRESENTATION OF RESULTS IN THIS REPORT

Tables 6, 7 and 8 provide a key for interpretation of results, which can be used to assist with reviewing results for each site.

Table 5. 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 6. 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 7. 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 8. 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 OTAUTAU STREAM AT WAIKOURO

A total of seven water samples were collected from the Otautau Stream at Waikouro, between December 2014 and June 2015 (Figure 25). Five were collected under base flow conditions, with two samples collected on the same date, but at different times of the day (7.15am and 9.45am 9th March). Two samples were collected following rainfall. Microbial water quality was observed to differ between rainfall and base flow conditions (Table 9).

Figure 25. The Otautau Stream at Waikouro Shortcut.

E. coli levels were generally elevated under base flow conditions, exceeding 1,000 cfu/100 ml in 4 of 5 samples (maximum 2,700 cfu/100 ml). A lower concentration of 260 cfu/100 ml was observed in June. *Campylobacter* was detected in all five base flow samples, with concentrations between 4.3 and 46 MPN/100 ml, except for one of the two samples collected on 9 March that contained 1,100 MPN/100 ml. Such high concentrations of *Campylobacter* are often associated with fresh faeces, deposited in or in close proximity to the water, providing

little to no opportunity for microbial die-off. Genotype analysis determined that *C. jejuni* was present in all of the samples. MBiT source attribution found that 4 of 5 samples contained a wildfowl *Campylobacter* source, and that three also contained an unknown source. Isolates from the 1,100 MPN/100 ml sample were determined to be of ruminant and unknown origin. The cause of the difference in *Campylobacter* concentration between the two March samples is unknown, but could reflect methodological difference or error, heterogeneous distribution of bacteria in the water, or UV (i.e. sunlight) inactivation of *Campylobacter* in the sample collected later in the morning. However, the two corresponding samples collected downstream of this site also contain elevated levels of *Campylobacter* (Table 11), suggesting levels were indeed elevated on this date. It is of note that the high concentration of *Campylobacter* is not associated with disproportionately high levels of *E. coli*; the relationship between *E. coli* and pathogens (and in turn, health risk) is thus not always clear.

Faecal source tracking analysis found that ruminant animals accounted for less than 50% of the pollution present at the site under base flow conditions. Wildfowl-specific PCR markers were detected in all base flow samples, and ovine markers from 4 of 5 samples. Bovine markers were present in the sample collected in June.

Following rainfall, there was a significant increase in *E. coli* concentrations in the water (15,000-19,000 cfu/100 ml). High levels of *Campylobacter* were also present, at up to 240 MPN/100 ml. *Campylobacter* isolates were determined to be *C. jejuni* from wildfowl and unknown sources, with *C. coli* also present in the April sample. Faecal source tracking determined that ruminant pollution was dominant following rainfall (50-100%). Specific markers for both sheep and cattle, as well as wildfowl were present in both samples. Human contamination was also detected in the February sample.

Faecal sterol analysis (four samples only) found a ruminant signature present in four samples, but no wildfowl, human or plant signatures.

A review of land use in the sub-catchment for this site shows a mixture of dairy (34%) and associated support (8%), sheep and beef farming (22%, including sheep only), and non-agricultural use (34%). Non-agricultural use is a combination of indigenous and plantation forestry (Figure 26, Figure 27). Consented discharges to land and water relate to dairy and meat works effluents (Table 10).

The data suggests that a background level of wildfowl contamination is present at this site under all conditions, and that this contamination is often associated with the presence of *Campylobacter*. Ruminant contamination, mostly of an ovine source, is also present at low levels the majority of the time. Following rainfall, significant run-off of faecal material into the stream occurs, and *E. coli* levels increase approximately 10-fold. Ruminant pollution, both ovine and bovine, dominates the pollution signal under these conditions. There is no clear source for the human contamination (e.g. no consented discharge for sewage or stormwater); this, together with the large number of *Campylobacter* isolates with an unidentifiable source, is cause for further site inspections and sanitary surveys to be undertaken.

Table 9. Results for microbial and FST analysis of water samples collected from the Otautau Stream at Waikouro.

Site		Otautau Stream at Waikouro						
Sample #		CMB140899	CMB150001	CMB150217	CMB150192	CMB150788	CMB150121	CMB150357
Client #		20145164	20145299	20150987	20151023	20152047	20150688	20151549
Date Sampled		9/12/2014	12/01/2015	9/03/2015	9/03/2015	8/06/2015	9/02/2015	13/04/2015
Rainfall		No	No	No	No	No	Yes	Yes
		Microbial Properties						
Faecal coliforms		2,900	1,100	1,400	1,400	280	20,000	21,000
E. coli		2,700	1,100	1,100	1,300	260	19,000	15,000
Campylobacter		46	4.3	24	>1,100	4.3	240	43
***Campylobacter* Species**		*C. jejuni* & Thermo	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni* & Thermo	*C. jejuni*	*C. jejuni* & *C. coli*
MBiT *Campylobacter* Source	**Wildfowl**	4	2	2		1	1	2
	Ovine/Bovine/Deer				1			
	Poultry							
	Not Wildfowl							
	Unknown	3		1	3		1	
		Faecal Source Tracking						
General - GenBac3		++++	++++	++++	++++	++++	++++	++++
Ruminant		10-50%	10-50%	1-10%	1-10%	10-50%	50-100%	50-100%
Human - BacH		+	-	-	-	+	+	+
Human - BiADO		-	-	+	+	-	+	-
Cow		+	-	-	-	-	+	+
Sheep		+	+	-	+	+	+	+
Wildfowl - GFD		+	+	+	+	+	+	+
Wildfowl - E2		-	+	+	+	-	+	+
		Sterol Properties						
Total Sterols		13,059	4,023	nt	nt	1,408	13,471	nt
Coprostanol		448	137			51	245	
Faecal		F1+F2	F1+F2			F1+F2	F1+F2	
Human		No	No			No	No	
Ruminant		Yes	Yes (2)			Yes (2)	Yes (2)	
Wildfowl		No	No			No	No	
Plant		No	No			No	No	

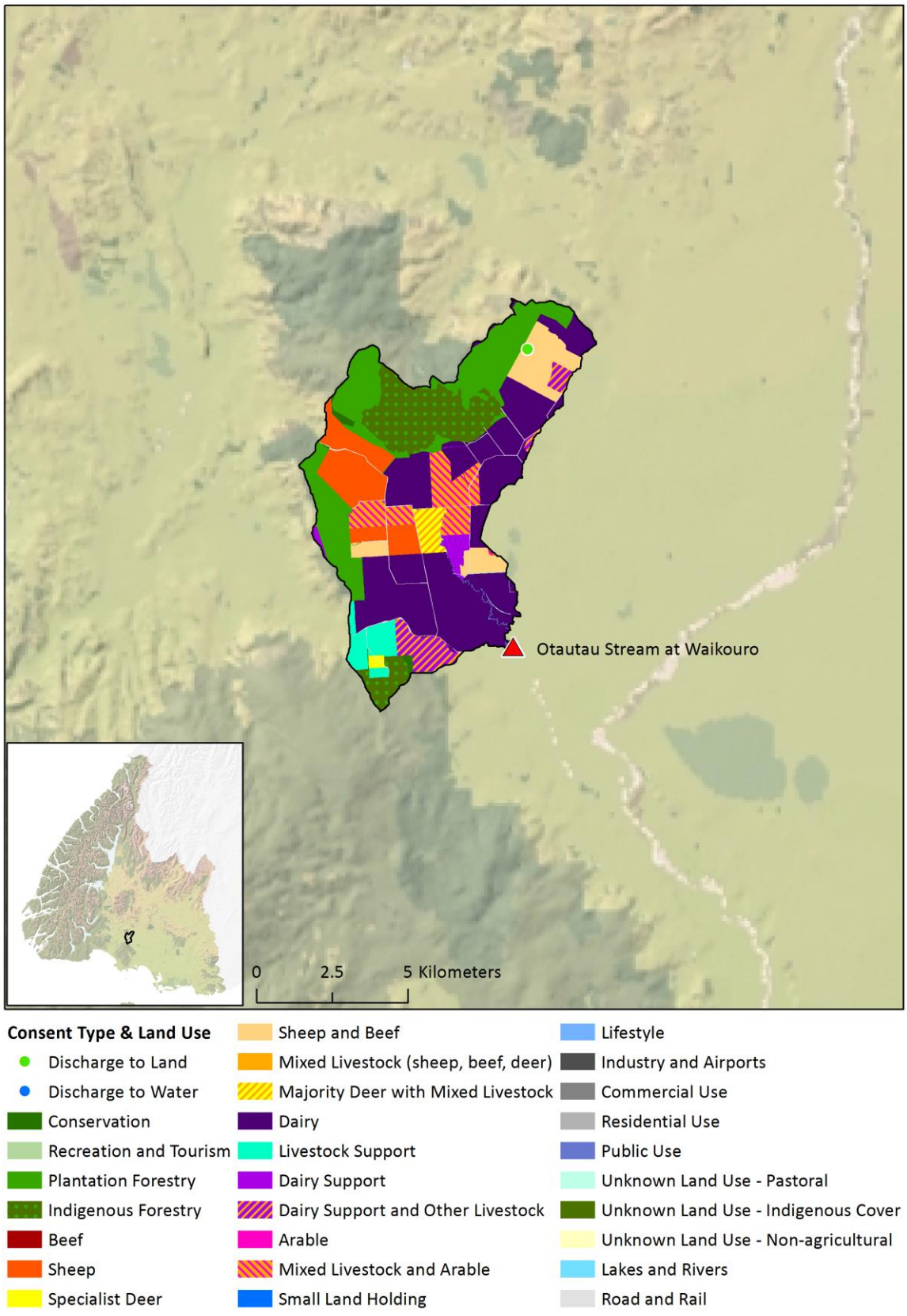


Figure 26. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Otautau Stream at Waikouro sampling site.

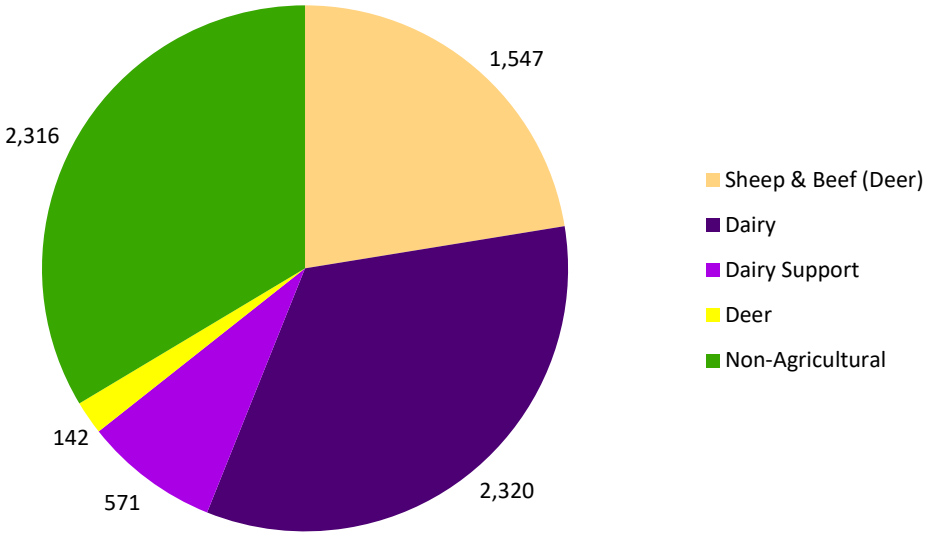


Figure 27. Land use (in hectares) in the catchment for the Otautau Stream at Waikouro.

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 Use Map.

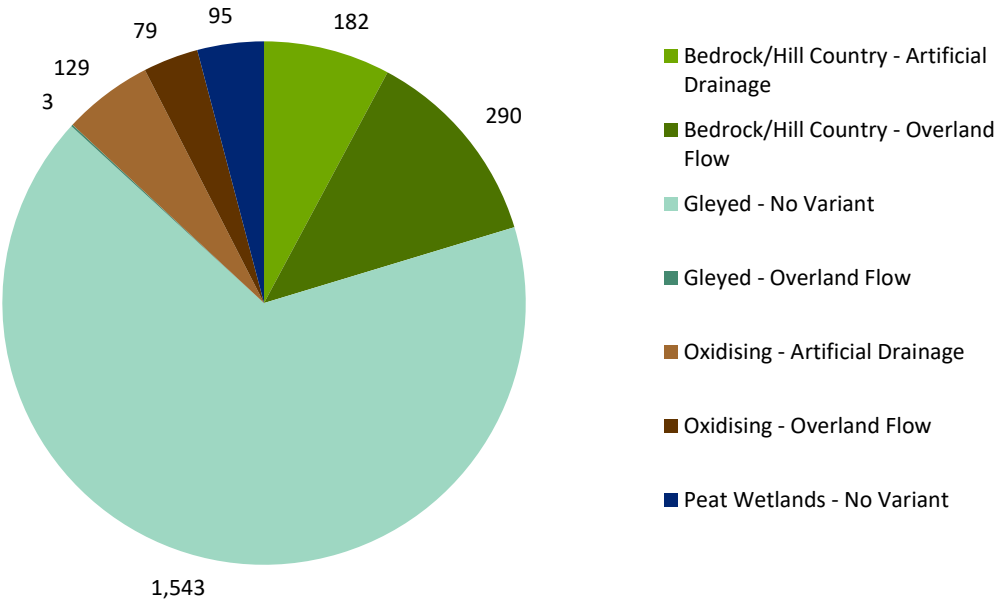


Figure 28. Dairying land (in hectares) in the catchment for the Otautau Stream Waikouro, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 10. Number and type of consented discharges to land and water in the catchment for the Otautau Stream at Waikouro.

Otautau Stream at Waikouro		
Discharge	Contaminant	Total
To Land	Dairy Shed Effluent (land)	5
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	2
	Meat Works Effluent, Waste Water	1
To Land Total		8
Grand Total		8

Note: Consent information accurate as of April 2017

B.2 OTAUTAU STREAM AT OTAUTAU-TUATAPERE ROAD

The Otautau Stream was also sampled downstream at Otautau-Tuatapere Road (Figure 29). Environment Southland advise that this site typically has high levels of *E. coli*. A total of 7 samples were collected between December 2014 and June 2015 – 5 under base flow conditions and 2 following rainfall. Similarly to the site at Waikouro, two samples were collected on 9 March, one at 6.10am and one at 10.20am (Table 11).

Figure 29. Sample collection from the Otautau Stream at Otautau-Tuatapere Road.

E. coli levels during base flow conditions varied between 270 and 3,900 cfu/100 ml. *Campylobacter* was detected in all samples: concentrations were between 0.4 and 4.3 MPN/100 ml for three samples, with the two March samples containing 1,100 MPN/100 ml. These high levels are consistent with those observed upstream at Waikouro. Isolates from four samples were identified as *C. jejuni*, with the isolates from the fifth sample identified only as a thermophyllic *Campylobacter*. MBiT source attribution identified a wildfowl source in each sample, with a ruminant source also present in the December sample.

Faecal source tracking analysis found that the impact of ruminant pollution during base flow conditions was varied: ruminant animals accounted for more than half of the pollution present in two samples, 10-50% in one, and less than 10% in two samples. Ovine PCR markers were detected in 4 of 5 samples, and bovine maker in 2 samples. Wildfowl markers were present in all base flow samples. Human faecal contamination was detected in one of the March samples.

Following rainfall, *E. coli* levels increased (4,000-8,000 cfu/100 ml). *Campylobacter* was present in both samples (43-46MPN/100 ml), and determined to be *C. jejuni*. A source could be identified for one sample only, which was found to be wildfowl. Faecal source tracking showed that ruminant pollution was the dominant pollution type (50-100%), with bovine marker detected in both samples. Wildfowl markers were also present in both samples. Human contamination was detected in the April sample.

Faecal sterol analysis found a ruminant signature to be present in all samples (base flow and post-rainfall), but did not detect any cases of wildfowl or human signatures.

