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# Application of Microbial Source Tracking (MST) Technologies for Identifying the Source of Microbial Contamination in the Lower Maitai River and Little Sydney Stream

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# Application of Microbial Source Tracking (MST) Technologies for Identifying the Source of Microbial Contamination in the Lower Maitai River and Little Sydney Stream

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# EXECUTIVE SUMMARY

Poor water quality conditions due to the presence of faecal bacteria have been a chronic problem within urban sections of the lower Maitai River in the city of Nelson (Sinton 2007). Information on the primary source(s) contributing to the contamination is required in order to successfully manage the problem. It has not been possible to determine the primary sources of contamination to date using conventional microbiological testing procedures; therefore, the primary objective of the study was to validate and implement an array of microbial source tracking (MST) techniques for identifying sources of faecal contamination in New Zealand freshwater environments, focusing on elevated faecal indicator bacteria concentrations in the lower Maitai River.

Prior to their application, a suite of markers (*Enterococcus faecium esp* gene, human- and ruminant-associated *Bacteroides sp.*, human polyomaviruses JCV and BKV markers) were validated on an array of faecal samples collected from local animals (99 individuals) and a sewage treatment facility. Following validation of the markers, they were then applied to water samples collected along the Maitai River. A less urbanised site in the Motueka/Riwaka Catchment (Little Sydney stream) was also surveyed for comparison with results from the Maitai River and in order to further validate the application of MST technologies in New Zealand.

Based on the validations, polyomavirus marker was found to be an excellent human-specific MST marker in New Zealand. However, cross-reactivity between the human-associated *Bacteroides sp.* marker and human-associated *Enterococcus faecium esp* gene marker and samples originating from marsupials (*e.g.* possum) and rabbits was detected. In other words, use of some these markers may incorrectly indicate the presence of human contamination in cases where possum and/or rabbit contamination is also present. A ruminant-specific *Bacteroides sp.* marker, formerly known to be specific to ruminant animals like cattle, sheep and goats, was also found to express some cross-reactivity with DNA extracted from possum and horse faeces.

Water samples were collected on 17 December 2007 (six Maitai River locations) and 19 December 2007 (three Maitai River locations and three Little Sydney Stream locations) and analysed for faecal indicator bacteria (*i.e.* faecal coliforms, enterococci) as well as for MST markers. All Maitai River samples expressed a signal of animal-associated (ruminant and/or possum) faecal contamination, however a strong human component was also detected in all samples collected downstream of the Halifax Street footbridge. The human-associated faecal contamination was supported by a minimum of two different human markers (polyomaviruses JCV and BKV markers) simultaneously detected at those sites. Unlike the Maitai River, there was no human contamination detected in Little Sydney Stream, which is surrounded primarily by agricultural and horticultural land.

While land runoff was probably the major source of animal-associated faecal contamination, leaking sewage collection systems or cross-connections that impact the groundwater or stormwater systems was likely the major source of human-associated faecal contamination in the Maitai River. Stormwater outfalls can transfer contamination to the river, as well as providing a sunlight-protected environment for faecal indicator bacteria to survive and potentially replicate. Stormwater runoff at the

Trafalgar Street Bridge site had concentrations of indicator bacteria similar to raw sewage. This stormwater outfall continued to flow after several days of no precipitation.

In summary, the source of high faecal indicator bacteria counts in the lower Maitai River was determined to be a combination of animal and human faecal contamination, while only animal input was detected in the Little Sydney Stream. Application of more specific markers (wildlife, livestock, birds, pets *etc*.) and quantitative real-time DNA amplification techniques would be required to provide a more complete understanding of the sources associated with reduced microbiological water quality in the lower Maitai River. The robust validation carried out in this study indicates that the MST technologies used on the Maitai River can also be applied elsewhere in New Zealand for assisting with water contamination problems.

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| | |
|---|---|
| CFU | Colony forming unit |
| ESP | *Enterococcus faecium esp* gene |
| GBAC | General *Bacteroides sp.* |
| HBAC | Human-associated *Bacteroides sp*. |
| HPYV | Human polyomaviruses JCV and BKV |
| MST | Microbial source tracking |
| MWM | Molecular weight marker |
| PBS | Phosphate buffered saline |
| PCR | Polymerase chain reaction |
| RBAC | Ruminant-associated *Bacteroides sp.* |
| NTC | No template control |

# 1. INTRODUCTION

Poor water quality conditions due to the presence of faecal bacteria have been a chronic problem within urban sections of the lower Maitai River in the city of Nelson (Sinton 2007). Information on the primary source(s) contributing to the contamination is required in order to successfully manage the problem. However, it has not been possible to determine the primary source(s) of contamination to date using conventional microbiological testing procedures. Therefore, the primary objective of the study was to validate and implement an array of microbial source tracking (MST) techniques for identifying sources of faecal contamination in New Zealand freshwater environments, focusing on elevated faecal indicator bacteria concentration in the lower Maitai River. The work was undertaken through funding from the Foundation for Research Science and Technology, Envirolink (NLCC8).

To our knowledge, this is the first time this type of microbial source tracking study has been applied in a New Zealand context and therefore an array of source tracking markers was initially validated using samples originating from local animals and a municipal sewage treatment facility. The validated suite of MST markers was then applied to samples collected from the Maitai River (high residential). For further tests of the applicability of MST markers in a New Zealand context, we also applied the markers to samples collected in a tributary of the Riwaka River (Little Sydney Stream). This waterway has a lower residential influence than the Maitai, and therefore provides information on the wider applicability of the MST markers within the Nelson/Tasman Bay region.

Objectives of the study were as follows:

1. Provide an overview of lower Maitai bacteriological monitoring results previously described by Sinton (Sinton 2007).
2. Validate an array of MST markers using a set of samples of known origin, including common types of animals in the watershed as well as municipal waste samples.
3. Apply MST markers to samples collected along the lower Maitai River at locations previously monitored for water quality.
4. Further test the markers using samples collected from Little Sydney Stream, a waterway that has low residential influence.

By meeting the above objectives, we aim to assist in managing this regionally important freshwater resource, and validate the transferability of overseas MST technology to complex catchment applications in New Zealand.

## 1.1. Study locations

The Maitai River is roughly 15 km long – initially gathering its water from springs and streams along the Bryant Range, then meandering between picturesque mountains of the Maitai valley,

eventually passing through the Nelson city and discharging to the Nelson Haven, Tasman Bay (Figure 1).

The upper Maitai River is surrounded by native bush and regenerating forest. Land usage along the middle reaches of Maitai River (downstream of Maitai Dam until Nelson City) involves low-intensity cattle and sheep farming and low-density residential, while the lower Maitai is surrounded by dense urban development. The Maitai River is greatly valued by locals as a place for rest and relaxation (Sheridan 2004), and it is a source of drinking water for the city. Unfortunately, water quality of the lower Maitai River has been deteriorating over the years (Spencer & Ramsay 1978). In particular, there have been high levels of *Escherichia coli* (*E. coli*) detected in the lower Maitai River that regularly exceed MfE/DOH guidelines (Campbell *et al.* 2002) for freshwater recreational waters (Sinton 2007, Wilkinson 2007) . Extensive targeted monitoring undertaken in accordance with the Microbiological Water Quality Guidelines 2002 failed to isolate a source for this contamination (Sinton 2007).

Little Sydney Stream is about 7 km long and gathers water from the gentle slopes of Airstrip Mountain. An initial natural network of streams combine at the lower portion of the mountain into a modified road-side channel. The stream discharges to the sea near the Riwaka village. Land uses in the Little Sydney catchment include horticulture, cattle and sheep grazing, and low-density residential. The predominantly rural catchment of Little Sydney Stream is in contrast with the urban influenced lower Maitai catchment.

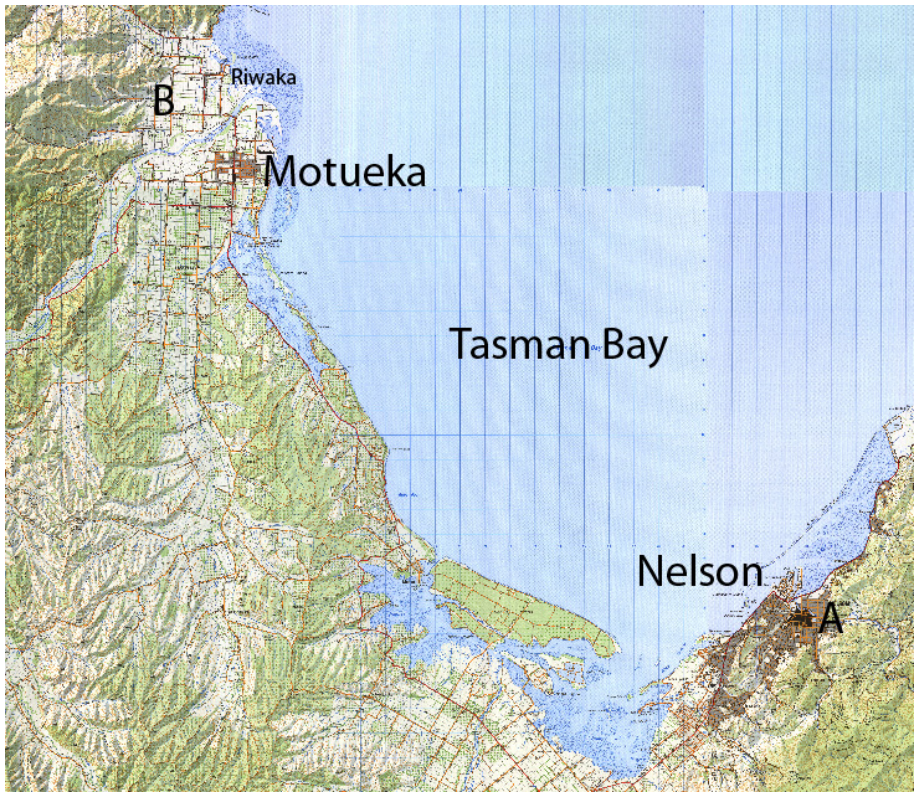


**Figure 1.** Study locations. A – Maitai River, B – Little Sydney Stream.

## 1.2. Overview of historical data

The Nelson area enjoys warm summers and mild winters with precipitation in the form of rain relatively evenly distributed throughout the year (roughly 70-90 mm/month, Figure 2). Major rain events, yielding above 50 mm of rain per day, are rare, but they do occur 1-2 times in most years.