Land use in the sub-catchment is dominated by dairy (39%) and associated support activity (8%), with mixed agriculture (sheep, sheep and beef, livestock and arable; 29%) also a significant activity. Non-agricultural use (indigenous and plantation forestry) comprises 21% of the sub-catchment, with a small amount of deer farming also present (2%) (Figure 30, Figure 31). Consented discharges are associated with agricultural and industrial activities, including dairy seed and related effluents, meat works effluent, mine water and stormwater (Table 12).

Similarly to the site upstream at Waikouro, the data suggests that a background level of wildfowl contamination is present in the Otautau Stream at the Otautau-Tuatapere Road site, and that this contamination is associated with the presence of *Campylobacter*. The impact of ruminant contamination under base flow conditions is varied, and mostly of ovine origin, with an intermittent bovine signature. Following rainfall, *E. coli* concentrations increase (though not by the magnitude observed upstream), and cattle become the dominant pollution source. Human contamination was observed under base flow and post-rainfall, although the source fof this remains unclear and warrants further investigation.

Table 11. Results for microbial and FST analysis of water samples collected from the Otautau Stream at Otautau-Tautapere Road.

Site		Otautau Stream at Otautau-Tautapere Road						
Sample #		CMB140898	CMB150002	CMB150186	CMB150218	CMB150789	CMB150122	CMB150358
Client #		20145163	20145300	20151007	20150988	20152048	20150689	20151550
Date Sampled		9/12/2014	12/01/2015	9/03/2015	9/03/2015	8/06/2015	9/02/2015	13/04/2015
Rainfall		No	No	No	No	No	Yes	Yes
		Microbial Properties						
Faecal coliforms		4,500	700	1,600	1,500	270	8,000	5,000
E. coli		3,900	500	1,400	1,300	270	8,000	4,000
Campylobacter		2.3	0.4	1,100	1,100	4.3	46	43
***Campylobacter* Species**		*C. jejuni*	Thermo	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni* & Thermo	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	2	1	3	4		2	
	Ovine/Bovine/ Deer	1						
	Poultry							
	Not Wildfowl							
	Unknown							
		Faecal Source Tracking						
General - GenBac3		++++	++++	+++	+++	++++	++++	++++
Ruminant		50-100%	10-50%	1-10%	1-10%	50-100%	50-100%	50-100%
Human - BacH		-	-	+	+	+	+	+
Human - BiADO		-	-	+	-	-	-	+
Cow		+	-	-	-	+	+	+
Sheep		+	+	+	+	-	-	-
Wildfowl - GFD		+	+	++	++	+	+	+
Wildfowl - E2		+	+	++	++	+	+	+
		Sterol Properties						
Total Sterols		24,754	2,515	5,554	4,596	2,204	5,369	9,151
Coprostanol		1,238	75	137	155	100	164	365
Faecal		F1+F2	F1+F2	F1+F2	F1+F2	F1+F2	F1+F2	F1 + F2
Human		No	No	No	No	No	No	No
Ruminant		Yes (2)	Yes (2)	Yes (2)	Yes (1)	Yes (2)	Yes (2)	Yes (3)
Wildfowl		No	No	No	No	No	No	No

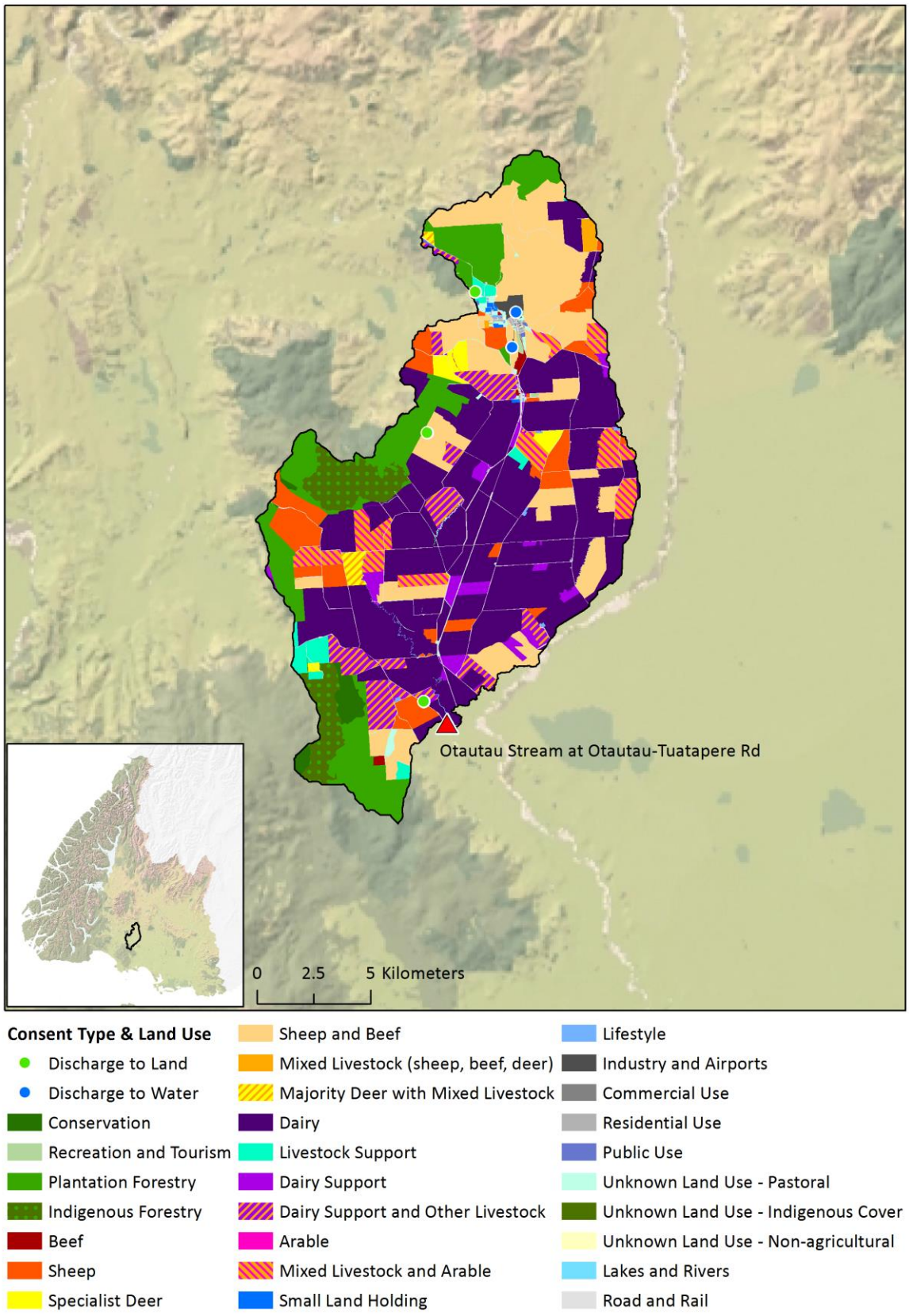


Figure 30. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Otautau Stream at Otautau-Tuatapere Road sampling site.

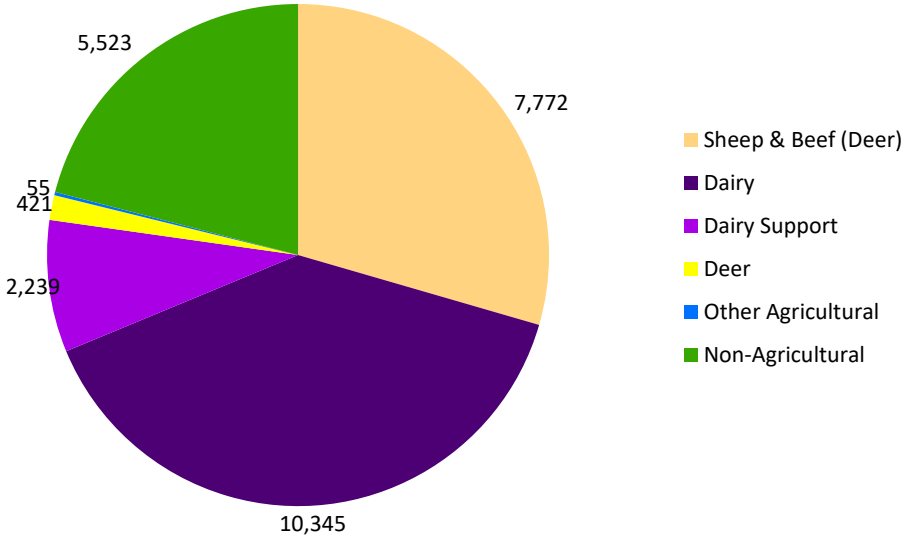


Figure 31. Land use (in hectares) in the catchment for the Otautau Stream at Otautau-Tuatapere Road.

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 Use Map.

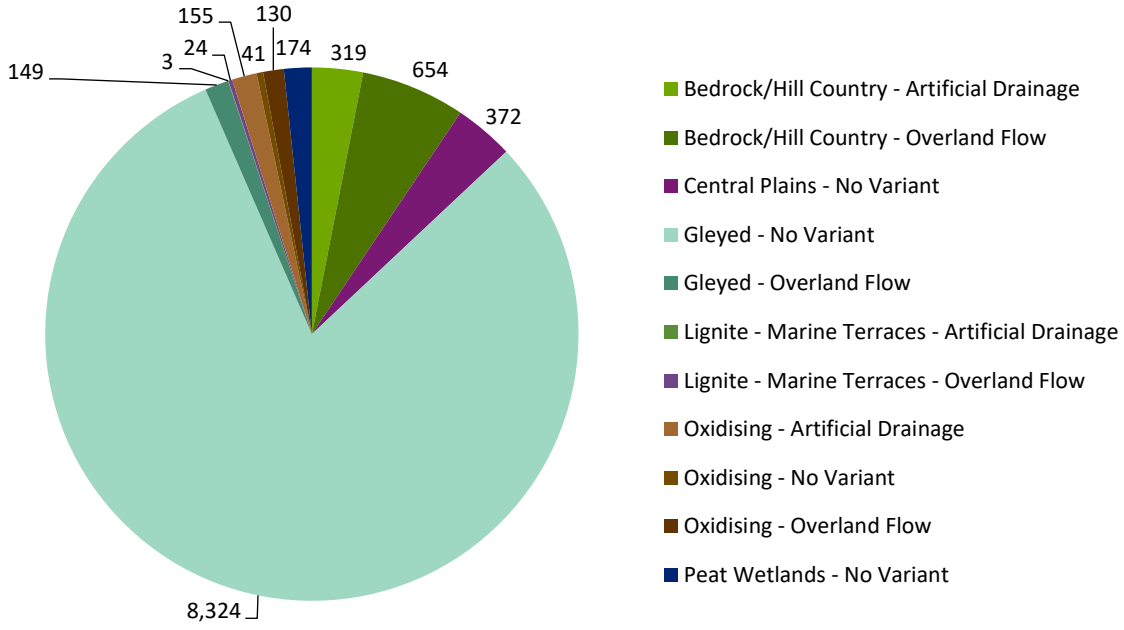


Figure 32. Dairying land (in hectares) in the catchment for the Otautau Stream at Otautau-Tuatapere Road, separated into physiographic units.

Southland Physiographic information accurate as of June 2016.

Table 12. Number and type of consented discharges to land and water in the catchment for the Otautau Stream at Otautau-Tuatapere Road.

Otautau Stream at Otautau-Tuatapere Road Catchment		
Discharge	Contaminant	Total
To Land	Other (dust suppressant)	2
	Ash	1
	Dairy Shed Effluent (land)	37
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	11
	Meat Works Effluent, Waste Water	1
	Mine water, Stormwater, Wash Water	1
	Sawmilling Waste	1
	Stockyard Effluent, Wintering Pad/Feedlot Effluent (land)	1
	Wash Down Effluent, Wash Water	1
To Land Total		56
To Water	Mine water	2
	Stormwater	1
	Waste Water	1
To Water Total		4
Grand Total		60

Note: Consent information accurate as of April 2017

B.3 APARIMA RIVER AT DUNROBIN

A single sample was collected from the Aparima River at Dunrobin in February 2015. The sample was collected following rainfall (Table 13). High levels of *E. coli* were present (3,400 cfu/100 ml), although no *Campylobacter* was detected. Faecal source tracking analysis found that ruminant pollution was the dominant pollution type present at this site, with PCR markers suggesting sheep as the source of the pollution. Faecal sterol analysis also identified a ruminant signature, with a weak wildfowl signature also detected.

The findings of the microbial and tracking analysis are consistent with land use in the sub-catchment for this site. Conservation land dominates the catchment (63%), with 27% used for beef and sheep or mixed (beef/sheep/deer) agriculture and 6% for deer. That sheep appear to be the most significant faecal source, and no *Campylobacter* were detected despite high *E. coli* levels, may indicate an aged pollution source, whereby *E. coli* persists but *Campylobacter* is no longer viable. This material is then washed into the Aparima River following rainfall.

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

Site		Aparima at Dunrobin
Sample #		CMB150119
Client #		20150686
Date Sampled		9/02/2015
Rainfall		Yes
		Microbial Properties
Faecal coliforms		3,500
E. coli		3,400
Campylobacter		<0.3
Campylobacter Species		nt
MBiT *Campylobacter* Source	Wildfowl	
	Ovine/Bovine/Deer	
	Poultry	
	Not Wildfowl	
	Unknown	
		Faecal Source Tracking
General - GenBac3		++++
Ruminant		50-100%
Human - BacH		+
Human - BiADO		-
Cow		-
Sheep		+
Wildfowl - GFD		-
Wildfowl - E2		-
Canine		-
		Sterol Properties
Total Sterols		8,111
Coprostanol		79
Faecal		F1+F2
Human		No
Ruminant		Yes (2)
Wildfowl		(Yes)
Plant		Yes

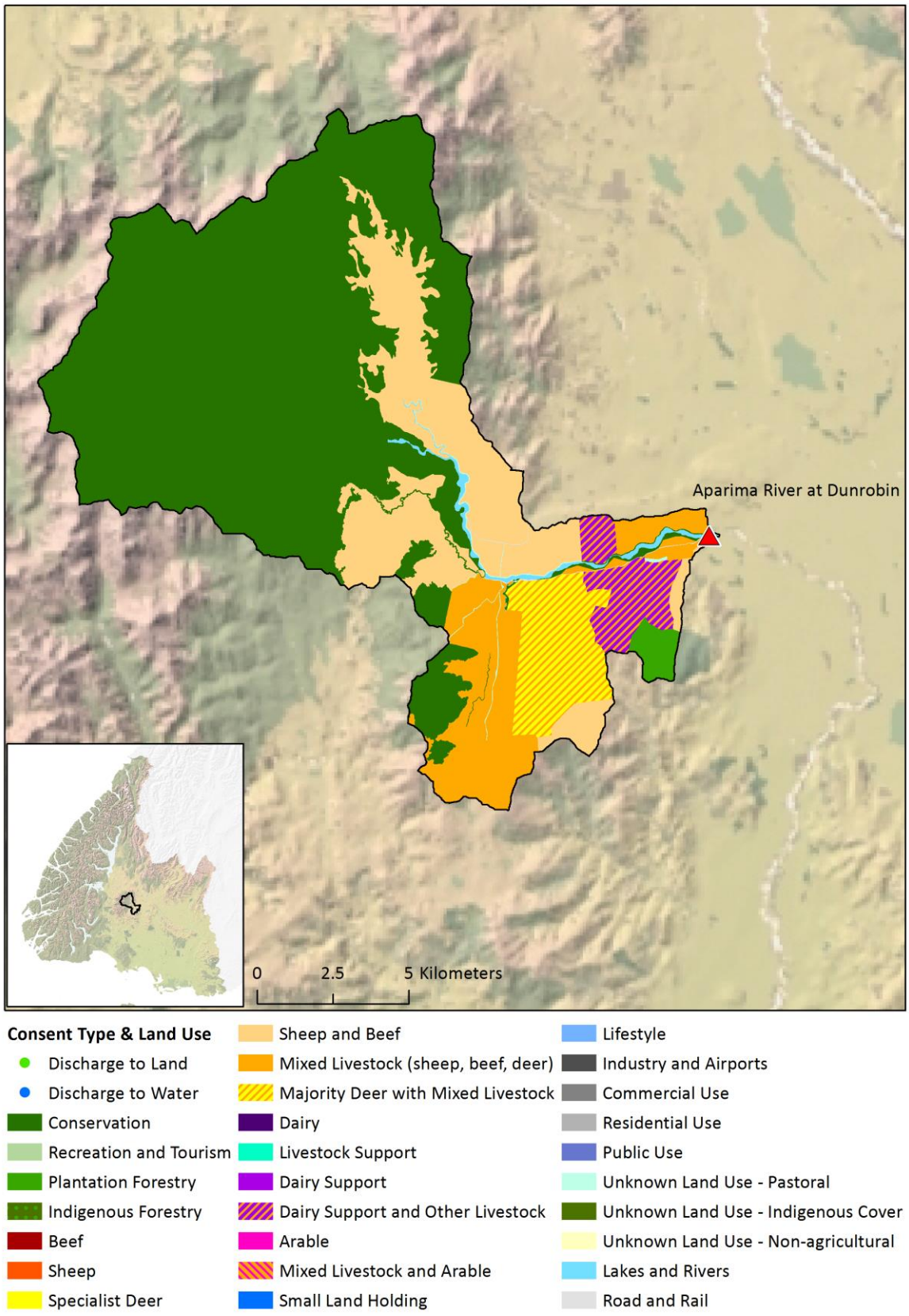


Figure 33. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Aparima River at Dunrobin sampling site.