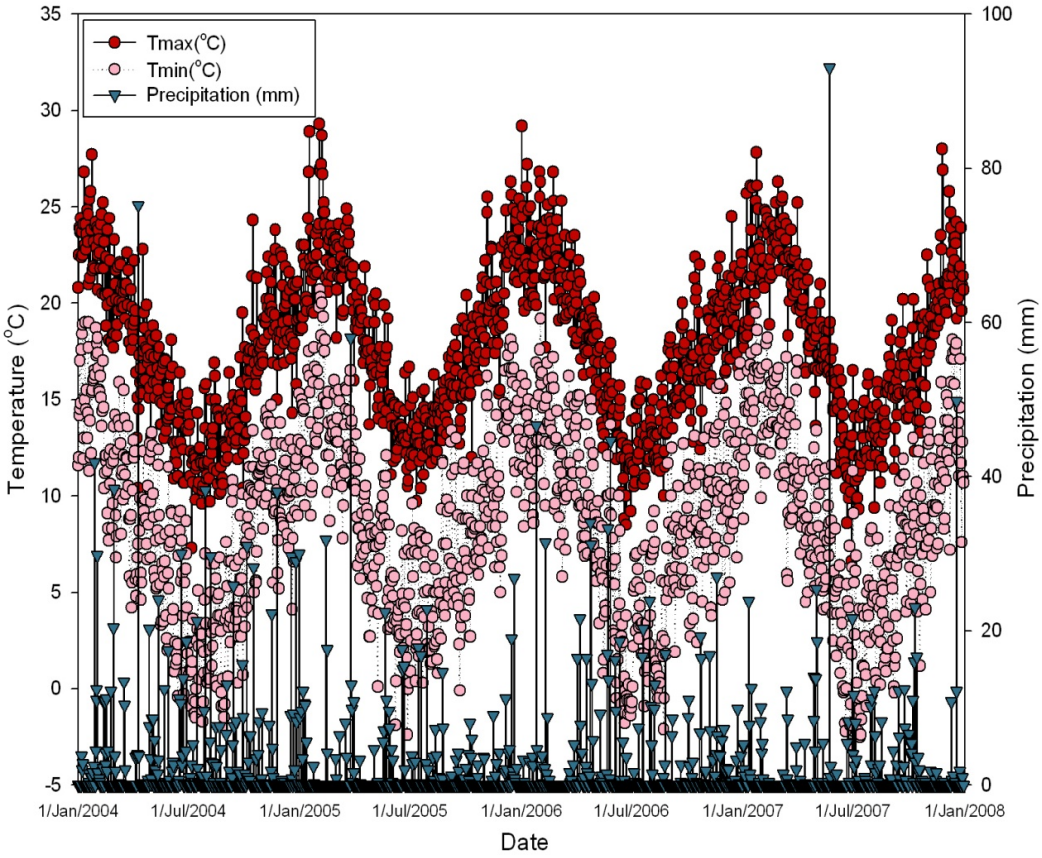


**Figure 2.** Daily precipitations and minimum and maximum temperatures at Nelson AWS (4271, Metservices).

Based on data supplied by Mr Paul Sheldon (Nelson City Council) for the 2005-2006 and 2006-2007 summer seasons, *E. coli* concentrations at regular monitoring sites in the lower Maitai River (Figure 3) fluctuate greatly over time (Figure 4), though a large portion remain in the acceptable/green surveillance mode range of 0 to 260 CFU per100 ml as specified by the Ministry of Environment (Campbell *et al.* 2002). Exceptions were samples from the downstream reaches collected near the Collingwood and Trafalgar Street bridges and occasional upstream samples at Sunday Hole and Maitai Camp (Figure 4) which at times exceeded alert/amber threshold concentrations specified as 260 CFU per 100 ml and action/red threshold (specified as 550 CFU per 100 ml (Campbell *et al.* 2002). Most notably, the high bacterial counts near the Collingwood Street Bridge were of concern. It should also be noted, however, that little or no data was available from April to October, and high indicator bacteria

counts could theoretically have occurred during this period as well. Greater variability was apparent in *E.coli* counts in samples from the tidally-mixed area compared to the areas further upstream (Figure 5), indicating dilution and/or salinity effects.

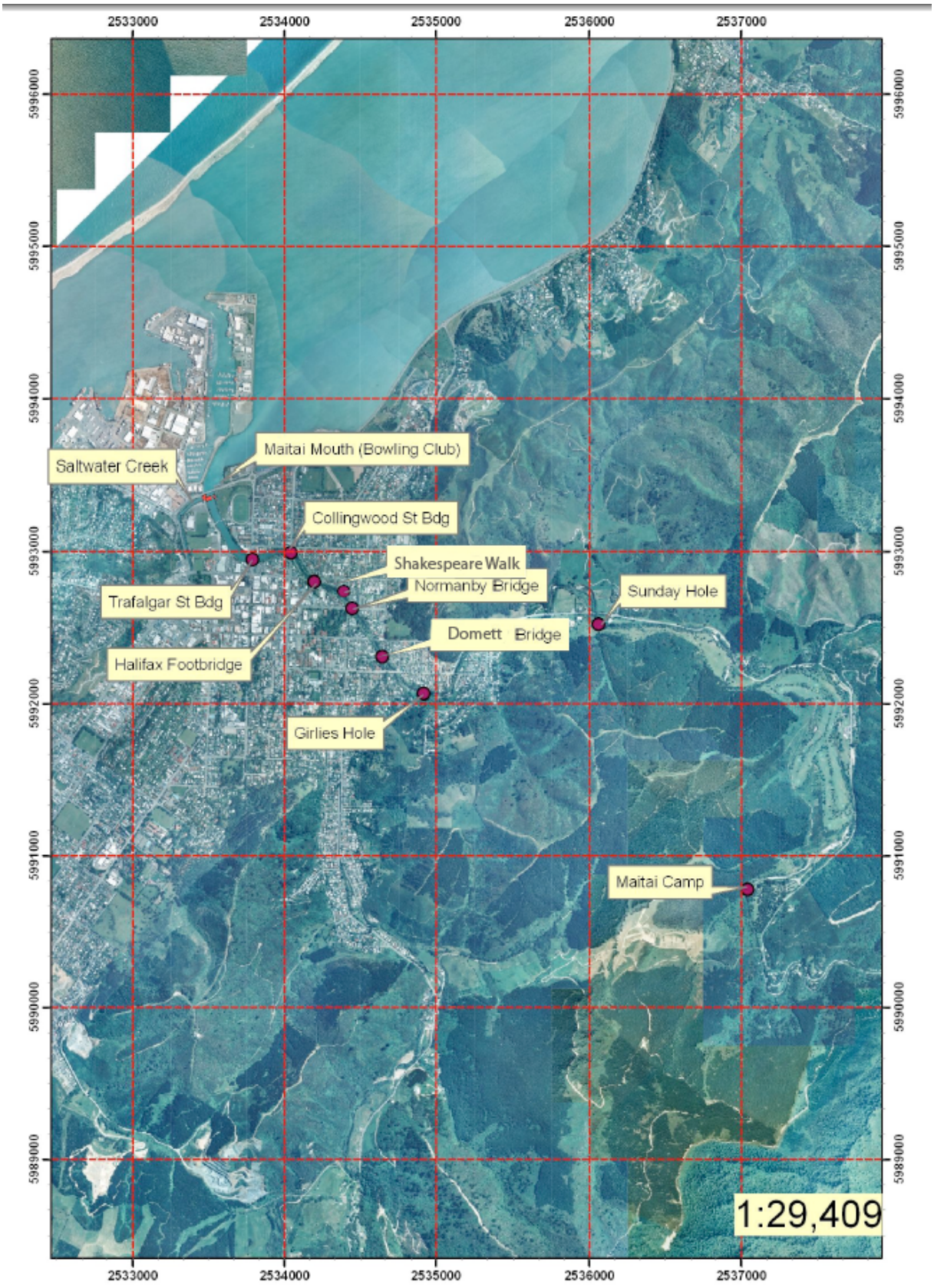


**Figure 3.** Maitai River routine sampling sites (figure from Sinton *et al.* 2007).

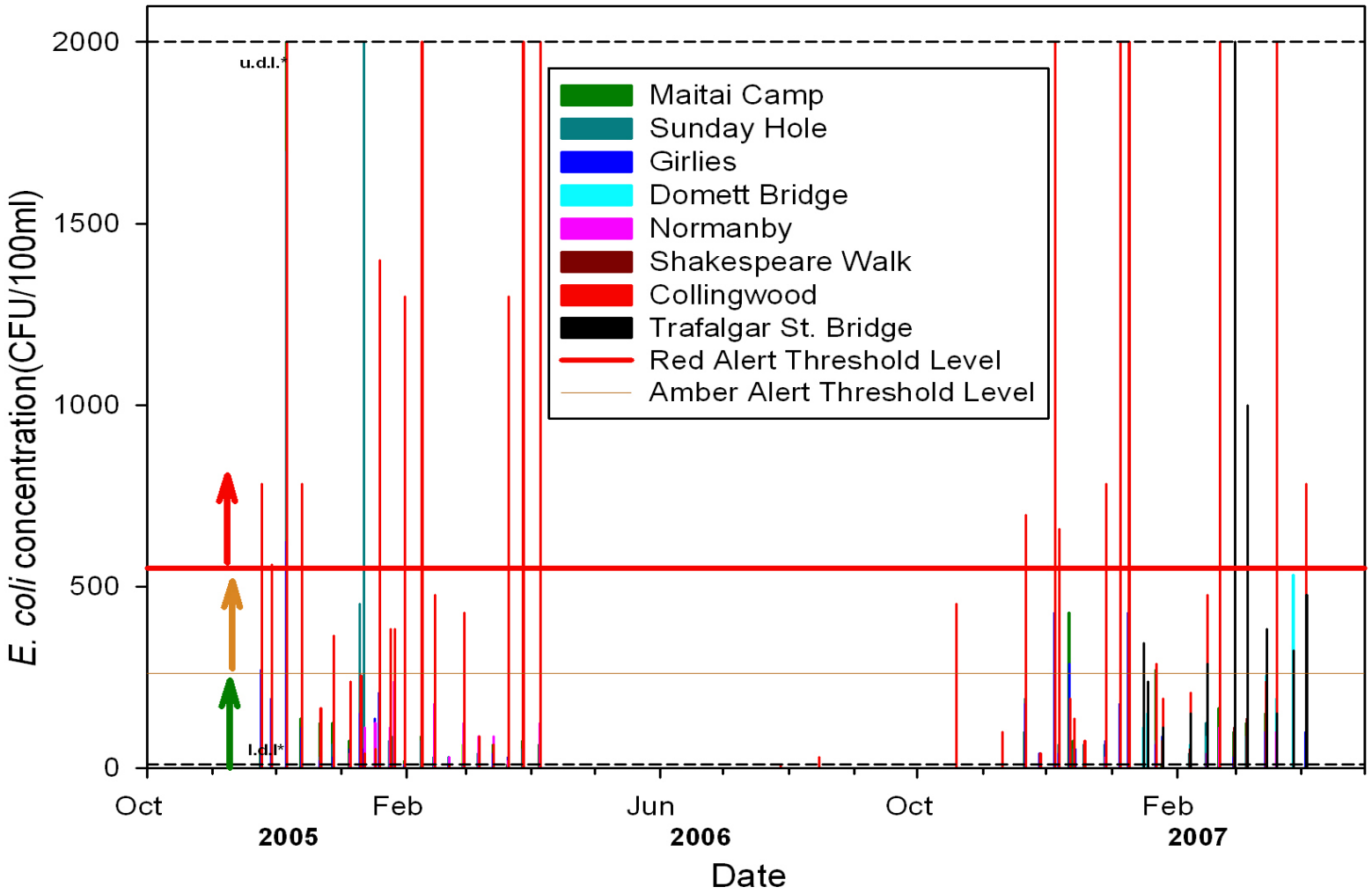


**Figure 4.** *E. coli* concentrations in the lower Maitai River (u.d.l. – upper detection limit, l.d.l. – lower detection limit, arrows indicate alert levels as specified in Campbell *et al.* 2002).

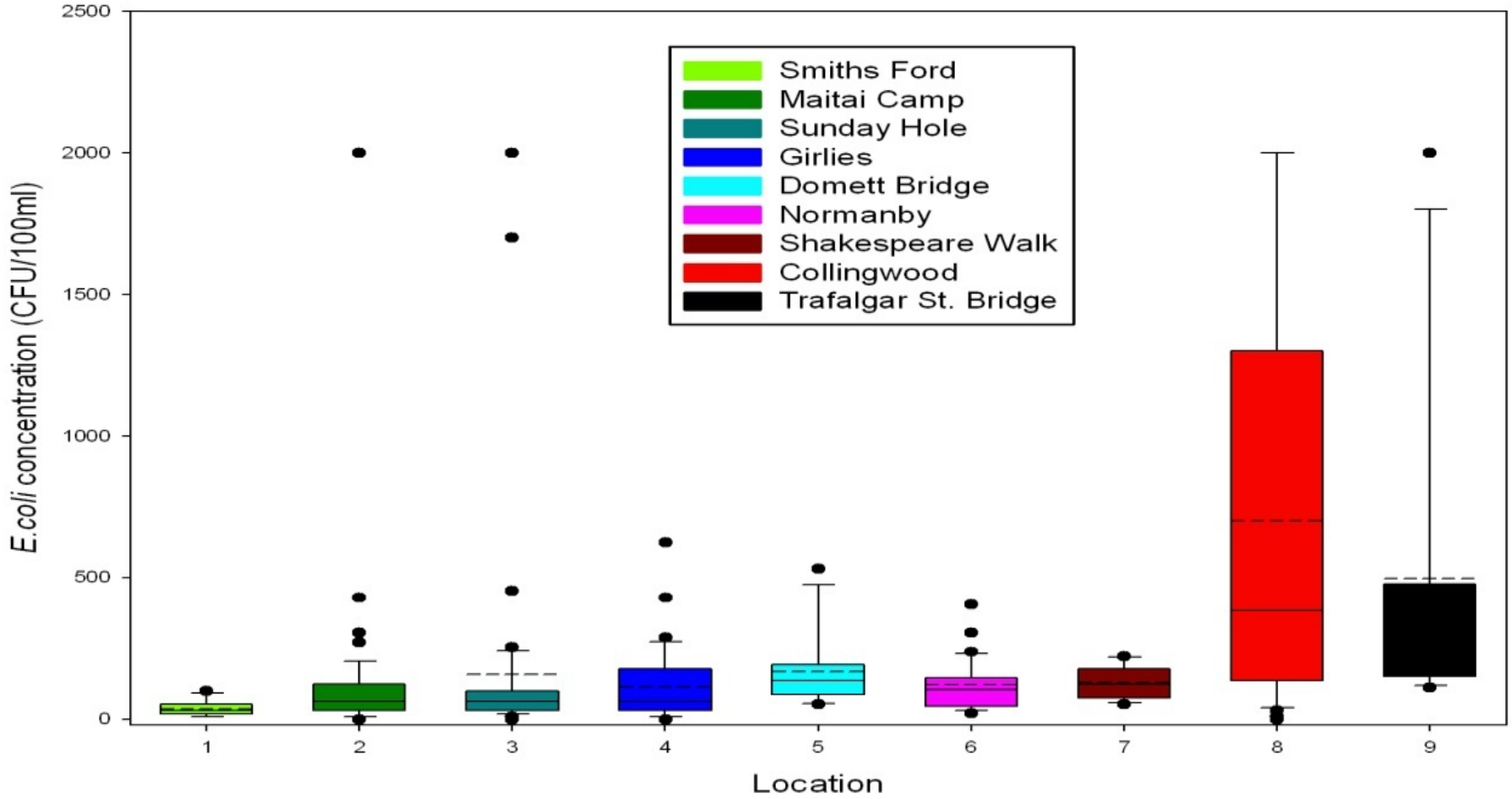


**Figure 5.** Variation of the *E.coli* concentrations during the period of 2005-2007. The boundary of the box closest to zero indicates the 25$^{th}$ percentile, a line within the box marks the median, dashed line indicates the mean, and the boundary of the box farthest from zero indicates the 75$^{th}$ percentile. Whiskers above and below the box indicate the 90$^{th}$ and 10$^{th}$ percentiles. Outlying samples are indicated as black dots. Sites closest to tidal influence include Collingwood and Trafalgar Street Bridge.

More intensive sampling was conducted on two separate occasions (22 December 2006 and 22 January 2007) by the Nelson City Council. There was positive correlation between the *E.coli* counts at Trafalgar Street Bridge and Collingwood Street Bridge and negative correlation between *E. coli* counts and tide height up to Normanby Bridge (Figure 6). This pattern was particularly evident on 22 January 2007 (see Figure 6). As *E. coli* concentrations decreased towards the period of high tide, it was unlikely that the source of high indicator bacteria was the incoming marine water from Nelson Haven. As the lower Maitai reach is tidally influenced, caution should be used when interpreting results based on *E. coli* concentrations alone, especially since the analyses were based on the Colilert kit (IDEXX, Westbrook, ME) which is not useful in marine waters (Dr. Harwood, pers. comm.). Therefore, testing for faecal coliforms and enterococci would probably result in more comparable results in tidally-mixed reaches of the Maitai River. It should also be noted that there was a small scale (<5 mm/day) rain event just before the December sampling.

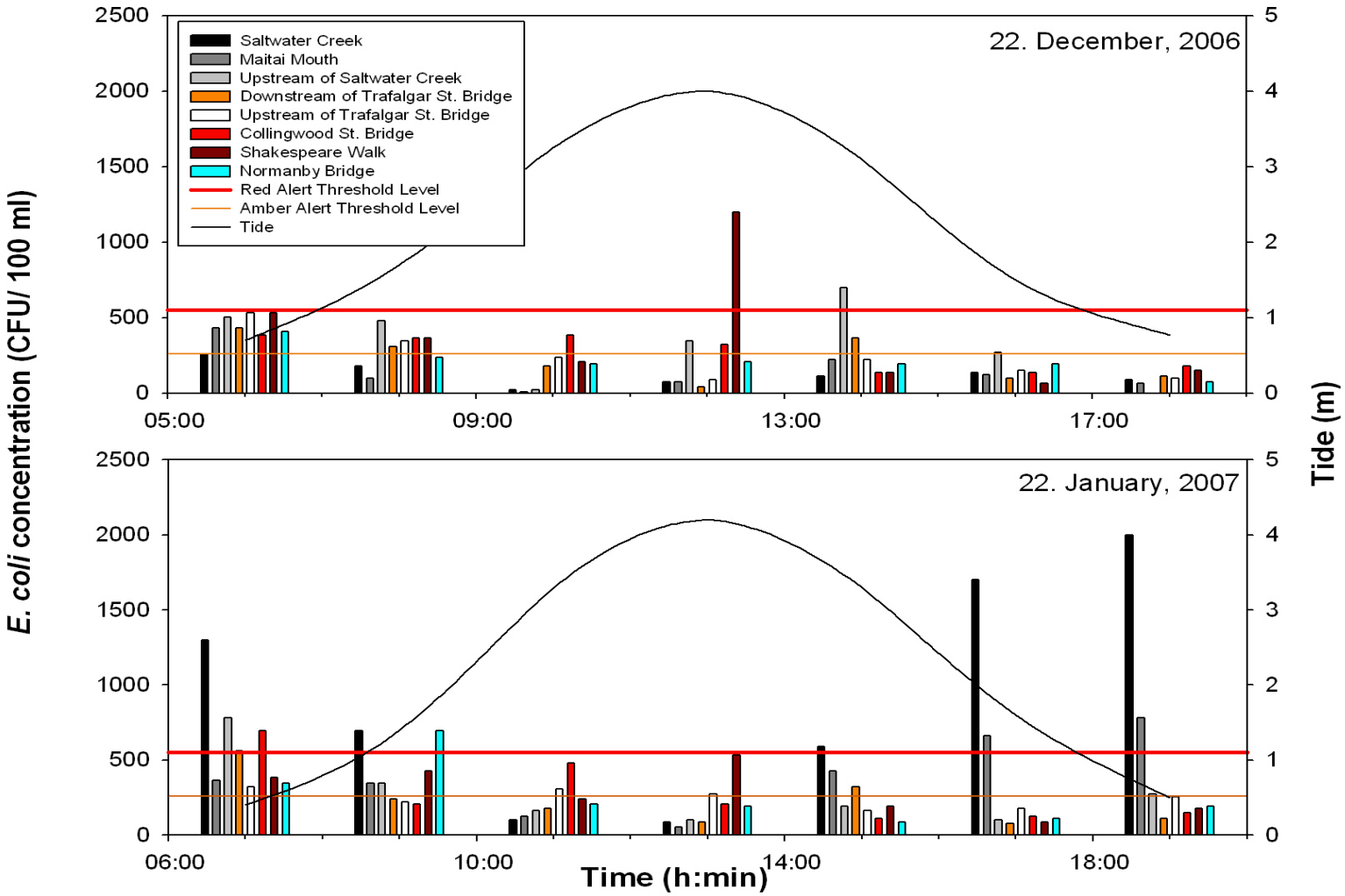


**Figure 6.** Tidal cycle and *E.coli* concentrations in Maitai River (adapted from Sinton 2007).

When the flood tide progresses, seawater can dilute faecal coliform concentrations in the river while during a receding tide the influence of stormwater runoff is more pronounced. Rain events in turn wash bacteria into the system from land and are a likely significant factor contributing to high counts at upstream sites that are surrounded by agricultural land use activities.

Faecal coliforms can persist in sediments where they are protected from the sunlight (Dale 1974). It is also known that *E. coli* (a subset of faecal coliforms) can establish viable populations in subtropical and tropical coastal sediments (Solo-Gabriele *et al.* 2000; Desmarais *et al.* 2002). Relatively recently it has been shown that *E. coli* populations can establish and replicate to high densities in beach sand in temperate climates (Ishii *et al.* 2006; Beversdorf *et al.* 2007). Potentially naturalised populations of *E.coli* have also been observed within epilithic (attached to stone) periphyton communities (Ksoll *et al.* 2007). Therefore it is possible that the sediments and epilithic periphyton communities could act as reservoirs and sustain growth of indicator bacteria, which are then released when disturbed during rain events or by tidal action downstream.

In summary, high counts of faecal coliforms have been measured in the Maitai River and temporal patterns generally correspond to the timing of rain events and within a given day on the stage of the tide. Sources contributing to these high counts are unknown, but could be associated with bacteria entering the system with catchment runoff and to some extent bacteria that have persisted in the system.

## 1.3. Possible sources

Although bacterial concentrations exceeding the amber and red action modes were frequent in the lower Maitai River, the source of those high counts is unknown. It has been speculated that the sediments, surface reticulation or leakage, and contaminated groundwater could be contributing sources (Sinton 2007). It is relatively unlikely that the saline wedge could transport tidally-sourced *E .coli* upstream as bacterial counts appear to be negatively correlated with tidal height.

While any of the listed sources could be a vector, initially bacteria had to have originated from host animals. Microbial source tracking can be effective in identifying the source(s) of microbial contamination (Stoeckel & Harwood 2007). Information gained by employing MST technology is necessary to evaluate the level of actual health risk and to enable effective management and remediation strategies to be implemented. Previously it had not been determined if the source of the high faecal bacteria counts was livestock or domestic animals, wildlife, or human sewage. If the dominant source is determined to be human, the possibility of a leaking sewage system could be addressed. If the dominant source is determined to be animal, management of local wildlife and/or livestock could be considered.

# 2. MICROBIAL SOURCE TRACKING

## 2.1. Validation of microbial source tracking markers

Microbial source tracking markers are used to identify the source of the faecal bacteria. They can be divided into two broad categories - library dependent and library independent. Library-dependent methodologies rely on the development of collections of faecal indicator bacteria isolates for a known source for comparison of genetic or phenetic patterns (fingerprints) of isolates from environmental samples. These libraries are location specific, and their utility is hampered by the diversity and temporal variability of faecal indicator organisms (Gordon 2001; Anderson *et al.* 2006). Library independent methods target source-specific markers which could be a source-specific organism, specific gene *etc*. In theory, library independent markers can be used at any location without a need to verify the specificity, while library dependent markers need to be established and verified on each location by sampling local animal populations, substantially adding to the cost and time needed for the analyses.