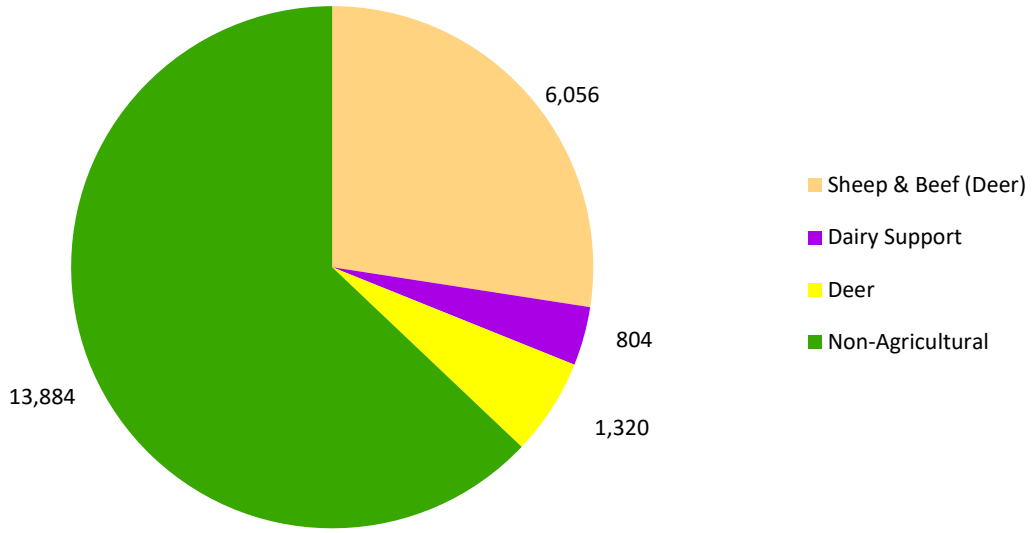


Figure 34. Land use (in hectares) in the catchment for the Aparima River at Dunrobin.

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 Use Map.

There is no dairying in this sub-catchment.

There are no consented discharges in this sub-catchment.

B.4 APARIMA RIVER AT THORNBURY

The Aparima River was also sampled at Thornbury. Three samples were collected under base flow conditions, and three following rainfall (Figure 35, Table 14).

Figure 35. Sample collection from the Aparima River at Thornbury.

E. coli concentrations under base flow were reasonably low (140-310 cfu/100 ml), with a low level of *Campylobacter* detected on one occasion (0.4 MPN/100 ml in March). Isolates were identified as *C. jejuni*, although their source could not be determined. Low levels of ruminant pollution were detected in two samples, with none detected in the third. Wildfowl-specific PCR markers were detected in the February sample.

Following rainfall, *E. coli* levels increased up to 10-fold over base flow levels (600-4,000 cfu/100 ml). Low levels of *Campylobacter* were detected in the two samples that contained the highest *E. coli* levels. These isolates were also determined to be *C. jejuni* of unknown origin. Faecal source tracking found that for two of the samples (February and April), ruminant pollution accounted for less than half (10-50%) of the contamination at the site, increasing to 50-100% in September. Ovine markers were detected in the February sample, and bovine markers in April and September. Wildfowl markers were detected in all three post-rain samples.

Land use in the sub-catchment for the Aparima at Thornbury site includes a mixture of sheep and beef (41%), dairy (21%), and deer (2%) farming. Conservation land and plantation forestry comprise 31% of the catchment Figure 36, Figure 37). There are also a number of consented discharges to land and water, primarily dairy-related, but also including sewage and septic tank effluents, mine water and stormwater (Table 15).

Table 14. Results for microbial and FST analysis of water samples collected from the Aparima River at Thornbury.

Site		Aparima River at Thornbury					
Sample #		CMB140895	CMB150015	CMB150220	CMB150176	CMB150362	CMB151538
Client #		20145160	20145305	20150993	20150694	20151555	20153067
Date Sampled		9/12/2014	12/01/2015	9/03/2015	9/02/2015	13/04/2015	7/09/2015
Rainfall		No	No	No	Yes	Yes	Yes
		Microbial Properties					
Faecal coliforms		490	210	140	600	1,900	4,000
E. coli		310	210	140	600	1,700	4,000
Campylobacter		<0.3	<0.3	0.4	<0.3	0.9	2.3
***Campylobacter* Species**		nt	nt	*C. jejuni*	nt	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**						
	Ovine/Bovine/ Deer						
	Poultry						
	Not Wildfowl						
	Unknown			1		1	
		Faecal Source Tracking					
General - GenBac3		++++	++	++++	++++	++++	++++
Ruminant		10-50%	ND	1-10%	10-50%	10-50%	50-100%
Human - BacH		-	-	+	-	-	-
Human - BiADO		-	-	-	-	-	-
Cow		-	-	-	-	+	+
Sheep		-	-	-	+	-	-
Wildfowl - GFD		-	-	+	+	+	+
Wildfowl - E2		-	-	+	+	+	+
		Sterol Properties					
Total Sterols		5,653	Sterols too Low	nt	nt	nt	nt
Coprostanol		119					
Faecal		F1+F2					
Human		No					
Ruminant		Yes (2)					
Wildfowl		No					

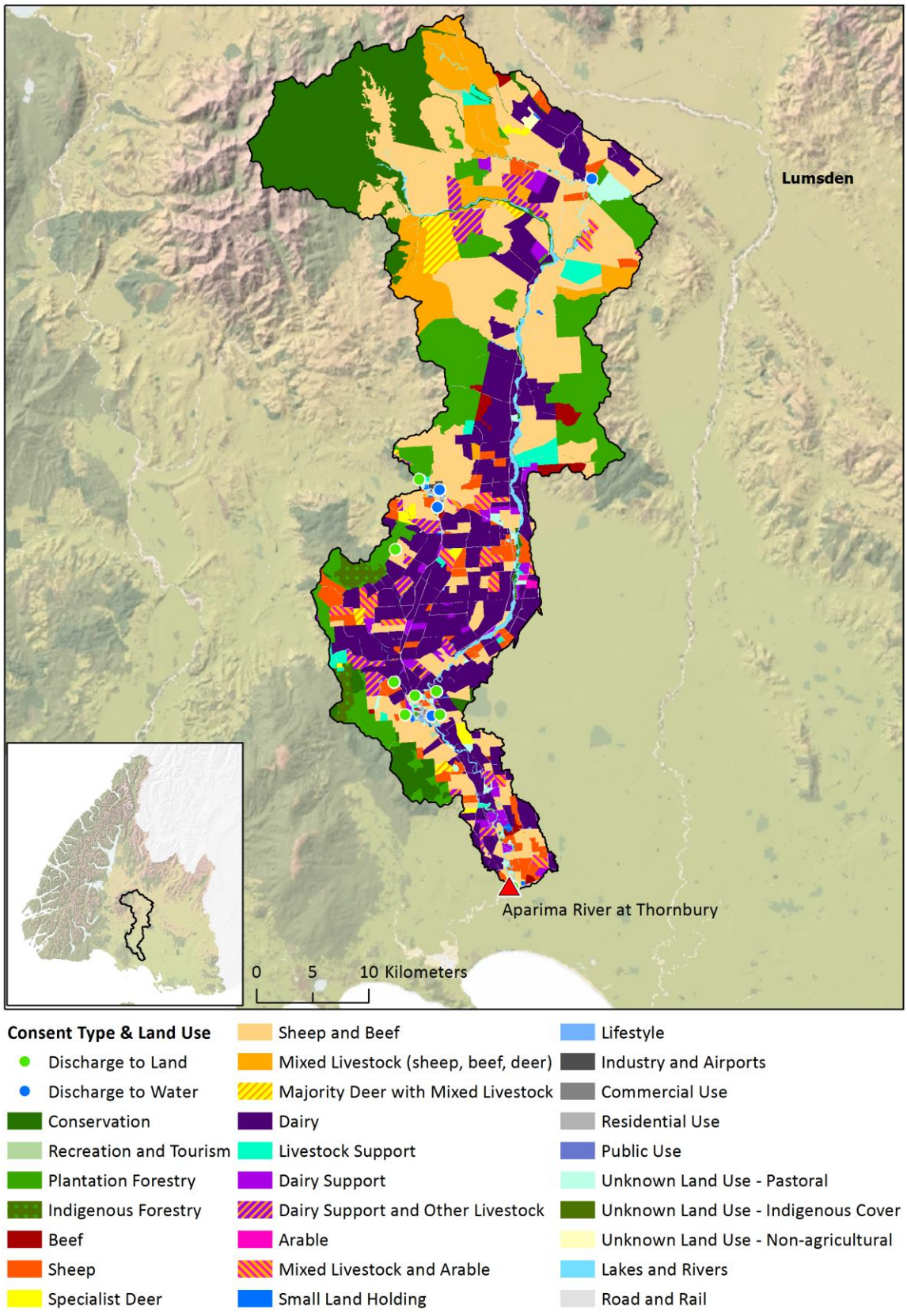


Figure 36. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Aparima River at Thornbury sampling site.

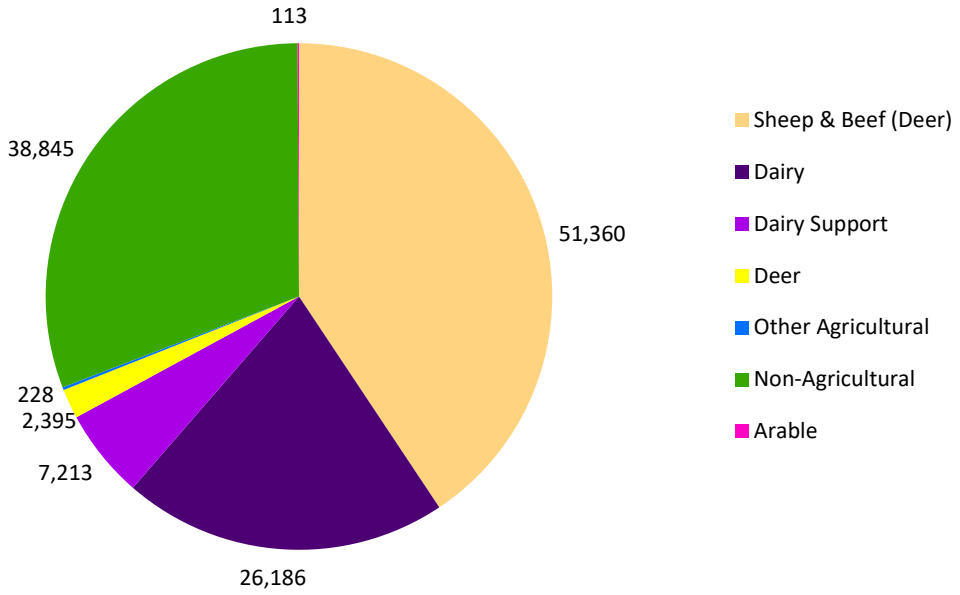


Figure 37. Land use (in hectares) in the catchment for the Aparima River at Thornbury.

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

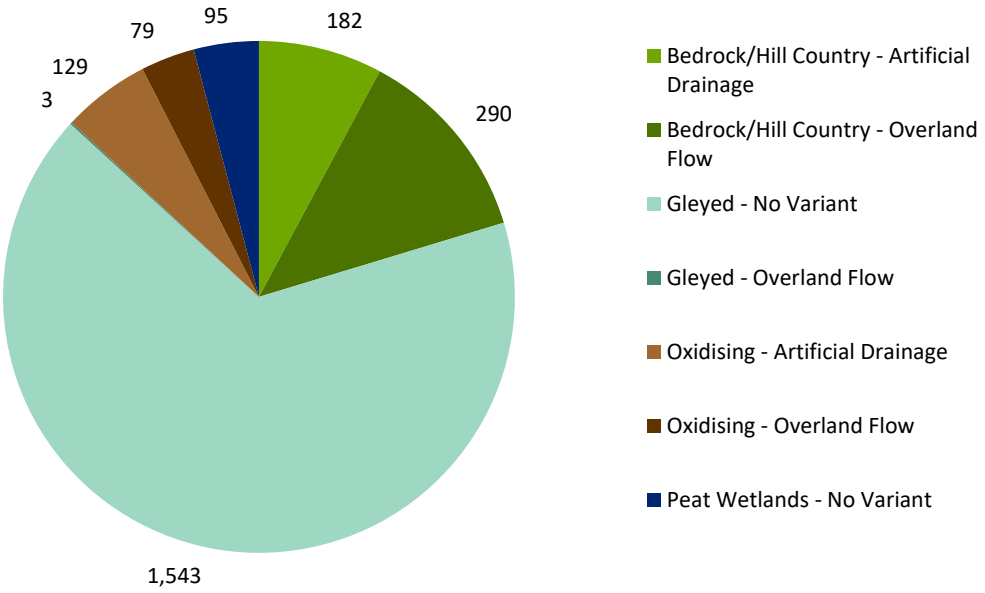


Figure 38. Dairying land (in hectares) in the catchment for the Aparima River at Thornbury, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 15. Number and type of consented discharges to land and water in the catchment for the Aparima River at Thornbury.

Aparima River at Thornbury		
Discharge	Contaminant	Total
To Land	Other (whey to pasture 7, dust suppressant 2)	9
	1080	2
	Ash	1
	Dairy Shed Effluent (land)	94
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	25
	Filter Backwash, Silt	1
	Leachate	1
	Meat Works Effluent, Waste Water	1
	Mine water, Stormwater, Wash Water	1
	Sawmilling Waste	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
	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		145
To Water	Mine water	2
	Stormwater	3
	Waste Water	1
To Water Total		6
Grand Total		151

Note: Consent information accurate as of April 2017

B.5 POURAKINO RIVER AT TRAILL ROAD

The Pourakino River was sampled on three occasions at Traill Road. One sample was collected under base flow, and two following rainfall (Figure 39, Table 16).

Figure 39. The sampling site at Pourokino River at Trail Road

Under base flow conditions, *E. coli* concentration was relatively low (300 cfu/100 ml), with a low level of *Campylobacter* also detected (0.9 MPN/100 ml). Isolates were determined to be *C. jejuni* from wildfowl. Ruminant pollution accounted for half (10-50%) of the overall pollution under these conditions, with ovine-specific markers detected.

Following rainfall, *E. coli* levels increased (up to 1,500 cfu/100 ml), as did *Campylobacter* concentration (2.3-9.3 MPN/100 ml). All *Campylobacter* isolates were determined to be *C. jejuni*, with a wildfowl source present in the February sample, and wildfowl, ruminant and 'not wildfowl' present in April. Ruminant animals became the dominant source of pollution (50-100%), although no specific ovine or bovine markers were detected to further identify a source.