Good MST markers should occur exclusively in the group of animals it is intended to track and be consistently shed in faeces of that group. General markers may be preferable at an initial phase of the study, while more specific markers could be applied later for verification. Well established, broad-range library independent markers that frequently occur in faeces were selected for analyses of environmental samples. These were: *Enterococcus faecium esp* gene (ESP) (Scott *et al.* 2005), human- and ruminant-associated *Bacteroides sp.*(RBAC and HBAC*)* (Bernhard & Field 2000), human polyomaviruses JCV and BKV (HPYV) (McQuaig *et al.* 2006).

Even though the selected markers have wide and long-term potential, they have not previously been used in New Zealand. Therefore it was decided that the selected set of markers needed to be validated in the New Zealand context.

### *2.1.1. Methods*

Composite faecal samples were collected from 99 animals. A water sample was collected from the oxidation pond at Motueka Wastewater Treatment Facility as a human sewage proxy (Table 1).

All samples, except marsupial samples from Wellington, were stored on ice and processed within three hours of sampling. The marsupial samples were shipped by mail and were processed two days after the collection. Upon arrival, faecal samples (0.2 g wet weight) were hydrated in 5 ml phosphate buffered saline (PBS). DNA was extracted using QIAamp Stool kit (Qiagen). For primers and PCR conditions see Appendix 1 and 2.

**Table 1.** Distribution of MST markers in different animals. n – number of individuals in the composite sample. Red indicates unexpected positive result. NA – not available.

| # | Animal | Location | n | Enterococci CFU/g | Marker GBAC | RBAC | HBAC | ESP | HPYV |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Cat | SPCA, Nelson | 2 | na | + | +? | - | - | - |
| 2 | Dog | SPCA, Nelson | 3 | 8.25E+06 | + | - | - | - | - |
| 3 | Goat | SPCA, Nelson | 1 | 7.25E+04 | + | + | - | - | - |
| 4 | Cow | SPCA, Nelson | 3 | 3.00E+04 | + | + | - | - | - |
| 5 | Rabbit | SPCA, Nelson | 1 | 9.00E+04 | + | - | + | - | - |
| 6 | Chicken | SPCA, Nelson | 2 | 2.88E+07 | + | - | - | - | - |
| 7 | Sheep | SPCA, Nelson | 1 | 1.30E+05 | + | - | - | - | - |
| 8 | Alpaca | SPCA, Nelson | 1 | 1.60E+07 | + | - | - | - | - |
| 9 | Pigeon | SPCA, Nelson | 1 | 7.45E+05 | + | - | - | - | - |
| 10 | Duck | SPCA, Nelson | 1 | 2.23E+08 | + | - | - | - | - |
| 11 | Rat | SPCA, Nelson | 3 | 1.58E+07 | + | - | - | - | - |
| 12 | Bird (pigeon?) | Natureland Zoo, Nelson | 2 | 1.13E+05 | + | - | - | - | - |
| 13 | Goat | Natureland Zoo, Nelson | 1 | 6.98E+05 | + | - | - | - | - |
| 14 | Duck | Natureland Zoo, Nelson | 1 | 9.80E+05 | + | - | - | - | - |
| 15 | Chicken | Natureland Zoo, Nelson | 3 | 6.05E+07 | + | - | - | - | - |
| 16 | Guinea Pig | Natureland Zoo, Nelson | 4 | 1.53E+05 | + | - | - | - | - |
| 17 | Sheep | Natureland Zoo, Nelson | 1 | 7.95E+05 | + | + | - | - | - |
| 18 | Rabbit | Natureland Zoo, Nelson | 3 | 3.05E+05 | + | - | - | + | - |
| 19 | Mice | Natureland Zoo, Nelson | 7 | 1.50E+06 | + | - | - | - | - |
| 20 | Rat | Natureland Zoo, Nelson | 3 | 1.03E+06 | + | - | - | - | - |
| 21 | Deer | Natureland Zoo, Nelson | 1 | 3.75E+04 | + | - | - | - | - |
| 23 | Cow | Motueka | 11 | 7.50E+06 | + | + | - | - | - |
| 24 | Horse | Motueka | 4 | 1.00E+07 | + | + | - | - | - |
| 25 | Possum | Wallaceville Animal Research Cen | 12 | na | + | + | + | NA | - |
| 26 | Kangaroo | Wellington Zoo | 1 | 7.00E+06 | + | + | + | - | - |
| 27 | Parma Wallaby | Wellington Zoo | 1 | 1.20E+07 | + | + | - | + | - |
| 29 | Parma Wallaby handraised | Wellington Zoo | 1 | 2.80E+06 | + | + | - | + | - |
| 28 | Red Neck Wallaby | Wellington Zoo | 1 | 8.00E+06 | + | + | + | - | - |
| 30 | Parma and Tammar Wallaby | Wellington Zoo | 1 | 4.00E+06 | + | + | + | - | - |
| 31 | Possum | Wellington Zoo | 18 | 1.20E+07 | + | + | + | + | - |
| 32 | Rock Wallaby | Wellington Zoo | 1 | 1.86E+07 | + | + | - | + | - |
| 33 | Possum | Natureland Zoo, Nelson | 1 | 3.25E+04 | + | + | + | - | - |
| 34 | Sheep | Natureland Zoo, Nelson | 2 | na | + | + | - | - | - |
| 35 | Sewage | Motueka | 100m | 1.50E+05 | + | - | + | + | + |

### *2.1.2. Results*

Concentrations of enterococci varied between the animals, ranging from $3x10^4$ CFU to $2.23x10^8$ CFU/g wet weight (Table 1.). It should be noted that different animals release different amount of faecal indicator bacteria, though considerable variation also appears to exist between individuals of the same species (*e.g.* ducks in Table 1). However we do not

have information on how long the samples had been exposed to the outside environment and therefore the given enterococci concentrations are rough estimates.

The general bacteroides marker was amplified in all samples studied. We consider it to be a good indicator because the PCR reactions were not inhibited. The ruminant specific RBAC primers strongly amplified cow, sheep and goat samples and weakly amplified horse and marsupial (possum, kangaroo, wallaby) faecal samples, showing some cross-reactivity with other animals than ruminants. However, most importantly, they did not amplify DNA isolated from the sewage samples, as expected.

The only human marker, which was consistently absent from animal faecal samples, but was detected in sewage samples, was human HPYV marker, indicating that this marker could be used as an indicator of human sewage input. The human specific HBAC and ESP primers also amplified the sewage samples. Unfortunately, these markers also amplified DNA isolated from marsupials (*e.g.* possums) as well as rabbits and therefore can not be considered as human-specific markers. If applied, they should be used in conjunction with other human specific markers (*e.g.* HPYV).

### *2.1.3. Synopsis*

While human HPYV marker appears to be host specific in New Zealand, there was strong cross reactivity between human HBAC and ESP markers and DNA isolated from marsupial and rabbit faecal samples. Some cross-reactivity was detected between ruminant RBAC and DNA extracted from horse and marsupial faeces. The former is also known for samples collected in Florida (Dr. V. Harwood, unpublished data) and Nebraska (Vogel *et al.* 2007). RBAC marker has also been reported in 96% of pig faeces in southern France (Gourmelon 2007). Therefore RBAC marker would probably be better considered as animal, rather than ruminant specific. Overall this five marker MST suite would be suitable for source tracking purposes in New Zealand (Table 2).

**Table 2.** Results of MST marker evaluations.

| Marker | Humans | Animals | Evaluation outcome |
|---|---|---|---|
| GBAC | YES | YES | Good PCR control |
| RBAC | NO | YES | Good marker, mostly ruminants |
| HBAC | YES | YES (rabbits, possums) | Only useful with other supporting markers |
| ESP | YES | YES (rabbits, possums) | Only useful with other supporting markers |
| HPYV | YES | NO | Good marker, highly human specific |

## 2.2. Applications of markers in the Maitai River

### *2.2.1. Methods*

Water samples were collected from the Maitai River on 17 and 19 December 2007. There was a six day period without any substantial precipitation before the first sampling date, and a heavy rain event (62 mm) before the last sampling date (Figure 3)

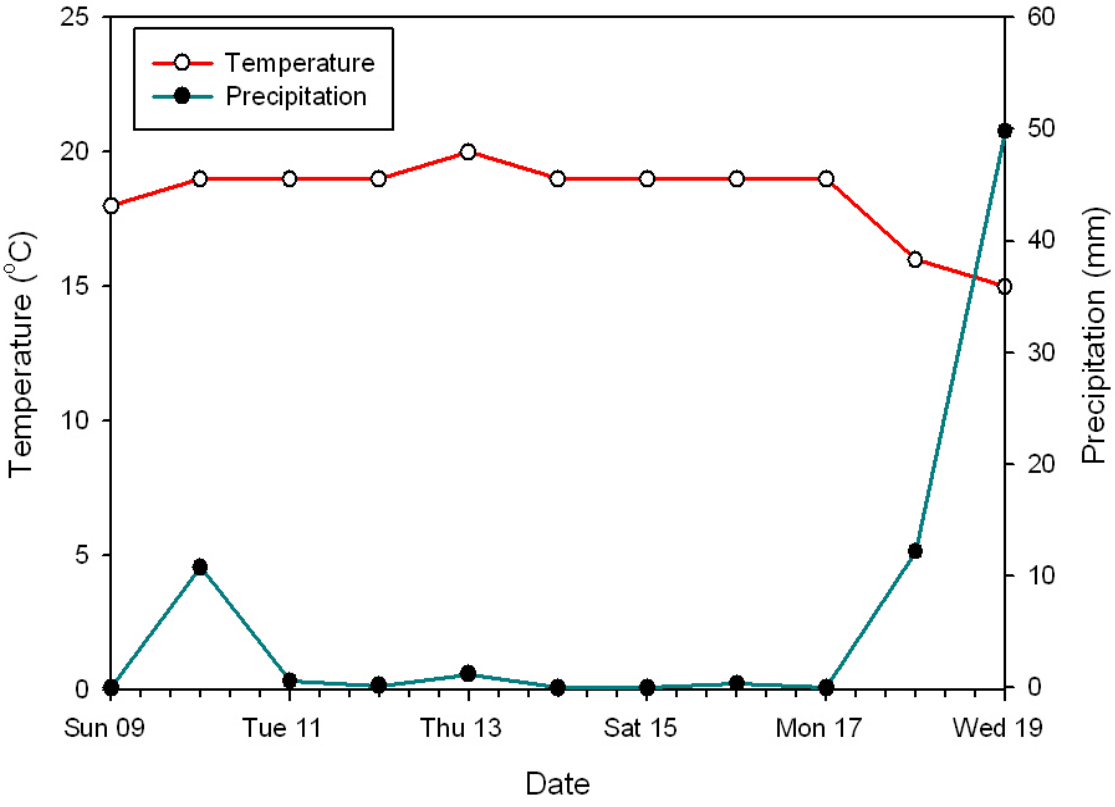


**Figure 7.** Temperature and precipitation during the sampling period.

Eight sites (Figure 4) were sampled for MST and indicator bacteria counts and four more supplementary sites (A, B, C, D) were sampled for indicator bacteria only. Sites 4 and 6 were not sampled during the first sampling event, and only Sites 2, 4 and 6 were sampled during the second sampling. It was noted that there was continuous flow from the stormwater runoff at Site 5, after several days of no precipitation. All sites were sampled at low tide.