The Paurokino River flows through native and plantation forest and conservation land (82% sub-catchment area), before reaching low intensity sheep and beef farming upstream of this site. There is also a small amount of dairy activity in the sub-catchment (Figure 40, Figure 41). Sampling staff noted that sheep were present in the field adjacent to the sampling site, and had easy access to the river bank. These observations support the FST findings that sheep appear to be the primary source of faecal contamination at this site.

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

Site		Pourakino River at Traill Rd		
Sample #		CMB140897	CMB150124	CMB150360
Client #		20145162	20150691	20151552
Date Sampled		9/12/2014	9/02/2015	13/04/2015
Rainfall		No	Yes	Yes
		Microbial Properties		
Faecal coliforms		370	1,500	900
E. coli		300	1,400	900
Campylobacter		0.9	9.3	2.3
Campylobacter Species		*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	Wildfowl	1	3	2
	Ovine/Bovine/ Deer			1
	Poultry			
	Not Wildfowl			2
	Unknown			
		Faecal Source Tracking		
General – GenBac3		+++	++++	++++
Ruminant		10-50%	50-100%	50-100%
Human - BacH		+	+	+
Human - BiADO		-	-	-
Cow		-	-	-
Sheep		+	-	-
Wildfowl - GFD		-	-	-
Wildfowl - E2		-	-	-
		Sterol Properties		
Total Sterols		nt	5,478	nt
Coprostanol			28	
Faecal			No	
Human			No	
Ruminant			<30	
Wildfowl			Yes	

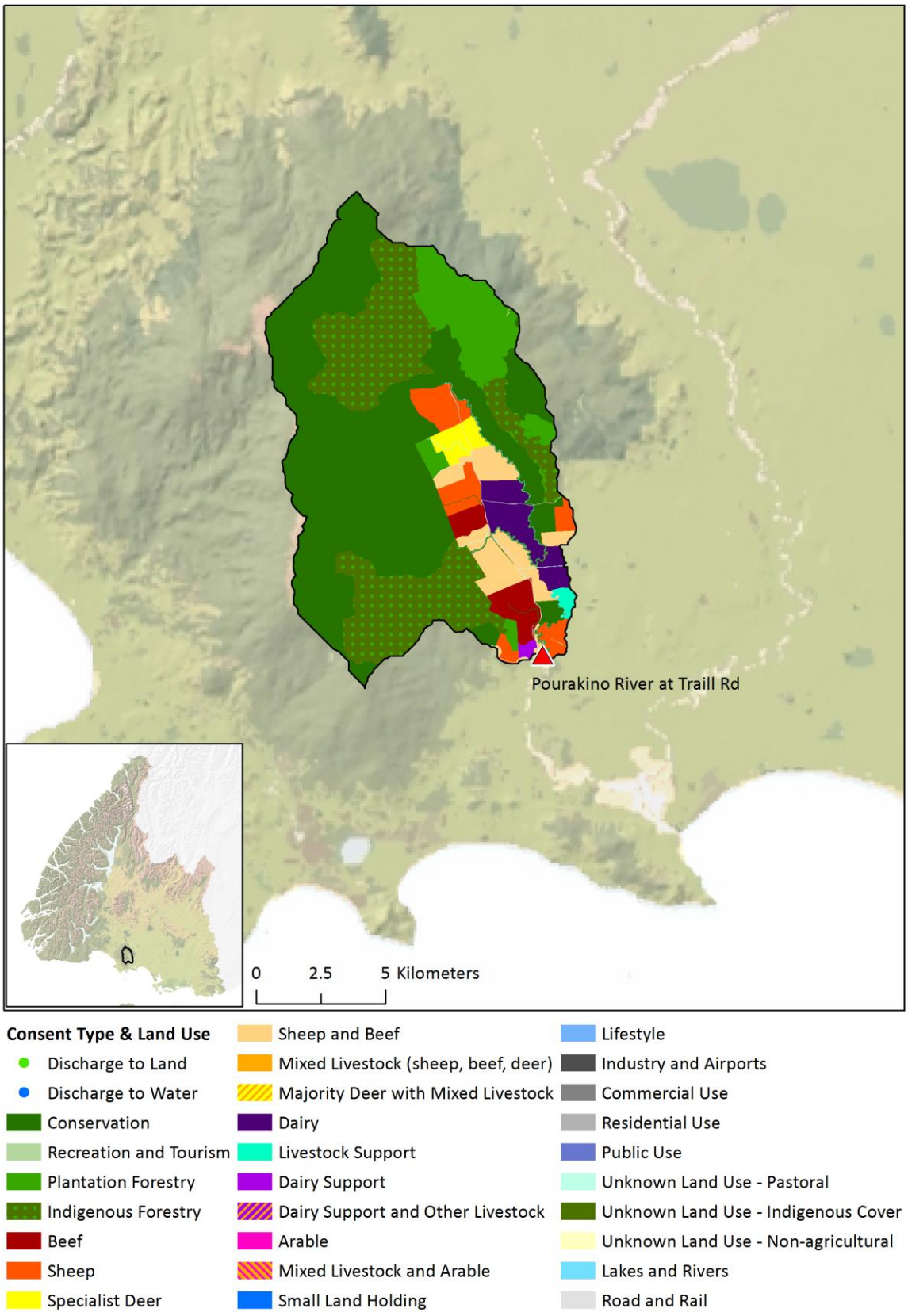


Figure 40. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Pourakino River at Traill Road sampling site.

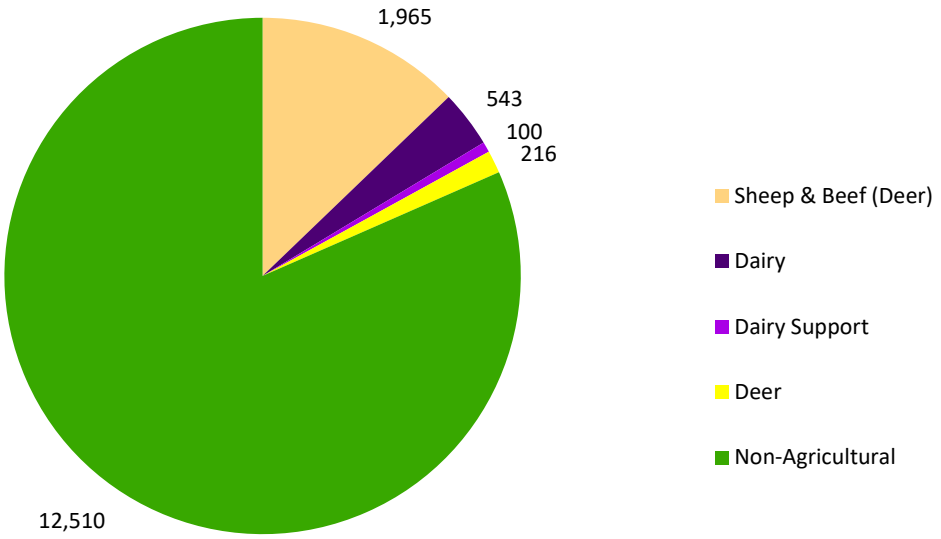


Figure 41. Land use (in hectares) in the catchment for the Pourakino River at Traill Road.

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 Use Map.

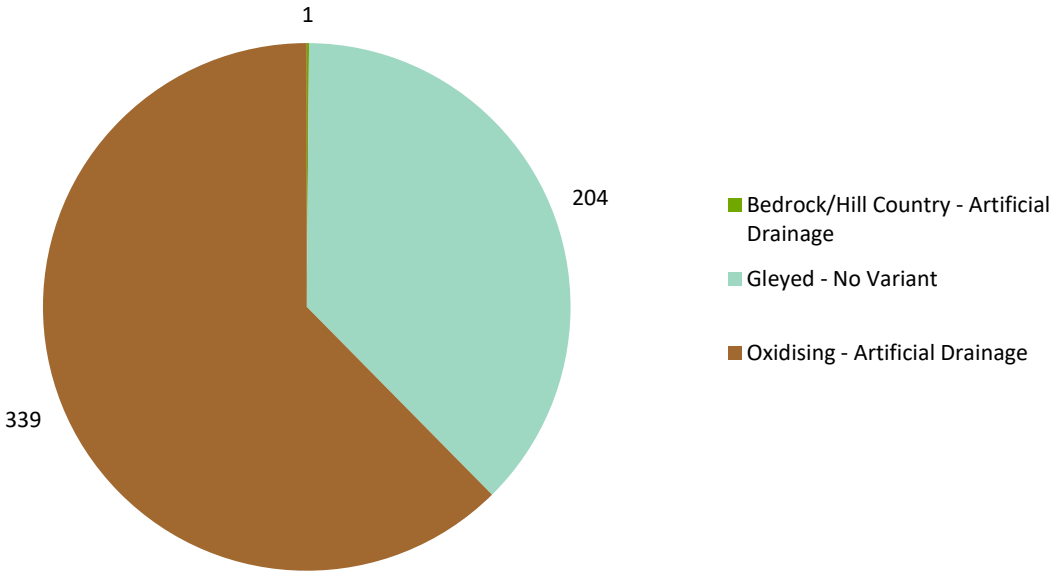


Figure 42. Dairying land (in hectares) in the catchment for the Pourakino River at Traill Road, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 17. Number and type of consented discharges to land and water in the catchment for the Pourakino River at Traill Road.

Pourakino River at Traill Road		
Discharge	Contaminant	Total
To Land	Dairy Shed Effluent (land)	2
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	1
To Land Total		3
Grand Total		3

Note: Consent information accurate as of April 2017

B.6 CASCADE STREAM AT POURAKINO VALLEY ROAD

A single water was collected from the Cascade Stream at Pourakino Valley Road, under base flow conditions. High levels of *E. coli* were present, and no *Campylobacter* was detected. Faecal source tracking determined that ruminant animals were the dominant source of pollution at this site, accounting for 50-100% of the pollution present. More specifically, ovine markers were also detected. Sterol analysis identified only a wildfowl signature; however, the coprostanol level (4ng/L) is lower than is required for accurate assessment. Further sampling of the site is suggested to clarify the possible significance of wildfowl contamination.

That sheep are the primary source of contamination of the Cascade Stream at this site is consistent with the land use in the sub-catchment. Ninety-seven percent of the sub-catchment is native forest or other conservation land; the remaining three percent, which is in the vicinity of the sampling location, is used in sheep and beef agriculture (Figure 43, Figure 44).

Table 18. Results for microbial and FST analysis of water samples collected from the Cascade Stream at Pourakino Valley Road.

Site		Cascade Stream at Pourakino Valley Rd
Sample #		CMB150123
Client #		20150690
Date Sampled		9/02/2015
Rainfall		Yes
		Microbial Properties
Faecal coliforms		2,000
E. coli		1,600
Campylobacter		<0.3
***Campylobacter* Species**		nt
MBiT *Campylobacter* Source	**Wildfowl**	
	Ovine/Bovine/Deer	
	Poultry	
	Not Wildfowl	
	Unknown	
		Faecal Source Tracking
General - GenBac3		++++
Ruminant		50-100%
Human - BacH		+
Human - BiADO		-
Cow		-
Sheep		+
Wildfowl - GFD		-
Wildfowl - E2		-
Canine		-
		Sterol Properties
Total Sterols		2,897
Coprostanol		4
Faecal		No
Human		No
Ruminant		No
Wildfowl		Yes
Plant		nt

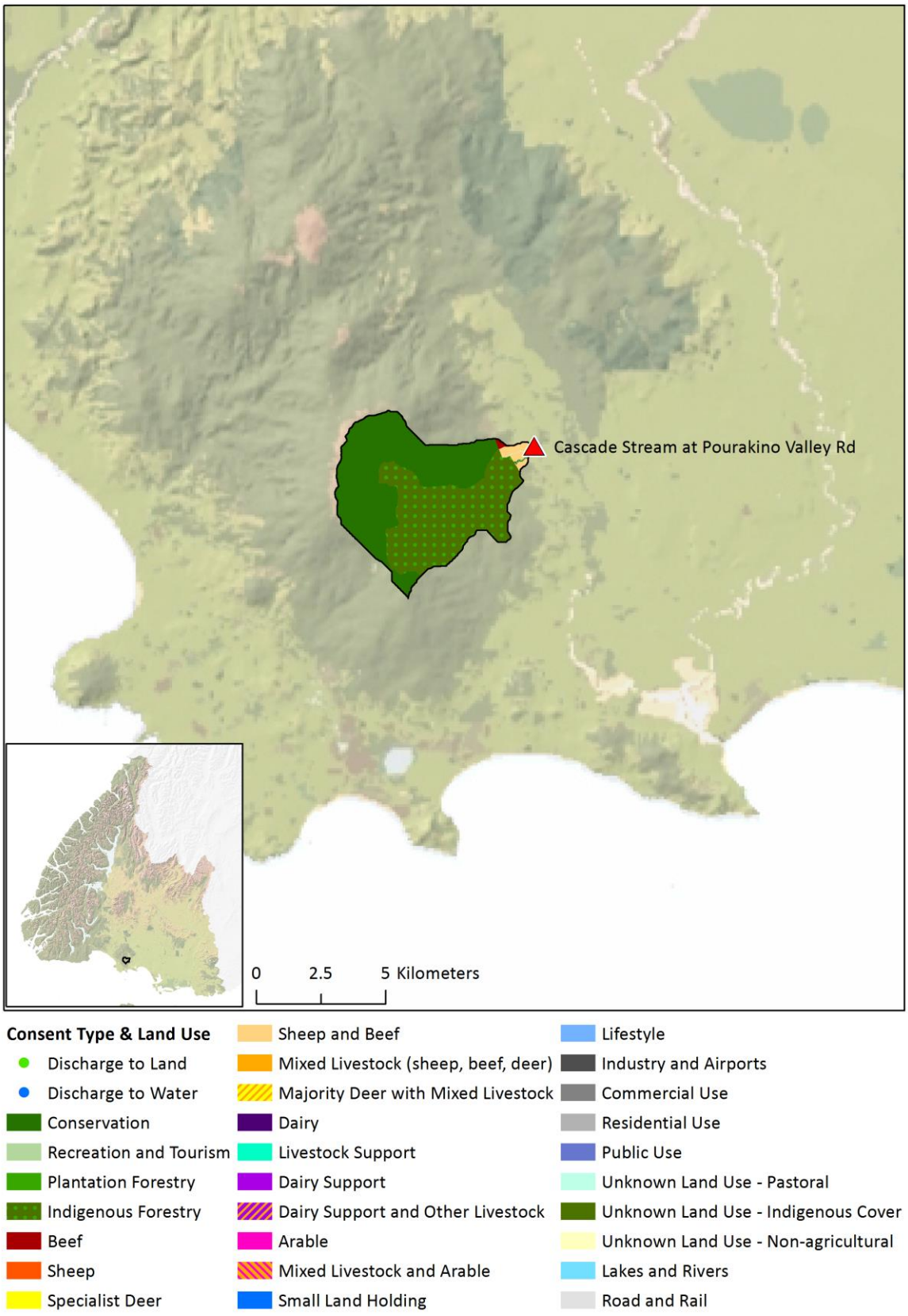


Figure 43. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Cascade Stream at Pourakino Valley Road sampling site.

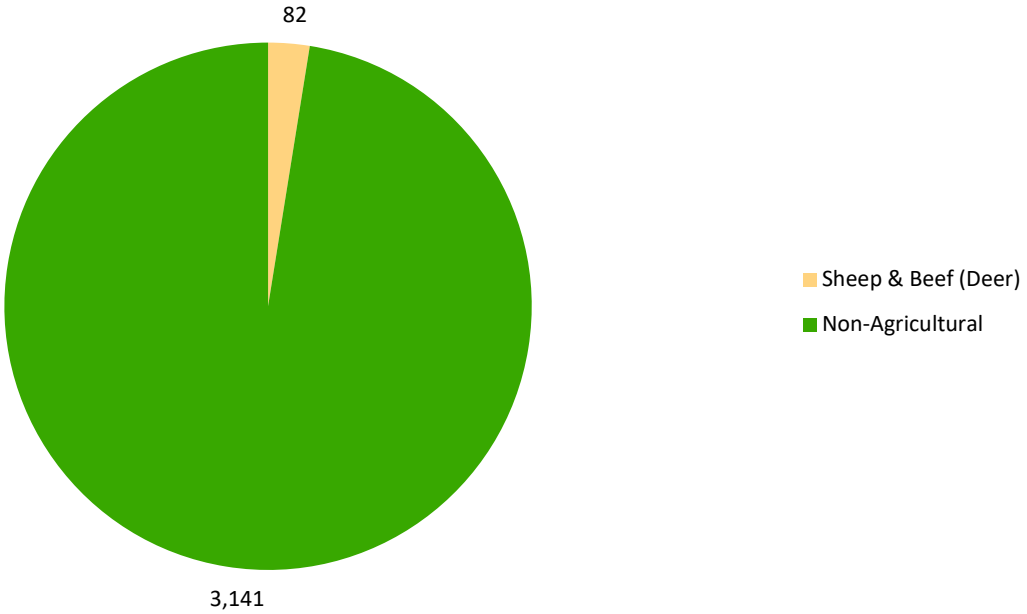


Figure 44. Land use (in hectares) in the catchment for the Cascade Stream at Pourakino Valley Road.