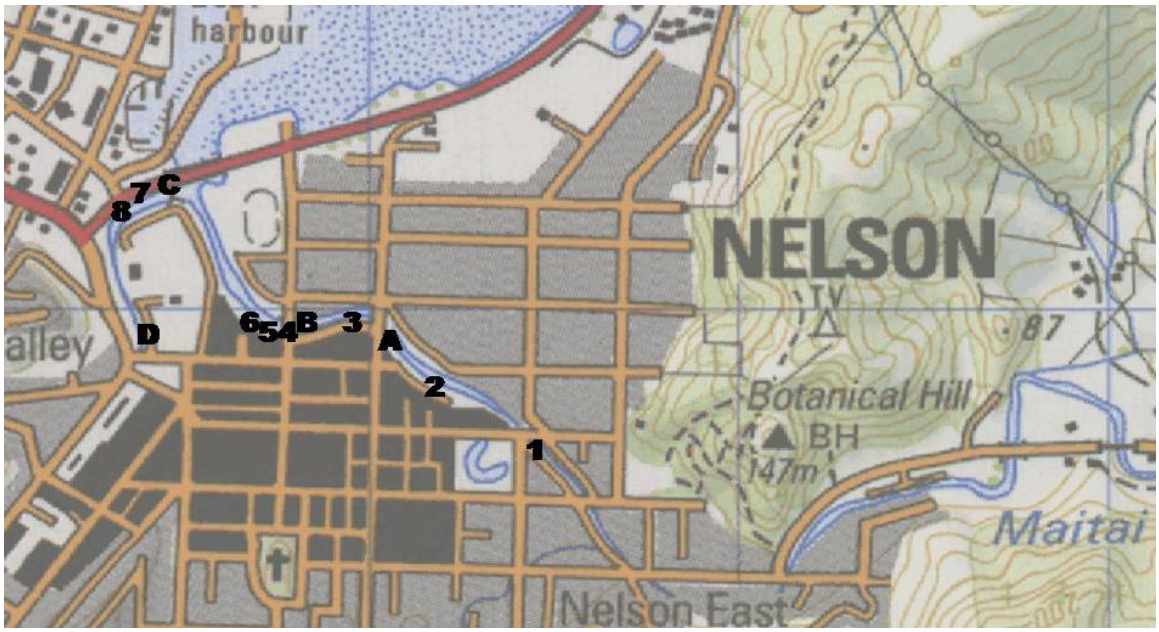


**Figure 8.** Maitai River sampling sites (1 - Upstream of construction at Bridge St., 2 - Upstream of Halifax St. footbridge, 3 - Downstream of Collingwood stormwater outfalls in river, 4 - Maitai River at Trafalgar St. Bridge, 5 - Downstream of Trafalgar St. bridge directly from stormwater outfall, 6- Maitai River, Trafalgar St. outfall, murky-soapy looking water, 7 - At stormwater outfall Saltwater Creek, 8 - Just upstream of stormwater outfall in Saltwater Creek. A - Upstream of stormwater outfall at Collingwood bridge, B - Upstream of Trafalgar St. bridge, C - Saltwater Creek mouth at boat ramp, D - Saltwater Creek at Halifax St.).

Samples were kept on ice and analysed within three hours of collection. Faecal coliform concentrations were determined using mFC agar and enterococci concentrations using mEI agar. Membrane filters from the mEI assay were used for the enrichment in azide dextrose broth followed by DNA extraction for the ESP assay as specified above. Protocol for the HPYV was modified by immediate pH adjustment to 4.5, followed by the filtration and DNA extraction using bead-beating and PowerSoil DNA kit (Mo Bio Laboratories Inc., Carlsbad, CA). Extracted DNA was used for GBAC, RBAC, HBAC and HPYV PCR assays as specified in Appendix 2.

### *2.2.2. Results*

Substantially higher faecal coliform and enterococci concentrations were detected at the lower end of the Maitai River from the stormwater runoff discharging at Trafalgar Street Bridge (Site 5) as well as from the Maitai River near Trafalgar Street (Site 4) (Table 3). Based on the unusually high bacterial counts and constant water flow even during the low tide, it is likely that some sewage leakage occurs into the stormwater runoff discharging at Site 5. Probable human impact was also evident in the adjacent stormwater runoff (Site 6) which had a murky appearance and high faecal coliform and enterococci concentrations. It is possible that faecal contamination from those sources remained undetected, if 'historical' Trafalgar Street Bridge samples were collected upstream from the bridge at low tide. Enterococci and faecal coliform

concentrations near the Collingwood Street Bridge (Site 3) were comparable to upstream sites (1, 2, A) studied.

A strong rainfall event, accumulating 62 mm of precipitation over 24 hour period, occurred just before the 19 December 2007 sampling. Although this research was not designed to study the impact of rainfall, the heavy rain probably had a diluting effect based on the parallel samples collected at Site 2 that exhibited over a two-fold decrease of faecal coliforms and enterococci counts after the rain.

**Table 3.** Concentrations of faecal coliform and enterococci in the lower Maitai River.

| Indicator | Sampling Event | Site 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | A | B | C | D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Faecal coliforms | 17/12/2007 | 1400 | 700 | 540 | | >25,000 | | 150 | 92 | 600 | 390 | 65 | 440 |
| (CFU/100ml) | 19/12/2007 | | 300 | | 2500 | | 1400 | | | | | | |
| Enterococci | 17/12/2007 | 290 | 320 | 190 | | >10,000 | | 80 | 63 | 170 | 230 | 53 | 170 |
| (CFU/100ml) | 19/12/2007 | | <100 | | 1700 | | 1800 | | | | | | |

While particularly high faecal coliform and enterococci counts are important to identify the health risk and faecal contamination 'hot spots', it is the MST markers which allow identification of the source of faecal contamination and suggest possible remediation strategies. Composite gel images (Appendix 3 and 4) show the results of the PCR analyses for MST markers in the Maitai River. Interpretation of the images is presented in Table 4.

**Table 4.** Interpretation of composite gel images (Maitai River, Appendix 3 and 4).

| | Site 1 | 2 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| **Marker** | **17/12** | **17/12** | **19/12** | **17/12** | **19/12** | **17/12** | **19/12** | **17/12** | **17/12** |
| GBAC | + | + | + | + | + | + | + | + | + |
| RBAC | + | + | + | + | + | + | + | + | + |
| HBAC | + | + | | + | + | + | | + | + |
| ESP | | | NA* | | + | | + | + | + |
| HPYV | | | | + | + | + | + | + | |
| Source | | | | | | | | | |
| Human-associated | ND** | ND | ND | **YES** | **YES** | **YES** | **YES** | **YES** | **YES** |
| Animal-associated | **YES** | **YES** | **YES** | **YES** | **YES** | **YES** | **YES** | **YES** | **YES** |

*NA –enterococci concentrations where under detectable limit, therefore the ESP assay was not available, **ND – not detected.

All of the Maitai River samples were very strongly positive for the GBAC marker, which is a general marker of faecal pollution from any animal or sewage source. All of the Maitai River samples also expressed a positive signal for RBAC marker (Table 3). This marker is reported to be specific to faecal pollution originating from ruminant animals, but was also identified in samples collected from horse and possum faeces (Section 2.1.2). Based on the visual inspection of gels, the signal was weaker in the samples collected before the rain event, indicating contamination from land runoff, probably originating from local wildlife and/or farming activities. The application of real-time PCR would provide quantitative results to enable a more focused management response by distinguishing between primary and secondary sources.

A human HBAC marker was detected in 75% of the samples. As was shown (Section 2.1.2), this marker exhibited strong reactivity with DNA isolated from possum faecal material. Therefore positive results for this marker could not be used as an explicit indicator of human faecal contamination without application of additional markers. In this study HPYV and ESP markers were used as additional human specific markers. The HPYV marker was identified at Sites 3-7 and ESP marker at Sites 4 and 6-8. Due to the presence of at least two human markers, all downstream sites (Sites 3-8) appeared to have a strong human faecal contamination component, while two upstream sites from Halifax Street Footbridge (Sites 1 and 2) appeared to have been contaminated only by animal-associated faecal material.

### *2.2.3. Synopsis*

The lower Maitai River appears to carry a constant signature of animal faecal contamination (Figure 5). As it is not clear whether it is from livestock, pets and/or wildlife, application of more specific markers is warranted. Explicit human faecal impact was detected in all samples downstream of the Halifax Street Footbridge (Figure 5). The stormwater runoff line at Site 5 warrants investigation for possible direct sewage leakage due to the high bacterial counts and presence of human markers. The origin of human faecal contamination is not clear, but leaking sewage collection systems or cross-connections that impact the groundwater and/or stormwater systems are likely pathways. Quantitative real-time amplification techniques would be warranted to provide added focus for remedial action. This is the first case where HPYV marker tests have been applied and detected in New Zealand surface waters.

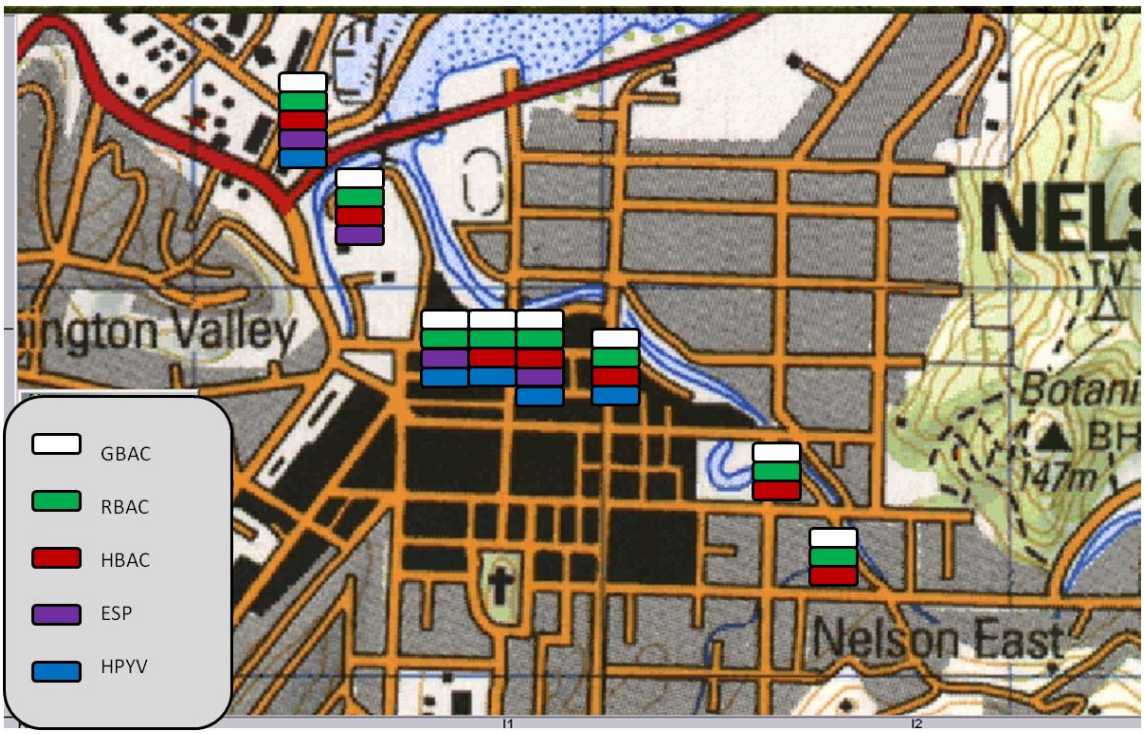


**Figure 9.** Distribution of MST markers in the Maitai River.

## 2.3. Application of markers in the Little Sydney Stream

The Little Sydney Stream, near the town of Motueka, was sampled in order to extend the evaluation of MST markers to a contrasting, predominantly rural catchment as compared to the urban-influenced lower Maitai catchment. Land uses in the Little Sydney catchment include horticulture, cattle and sheep grazing, and low-density residential.