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 Use Map.

There is no dairy activity in this sub-catchment.

There are no consented discharges in this sub-catchment.

B.7 OPOURIKI STREAM AT TWEEDIE ROAD

A total of seven samples were collected from the Oporiki Stream at Tweedie Road, between December 2014 and September 2015 (Table 19). Four samples were collected under base flow conditions, and three following rainfall. Water at the site was fast-flowing, with fencing present. However, sheep have access to the river. Macrophytes and filamentous green algae were visible in the river, and a small riparian strip was present close to the river bank (Figure 45).

Figure 45. Environment Southland staff conducting water sampling in the Opouriki Stream at Tweedie Rd.

E. coli levels under base flow conditions were highly variable, ranging between 280 and 1,100 cfu/100 ml. *Campylobacter* was present in three of the four samples, at between 0.3 and 15 MPN/100 ml. Interestingly, the sample that contained the lowest *E. coli* concentration contained the highest *Campylobacter* concentration, again demonstrating the potential for a lack of correlation between the two groups. Genotype analysis of the *Campylobacter* isolates found that *C. jejuni* was present in two of the three samples, and a thermophyllic species also present in all three samples. MBiT source attribution identified a mixture of wildfowl, ruminant and unknown sources.

Faecal source tracking found that under base flow conditions, the impact of ruminant pollution was varied, but was always less than half of the pollution present: levels were <1% of pollution in the two summer sample, increasing to 1-10 and 10-50% in subsequent samples. However, an ovine PCR marker was detected only in the December sample. Wildfowl markers were detected in all four samples, although this was not reflected in the sterol analysis.

Following rainfall, *E. coli* level increased, with a maximum concentration of 16,000 cfu/100 ml observed in the April sample. This was not reflected in *Campylobacter* concentration, which was detected at only low levels in all three samples (0.4-4.3 MPN/100 ml). *C. jejuni* was present in all three samples, and an unspeciated thremophyllic *Campylobacter* also present in the September sample. Sources were found to be ruminant and 'not wildfowl.' Faecal source tracking showed that after rainfall, ruminant pollution came to dominate the site, accounting for 50-100% of pollution present. Ovine, bovine and wildfowl markers were all identified in each of the three samples. Human contamination markers were also detected in the February sample, although human sterols were not (it should be noted however that coprostanol levels were lower than the levels required for accurate assessment).

The sub-catchment for this site is reasonably small, and contains a mixture of sheep, sheep and beef (together with sheep, 45%), and dairy farming (37% including support) (Figure 46, Figure 47). Conservation land makes up 18% of land use. Consented discharge to land and water are few and relate to dairy farming (Table 20). There is no obvious source for the human contamination based on the available information, and this warrants further investigation.

Table 19. Results for microbial and FST analysis of water samples collected from the Opouriki Stream at Tweedie Road.

Site		Opouriki Stream at Tweedie Road						
Sample #		CMB140896	CMB150003	CMB150219	CMB150790	CMB150125	CMB150361	CMB151537
Client #		20145161	20145303	20150991	20152051	20150692	20151553	20153065
Date Sampled		9/12/2014	12/01/2015	9/03/2015	8/06/2015	9/02/2015	13/04/2015	07/09/2015
Rainfall		No	No	No	No	Yes	Yes	Yes
		Microbial Properties						
Faecal coliforms		1,500	800	470	280	5,000	16,000	600
E. coli		1,100	800	360	280	4,000	16,000	600
Campylobacter		9.3	0.3	<0.3	15	0.4	4.3	0.4
***Campylobacter* Species**		*C. jejuni* & Thermo	*Thermo*	nt	*C. jejuni* & Thermo	*C. jejuni*	*C. jejuni*	*C. jejuni* & Thermo
MBiT *Campylobacter* Source	**Wildfowl**		1		2			
	Ovine/Bovine/ Deer				1		1	
	Poultry							
	Not Wildfowl					1		
	Unknown	2			1			
		Faecal Source Tracking						
General – GenBac3		++++	++++	++++	++++	++++	++++	++++
Ruminant		≤1%	≤1%	1-10%	10-50%	50-100%	50-100%	50-100%
Human - BacH		+	-	+	+	+	+	+
Human - BiADO		-	-	-	-	+	-	-
Cow		-	-	-	-	+	+	+
Sheep		+	-	-	-	+	+	+
Wildfowl - GFD		+	+	+	+	+	+	+
Wildfowl - E2		+	+	+	+	+	+	-
		Sterol Properties						
Total Sterols		8,555	1,677	2,164	1,459	5,136	nt	nt
Coprostanol		169	38	29	47	226		
Faecal		F1+F2	F1+F2	F2	F1+F2	F1+F2		
Human		No	No	No	No	No		
Ruminant		Yes (2)	Yes (2)	Yes (2)	Yes (2)	Yes (2)		
Wildfowl		No	No	No	No	No		

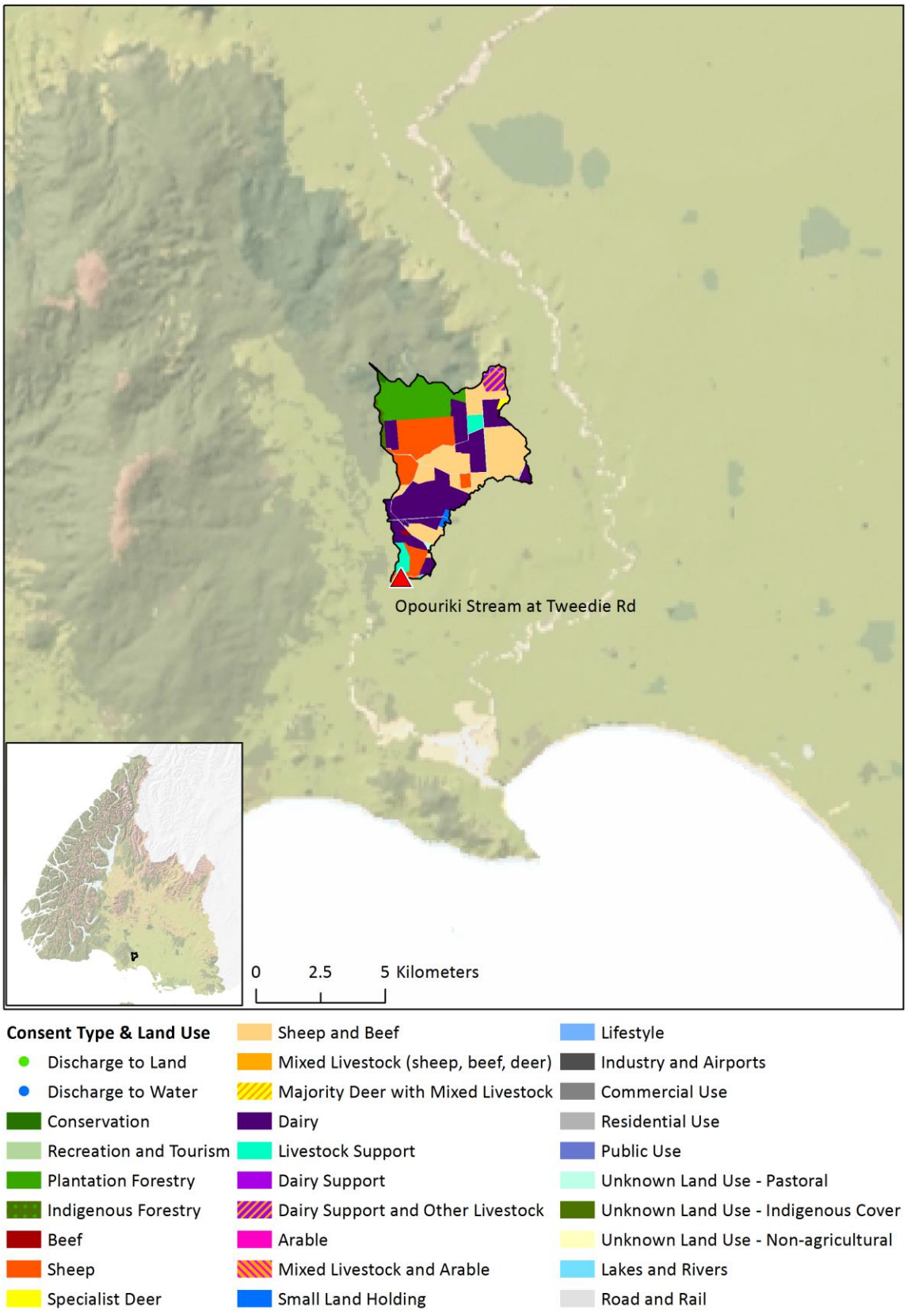


Figure 46. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Opouriki Stream at Tweedie Road sampling site.

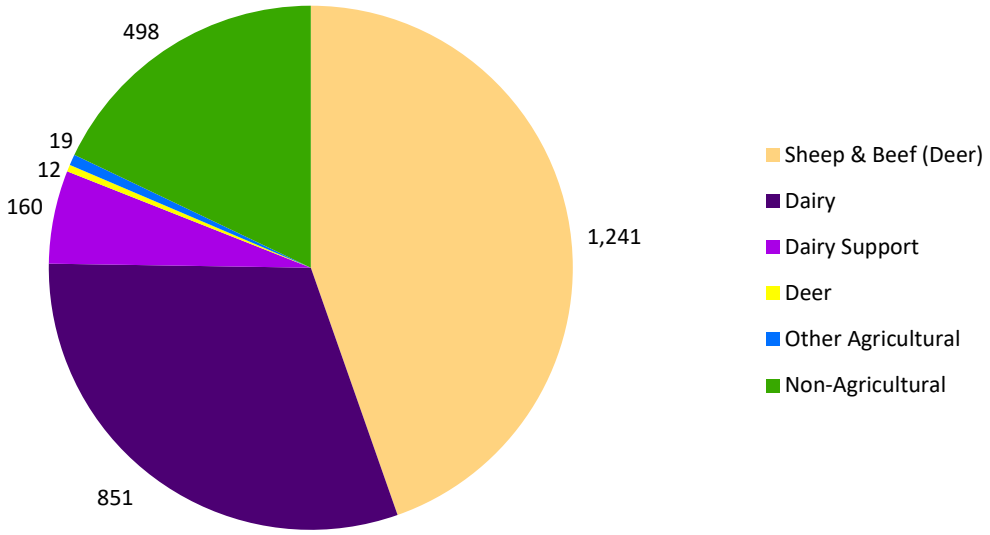


Figure 47. Land use (in hectares) in the catchment for the Opouriki Stream at Tweedie Road.

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 Use Map

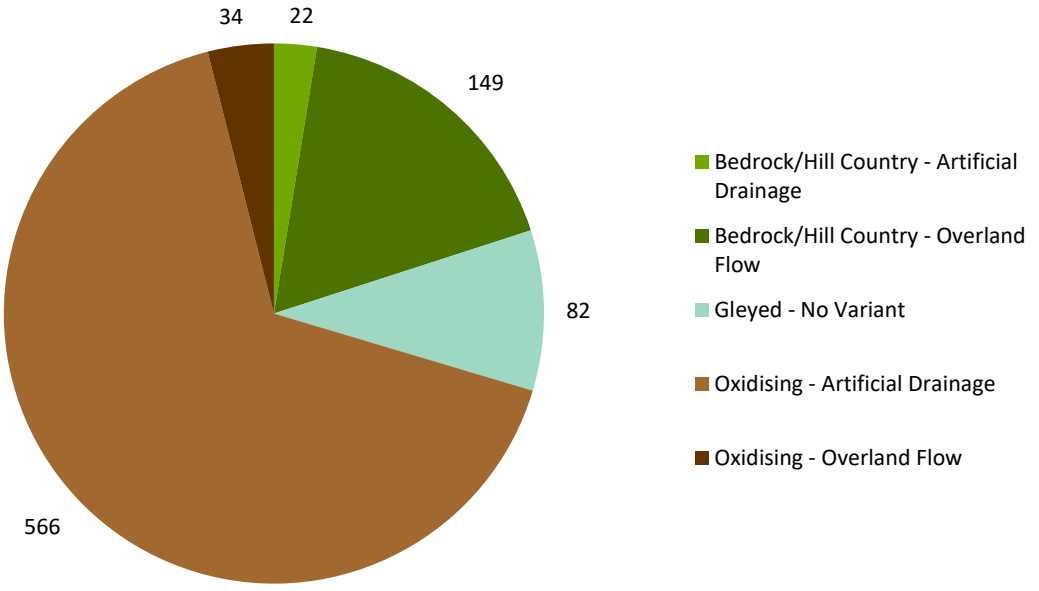


Figure 48. Dairying land (in hectares) in the catchment for the Opouriki Stream at Tweedie Road, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 20. Number and type of consented discharges to land and water in the catchment for the Opouriki Stream at Tweedie Road.

Opouriki Stream at Tweedie Road		
Discharge	Contaminant	Total
To Land	Dairy Shed Effluent (land)	3
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	2
To Land Total		5
Grand Total		5

Note: Consent information accurate as of April 2017

B.8 WAIMATUKU STREAM AT LORNEVILLE-RIVERTON HIGHWAY

The Waimatuku Stream was sampled at the Lorneville-Riverton Highway on five occasions; three samples were collected during dry weather and two following rainfall (Table 21).

Figure 49. Environment Southland staff collecting water samples from the Waimatuku Stream at Lorneville-Riverton Highway.

Under base flow conditions, *E. coli* levels varied between 160 and 220 cfu/100 ml. *Campylobacter* was present in two of the three samples (0.9-9.3 MPN/100 ml), with none detected in the sample with the lowest *E. coli* level. Isolates were determined to be *C. jejuni* from wildfowl, 'not wildfowl' and unknown sources. Ruminant pollution was found to account for less than 10% of the overall pollution at the site, with sheep markers identified in the June sample. Wildfowl contamination markers were present in all three base flow samples. Human contamination was also detected in the June sample.

Following rainfall, *E. coli* concentrations increased (560-1,200 cfu/100 ml), and whilst *Campylobacter* was present in both samples, concentrations were not dissimilar to base flow samples (0.4-0.7 MPN/100 ml). Both samples contained *C. jejuni* from a wildfowl source, with

C. jejuni from an unknown source also present in the more contaminated February sample. Faecal source tracking showed that the relative impact of ruminant pollution increased after rainfall, but still accounted for less than half of overall pollution; neither ovine nor bovine-specific markers were detected. Wildfowl markers were present in both samples, with human contamination also detected in the April sample.

It is interesting that there is no specific indication of contamination from cattle, as dairy farming dominates the sub-catchment (53%, plus 3% dairy support). Sheep and sheep and beef farming make up much of the remainder of land use in the area (38%) (Figure 50, Figure 51). Consented discharges include dairy shed effluents, wash down effluents and oil and grease; there is no obvious source of the human contamination detected under base flow or post-rain conditions (Table 22).

Table 21. Results for microbial and FST analysis of water samples collected from the Waimatuku Stream at the Lorneville-Riverton Highway.