### *2.3.1. Methods*

Water samples were collected at three sites on 19 December 2007 (Figure. 6). The historic sampling point on Factory Road (Figure 10) is a roadside ditch where elevated faecal coliform concentrations had previously been observed (Young *et al.* 2005), therefore ensuring a strong signal. The tributary was flowing well due to heavy rainfall on the previous night. Samples were kept on ice and analysed within three hours of collection. Samples were analysed for bacteriological indicators and MST markers as in Section 2.2.1

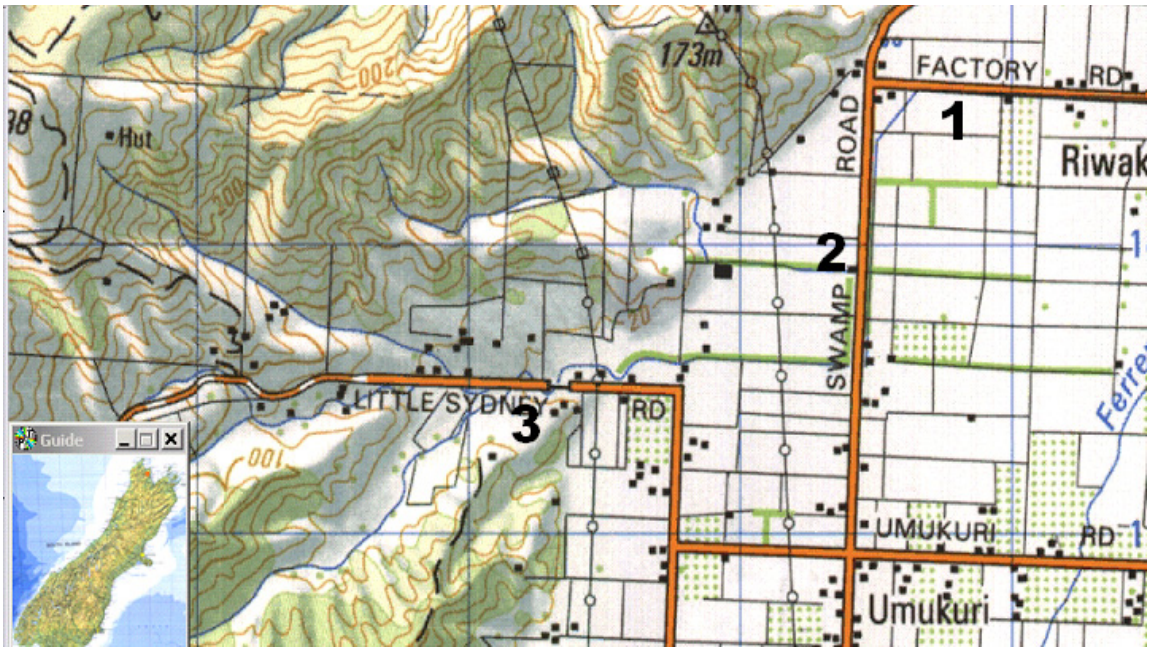


**Figure 10.** Little Sydney Stream sampling sites (1 – Little Sydney at Factory Road., 2 - Little Sydney at Swamp Road., 3 - Little Sydney at Little Sydney Road.)

### *2.3.2. Results*

At all sites studied, elevated concentrations of faecal coliforms and enterococci were detected (Table 5). There was a strong signal of animal-associated faecal contamination in all the samples collected, based on the ruminant RBAC primer sets (Appendix 5, Table 6). Although HBAC marker was found at Little Sydney Sites 1 and 2, the human component could not be confirmed at those locations due to possible cross-reactivity (see Section 2.1.2). The human ESP and HPYV markers were not detected.

**Table 5.** Concentrations of faecal coliforms and enterococci in the Little Sydney Stream.

| Indicator | Event | Site | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Faecal coliforms (CFU/100 ml) | 19/12/2007 | 3900 | 4000 | 6200 |
| Enterococci (CFU/100 ml) | 19/12/2007 | 1600 | 1200 | 800 |

It should be noted that the strongest RBAC signal was detected at Site 2, which was located in close proximity to the cattle farm. Again, quantitative real-time DNA amplification techniques would be required to track the signal to the specific location. The strong animal component was expected at Little Sydney sites due to the rural location sampled. The presence of

ruminant-associated signal and lack of human-associated faecal contamination suggests that these markers could have high discriminative power in the field.

**Table 6.** Interpretation of composite gel image (Little Sydney Stream, Appendix 5).

| | Site | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| **GBAC** | + | + | + |
| **RBAC** | + | + | + |
| **HBAC** | + | + | - |
| **ESP** | - | - | - |
| **HPYV** | - | - | - |
| **Human-associated** | **ND*** | **ND** | **ND** |
| **Animal-associated** | **YES** | **YES** | **YES** |

*ND - not detected

### 2.3.3. *Synopsis*

Animal-associated faecal contamination was identified as a major source in this rural environment (Table 6, Figure 7) indicating the effectiveness of the MST markers used.

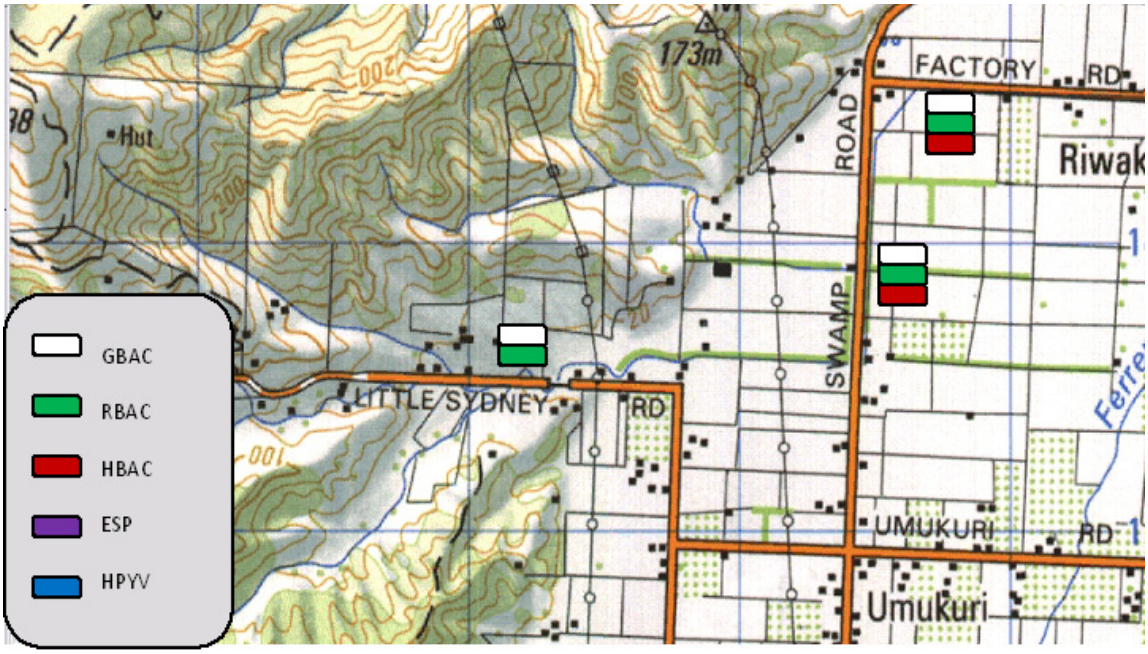


**Figure 11.** Distribution of MST markers in the Little Sydney Stream.

# 3. SUMMARY AND RECOMMENDATIONS

Based on the animals tested, the selected suite of MST markers (GBAC, RBAC, HBAC, ESP, HPYV) could be used in New Zealand to distinguish between animal and human faecal contamination. These markers were used to identify sources of faecal contamination in the lower Maitai River and Little Sydney Stream. While faecal contamination of animal (ruminants and/or rabbits and/or possums and/or horse) origin was detected at all the sites studied, human faecal contamination was detected in all the samples collected downstream of Halifax Street Footbridge in the lower Maitai River.

Primary findings include the following:

- A suite of MST markers previously applied overseas was validated in New Zealand. While RBAC and HPYV primer sets appear to be excellent MST markers, the human HBAC and ESP markers were also detected in samples originating from rabbits and possums indicating some cross reaction.
- A continuous animal signal was detected throughout the lower Maitai River and Little Sydney Stream.
- Human- associated faecal contamination was detected in all the samples collected downstream of Halifax Street Footbridge in the lower Maitai River.
- The origin of human faecal contamination in the Maitai River was not clear, but leaking sewage collection systems or cross-connections that impact the groundwater and/or stormwater systems may have been responsible.

This initial MST investigation provides information on the usefulness of MST markers in the New Zealand context and discriminates between faecal contamination of human and non-human origin in two systems studied. Further, it is important to identify the vectors (stormwater, groundwater *etc*.) via which human faecal contamination enter, as well as locate the initial origin of the problem. It is likely that slow leakage to groundwater from the sewage lines was present, especially when considering the seismic activity of the area. Based on the MST marker as well as bacteriological data, it is also likely that a considerable amount of faecal contamination was flushed from stormwater runoff lines by tides. It is also possible that some human sewage was leaking directly into stormwater lines. Particularly the stormwater line discharging at Site 5 should be further investigated. It should be noted that contaminated groundwater is not an uncommon problem in New Zealand. Nationwide roughly 20% of groundwater monitoring sites (n=520) were reported to be contaminated by faecal material (van Bunnick *et al.* 2007).

Although visual inspection of gels and software-based quantification of signal on gel images can be obtained, the results achieved by those means are semi-quantitative at best. True quantitative results could only be achieved by application of quantitative real-time DNA or RNA amplification techniques. If one wishes to study the quantitative distribution of these markers, application of quantitative RNA and DNA amplification techniques (real-time PCR,

NASBA, TMA *etc*) is strongly recommended. Quantitative results could also be more useful where system-wide modelling and risk estimates are needed. Also more specific animal markers could be used to identify the major groups of animals contributing to the faecal contamination in the systems studied.

The following is recommended:

- Samples for faecal indicator bacteria should ideally be collected year round (*e.g.* on a weekly basis).
- River sediments should also be sampled for faecal indicator bacteria as their replication in sunlight-protected environments is a possibility.
- Groundwater at various locations adjacent to the Maitai River should be tested for faecal indicator bacteria and the presence/absence of MST markers.
- Stormwater runoff linkages discharging to Maitai River at Site 5 on Trafalgar Street should be inspected.
- Application of more specific MST markers (when validated) could further differentiate animal sources.
- Application of real-time quantitative DNA amplification techniques to study the quantitative distribution of MST markers is highly recommended.
- Further MST should be integrated closely with on-going water quality monitoring.
- Further detailed inspection of Maitai River banks could identify additional contaminant pathways.
- Joint analyses and discussion of GIS information with respect to bacteriological and additional field data would provide direction for follow up investigation.

# 4. ACKNOWLEDGEMENTS

This study was facilitated by discussions with Mr Paul Sheldon, Nelson City Council Environmental Monitoring Co-ordinator. We are grateful to Dr Doug Eckery (School of Biological Sciences, Victoria University, Wellington) for assistance with sample collection and to Dr Rachel Noble and Denene Blackwood (Institute of Marine Science, University of North Carolina at Chapel Hill) for guidance on molecular techniques. Funding was provided by the Foundation for Research Science and Technology through Envirolink (NLCC8).

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# 6. APPENDICES

## Appendix 1. Primers

| Assay | Primer Name | Sequence (5'-3') | Amplicon size (bp) |
|---|---|---|---|
| HBAC | HF183 | ATC ATG AGT TCA CAT GTC CG | 525 |
| | Bac708 | CAA TCG GAG TTC TTC GTG | |
| RBAC | CF128 | CCA ACY TTC CCG WTA CTC | 580 |
| | Bac708 | CAA TCG GAG TTC TTC GTG | |
| GBAC | Bac32 | AAC GCT AGC TAC AGG CTT3 | 676 |
| | Bac708 | CAA TCG GAG TTC TTC GTG | |
| ESP | ESPF | TAT GAA AGC AAC AGC ACA AGT T | 680 |
| | ESPR | ACG TCG AAA GTT CGA TTT CC | |
| HPYV | HPyVsF | AGT CTT TAG GGT CTT CTA CC | 172 |
| | HPyVsR | GGT GCC AAC CTA TGG AAC AG | |

## Appendix 2. PCR Conditions

a) *Bacteroides* (General) Assay (HBAC).

| | | | |
|---|---|---|---|
| **Assay:** | *Bacteroides* (General) | **Thermocycler:** | iCycler (Bio-Rad, Hercules, CA) |
| **PCR primers:** | Bac32F<br>Bac708R<br>(Bernhard and Field, 2000) | **Program conditions:** | |
| **PCR mix:** | GoTaq Green Mastermix (Promega, Madison, WI) | **Denaturing:** | 94˚C 45s |
| **PCR rxn volume:** | 25 µl | **Annealing:** | 55˚C 45s |
| **Template volume:** | 2 µl | **Extension:** | 72˚C 30s |
| | | **# of cycles:** | 30 |
| **Other conditions:** | Touchdown: Annealing: initially 65-62˚C: 1˚C/2 cycles, then 62˚C-55˚C: 1˚C/cycle, then as above. | | |
| **Gel conditions:** | Agarose strength: 2%, TAE buffer (1X), Voltage: 5V/cm, MW ladder: GeneRuler 100bp Ladder (Fermentas, Ontario, Canada) | | |

b) *Bacteroides* (Human) Assay (HBAC)

| | | | |
|---|---|---|---|
| **Assay:** | *Bacteroides* (Human) | **Thermocycler:** | iCycler (Bio-Rad, Hercules, CA) |
| **PCR primers:** | HF183F<br>Bac708R<br>(Bernhard and Field, 2000) | **Program conditions:** | |
| **PCR mix:** | GoTaq Green Mastermix (Promega, Madison, WI) | **Denaturing:** | 94˚C 45s |
| **PCR rxn volume:** | 25 µl | **Annealing:** | 55˚C 45s |
| **Template volume:** | 2 µl | **Extension:** | 72˚C 30s |
| | | **# of cycles:** | 30 |
| **Other conditions:** | Touchdown: Annealing: initially 65-62˚C: 1˚C/2 cycles, then 62˚C-55˚C: 1˚C/cycle, then as above. | | |
| **Gel conditions:** | Agarose strength: 2%, TAE buffer (1X), Voltage: 5V/cm, MW ladder: GeneRuler 100bp Ladder (Fermentas, Ontario, Canada) | | |

c) *Bacteroides* (Ruminant) Assay (RBAC)

| | | | |
|---|---|---|---|
| **Assay:** | *Bacteroides* (Ruminant) | **Thermocycler:** | iCycler (Bio-Rad, Hercules, CA) |
| **PCR primers:** | CF128F<br>Bac708R<br>(Bernhard and Field, 2000) | **Program conditions:** | |
| **PCR mix:** | GoTaq Green Mastermix (Promega, Madison, WI) | **Denaturing:** | 94˚C 45s |
| **PCR rxn volume:** | 25 µl | **Annealing:** | 55˚C 45s |
| **Template volume:** | 2 µl | **Extension:** | 72˚C 30s |
| | | **# of cycles:** | 30 |
| **Other conditions:** | Touchdown: Annealing: initially 65-62˚C: 1˚C/2 cycles, then 62˚C-55˚C: 1˚C/cycle, then as above. | | |
| **Gel conditions:** | Agarose strength: 2%, TAE buffer (1X), Voltage: 5V/cm, MW ladder: GeneRuler 100bp Ladder (Fermentas, Ontario, Canada) | | |

d) *esp* Gene (Human) Assay (ESP)

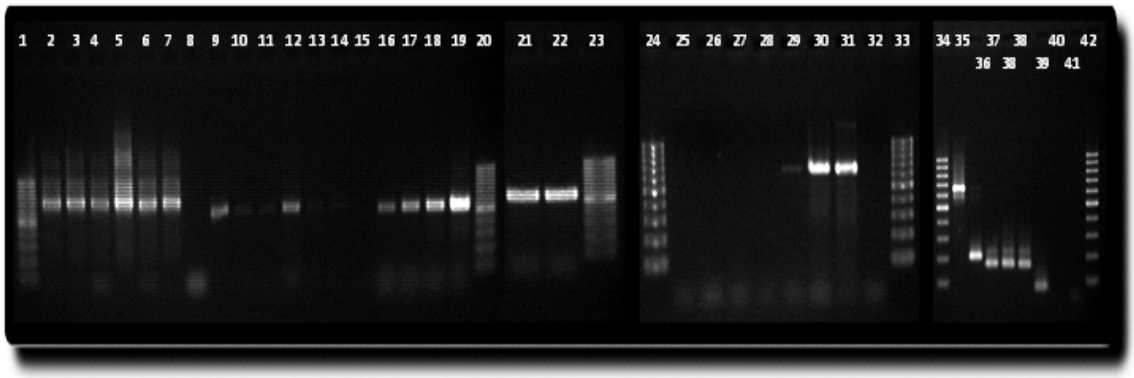
| | | | |
|---|---|---|---|
| **Assay:** | *esp* Gene (Human) | **Thermocycler:** | iCycler (Bio-Rad, Hercules, CA) |
| **PCR primers:** | ESPF<br>ESPR<br>(Scott, et al., 2005) | **Program conditions:** | |
| **PCR mix:** | GoTaq Green Mastermix (Promega, Madison, WI) | **Denaturing:** | 94˚C 60s |
| **PCR rxn volume:** | 25 µl | **Annealing:** | 58˚C 60s |
| **Template volume:** | 2 µl | **Extension:** | 72˚C 60s |
| | | **# of cycles:** | 35 |
| **Other conditions:** | | | |
| **Gel conditions:** | Agarose strength: 2%, TAE buffer (1X), Voltage: 5V/cm, MW ladder: GeneRuler 100bp Ladder (Fermentas, Ontario, Canada) | | |

e) Human Polyomavirus JCV and BKV Assay (HPYV).

| | | | |
|---|---|---|---|
| **Assay:** | Human Polyomaviruses | **Thermocycler:** | iCycler (Bio-Rad, Hercules, CA) |

| | | | |
|---|---|---|---|
| **PCR primers:** | HPyVsF<br>HPyVsR<br>(McQuaig, et al., 2007) | **Program conditions:** | |
| **PCR mix:** | Blue Mastermis (Invitrogen) | **Denaturing:** | 94˚C 20s |
| **PCR rxn volume:** | 45 µl | **Annealing:** | 55˚C 20s |
| **Template volume:** | 4 µl | **Extension:** | 72˚C 20s |
| | | **# of cycles:** | 45 |
| **Other conditions:** | "Nested": 1 µl of PCR product is 'run' yet again using identical primers and PCR conditions. | | |
| **Gel conditions:** | Agarose strength: 3%, TAE buffer (1X), Voltage: 5V/cm, MW ladder: GeneRuler 100bp Ladder (Fermentas, Ontario, Canada) | | |

## Appendix 3. Composite gel images of PCR analyses of the Maitai River samples collected 17 December 2007.




**Sample Legend:**

1. MWM
2. Maitai 1
3. Maitai 2
4. Maitai 3
5. Maitai 5
6. Maitai 7
7. Maitai 8 — **GBAC primers**
8. NTC
9. Maitai 1
10. Maitai 2
11. Maitai 3
12. Maitai 5
13. Maitai 7
14. Maitai 8
15. No template control — **RBAC primers**
16. Maitai 1
17. Maitai 2
18. Maitai 3
19. Maitai 5 — **HBAC primers**
20. MWM
21. Maitai 7
22. Maitai 8

23. MWM
24. MWM
25. Maitai 1
26. Maitai 2
27. Maitai 3
28. Maitai 5
29. Maitai 7
30. Maitai 8
31. Positive control
32. NTC

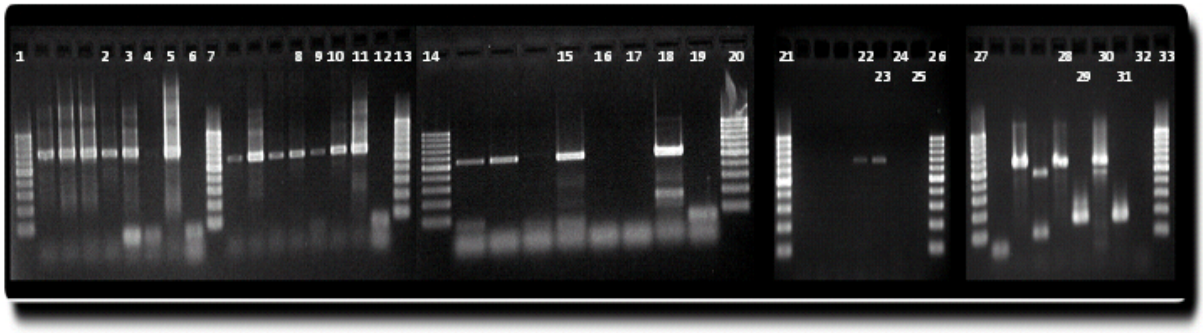
**ESP primers**

33. MWM
34. MWM
35. Maitai 1
36. Maitai 2
37. Maitai 3
38. Maitai 5
39. Maitai 7
40. Maitai 8
41. NTC

**HPYV primers**

42. MWM

## Appendix 4. Composite gel images of PCR analyses of the Maitai River samples collected 19 December 2007

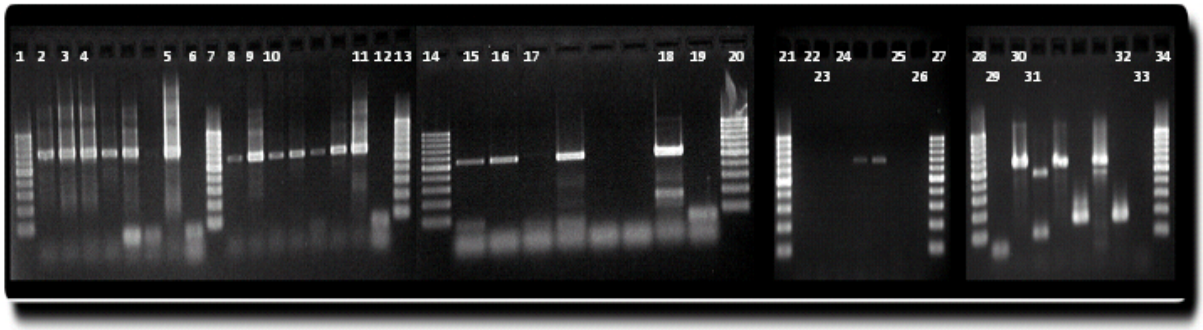



**Sample Legend:**

| | | |
|---|---|---|
| 1 | MWM | |
| 2 | Maitai 4 | **GBAC primers** |
| 3 | Maitai 6 | |
| 4 | Maitai 2 | |
| 5 | Positive control | |
| 6 | NTC | |
| 7 | MWM | |
| 8 | Maitai 4 | **RBAC primers** |
| 9 | Maitai 6 | |
| 10 | Maitai 2 | |
| 11 | Positive control | |
| 12 | NTC | |
| 13 | MWM | |
| 14 | MWM | |
| 15 | Maitai 2 | **HBAC primers** |
| 16 | Maitai 6 | |
| 17 | Maitai 4 | |
| 18 | Positive control | |
| 19 | NTC | |
| 20 | MWM | |

| | | |
|---|---|---|
| 21 | MWM | |
| 22 | Maitai 2 | **ESP primers** |
| 23 | Maitai 6 | |
| 24 | Extraction blank | |
| 25 | NTC | |
| 26 | MWM | |
| 27 | MWM | |
| 28 | Maitai 2 | **HPYV primers** |
| 29 | Maitai 6 | |
| 30 | Maitai 4 | |
| 31 | Positive control | |
| 32 | NTC | |
| 33 | MWM | |