Site		Waimatuku Stream at Lorneville-Riverton Highway				
Sample #		CMB150005	CMB150241	CMB150791	CMB150127	CMB150363
Client #		20145306	20150994	20152054	20150695	20151556
Date Sampled		12/01/2015	9/03/2015	8/06/2015	9/02/2015	13/04/2015
Rainfall		No	No	No	Yes	Yes
		Microbial Properties				
Faecal coliforms		160	490	220	1,400	670
E. coli		160	380	220	1,200	560
Campylobacter		<0.3	0.9	9.3	0.7	0.4
***Campylobacter* Species**		nt	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**			2	1	1
	Ovine/Bovine/ Deer					
	Poultry					
	Not Wildfowl		1			
	Unknown			1	1	
		Faecal Source Tracking				
General - GenBac3		++++	++++	++++	++++	++++
Ruminant		≤1%	1-10%	1-10%	10-50%	10-50%
Human - BacH		-	-	+	-	+
Human - BiADO		-	-	+	+	+
Cow		-	-	-	-	-
Sheep		-	-	+	-	-
Wildfowl - GFD		+	+	+	+	+
Wildfowl - E2		-	+	+	+	+
		Sterol Properties				
Total Sterols		4,549	nt	1,337	nt	4,925
Coprostanol		48		38		108
Faecal		F2		F1+F2		(F1)+F2
Human		No		No		No
Ruminant		<30		Yes (2)		Yes (2)
Wildfowl		Yes		No		No

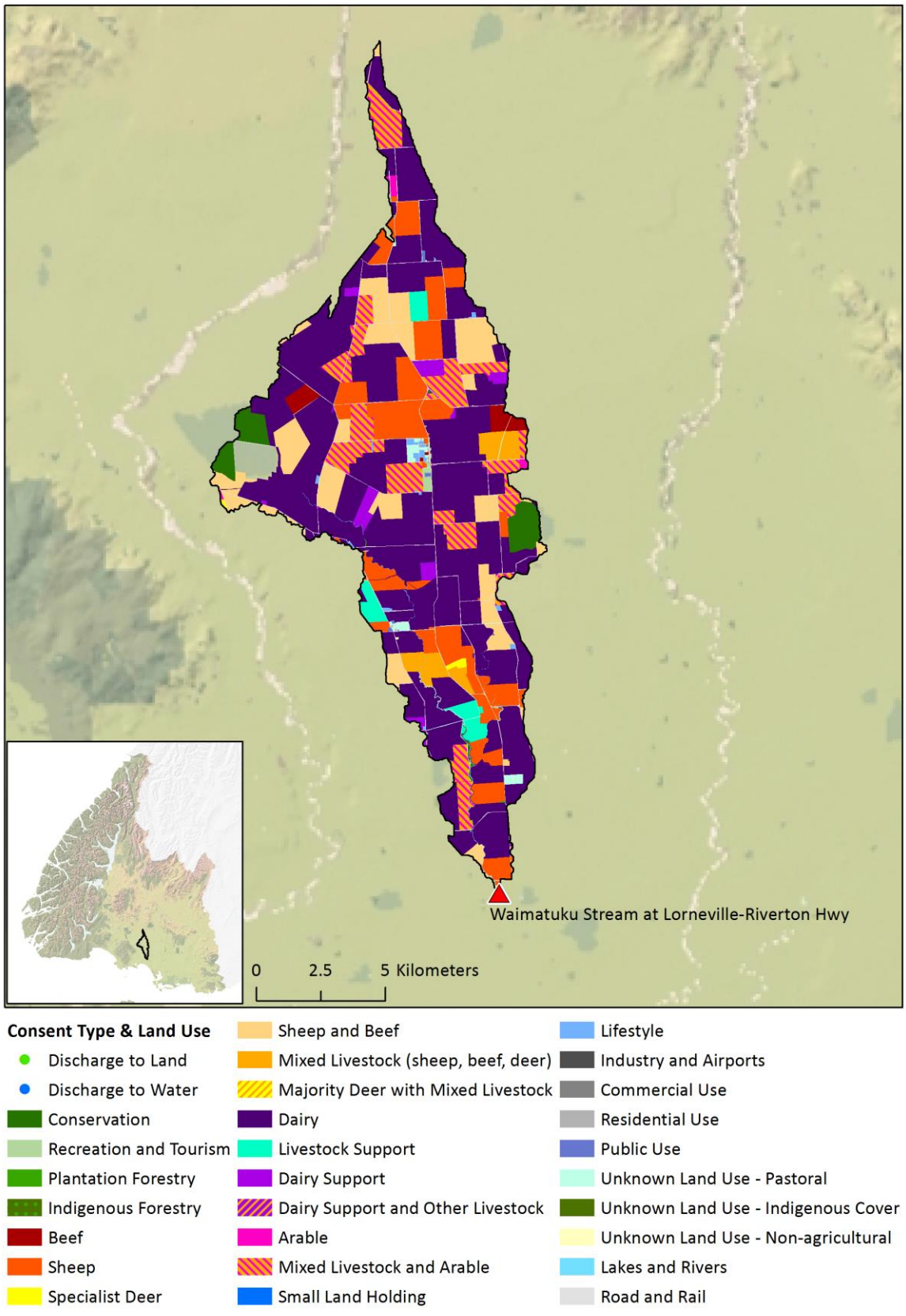


Figure 50. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Waimatuku Stream at Lorneville-Riverton Highway sampling site.

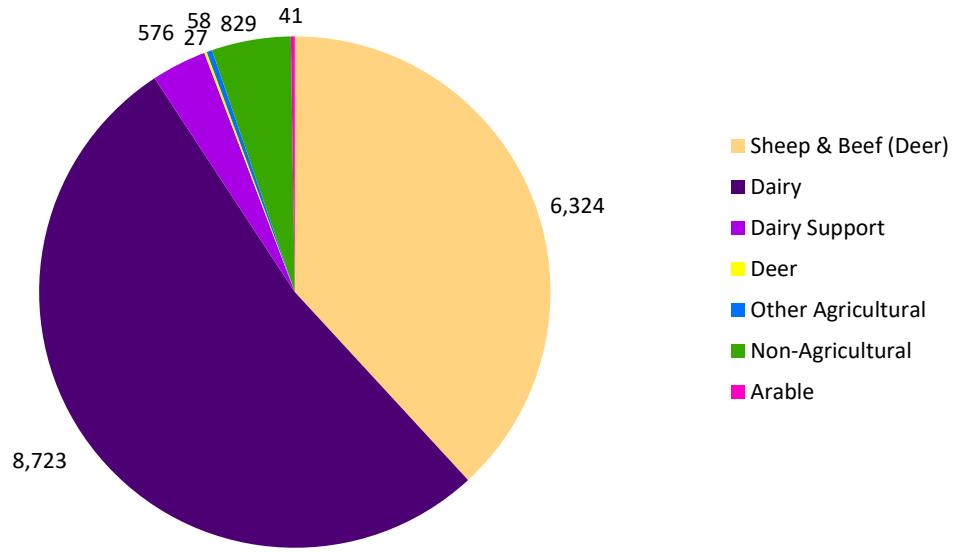


Figure 51. Land use (in hectares) in the catchment for the Waimatuku Stream at Lorneville-Riverton Highway.

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 Use Map.

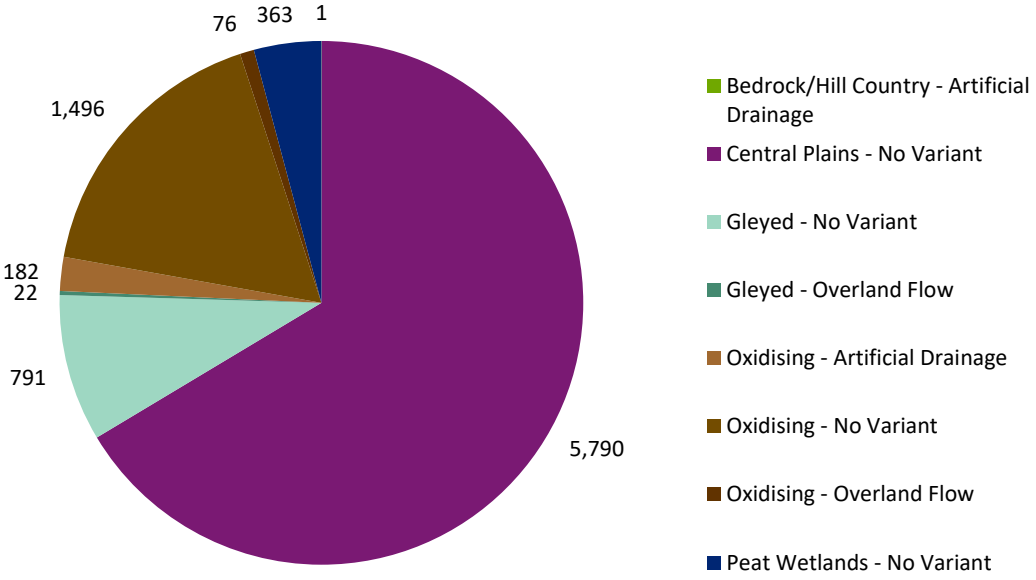


Figure 52. Dairying land (in hectares) in the catchment for the Waimatuku Stream at Lorneville-Riverton Highway, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 22. Number and type of consented discharges to land and water in the catchment for the Waimatuku Stream at Lorneville-Riverton Highway.

Waimatuku Stream at Lorneville-Riverton Hwy		
Discharge	Contaminant	Total
To Land	Other (whey to pasture)	7
	Dairy Shed Effluent (land)	30
	Dairy Shed Effluent (land), Wintering Pad/Feedlot Effluent (land)	8
	Oil/Grease	1
	Wash Down Effluent, Wash Water	1
To Land Total		47
Grand Total		47

Note: Consent information accurate as of April 2017

B.9 HAMILTON BURN AT AFFLECK ROAD

Hamilton Burn was sampled at Affleck Road on four occasions between February and June 2015 – twice under base flow, and twice after rainfall (Table 23).

Samples collected under base flow conditions had low levels of *E. coli* (120-210 cfu/100 ml), although a low level of *Campylobacter* was also present (2.30-4.3 MPN/100 ml). Isolates were determined to be *C. jejuni*, with an unspeciated thermophilic *Campylobacter* also present in the June sample. MBiT source attribution identified wildfowl and an unknown source. Faecal source tracking found that ruminant pollution made up less than half of the pollution at this site. Wildfowl markers were present in both samples and are likely to be a significant faecal source.

The samples collected following rainfall were similar to base flow samples. *E. coli* levels were slightly higher (340-400 cfu/100 ml), and low levels of *Campylobacter* were recovered from one samples (4.3 MPN/100 ml in April). Further analysis confirmed *C. jejuni* from a wildfowl source. Ruminant pollution was determined to account for 10-50% of pollution in the February samples, increasing to the dominant source in April, although a specific ruminant source could not be identified. A wildfowl signature was again present in both samples.

A review of land use shows a mixture of sheep, sheep and beef (together 62%), conservation (21%) and dairy (12%) applications within the Hamilton Burn sub-catchment (Figure 53, Figure 54).

Table 23. Results for microbial and FST analysis of water samples collected from Hamilton Burn at Affleck Road.

Site		Hamilton Burn at Affleck Road			
Sample #		CMB150216	CMB150787	CMB150120	CMB150356
Client #		20150986	20152046	20150687	20151548
Date Sampled		9/03/2015	8/06/2015	9/02/15	13/04/2015
Rainfall		No	No	Yes	Yes
		Microbial Properties			
Faecal coliforms		270	130	470	460
E. coli		210	120	400	340
Campylobacter		2.3	4.3	<0.3	4.3
***Campylobacter* Species**		*C. jejuni*	*C. jejuni* & Thermo	nt	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**		4		4
	Ovine/Bovine/Deer				
	Poultry				
	Not Wildfowl				
	Unknown	1			
		Faecal Source Tracking			
General - GenBac3		++++	++++	++++	+++
Ruminant		1-10%	10-50%	10-50%	50-100%
Human - BacH		-	-	-	-
Human - BiADO		-	-	+	-
Cow		-	-	-	-
Sheep		-	-	-	-
Wildfowl - GFD		+	+	+	+
Wildfowl - E2		+	+	+	+
		Sterol Properties			
Total Sterols		nt	395	nt	nt
Coprostanol			14		
Faecal			-		
Human			-		
Ruminant			-		
Wildfowl			-		

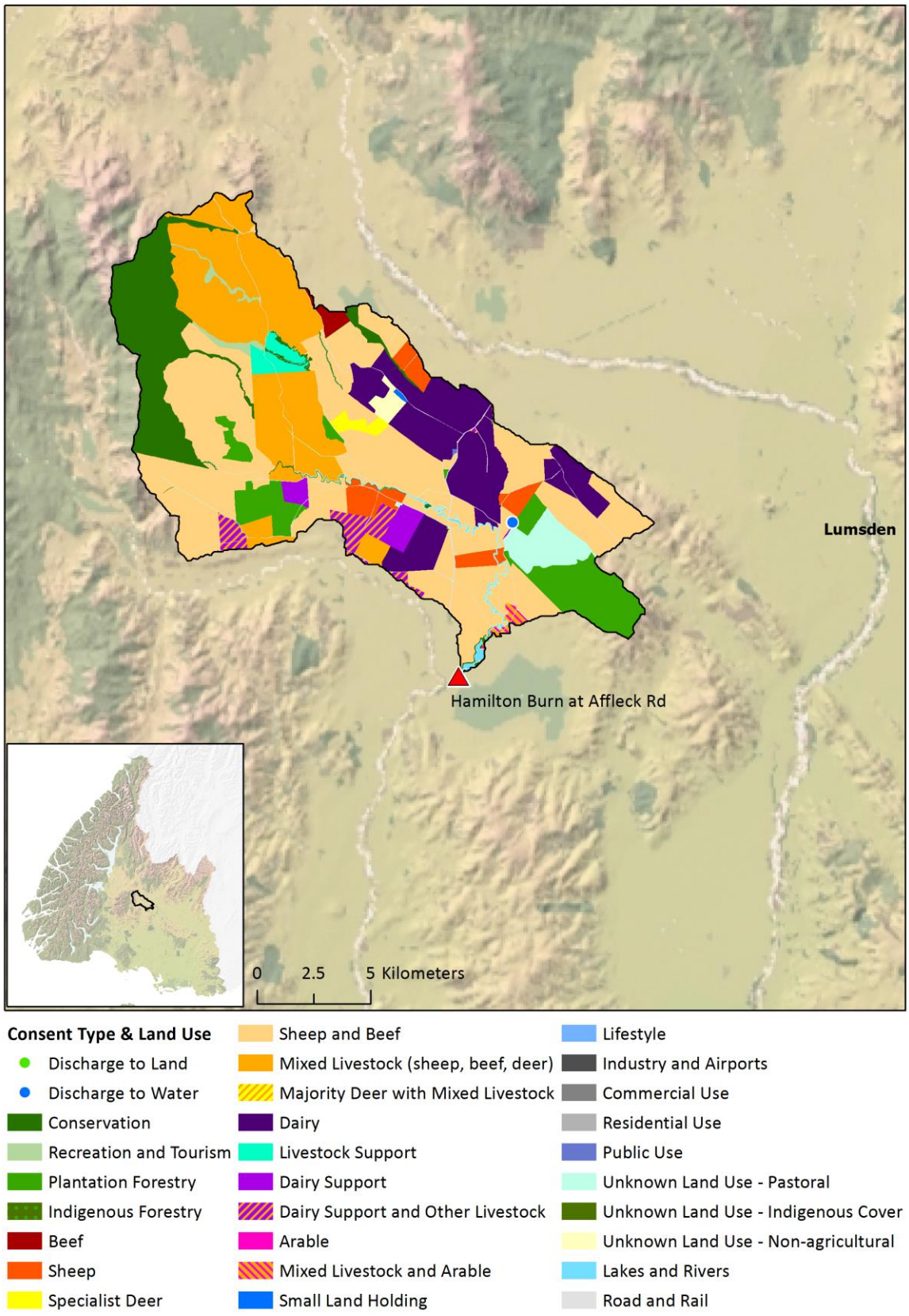


Figure 53. Land use and consents with potential *E. coli* contamination risk (non-dairy) in the catchment for the Hamilton Burn at Affleck Road sampling site.