## Appendix 5. Composite gel images of PCR analyses of the Little Sydney Stream samples collected 19 December 2007.




**Sample Legend:**

1. MWM
2. Little Sydney Stream 1
3. Little Sydney Stream 2
4. Little Sydney Stream 3 — **GBAC primers**
5. Positive control
6. No template control

7. MWM
8. Little Sydney Stream 1
9. Little Sydney Stream 2
10. Little Sydney Stream 3 — **RBAC primers**
11. Positive control
12. No template control
13. MW ladder
14. MW ladder
15. Little Sydney Stream 1
16. Little Sydney Stream 2 — **HBAC**
17. Little Sydney Stream 3
18. Positive control
19. No template control
20. MW ladder

21. MW ladder
22. Little Sydney Stream 1
23. Little Sydney Stream 2
24. Little Sydney Stream 3 — **ESP primers**
25. Extraction blank
26. No template control
27. MW Ladder
28. MW ladder
29. Little Sydney Stream 1
30. Little Sydney Stream 2
31. Little Sydney Stream 3 — **HPYV primers**
32. Positive control
33. No template control
34. MW Ladder

**Appendix 6. Envirolink medium advice grant application.**

FOUNDATION FOR RESEARCH SCIENCE & TECHNOLOGY
Tūāpapa Rangahau Pūtaiao

# Envirolink application form for medium advice grants

Please fill out this form if you are applying for a medium advice grant.

This grant is available to the trial Regional Councils for

- expert consultation/ advice for a discrete project; or
- for the second phase of an initial small grant Envirolink project.

The form will be used by the Envirolink Governance Committee to screen the project and by the Foundation to consider the proposal for funding

The Regional Council may prepare this form independently or jointly with the selected research organisation.

The grant covers expenses of up to $20,000, excluding GST and does not cover capital purchases.

Please fill in all sections of this form. Typically the application will not exceed two pages of written text. Please note the following points:

- The answers to questions one to four should contain sufficient information to allow the work to be assessed against the following two criteria - Environmental Benefits and Path to Implementation. Details are explained in the eligibility and assessment criteria for medium advice grants, visit http://www.frst.govt.nz/research/Envirolink_medium.cfm

- In the budget section, please itemise hourly rates and number of hours for each external resource, including costs for external facilitators or experts and any other external costs such as travel and materials.

The Regional Council Advice number allows tracking of the advice path. Please use the designated code for your council name (listed below) and a unique number.

Northland Regional Council — (NLRC)
Gisborne District Council — (GSDC)
Hawkes Bay Regional Council — (HBRC)
Horizons Regional Council — (HZLC)
Nelson City Council - (NLCC)
Marlborough District Council — (MLDC)

Tasman District Council — (TSDC)
West Coast Regional Council — (WCRC)
Environment Southland — (ESRC)

**Envirolink application for medium advice grants (up to $20,000 excluding GST)**

Regional Council Advice number: NLCC9 Date: 27 June 2007

Regional Council: Nelson City Council

Advice requested by: Paul Sheldon

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Research organisation: Cawthron Institute

Primary contact providing advice: Dr Paul Gillespie

Phone number: 03 5482319 Email address: paul.gillespie@cawthron.org.nz

Type of ecosystem involved _Freshwater

Did this request arise from prior advice received under this scheme? No *(although associated with a previous project (NLCC8)*

Name any other councils who are directly linked to this request:

Please answer all questions so that your application can be fully considered.

| **Short Title**<br>Please title the environmental management issue you are seeking advice on. |
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| Suitability of newly developed microbial source tracking (MST) technologies as a tool for identifying the source of microbial contamination in the lower Maitai River. |

| **1. Work Plan** |
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| Please give an overview of the project that you are proposing and identify the deliverables from this project. (*e.g.* seminar, training, collating research, informal |

verbal consultation, literature survey, or other services)

**Background:**

NCC currently struggle with the ability to improve poor microbial water quality conditions within urban regions of the lower Maitai River. Critical to successful management of the problem, information is required to identify the primary contaminant source(s). This information has not been achievable to date using conventional microbiological testing procedures available in New Zealand.

Dr Valerie Harwood (University of South Florida), who has been largely responsible for developing microbial source tracking standards and capabilities in her home state of Florida and in a number of locations in the USA and Europe, visited Cawthron (March 2007) to assist in development of parallel capabilities in New Zealand and to test the global applicability of a range of genetic markers that have been shown to be effective for identification of microbial contaminant sources in northern hemisphere environments. Four microbial source tracking methods were validated using faecal and water samples from New Zealand. Analytical procedures are now in place and preliminary tests were successful.

**Proposed work:**

Dr Harwood is prepared to return to Cawthron within the next few months (travel funded elsewhere) to facilitate application of the MST procedures to the Maitai case study. In addition to application of the markers previously validated, Dr Harwood will bring newly developed markers (*e.g.* the PCR-based human polyomavirus assay (McQuaig *et al.* (2006) Appl & Env. Microbiol. 72: 7567-7574) for validation in the lower Maitai case study.

Deliverables will include:

- Testing applicability of overseas technology in the New Zealand context,
- Report to NCC and
- Seminar on recent advances in MST technology and its applicability to environmental management in New Zealand.

**2. Context**

Please provide context for why the work is being proposed. To do this you may answer some or all of the following questions:

- How does work fit with other developments being undertaken by your council?
- Does it align with the council's strategy?
- Does it link to previous Envirolink funding or activities undertaken by other councils?

- Why is this beyond business as usual?
- Are there any additional aspects of the issue that needs to be covered?
- Is the information already available elsewhere?

Nelson City Council is a unitary authority and has responsibilities under both the Resource Management Act and Health Act to manage water quality. The Lower Maitai River regularly exceeds MfE/DOH guidelines for contact recreation. Extensive targeted monitoring undertaken in accordance with the Microbiological Water Quality Guidelines 2002 failed to isolate a source for this contamination.

A review of lower Maitai bacteriological monitoring results was completed in May 2007 (Sinton 2007) through Envirolink NLCC8. The review drew attention to a number of potential contaminant sources and concluded that further amelioration could not be considered until the primary source or sources had been identified. Microbial source tracking investigation was specifically recommended.

Within the New Zealand context microbial source tracking is still in its infancy. It is beyond the financial and technical ability of Nelson City Council to pursue without support of the science community.

Introduction and trial of microbial source indicators will provide valuable tools for other regional and district councils struggling with water contamination issues. Many have situations which they have been unable to resolve and improved techniques allowing separation of sources of contamination may assist.

The following two questions are used to help us assess and score the proposed work. The project will be scored against key points which are provided on the Envirolink website - http://www.frst.govt.nz/research/Envirolink_medium.cfm

**3. Environmental benefits of project**

Please explain how the advice sought will contribute to enhanced environmental management by the council, or assist the council to help others to improve their environmental management.

The following questions should be addressed in your explanation:

- If good advice is received and used effectively, how will the environment benefit?
- When might that benefit come about? Will the benefit be sustainable and if so, to what extent? (For example, The advice might affect decision making for all future aquaculture developments in Southland)
- Will the advice stimulate a positive change in how your council operates?

Determination of primary contaminant source(s) in the lower Maitai River will enable Council to focus management efforts more efficiently to improve a long-standing water

quality problem. Depending on what sources are identified, this will provide options for interrupting contaminant pathways and ultimately improving environmental condition. This has not previously been possible using the normal range of faecal indicator analyses that has been standard practice for councils.

Successful application of USA-developed genetic markers and related cutting edge MST technologies will provide sustainable benefit by accessing those that prove to be most appropriate for use in addressing similar (and more complex) water quality issues in the New Zealand environment. Information transfer will be achieved through a seminar, open to all interested parties, describing recent advances in MST technologies and a report to NCC describing the application of MST methods and case study results. A key component of the information transfer will be discussion of the potential of MST to improve water quality management in New Zealand.

Successful completion of this project will also provide a basis for an ongoing contractual relationship with leading MST experts in New Zealand and the USA. Thus Council's enhanced ability to address water quality issues will benefit over the long term.

**4. Implementation of project**

Consider how the new information will be used to influence change and achieve outcomes as discussed in question three above. Show that you have identified a plausible pathway in which the advice sought will be used or passed onto others for use. You should explain and justify your choice of pathway.

We suggest you address the following questions:

- How will the council realise the benefits?
- What happens next with the advice? Who will use it? What will it influence?
- What might it lead to?
- Who will take it up?
- Will you be training others as a result of receiving the advice?
- Have future users made a commitment to use the advice and are they fully aware of its nature?
- Is there any budget commitment to use this advice?

Focus on a long-standing question will enable Council to proceed with the most appropriate method(s) of identifying and managing the offending source(s).

Testing of microbial source markers accessed from the USA and selecting those that are most effective in the New Zealand environment will pave the way for further development of standardised protocols as tools that will potentially change (and greatly improve) the way water quality problems are dealt with by councils throughout New Zealand. In addition, guidance for the use of MST will enable mussel aquaculture developers and wild shellfish harvesters to more effectively assess risks of product

contamination and harvest restriction. Effective tracking abilities will lead to more efficient focus on mitigation of major offending sources.

There are a number of steps required to achieve these changes. The first preliminary step has already been completed. This involved the setting up and calibration of PCR analytical procedures in Cawthron's molecular genetics and microbiology laboratories. The second step involved review of bacteriological monitoring results from the lower Maitai River (Envirolink NLCC8). Microbial source tracking investigation was specifically recommended. These preliminary steps provide lead-in to the work proposed here.

Successful completion of the work proposed here will, in turn, provide lead-in to a subsequent tools development Envirolink application aimed at canvassing the relevant needs of New Zealand councils and providing guidance for application of MST to the range of water quality issues identified. This will include a scoping workshop involving not only councils but also government departments, industry representatives and other interested parties. Preliminary discussions with many of these groups have indicated universal interest in using MST advice.

Estimated budget:

| **Item** | **Description** | **Cost** | **Qty.** | **Total** |
|---|---|---|---|---|
| FTEs | Labour | | 0.1 FTE | 16,000 |
| Subcontractor | | | | 0 |
| Materials | Consumables for PCR analyses | | | 2,000 |
| Travel/ Accommodation | Jodie Harwood's travel is funded by ISAT | | | 0 |
| Other | | | | |
| | | | | |
| | | | | |
| | | | | |
| **Total (GST excluded)** | | | | **20,000** |

Has application has been sighted by your Council's Envirolink Coordinator? Yes

Name of person completing form: Paul Sheldon

The Regional Council Governance Committee will screen your application before it is submitted to the Foundation. Please contact your Regional Council Envirolink coordinator for next steps.

| Comments from Governance Board to Foundation.<br><br>Are there any additional aspects or information the Foundation needs to consider when evaluating this project? |
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