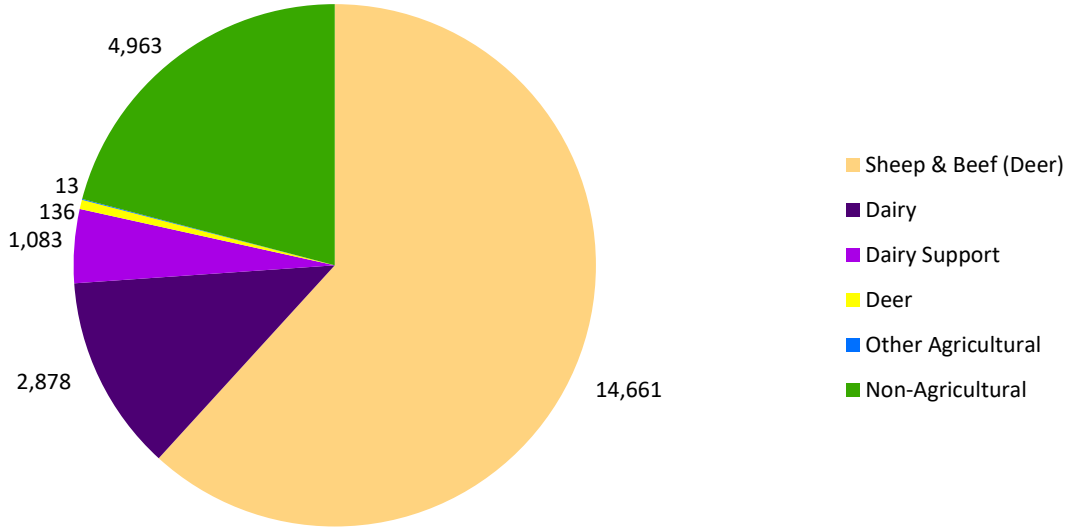


Figure 54. Land use (in hectares) in the catchment for Hamilton Burn at Affleck Road.

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 Use Map.

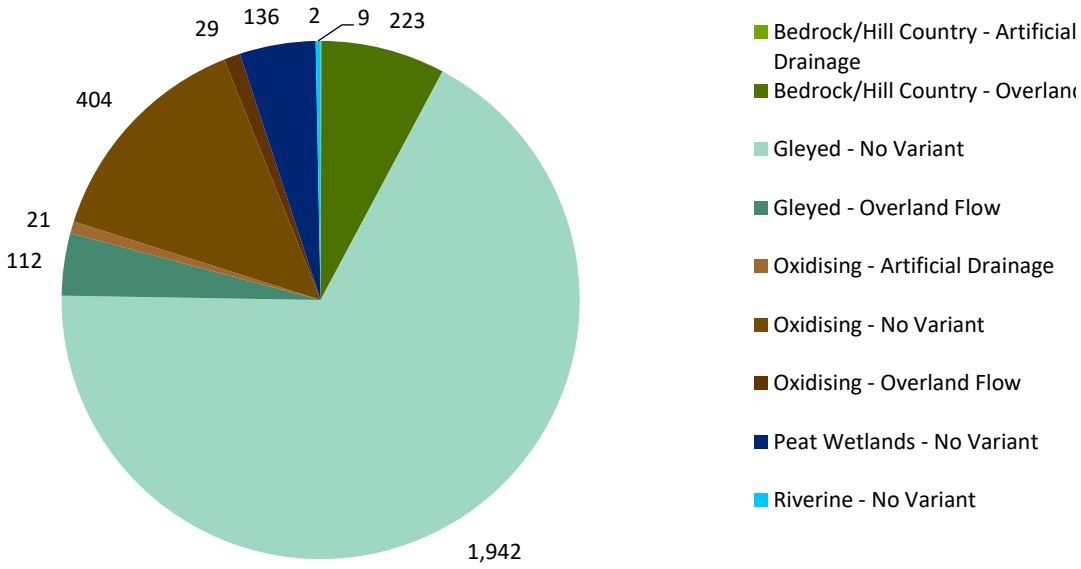


Figure 55. Dairying land (in hectares) in the catchment for Hamilton Burn at Affleck Road, separated into physiographic units.

Southland Physiographic information accurate as of June 2016

Table 24. Number and type of consented discharges to land and water in the catchment for Hamilton Burn at Affleck Road

Hamilton Burn at Affleck Road		
Discharge	Contaminant	Total
To Land	Other (whey to pasture) 1080 Dairy Shed Effluent (land)	1 2 9
To Land Total		12
To Water	Stormwater	1
To Water Total		1
Grand Total		13

Note: Consent information accurate as of April 2017

B.10 OTAUTAU PLAINS DETAILED STUDY

B.10.1 Wairio Stream

The Wairio Stream was tested at four sites on the 9 March 2015 (Figure 56; Table 25). These were all one-off samples, collected under base flow conditions. Wairio Stream feeds into the Otautau Stream, close to the Waikouro Shortcut sampling site.

Figure 56. Wairio Stream sampling sites used in the detailed Otuatua stream study. a) Wairio Stream at 20 Birchwood-Wairirio Road; b) Wairio Stream at 55 Collie Road; c) Wairio Stream at Waicolo Road East; d) Wairio Stream at 100 Line Rd West 1050-1076.

The sampling site at 20 Birchwood-Wairirio Road had very high levels of *E. coli* (7,000 cfu/100 ml), with low levels of *Campylobacter* detected (2.3 MPN/100 ml). Further analysis identified *C. jejuni* of wildfowl origin. Faecal source tracking analysis showed that ruminant pollution was dominant at this site, accounting for 50-100% of pollution present, and with both ovine and bovine PCR markers detected. Wildfowl markers were also detected. Faecal sterol analysis also supported the presence of ruminant contamination. The land surrounding the site is agricultural with evidence of animals in the vicinity of the stream. There also appears to be a number of houses and a tavern in the vicinity.

Downstream at 55 Collie Road, the water quality was significantly better, with *E. coli* levels of 230 cfu/100 ml, and no detectable *Campylobacter*. Faecal source tracking identified low levels of ruminant contamination (1-10%), with no specific PCR markers detected.

The site at Waicolo Road East was found to have slightly higher levels of faecal indicators (800 cfu *E. coli*/100 ml), with *Campylobacter* again undetected. Ruminant pollution was more prevalent here than at Collie Road, accounting for 10-50% of the overall pollution present, although no specific source markers were identified. Wildfowl markers were detected.

E. coli concentration increased again at the final site at Hundred Line Road West (1,100 cfu/100 ml), and low levels of *Campylobacter* were detected. Further analysis identified *C. jejuni* of wildfowl origin. Faecal source tracking showed ≤1% of the faecal pollution detected was of ruminant origin, which represents a dilution from the previous site. This may be due to an input of clean water to the stream, as the flow rate (0.1 cumecs) was the highest of the four sites. Wildfowl pollution appears to be the dominant pollution type, with an especially high concentration of E2 markers, which are most commonly associated with ducks.

Table 25. Results for microbial and FST analysis of water samples collected from the four sites along the Wairio Stream, as part of a detailed study of pollution in the Otautau Plains area.

Site		Wairio Stream at 20 Birchwood-Wairirio Road	Wairio Stream at 55 Collie Road	Wairio Stream East Trib. at Waicola Road	Wairio Stream at 1050-1076 Hundred Line Rd West
Sample #		CMB150214	CMB150213	CMB150207	CMB150203
Client #		20151033	20151032	20151026	20151022
Date Sampled		9/03/2015	9/03/2015	9/03/2015	9/03/2015
Map Reference		B	C	D	E
Rainfall		No	No	No	No
		Microbial Properties			
Faecal coliforms		8,000	300	800	1,400
E. coli		7,000	230	800	1,100
Campylobacter		2.3	<0.3	<0.3	1.5
***Campylobacter* Species**		*C. jejuni*	nt	nt	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	1			2
	Ovine/Bovine /Deer				
	Poultry				
	Not Wildfowl				
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	++++	++++
Ruminant		50-100%	1-10%	10 - 50%	≤1%
Human - BacH		+	-	-	-
Human - BiADO		+	-	-	+
Cow		+	-	-	-
Sheep		+	-	-	-
Wildfowl - GFD		+	-	+	+
Wildfowl - E2		-	-	+	++
Canine		-	-	-	-
		Sterol Properties			
Total Sterols		8,556	nt	nt	nt
Coprostanol		911			
Faecal		F1+F2			
Human		No			
Ruminant		Yes (2)			
Wildfowl		No			
Plant		No			

B.10.2 North Head Stream and Upper Otautau River sites

Water samples were collected from the North Head Stream and Upper Otautau Stream on the 9 March 2015, under base flow conditions. These streams are in the northwest of the catchment, and were sampled on a single occasion under base flow conditions.

North Head stream was sampled at two sites: 267 Waicola Road and 1015-1047 Hundred Line Road West (Figure 57, Table 26).

Figure 57. ESR staff carrying out sampling at North Head Stream sites: a) 267 Waicola Road; b) 100 Line Rd West 1015-1047.

The North Head stream site at 267 Waicola Road had relatively low levels of indicator bacteria (100 cfu *E. coli*/100 ml). No *Campylobacter* was detected. Faecal source tracking found only a weak ruminant signal, accounting for less than 1% of overall pollution. No source-specific PCR markers were detected, possibly as a consequence of the low levels of pollution present.

Downstream at 100 Line Road West, *E. coli* levels increased to 600 cfu/100 ml, and low levels of *Campylobacter* were detected (0.7 MPN/100 ml). Further analysis identified isolates as being *C. jejuni* from a wildfowl source. Similarly to the Waicola Road site, ruminant pollution accounted for less than 1% of overall pollution. The presence of both GFD and E2 markers show that wildfowl are the source of contamination at this site.

Table 26. Results for microbial and FST analysis of water samples collected from two sites along North Head Stream, as part of a detailed study of pollution in the Otautau Plains area.

Site		North Head Stream at 267 Waicola Road	North Head Stream at 1015-1047 Hundred Line Rd West
Sample #		CMB150209	CMB150202
Client #		20151028	20151030
Date Sampled		9/03/2015	9/03/2015
Map Reference		F	G
Rainfall		No	No
		Microbial Properties	
Faecal coliforms		100	700
E. coli		100	600
Campylobacter		<0.3	0.7
***Campylobacter* Species**		nt	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**		2
	Ovine/Bovine/ Deer		
	Poultry		
	Not Wildfowl		
	Unknown		
		Faecal Source Tracking	
General - GenBac3		++++	++++
Ruminant		≤1%	≤1%
Human - BacH		-	-
Human - BiADO		-	-
Cow		-	-
Sheep		-	-
Wildfowl - GFD		-	+
Wildfowl - E2		-	+
Canine		-	-
		Sterol Properties	
Total Sterols		nt	nt
Coprostanol			
Faecal			
Human			
Ruminant			
Wildfowl			
Plant			

Further downstream, the upper Otautau Stream was sampled in four locations (Figure 58, Table 27). The four sites had similar levels of *E. coli*, ranging between 800 and 1,200 cfu/100 ml. *Campylobacter* was detected at three of the sites – Hundred Line Road, Upper Scott's Gap/Symonds Road, and Lower Scott's Gap/Symonds Road – at concentrations between 4.3 and 24 MPN/100 ml. Genotype analysis identified the *Campylobacter* isolates from Hundred Line Road and Upper Scott's Gap as *C. jejuni*, and those from Lower Scott's Gap as being a thermophyllic *Campylobacter*. MBiT source attribution analysis found a number of different sources between sites. Faecal source tracking analysis also suggested that faecal sources were different between sites.

The site at Hundred Line Road was impacted predominantly by wildfowl pollution; ruminant pollution accounted for <1% of pollution. Wildfowl were also determined to be the most likely source of the *Campylobacter* present. Canine PCR markers were also detected. Faecal sterol analysis identified wildfowl and plant sterol signatures.

Up to half of the pollution present at the Upper Scott's Gap site (10-50%) was determined to be of ruminant origin, with ovine PCR markers detected. Ruminant sterols were also detected. Although no wildfowl PCR markers were detected, faecal sterols suggested the presence of wildfowl pollution.

The site at Upper Scott's Gap/Symon's Road was impacted largely by ruminant pollution (50-100%), with bovine PCR markers and ruminant faecal sterols present. Wildfowl pollution was also evident. The *Campylobacter* could be identified only as 'not wildfowl,' and could therefore be of ruminant, human or poultry origin.

At Lower Scott's Gap, up to half of the pollution present was of ruminant origin, likely cattle. Wildfowl markers were also detected. The *Campylobacter* isolates were determined to be of a mixture of wildfowl and 'not wildfowl' origin.

Figure 58. Sites along the Otautau Stream sampled as part of the detailed study: a) Otautau at 100 Line Road 1248-1346; b) 99 Upper Scott's Gap Upper; c) 122 Upper Scott's Gap/Symons Road; d) 232-402 Lower Scott's Gap/Symons Road.

Table 27. Results for microbial and FST analysis of water samples collected from four sites along the upper Otautau Stream, as part of a detailed study of pollution in the Otautau Plains area.

Site		Otautau at 1248-1346 Hundred Line Road	Otautau at 99 Upper Scott's Gap	Otautau at 122 Upper Scott's Gap/Symons Road	Otautau at 232-402 Lower Scott's Gap/Symons Road
Sample #		CMB150201	CMB150198	CMB150196	CMB150195
Client #		20151020	20151017	20151015	20151029
Date Sampled		9/03/2015	9/03/2015	9/03/2015	9/03/2015
Map Reference		H	I	J	K
Rainfall		No	No	No	No
		Microbial Properties			
Faecal coliforms		1,000	800	1,500	1,300
E. coli		900	800	1,000	1,200
Campylobacter		24	<0.3	4.3	9.3
***Campylobacter* Species**		*C. jejuni*	nt	*C. jejuni*	Thermo
MBiT *Campylobacter* Source	**Wildfowl**	2			1
	Ovine/Bovine/ Deer				
	Poultry				1
	Not Wildfowl			2	
	Unknown				
		Faecal Source Tracking			
General - GenBac3		++++	++++	++++	++++
Ruminant		≤1%	10-50%	50-100%	10-50%
Human - BacH		+	-	-	-
Human - BiADO		-	-	-	-
Cow		-	-	+	+
Sheep		-	+	-	-
Wildfowl - GFD		+	-	-	+
Wildfowl - E2		+	-	+	+
Canine		+	-	-	-
		Sterol Properties			
Total Sterols		3,438	3,354	1,456	nt
Coprostanol		45	31	38	
Faecal		F2	F2	F1+F2	
Human		No	No	No	
Ruminant		No	<30	Yes (2)	
Wildfowl		Yes	Yes	No	
Plant		Yes	Yes	No	

B.10.3 Confluence of Wairio Stream and Otautau Stream at Waikouro Shortcut

Samples were collected from the Wairio and Otautau Streams, upstream of their confluence at the Waikouro Shortcut (Figure 59, Table 28). Results found high levels of *E. coli* (1,100-1,500 cfu/100 ml) and *Campylobacter* (240 MPN/100 ml) in both streams. Analysis of the *Campylobacter* determined that in both cases, isolates were *C. jejuni*, and were most likely of a wildfowl origin.

The impact of ruminant pollution on both the Wairio and Otautau Streams at this site was found to be relatively low (1-10% of overall pollution), although ovine PCR markers were detected in the Wairio Stream. Both streams had wildfowl markers present, particularly the E2 (duck) markers, which was present in high concentrations.

The Wairio Stream was also positive for human faecal contamination; there was no evidence of this in the Otautau Stream.

Faecal sterol analysis was only undertaken for the Wairio Stream, and found evidence of ruminant sterols only.

Figure 59. Otautau Stream and Wairio Stream confluence at Waikouro Shortcut.

Table 28. Results for microbial and FST analysis of water samples collected from the Wairio and Otautau Streams, just upstream of their confluence at the Waikouro Shortcut, as part of a detailed study of pollution in the Otautau Plains area.

Site		Wairio Stream upstream confluence with Otautau Stream	Otautau Stream upstream of confluence with Wairio Stream
Sample #		CMB150206	CMB150187
Client #		20151025	20151006
Date Sampled		9/03/2015	9/03/2015
Map Reference		L	M
Rainfall		No	No
		Microbial Properties	
Faecal coliforms		1,200	2,100
E. coli		1,100	1,500
Campylobacter		240	240
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	3	1
	Ovine/Bovine/ Deer		
	Poultry		
	Not Wildfowl		
	Unknown		
		Faecal Source Tracking	
General - GenBac3		++++	++++
Ruminant		1-10%	1-10%
Human - BacH		+	-
Human - BiADO		+	-
Cow		-	-
Sheep		+	-
Wildfowl - GFD		++	+
Wildfowl - E2		++	++
		Sterol Properties	
Total Sterols		7,367	nt
Coprostanol		273	
Faecal		F1+F2	
Human		No	
Ruminant		Yes (2)	
Wildfowl		No	

B.10.4 Waicolo Stream

The Waicolo Stream was sampled where it crosses Hundred Line Road, and further downstream where it crosses Waikouro Wairio Road (Figure 60). Waicolo Stream then connects with the Otautau Stream. The Opio Stream and North and South Fern Burns also join the Otautau Stream at this junction, which is just upstream of the Otautau-Tuatapere Road sampling site. Samples were collected from the Hundred Line Road site in March 2015, and from Waikouro in December 2014 and March 2015 (Table 29).

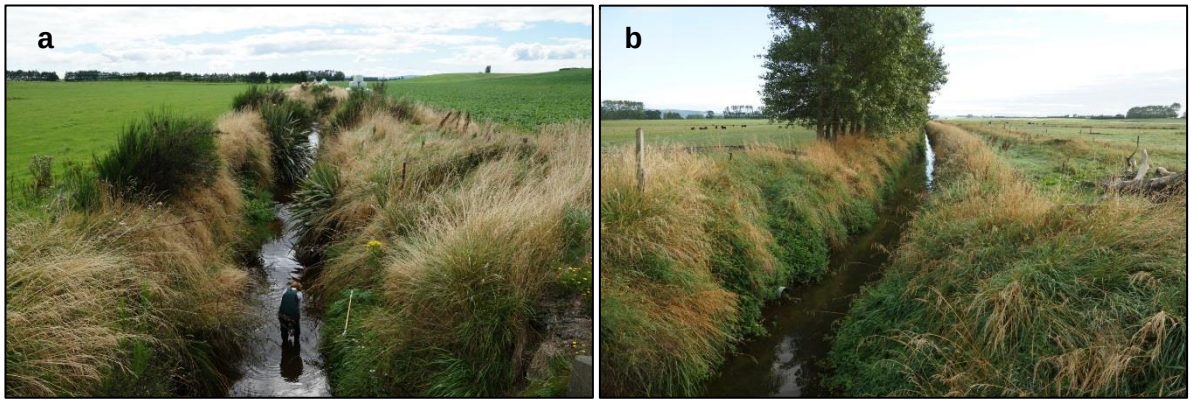


Figure 60. Sampling sites on Waicolo Stream at: a) 100 Line Rd East; b) Waicolo Stream at Waikouro.

The samples collected from both sites in March had similar *E. coli* concentrations, at 340 and 330 cfu/100 ml. *Campylobacter* was detected at both sites, but at significantly different concentrations: 4.3 MPN/100 ml at Hundred Line Road and 150 MPN/100 ml at Waikouro. All isolates were determined to be *C. jejuni*, and from a wildfowl source.

Faecal source tracking data was consistent for the two sites, showing that the impact of ruminant pollution was minimal (≤10%), and that wildfowl were likely the dominant pollution source.

Comparison of the two samples taken from the Waicolo Stream at Waikouro also found that ruminant pollution was minimal and that wildfowl were the most likely source of pollution. *C. jejuni* of wildfowl origin was isolated from both samples. However, the concentrations of *E. coli* and *Campylobacter* in the two samples are quite different: where the March sample had relatively low *E. coli* and high *Campylobacter* concentrations, the earlier sample had high *E. coli* (5,100 cfu/100 ml) and low *Campylobacter* (2.3 MPN/100 ml). The reasons for these different ratios of *E. coli* and *Campylobacter* are unclear, however the results highlight the potential that high pathogen concentrations are not always matched by high indicator levels.

Table 29. Results for microbial and FST analysis of water samples collected from the Waicolo Stream, as part of a detailed study of pollution in the Otautau Plains area.

Site		Waicolo Stream at Hundred Line Rd East	Waicolo at Waikouro	
Sample #		CMB150205	CMB140903	CMB150193
Client #		20151024	20145169	20151027
Date Sampled		9/03/2015	9/12/2014	9/03/2015
Map Reference		O	P	
Rainfall		No	No	No
		Microbial Properties		
Faecal coliforms		390	6,100	360
E. coli		340	5,100	330
Campylobacter		4.3	2.3	150
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**	1	2	4
	Ovine/Bovine/Deer			
	Poultry			
	Not Wildfowl			
	Unknown			
		Faecal Source Tracking		
General - GenBac3		++++	++++	++++
Ruminant		1-10%	1-10%	≤1%
Human - BacH		-	-	+
Human - BiADO		+	+	-
Cow		-	-	-
Sheep		-	-	-
Wildfowl - GFD		+	+	+
Wildfowl - E2		+	+	+
		Sterol Properties		
Total Sterols		nt	14,397	4,161
Coprostanol			426	134
Faecal			F1+F2	F1+F2
Human			No	No
Ruminant			Yes	Yes (2)
Wildfowl			No	No

B.10.5 Opio Stream at Otautau Nightcaps

Water samples were collected from the Opio Stream at Nightcaps in December 2014 and March 2015 (Figure 61, Table 30). Both samples were collected under base flow conditions. *E. coli* concentrations were elevated in December (3,200 cfu/100 ml), but were reduced in March (430 cfu/100 ml). *Campylobacter* was detected only in the March sample, at a concentration of 240 MPN/100 ml. Genotype analysis identified isolates as *C. jejuni*, with source attribution analysis identifying both wildfowl and ruminant sources. This concentration of *Campylobacter* would be regarded as significant in any water sample, but is of particular interest given the relatively low *E. coli* concentration. Further sampling would be useful to determine whether this concentration of *Campylobacter* is a regular occurrence or an outlier.

Faecal source tracking identified some differences in the faecal sources between the two samples. In December, ruminant pollution was dominant (50-100%), with ovine and bovine markers detected. Wildfowl markers were also present. In March, ruminant pollution accounted for less than half (10-50%) of the pollution, with bovine markers only being detected. Wildfowl markers were again present, with especially high levels of the E2 duck marker, suggesting wildfowl pollution was more prevalent in this sample.

Figure 61. Sample collection from the Opio Stream at Otautau Nightcaps.

Table 30. Results for microbial and FST analysis of water samples collected from the Opio Stream at Otautau Nightcaps, as part of a detailed study of pollution in the Otautau Plains area.

Site		Opio Stream at Otautau Nightcaps	
Sample #		CMB140902	CMB150194
Client #		20145168	20151013
Date Sampled		9/12/2014	9/03/2015
Map Reference		R	
Rainfall		No	No
		Microbial Properties	
Faecal coliforms		3,500	530
E. coli		3,200	430
Campylobacter		<0.3	240
***Campylobacter* Species**		nt	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**		2
	Ovine/Bovine/ Deer		1
	Poultry		
	Not Wildfowl		
	Unknown		
		Faecal Source Tracking	
General - GenBac3		++++	++++
Ruminant		50-100%	10-50%
Human - BacH		-	-
Human - BiADO		-	+
Cow		+	+
Sheep		+	-
Wildfowl - GFD		+	+
Wildfowl - E2		+	++
		Sterol Properties	
Total Sterols		25,214	nt
Coprostanol		1,239	
Faecal		F1+F2	
Human		No	
Ruminant		Yes	
Wildfowl		No	

B.10.6 North and South Fern Burn

Water samples were collected from North Fern Burn at Forestry Road and at Otautau-Tuatapere Road (Figure 62), and from South Fern Burn at Otautau-Tuatapere Road (Figure 63). A single sample was collected from the Forestry Road site, while the two Otautau-Tuatapere Road sites were each samples in both December 2014 and March 2015 (Table 31).

Figure 62. North Fern Burn sampling sites at: a) Forestry Road; and b) North-Fern Burn at Otautau-Tuatapere Road.

Figure 63. South Fern Burn at Otautau-Tuatapere Road.

E. coli levels in North Fern burn varied between 280 and 600 cfu/100 ml. *Campylobacter* was detected in the three samples from this stream, with higher concentrations present at Otautau-Tuatapere Road than at Forestry Road. Concentrations at the Otautau-Tuatapere Road site were higher in December (110 MPN/100 ml) than March (24 MPN/100 ml). In all cases isolates were identified as *C. jejuni*; isolates from Otautau-Tuatapere Road were of wildfowl origin, however the origin of those from Forestry Road were unknown.

Faecal source tracking found that ruminant pollution was the dominant pollution type at the Forestry Road site (50-100%), although no specific markers were detected. Faecal pollution was less dominant at the Otautau-Tuatapere Road site (10-50%), and again no specific markers were detected. Wildfowl markers were detected in the March sample from Otautau-Tuatapere Road. Faecal sterol analysis showed a wildfowl signature in all three samples.

At South Fern Burn, *E. coli* levels were higher than North Fern Burn, with the higher concentration present in December. *Campylobacter* was present at the same concentration in both samples (7.5 MPN/100 ml), and was determined to be *C. jejuni* of unknown origin. Faecal source tracking results for the two samples were consistent, showing ruminant pollution accounted for 10-50% of pollution at the site, with sheep and wildfowl markers present.

Table 31. Results for microbial and FST analysis of water samples collected from North Fern Burn and South Fern Burn, as part of a detailed study of pollution in the Otautau Plains area.

Site		North Fern Burn at Forestry Road (via Flett)	North Fern Burn at Otautau-Tautapere Road		South Fern Burn at Otautau-Tautapere Road	
Sample #		CMB150191	CMB140901	CMB150190	CMB140900	CMB150189
Client #		20151019	20145166	20151018	20145165	20151016
Date Sampled		9/03/2015	9/12/2014	9/03/2015	9/12/2014	9/03/2015
Map Reference		S	T		U	
Rainfall		No	No	No	No	No
		Microbial Properties				
Faecal coliforms		370	290	700	1,200	600
E. coli		300	280	600	1,200	600
Campylobacter		0.4	110	24	7.5	7.5
***Campylobacter* Species**		*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. jejuni*
MBiT *Campylobacter* Source	**Wildfowl**		3	2		
	Ovine/Bovine/Deer					
	Poultry					
	Not Wildfowl					
	Unknown	1			1	2
		Faecal Source Tracking				
General - GenBac3		++++	++++	++++	++++	++++
Ruminant		50-100%	10-50%	10-50%	10-50%	10-50%
Human - BacH		+	+	+	-	+
Human - BiADO		-	-	-	-	-
Cow		-	-	-	-	-
Sheep		-	-	-	+	+
Wildfowl - GFD		-	-	+	+	+
Wildfowl - E2		-	-	+	-	+
Canine		-		-		-
		Sterol Properties				
Total Sterols		2,894	4,812	3,575	6,372	2,920
Coprostanol		9	46	31	83	24
Faecal		F2	F1+F2	No	F1+F2	F2
Human		No	No	No	No	No
Ruminant		<30	<30	<30	Yes (2)	<30
Wildfowl		Yes	Yes	Yes	No	Yes

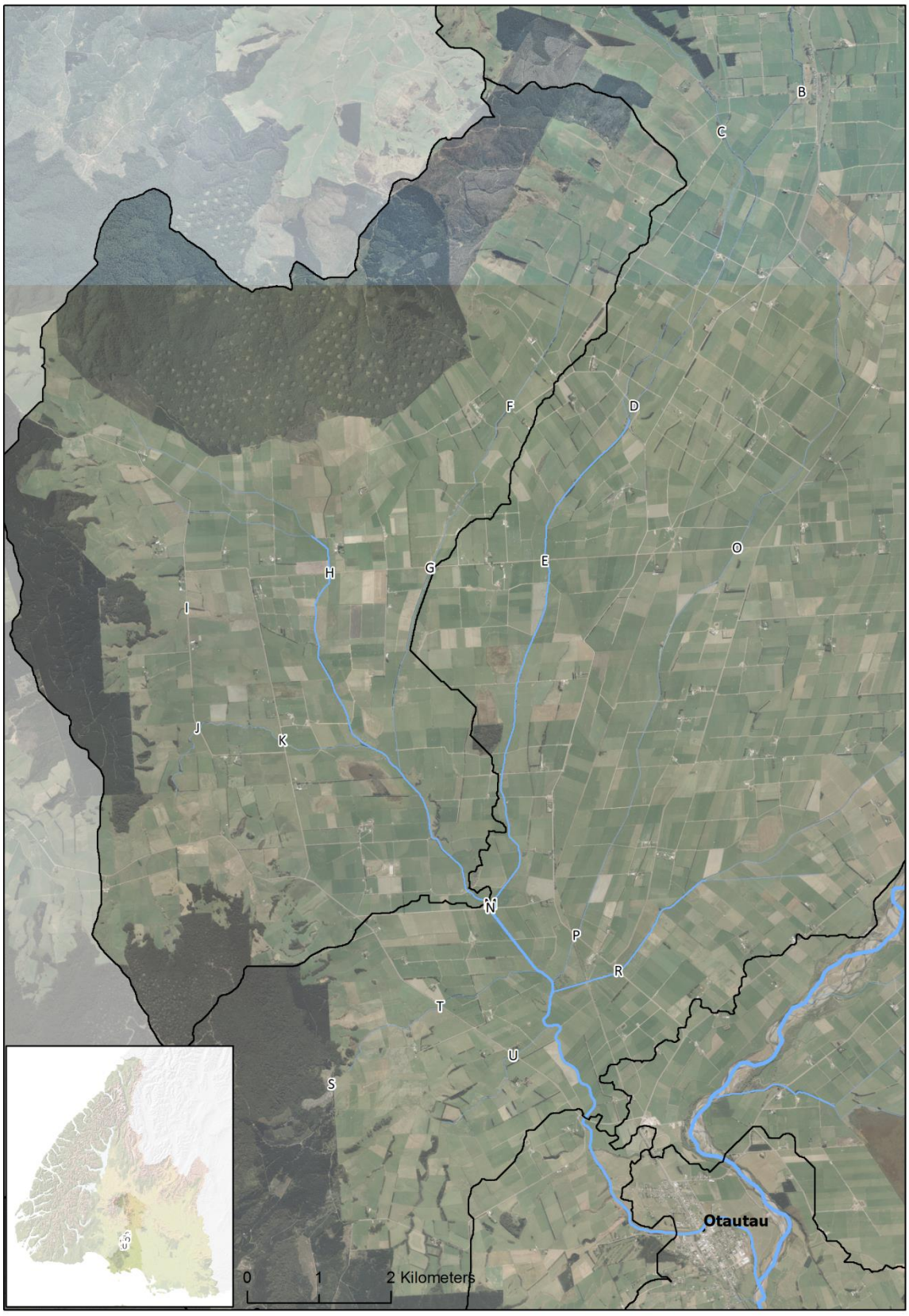


Figure 64. Investigation sample sites on the Otautau Plains, with river orders 4-7 shown.

REFERENCES

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

INSTITUTE OF ENVIRONMENTAL SCIENCE AND RESEARCH LIMITED

Kenepuru Science Centre
34 Kenepuru Drive, Kenepuru, Porirua 5022
PO Box 50348, Porirua 5240
New Zealand
T: +64 4 914 0700 **F:** +64 4 914 0770

Mt Albert Science Centre
120 Mt Albert Road, Sandringham, Auckland 1025
Private Bag 92021, Auckland 1142
New Zealand
T: +64 9 815 3670 **F:** +64 9 849 6046

NCBID – Wallaceville
66 Ward Street, Wallaceville, Upper Hutt 5018
PO Box 40158, Upper Hutt 5140
New Zealand
T: +64 4 529 0600 **F:** +64 4 529 0601

Christchurch Science Centre
27 Creyke Road, Ilam, Christchurch 8041
PO Box 29181, Christchurch 8540
New Zealand
T: +64 3 351 6019 **F:** +64 3 351 0010

www.esr.cri.nz